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CALLY Index Is Negatively Associated with Hepatic Steatosis and Liver Fibrosis in US Adults: A Population-Based Study

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Abstract

Background: The C-reactive protein-albumin-lymphocyte (CALLY) index is a novel inflammatory marker that combines CRP, albumin, and lymphocytes, and the development of hepatic steatosis and fibrosis is closely related to inflammation. However, the correlation between the CALLY index and hepatic steatosis and fibrosis is unclear. The aim of this study was to investigate the relationship between CALLY and hepatic steatosis and fibrosis. **Methods:** A dataset from the National Health and Nutrition Examination Survey (NHANES) 2017-2020 was used for a cross-sectional investigation. Multivariate linear regression models were used to examine linear associations between CALLY and Controlled Attenuation Parameters (CAP) and Liver Stiffness Measurement (LSM). Fitted smoothed curves and threshold effects analyses were used to characterize nonlinear relationships. **Results:** This population-based study included 6226 adults aged 20 - 80 years. Based on multivariate linear regression analysis, ln CALLY was negatively associated with liver fibrosis (LSM, $\beta = -0.35$, 95% CI: −0.48, −0.23, $p < 0.0001$) and hepatic steatosis (CAP, $\beta = -7.76$, 95% CI: −9.49, −6.02, $p < 0.001$). **Conclusion:** CALLY is negatively associated with CAP and LSM in US adults. The results suggest that CALLY may be a valuable biomarker for assessing the severity of liver fibrosis and hepatic steatosis in individuals with NAFLD.

Keywords

CALLY Index, Hepatic Steatosis, Liver Fibrosis, NAFLD

1. Background

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic

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liver diseases worldwide, affecting about 25% of the adult population, of which 10% - 30% may progress to non-alcoholic steatohepatitis (NASH) and hepatic fibrosis, ultimately leading to cirrhosis or hepatocellular carcinoma [1]. NAFLD is characterized by the rapid progression of abnormal accumulation of hepatic lipids, hepatocellular damage, necroinflammation, and fibrosis [2]. In the United States, the prevalence of NAFLD continues to climb with the obesity and metabolic syndrome epidemics and has become the second leading cause of liver transplantation [3]. Therefore, early identification of hepatic steatosis and liver fibrosis is critical to intervene in disease progression [4], but current clinical reliance on liver biopsy carries invasive risks. Vibration-controlled transient elastography (VCTE) is being increasingly used clinically as a non-invasive alternative. Recent observational studies have shown that VCTE has high accuracy in assessing hepatic steatosis grading and liver fibrosis staging [5] [6].

The C-reactive protein-albumin-lymphocyte (CALLY) index is a novel inflammatory biomarker designed based on CRP, albumin, and lymphocyte counts [7]. In existing studies, CALLY has been widely used for the prognosis of various malignant tumors, including colorectal cancer [8], esophageal cancer [9], gastric cancer [10], oral cancer [11], breast cancer [12], and non-small cell lung cancer [13]. In addition, the CALLY index has a high predictive value in cardiovascular diseases [14]-[16].

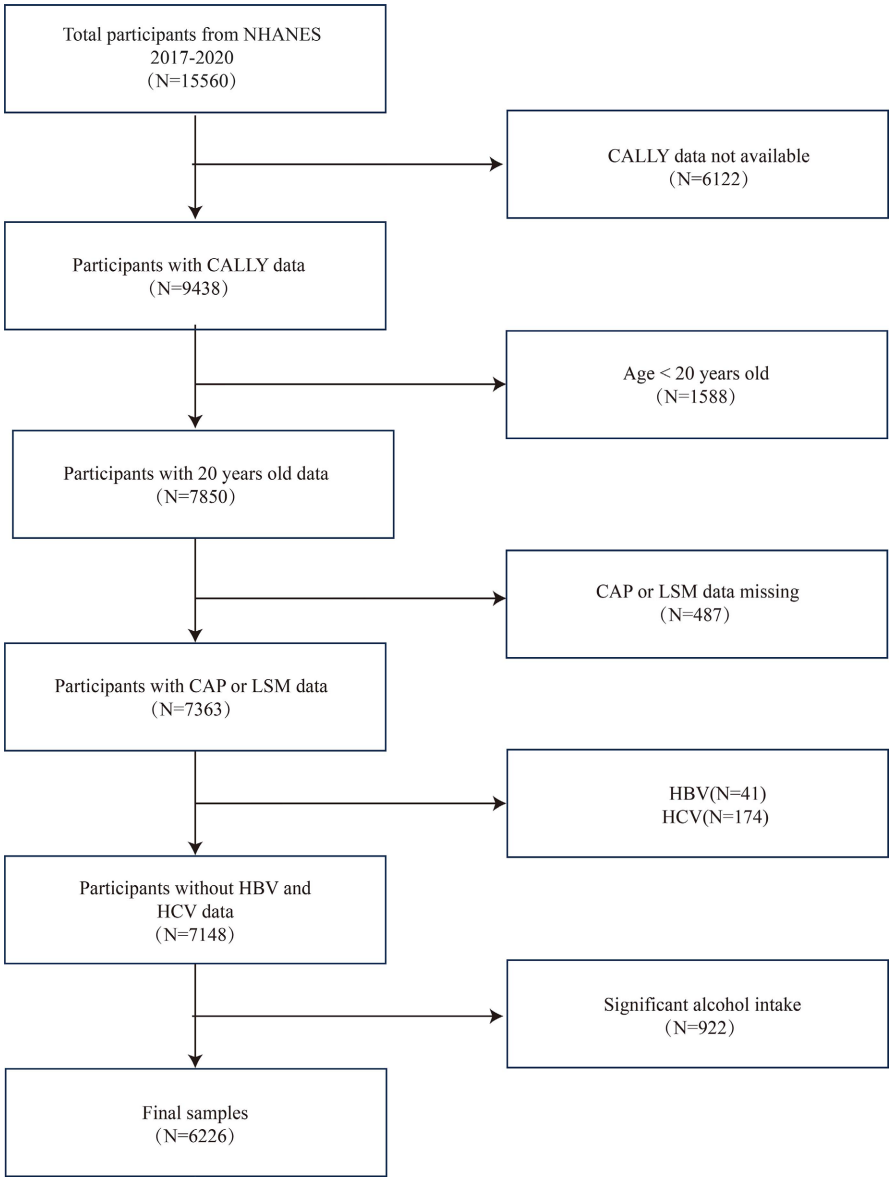
Inflammation is a hallmark of NAFLD progression [17], and the CALLY index plays a role in predicting hepatic steatosis and hepatic fibrosis due to the association between inflammation, immunity, and nutrition. However, currently, no studies have focused on the association between the CALLY index and hepatic steatosis and hepatic fibrosis. To address this research gap, we utilized the National Health and Nutrition Examination Survey (NHANES) to investigate the potential relationship between the CALLY Index and hepatic steatosis and liver fibrosis in adults.

2. Methods

2.1. Study Population

Information for this analysis was obtained from the National Health and Nutrition Examination Survey (NHANES), which is under the jurisdiction of the Centers for Disease Control and Prevention (CDC). The NHANES is overseen by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). A stratified, multistage random sampling strategy was used to ensure that the sample was representative of the national population. Participants underwent a detailed physical examination, completed health and nutrition questionnaires, and provided samples for laboratory analysis. The NHANES protocol was reviewed and approved by the NCHS Ethics Committee [15]. The 2017-2018 and 2019-March 2020 datasets were combined into one analytical sample. We used the NHANES 2-year integrated sample weight (WTMEC2YR), strata (SDMVSTRA), and primary sampling units (SDMVPSU) to account for the complex survey design. Analysis of the 2017 to 2020 NHANES dataset revealed data from 15,560 partici-

pants. Participants were excluded based on the following criteria: 6122 participants with missing CALLY index data, 1588 individuals younger than 20 years of age, 487 participants with missing CAP or LSM data, 41 hepatitis B antigen-positive and 174 hepatitis C antibody-positive or RNA-positive samples, and 922 participants who consumed a significant amount of alcohol (4, 5, or more drinks per day). Finally, this study included 6226 subjects (as shown in Figure 1).



Note: NHANES: National Health and Nutrition Examination Survey; CALLY: CRP-Albumin-Lymphocyte Index; CAP: Controlled Attenuation Parameter; LSM: Liver Stiffness Measurement.

Figure 1. Flowchart of participant selection.

2.2. Study Variables

The outcome variables in this study were CAP and LSM, and the exposure variable

was the CALLY index. NHANES staff evaluated participants for vibration-controlled transient elastography (VCTE) using the FibroScan® equipped Model 502 V2 Touch. According to a recent landmark study, a CAP value (also known as CAP) ≥ 274 dB/m is considered indicative of NAFLD status due to 90% sensitivity in detecting all degrees of hepatic steatosis [6]. CAP ≥ 302 dB/m was defined in this study as severe steatosis. Significant fibrosis was defined as LSM ≥ 8.2 kPa, advanced fibrosis as 9.7 ≤ LSM < 13.6 kPa, and cirrhosis as LSM ≥ 13.6 kPa. [18]. Lymphocyte count (10^9/L) × albumin concentration (g/dL) ÷ [CRP (mg/dL) × 10] was the formula for the CALLY index [8]. CRP values below the lower limit of detection (0.1 mg/dL) were replaced with 0.075 mg/dL. Zero or negative CALLY values were excluded before log transformation. Extreme CALLY values were Winsorized at the 1st and 99th percentiles. Covariates included: sex, age, education, race, household income to poverty ratio, diabetes mellitus, hypertension, smoking status, low-density lipoprotein cholesterol (LDL-C), direct high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, and triglycerides. Smoking status was defined based on the NHANES Smoking Data Questionnaire, and participants were categorized as smokers if they reported smoking at least 100 cigarettes in their lifetime. Diabetes mellitus was defined as a physician diagnosis, or HbA1c > 6.5%, or use of antidiabetic medication or insulin. Hypertension was defined as a physician diagnosis, or mean blood pressure ≥ 140/90 mmHg, or use of antihypertensive medications.

2.3. Statistical Analysis

Statistical analyses for this study were performed using the statistical computing and graphics software R (version 4.1.3) and EmpowerStats (version 2.0). Baseline characteristics of the study population were statistically described by CAP and LSM subgroups. Continuous variables were expressed as mean ± standard deviation (SD) and analyzed by weighted linear regression models. To assess the relationship between CALLY, CAP, and LSM, multivariate linear regression analyses were performed, and beta values and their 95% confidence intervals were calculated. Three models were used for the multivariate analyses: model 1 was an unadjusted model; model 2 adjusted for gender, age, and race; and model 3 adjusted for all covariates. Body mass index (BMI) and waist circumference were not included in the fully adjusted model because they are strongly collinear with diabetes, hypertension, and lipid parameters, which are already in the model. Including adiposity measures could lead to overadjustment and obscure the independent association of the CALLY index. Nevertheless, we acknowledge the absence of direct adiposity adjustment as a limitation of this study (see Discussion). With these adjustments, the models were able to simultaneously perform smooth curve fitting. In addition, a threshold effects analysis was conducted to explore the relationship between CALLY and CAP and its inflection points. The criterion for statistical significance was set at $P < 0.05$. Given the significant skewness of the CALLY

index, the study performed a natural logarithmic transformation (ln CALLY) and stratified the transformed data according to quartiles. This preprocessing method significantly improves the model fit and enhances the robustness and interpretability of the results. Meanwhile, the use of the weighting method effectively reduces the significant volatility of the dataset.

3. Results

In this study, 6226 adults were included based on inclusion and exclusion criteria, and the mean age of the participants was 47.668 years ± 17.297 years. Of these participants, 44.67% were male, 55.33% were female, 12.42% were Mexican American, 34.13% were non-Hispanic white, 24.99% were non-Hispanic black, and 28.46% were from other races. The mean (SD) for CAP was 263.351 dB/m ± 62.846 dB/m, for LSM was 5.757 kPa ± 4.601 kPa, and for CALLY was 1.530 ± 1.176. Table 1 lists all the clinical characteristics of the CAP participants as column-stratified variables. Compared to the non-NAFLD group, the severe steatosis group was more likely to have participants in the lowest quartile. Compared to the non-NAFLD group, the participants in the highest quartile were more likely to be older males, Mexican Americans, and non-Hispanic whites. They also exhibited good education, higher rates of smoking, rates of diabetes and hypertension, and elevated levels of ALT, AST, TC, TG, and LDL, compared with lower levels of HDL. Of particular note, individuals with severe hepatic steatosis had significantly lower ln CALLY values compared with those without NAFLD. In addition, a lower proportion of patients with severe steatosis was found in the highest ln CALLY quartiles (Q3 and Q4), which was significantly different ($P < 0.001$) compared with the non-NAFLD group.

Table 1. Weighted characteristics of the study population based on the controlled attenuated parameter (CAP).

	Non-NAFLD (CAP < 274, n = 3484)	NAFLD (274 ≤ CAP < 302, n = 968)	Severe Steatosis (CAP ≥ 302, n = 1774)	P-Value
Age (Years)	45.65±17.77	50.57±16.62	50.21±16.09	<0.001
Gender (%)				<0.001
Men	40.21	48.48	55.38	
Women	59.79	51.52	44.62	
Race/Ethnicity (%)				<0.001
Mexican American	6.47	11.07	11.52	
Non-Hispanic White	63.39	59.52	63.99	
Non-Hispanic Black	12.07	10.55	7.80	
Other Race	18.07	18.85	16.69	
Education Lever (%)				0.003
Less than High School	3.26	3.68	3.82	
High School	30.28	32.67	35.24	
More than High School	66.46	63.65	60.94	

Continued

Smoked at Least 100 Cigarettes				<0.001
Yes	36.24	33.54	41.95	
No	63.76	66.46	58.05	
Income to Poverty Ratio	3.25 ± 1.64	3.34 ± 1.60	3.15 ± 1.60	0.021
Diabetes				<0.001
Yes	6.39	13.83	28.38	
No	93.61	86.17	71.62	
Hypertension				<0.001
Yes	27.14	42.41	53.84	
No	72.86	57.59	46.16	
ALT (IU/L)	18.52 ± 11.51	22.32± 13.70	29.24 ± 20.09	<0.001
AST (IU/L)	20.08 ± 9.24	21.01 ± 9.94	23.62 ± 13.45	<0.001
Total Cholesterol (mmol/L)	4.79 ± 1.00	4.95 ± 1.07	4.89 ± 1.03	<0.001
Triglyceride (mmol/L)	0.98 ± 0.61	1.44 ± 1.51	1.66 ± 1.12	<0.001
HDL (mmol/L)	1.50 ± 0.40	1.34 ± 0.38	1.21 ± 0.34	<0.001
LDL (mmol/L)	2.81 ± 0.89	2.97 ± 1.04	2.97 ± 0.89	<0.001
Ln CALLY	1.78 ± 1.17	1.39 ± 1.09	1.09 ± 1.08	<0.001
Ln CALLY Quartile				<0.001
Q1	16.79	26.94	32.87	
Q2	20.43	27.28	31.35	
Q3	28.72	24.68	23.36	
Q4	34.06	21.10	12.42	

Mean ± SD for continuous variables: P-value was calculated by a weighted linear regression model. % for categorical variables: P-value was calculated by the weighted chi-square test. LDL-Cholesterol: Low-Density Lipoprotein Cholesterol; HDL-Cholesterol: High-Density Lipoprotein Cholesterol; ALT: Alanine Transaminase; AST: Aspartate Aminotransferase.

Table 2 lists all the clinical characteristics of LSM individuals as column-stratified variables. Compared to the normal group, the cirrhotic group was more likely to be well-educated males exhibiting a higher prevalence of hypertension and diabetes mellitus. As well as elevated ALT, AST, and TG levels, in contrast, they had lower TC and HDL levels. The cirrhosis group had significantly lower ln CALLY than individuals without liver fibrosis, with lower proportions found in the highest ln CALLY quartiles (Q3, Q4, $P < 0.001$).

3.1. Correlation Study between CALLY Index and Hepatic Steatosis

As shown in Table 3, CALLY was significantly and negatively associated with hepatic steatosis in multivariate linear regression models, and this association remained consistent across models: unadjusted (Model 1), partially adjusted (Model 2), and fully adjusted (Model 3). In the fully adjusted model, each 1-unit

Table 2. Weighted characteristics of the study population based on median liver stiffness measurement (LSM).

	Normal Group (LSM < 8.2, n = 6098)	Significant Fibrosis (8.2 ≤ LSM < 9.7, n = 283)	Advanced Fibrosis (9.7 ≤ LSM < 13.6, n = 223)	Cirrhosis (LSM ≥ 13.6, n = 188)	P-Value
Age (Years)	47.21 ± 17.35	51.92 ± 15.78	53.83 ± 16.17	51.61 ± 15.90	<0.001
Gender (%)					<0.001
Men	44.86	52.12	53.07	58.66	
Women	55.14	47.88	46.93	41.34	
Race/Ethnicity (%)					0.9138
Mexican American	8.56	8.94	9.02	8.38	
Non-Hispanic White	62.81	62.00	64.07	68.46	
Non-Hispanic Black	10.68	12.62	9.74	7.99	
Other Race	17.95	16.45	17.17	15.17	
Education Lever (%)					<0.001
Less than High School	3.40	3.89	6.51	2.43	
High School	31.12	38.53	40.57	46.00	
More than High School	65.48	57.57	52.91	51.57	
Smoked at Least 100 Cigarettes					0.0617
Yes	37.21	33.20	44.34	42.79	
No	62.79	66.80	55.66	57.21	
Income to Poverty Ratio	3.25 ± 1.63	3.17 ± 1.56	2.99 ± 1.58	2.93 ± 1.45	0.0253
Diabetes					<0.0001
Yes	11.37	33.03	37.75	42.47	
No	88.63	66.97	62.25	57.53	
Hypertension					<0.0001
Yes	34.97	52.78	60.22	59.11	
No	65.03	47.22	39.78	40.89	
ALT (IU/L)	21.28 ± 14.13	28.09 ± 17.32	31.81 ± 26.61	32.24 ± 26.49	<0.0001
AST (IU/L)	20.65 ± 8.85	24.89 ± 15.44	26.22 ± 16.44	30.18 ± 30.93	<0.0001
Total Cholesterol (mmol/L)	4.85 ± 1.02	4.65 ± 1.00	4.83 ± 1.12	4.69 ± 0.99	0.0075
Triglyceride (mmol/L)	1.21 ± 0.98	1.36 ± 1.11	1.61 ± 1.45	1.38 ± 0.89	0.0011
HDL (mmol/L)	1.41 ± 0.40	1.31 ± 0.37	1.25 ± 0.39	1.20 ± 0.40	<0.0001
LDL (mmol/L)	2.89 ± 0.91	2.73 ± 0.95	2.85 ± 1.13	2.78 ± 0.80	0.3363
Ln CALLY	1.59 ± 1.17	1.22 ± 1.14	0.94 ± 1.10	0.68 ± 1.08	<0.0001
Ln CALLY Quartile					<0.0001
Q1	21.19	29.10	41.09	51.07	
Q2	24.32	26.00	26.71	27.17	
Q3	27.00	30.67	21.11	14.17	
Q4	27.48	14.23	11.08	7.59	

Mean ± SD for continuous variables: P-value was calculated by a weighted linear regression model. % for categorical variables: P-value was calculated by the weighted chi-square test. LDL-cholesterol: Low-Density Lipoprotein Cholesterol; HDL-Cholesterol: High-Density Lipoprotein Cholesterol; ALT: Alanine Transaminase; AST: Aspartate Aminotransferase.

increase in the index was associated with a 7.76 dB/m decrease in CAP units [β = −7.76, 95% CI (−9.49, −6.02), p < 0.001]. Sensitivity analysis was performed by dividing the ln CALLY index into quartiles. In the fully adjusted model, the β coefficients of the ln CALLY index were −7.86, −16.27, and −28.04 for Q2, Q3, and Q4 subjects, respectively, compared to Q1.

Table 3. The association between CALLY and CAP.

	Model 1 β (95% CI)	P-Value	Model 2 β (95% CI)	P-Value	Model 3 β (95% CI)	P-Value
Ln CALLY	−14.85 (−16.12, −13.57)	<0.001	−14.94 (16.20, −13.69)	<0.001	−7.76 (−9.49, −6.02)	<0.001
Ln CALLY Quartile						
Q1	Reference		Reference		Reference	
Q2	−7.11 (−11.46, −2.77)	0.0014	−9.39 (−13.60, −5.19)	<0.001	−7.86 (−13.51, −2.22)	0.0064
Q3	−25.94 (−30.21, −21.68)	<0.001	−29.23 (−33.37, −25.08)	<0.001	−16.27 (−21.72, −10.82)	<0.001
Q4	−46.99 (−51.27, −42.70)	<0.001	−47.47 (−51.67, −43.28)	<0.001	−28.04 (−33.88, −22.20)	<0.001

Model 1: no covariates were adjusted. Model 2: age, gender, and race were adjusted. Model 3: age, gender, race, educational level, family income-to-poverty ratio, smoking status, ALT, AST, total cholesterol, triglyceride, LDL, HDL, diabetes, and hypertension were adjusted.

3.2. Correlation Study between CALLY Index and Liver Fibrosis

As shown in Table 4, the analysis revealed that for each unit increase in the ln CALLY index, there was a corresponding decrease in LSM of −0.35 kPa after adjusting for all covariates [β = −0.35, 95% CI (−0.48, −0.23) < 0.0001]. In addition, the adjusted β coefficients were −0.97, −1.05, and −1.28 for Q2, Q3, and Q4 subjects, respectively, compared with Q1.

Table 4. The association between CALLY and LSM.

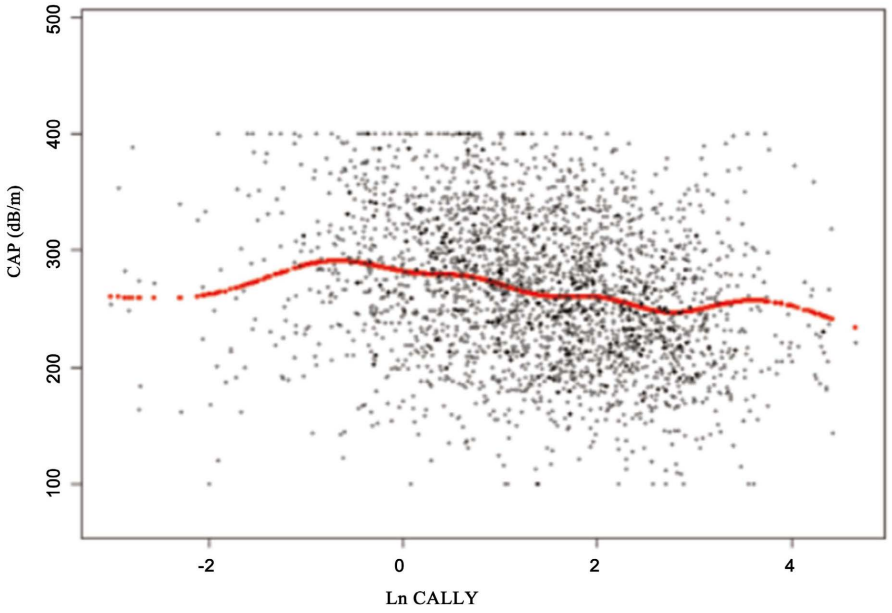
	Model 1 β (95% CI)	P-Value	Model 2 β (95% CI)	P-Value	Model 3 β (95% CI)	P-Value
Ln CALLY	−0.61 (−0.70, −0.51)	<0.001	−0.64 (−0.73, −0.54)	<0.001	−0.35 (−0.48, −0.23)	<0.001
Ln CALLY Quartile						
Q1	Reference		Reference		Reference	
Q2	−1.02 (−1.34, −0.69)	<0.001	−1.07 (−1.40, −0.75)	<0.001	−0.97 (−1.38, −0.56)	<0.001
Q3	−1.66 (−1.98, −1.34)	<0.001	−1.79 (−2.12, −1.47)	<0.001	−1.05 (−1.45, −0.65)	<0.001
Q4	−1.94 (−2.27, −1.62)	<0.001	−2.03 (−2.35, −1.70)	<0.001	−1.28 (−1.71, −0.86)	<0.001

Model 1: no covariates were adjusted. Model 2: age, gender, and race were adjusted. Model 3: age, gender, race, educational level, family income-to-poverty ratio, smoking status, ALT, AST, total cholesterol, triglyceride, LDL, HDL, diabetes, and hypertension were adjusted.

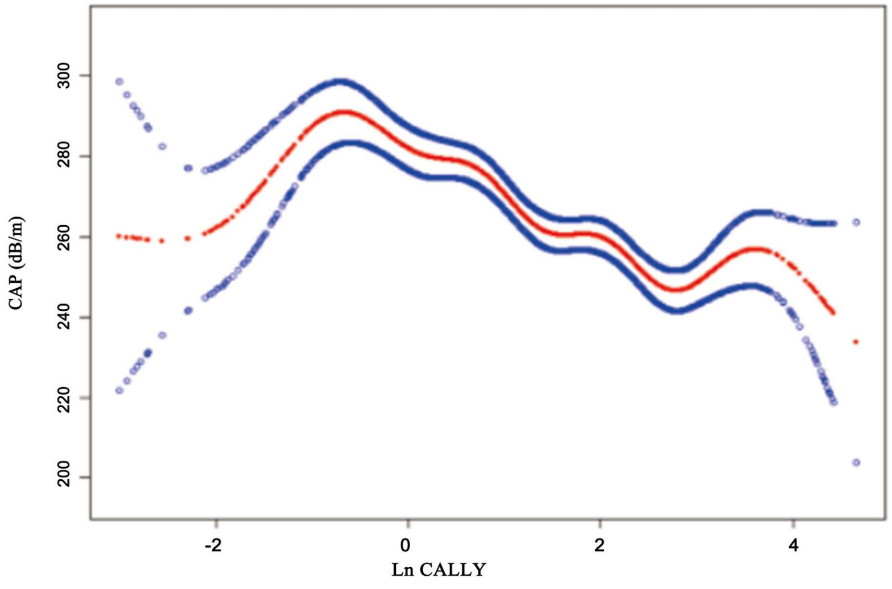
3.3. Nonlinear Relationship between CALLY Index and Hepatic Steatosis and Fibrosis

After adjusting for all variables, a nonlinear relationship between the CALLY in-

dex and CAP was determined, as shown in Figure 2. Specifically, an inverted U-shaped curve was observed between the lnCALLY index and CAP, with an inflection point of −0.59. When the ln CALLY index was below −0.59, the effect value was 15.18, while when the index exceeded −0.59, the effect value was −9.62 (Table 5). This positive slope at low ln CALLY (<−0.59) may be affected by sparse observations in the lower range and should be interpreted cautiously.



(a)



(b)

Figure 2. The smooth curve fit for the association between Ln CALLY index and CAP.

An L-shape between the ln CALLY index and the LSM was also observed when nonlinear modeling was performed (Figure 3). The inflection point was 2.59. The

effect value was −0.46 when the ln CALLY index was below 2.59, while the effect value was not statistically significant when the ln CALLY index exceeded 2.59 (Table 5).

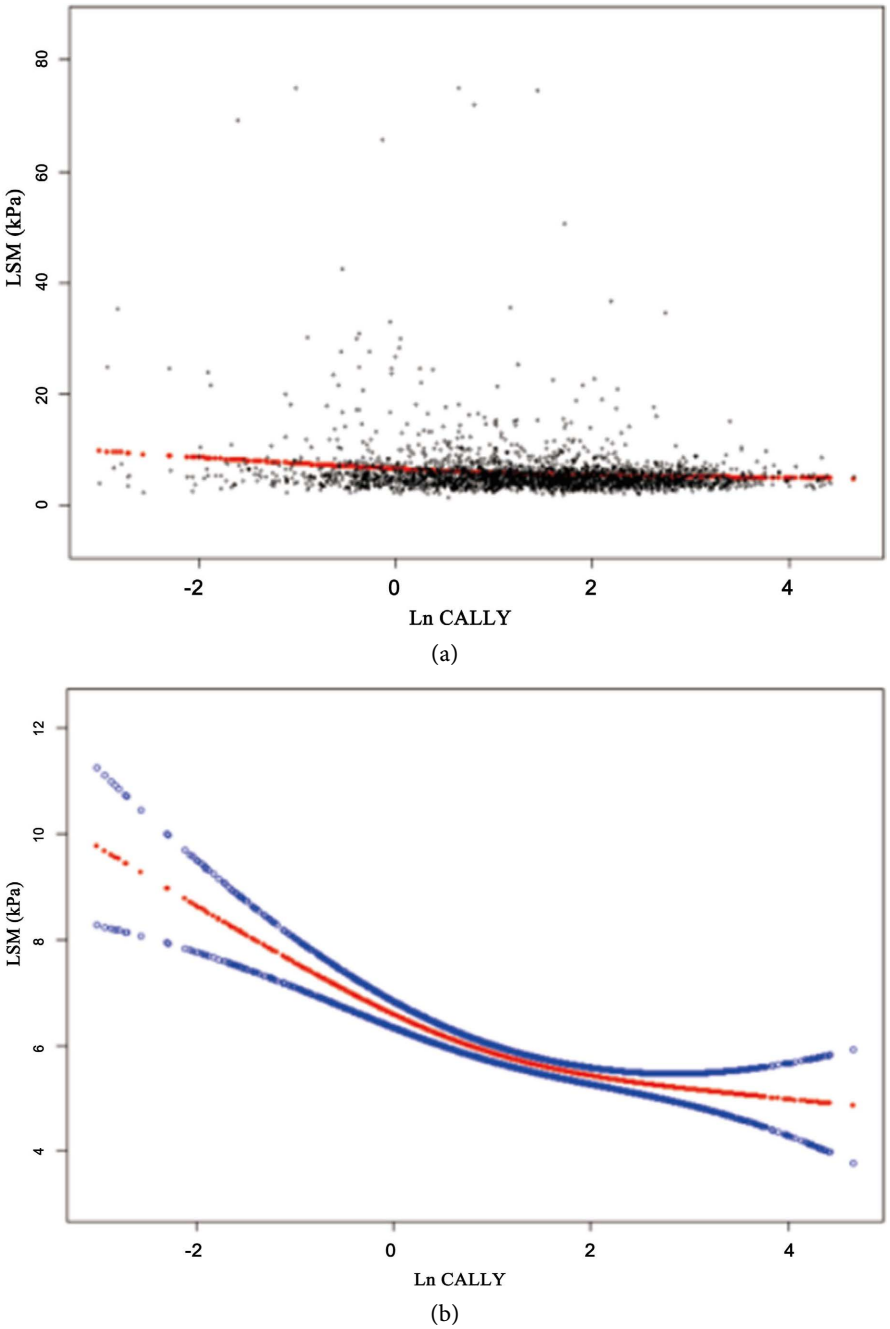


Figure 3. The smooth curve fit for the association between Ln CALLY index and LSM.

The positive association between ln CALLY and CAP below the inflection point (ln CALLY < −0.59) should be interpreted with caution, as it may be driven by sparse data at the lower tail of the distribution. This segment of the curve may not be reliable for clinical inference.

Table 5. Threshold effect analysis of CALLY on CAP and LSM using a two-piecewise linear regression model.

Ln CALLY	CAP Adjusted β (95% CI) P-Value	LSM Adjusted β (95% CI) P-Value
Fitting by the Standard Linear Model	−7.76 (−9.49, −6.02) < 0.0001	−0.35 (−0.48, −0.23) < 0.0001
Fitting by the Two-Piecewise Linear Model		
Inflection Point	−0.59	2.59
<K Segment Effect	15.18 (4.23, 26.13) 0.0066	−0.46 (−0.61, −0.31) < 0.0001
>K Segment Effect	−9.62 (−11.56, −7.68) < 0.0001	0.45 (−0.14, 1.03) 0.1345
Log Likelihood Ratio	<0.001	0.006

4. Discussion

This study provides a comprehensive analysis of the association between the CALLY Index and hepatic steatosis and liver fibrosis in US adults using NHANES data from 2017 to 2020. Analysis of data from 6226 participants showed that the CALLY index was negatively associated with hepatic steatosis and hepatic fibrosis in US adults, even after adjusting for potential confounders through multivariate linear regression. A higher CALLY index was associated with a lower likelihood of angina. Notably, there was an inverted U-shaped (inflection point −0.59) relationship between the ln CALLY index and hepatic steatosis. In addition, there was an L-shaped relationship between the ln CALLY index and hepatic fibrosis, with an inflection point of 2.59. When the ln CALLY index was below the inflection point, an increase in the ln CALLY index was able to reduce the prevalence of hepatic fibrosis, which had a protective effect. To the best of our knowledge, this is the first study to report the relationship between the CALLY index and liver fibrosis and steatosis.

The CALLY index is a multidimensional assessment system integrating inflammatory response, nutritional status, and immune function constructed by Lida's team, and its clinical value has been validated in the prognostic prediction of hepatocellular carcinoma (HCC) [7]. Among them, C-reactive protein (CRP), a classical inflammatory marker, has been shown to be closely associated with the severity of hepatic steatosis. A cohort study that included 393 patients showed that each elevated CRP level was positively associated with hepatic steatosis and fibrosis [19]. A study showed that CRP is an independent risk factor for the development of NAFLD [20]. Albumin is a medium-sized housekeeping protein with various functions such as osmoregulation, antioxidant, and anti-inflammatory, which accounts for more than half of the total serum composition of the human body. Diseases such as cirrhosis are not only associated with decreased albumin synthesis, but also with specific changes in its structure and function [2]. A study of 5.7 million Chinese adults showed that reduced Alb was positively associated with ≥F2, ≥F3, and F4 fibrosis [21]. In addition, existing studies have shown that lymphocytes play a unique role in the process of liver fibrosis and steatosis [22]-[24]. Distinguishing from traditional single biomarkers, the innovation of the CALLY

index lies in the systematic integration of multidimensional pathophysiological processes. For example, although CRP reflects the intensity of hepatic inflammation, it is unable to assess the synthetic function or immunoregulatory status; whereas a decrease in albumin, although suggestive of impaired hepatic function, may mask the underlying inflammatory activity. By combining the three, the CALLY index can more accurately identify people at high risk for the transition from hepatic steatosis to fibrosis, enabling early intervention and improved outcomes. This provides new perspectives and strong evidence for exploring its underlying mechanisms and clinical applications.

NAFLD comprises a disease continuum ranging from steatosis with or without mild inflammation (NAFLD) to non-alcoholic steatohepatitis (NASH), characterized by necroinflammation and a more rapid progression of fibrosis than in NAFLD [25]. However, the role that inflammation plays in the progression of NAFLD is unclear. Various factors, such as lipotoxicity and LPS, activate NLRP3 inflammatory vesicles through the TLR4/NF-κB pathway or a series of pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP), which in turn produce large amounts of IL-1β and IL-18, leading to inflammatory responses and cellular pyropoiesis, thus exacerbating liver injury [26]. Meanwhile, activation of inflammatory signaling pathways such as JNK, IKK/NF-κB, and JAK/STAT can cause insulin resistance and promote further development of hepatic steatosis and fibrosis [27]. Activation of innate immunity also promotes infiltration and aggregation of inflammatory cells in the liver, exacerbating liver inflammation and injury. For example, in the early stages of liver injury, Kupffer cells (KC) rapidly acquire a pro-inflammatory phenotype through the secretion of tumor necrosis factor and chemokines that trigger the recruitment of circulating monocyte-derived macrophages, which in turn exacerbate the onset and progression of NASH and liver fibrosis [28]. Neutrophils, on the other hand, drive hepatic metabolic inflammation by forming neutrophil extracellular traps (NETs) that interact with other pro-inflammatory immune cells [29]. In addition, NK cells have antifibrotic properties by secreting interferon γ to kill activated HSC and help to clear senescent activated hepatic stellate cells. Several studies have shown that the increased number of NK cells in NASH patients may be associated with elevated levels of NK cell-activating cytokines (e.g., IL-2, IL-12) [30]. Natural killer T (NKT) cells have a dual effect in NASH; they not only play an antifibrotic role by killing activated HSC, but may also accumulate in progressive NASH and promote the fibrotic process [31].

This study ensured national representativeness and broad generalizability of the sample through the use of the NHANES database and the assessment of the CALLY Index as a new non-invasive biomarker, which may provide strong support for the assessment of risk of hepatic fibrosis and steatosis. The application of the CALLY Index can assist primary care physicians in identifying high-risk individuals and provides a practical and cost-effective tool. However, despite the clinical relevance demonstrated in this study, some limitations remain. For example, because this

study was a retrospective cross-sectional analysis, causality could not be established, and it was susceptible to inherent bias, while the accuracy of the results may have been affected by the way the data were collected and selection bias. In particular, the study did not take into account the participants' medication use, which is particularly relevant for patients with NAFLD. In addition, the assessment of hepatic steatosis and hepatic fibrosis relies on transient elastography, which, although highly accurate, still differs from biopsy results and may therefore influence the judgment of the extent of liver disease. Furthermore, BMI and waist circumference were not included as covariates due to collinearity with metabolic variables; however, residual confounding by adiposity cannot be ruled out.

Nonetheless, the negative correlation of the CALLY index with hepatic fibrosis and steatosis suggests that individualized therapeutic approaches focusing on reducing inflammation and improving nutritional status in patients with lower indices may be helpful in enhancing metabolic health and preventing the progression of hepatic fibrosis. To further validate the potential of the CALLY index in assessing hepatic fibrosis and hepatic steatosis, future studies still need to overcome the limitations in the current study, such as the effects of temporality and potential confounders, and incorporate other more precise diagnostic methods for in-depth analysis.

5. Conclusion

In conclusion, our study demonstrates a negative association between the CALLY index and hepatic fibrosis and steatosis, supported by a large, nationally representative sample. However, further studies are needed to validate these findings in prospective studies and explore potential mechanisms.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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