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Original article

Non-linear associations of circulating total bilirubin concentration with the risk of nonalcoholic fatty liver disease and all-cause mortality

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ABSTRACT

Introduction and Objectives: Accumulating evidence has supported that mild elevated total bilirubin exerts antioxidant and anti-inflammatory properties in multiple metabolic diseases. We aimed to explore the association of circulating total bilirubin concentration with non-alcoholic fatty liver disease (NAFLD) risk and all-cause mortality and examine the potential nonlinear relationships between them.

Material and Methods: We used nationally representative data from the National Health and Nutrition Examination Survey (NHANES). NAFLD was assessed using the fatty liver index (FLI) and United States fatty liver index (USFLI), respectively.

Results: A total of 35 912 and 17 329 participants were included in FLI-NAFLD (case with NAFLD was diagnosed by FLI) and USFLI-NAFLD (case with NAFLD was diagnosed by USFLI) groups, respectively. The mean age of total population was 46.25 years, and 48.51% were male. Compared to participants with lowest quintile of total bilirubin concentration, those with highest quintile had lower risk of NAFLD in both FLI-NAFLD (OR: 0.48, 95% CI: 0.40, 0.59) and USFLI-NAFLD (OR: 0.55, 95% CI: 0.43, 0.70) groups. Compared to participants with lowest quintile of total bilirubin concentration, the association between total bilirubin concentration and all-cause mortality was not significant among those with highest quintile of total bilirubin concentration (HR: 0.89, 95% CI: 0.66, 1.20). The restricted spline curves showed the nonlinear U-shaped association of total bilirubin concentration with NAFLD risk and all-cause mortality. The segmented linear regression analysis showed negative associations between total bilirubin concentration and risk of NAFLD in both FLI-NAFLD (OR: 0.94, 95% CI: 0.93, 0.95) and USFLI-NAFLD (OR: 0.95, 95% CI: 0.93, 0.96) groups when total bilirubin concentration was below the turning point (FLI-NAFLD: 18.81 μmol/L; USFLI-NAFLD: 15.39 μmol/L) and these associations were not significant when total bilirubin concentration was higher than the turning point. Furthermore, all-cause mortality decreased (OR: 0.97, 95%CI: 0.95, 1.00) with increased total bilirubin concentration up to the turning point (11.97 μmol/L), and then all-cause mortality increased with increasing total bilirubin concentration (OR: 1.03, 95%CI: 1.02, 1.04).

Conclusions: We found that higher circulating total bilirubin concentration within the physiological range was associated with decreased risk of NAFLD and all-cause mortality among NAFLD patients.

1. Introduction

Over the past decades, nonalcoholic fatty liver disease (NAFLD) has become the most prevalent hepatic disease worldwide, affecting 25% of the adult population [1]. NAFLD, as a major cause of cirrhosis and hepatocellular carcinoma, has been determined to cause considerable liver-related and extrahepatic morbidity and mortality [2,3]. The conventional methods of diagnosing NAFLD have shortcomings. Liver biopsy is invasive, and imaging technologies such as

Abbreviations: NAFLD, nonalcoholic fatty liver disease; CT, computed tomography; MRI, magnetic resonance imaging; FLI, fatty liver index; USFLI, United States FLI; NHANES, the National Health and Nutrition Examination Survey; NCHS, the National Center for Health Statistics; CDC, Centers for Disease Control and Prevention; BMI, body mass index; WC, waist circumference; TG, triglycerides; GGT, gamma-glutamyl transferase; ORs, odds ratios; CIs, confidence intervals; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase

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ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) are expensive and not suitable for large-scale population-based studies [4]. Recently, noninvasive diagnostic methods based on clinical parameters with routine laboratory tests have been developed. Of these, the fatty liver index (FLI) and United States FLI (USFLI) are simple and reliable tools with high diagnostic accuracy for NAFLD [5,6].

The pathophysiology of NAFLD is highly complex and involves diverse factors, including metabolic disturbances, oxidative stress, and inflammation [7]. Bilirubin, the metabolic end product of heme catabolism, is known to have prominent antioxidant and anti-inflammatory properties [8–11]. Furthermore, bilirubin also has potent immunosuppressive effects associated with long-term pathological and physiological sequelae [12]. Therefore, the association between bilirubin and NAFLD has received considerable attention recently; however, most of observational studies have provided inconsistent evidence and have been limited to the Asian population or small sample size. For example, two recent studies involving the general population have demonstrated that serum bilirubin levels were negatively associated with the risk of NAFLD, indicating that bilirubin might be a protective marker for NAFLD [4,13]. By contrast, several cross-sectional studies have reported that serum bilirubin levels were unlikely to be associated with NAFLD in nonobese patients or obese children [14,15]. Furthermore, evidence from an Asian population indicated that total and indirect bilirubin were not associated with the risk of NAFLD [16,17].

For decades, bilirubin was believed to be an ominous sign of liver disease; however, recent accumulating evidence has supported the hypothesis of the nonlinear U-shaped relationships between circulating bilirubin concentration and oxidative stress-related disorders such as coronary heart disease, ischemic heart disease, diabetic complications, malignancy, and telomere length [18–21]. These findings indicate that bilirubin, when only moderately elevated, may exert a protective effect on oxidative stress-related disorders [22–24]. To our knowledge, whether the relationship between bilirubin concentration and the risk of NAFLD is nonlinear remains unclear.

To fill this knowledge gap, we explored the association between circulating total bilirubin concentration and risk of NAFLD and examined their potential nonlinear relationship by using nationally representative data from the National Health and Nutrition Examination Survey (NHANES) in the United States. In addition, given that NAFLD is one of the leading causes of death, we explored the association between total bilirubin concentration and mortality among patients with NAFLD.

2. Materials and Methods

2.1. Study design and population

This study was based on data from the NHANES 2005-2018, which was conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC). To ensure representative data of the health and nutritional status of the noninstitutionalized civilian United States population, NHANES data were collected with the use of a complex, multistage, and stratified sampling design. The NHANES interviews collected data on demographics, dietary intake information, physical examinations, and laboratory examinations. Data from the survey and operation details are publicly available from the CDC at https://www.cdc.gov/nchs/nhanes/.htm. Survey protocols were reviewed and approved by the NCHS Research Ethics Review Board, and all participants provided written informed consent.

Of the 70190 participants in NHANES 1999-2016, 28047 were excluded due to young age (<18 years). We then excluded 4342 with missing data on total bilirubin concentration and 1889 who had insufficient data for FLI. Finally, a total of 35912 participants were included in FLI-NAFLD (case with NAFLD was diagnosed by FLI) group. In addition, after excluding participants who had insufficient data for USFLI, data on 17329 participants were included in the USFLI-NAFLD (cases with NAFLD was diagnosed by USFLI) group.

2.2. Exposure measures

Based on the NHANES Laboratory Procedures, Total bilirubin concentration (μmol/L) was measured by using a timed endpoint diazo method on Beckman Synchron LX20. In the reaction, bilirubin reacts with diazo reagent in the presence of caffeine, benzoate, and acetate as promoters to produce azobilirubin. A colorimetric analysis at 520 nm at a fixed time interval was conducted by LX20. The change in absorbance is directly proportional to the total bilirubin concentration in the sample.

2.3. NAFLD definition

The following NAFLD-related markers were included in the current study: body mass index (BMI), waist circumference (WC), triglycerides (TG), gamma-glutamyl transferase (GGT), insulin, and glucose. The presence of NAFLD was predicted using FLI and USFLI. The FLI is a biochemical model that predicts the presence of NAFLD. It had been widely used in epidemiologic studies and was recommended by European guidelines for the definition of NAFLD [25]. A FLI cutoff value of ≥ 60 was used to denote NAFLD [26]. In addition, NAFLD was determined by using the improved FLI for the multiethnic U.S. population, and a USFLI cutoff value of ≥ 30 was used to denote NAFLD [27]. The FLI and USFLI were calculated using the following established formulas:

$$FLI = (e^{0.953} \times \log_e[TG] + 0.139 \times BMI + 0.718 \times \log_e[GGT] + 0.053 \times WC - 15.745) / (1 + e^{0.953} \times \log_e[TG] + 0.139 \times BMI + 0.718 \times \log_e[GGT] + 0.053 \times WC - 15.745) \times 100$$

$$USFLI = (e^{-0.8073} \times \text{Non-Hispanic Black} + 0.3458 \times \text{Mexican American} + 0.0093 \times Age + 0.6151 \times \log_e[GGT] + 0.0249 \times WC + 1.1792 \times \log_e[Insulin] + 0.8242 \times \log_e[Glucose] - 14.7812) / (1 + e^{-0.8073} \times \text{Non-Hispanic Black} + 0.3458 \times \text{Mexican American} + 0.0093 \times Age + 0.6151 \times \log_e[GGT] + 0.0249 \times WC + 1.1792 \times \log_e[Insulin] + 0.8242 \times \log_e[Glucose] - 14.7812) \times 100$$

2.4. Ascertainment of mortality

Mortality information was collected from the date of survey participation through 31 December 2018 and ascertained by linking the death certificate records to the National Death Index. Other sources of follow-up for mortality included the United States Social Security Administration, the Centers for Medicare and Medicaid Services, and death certificates.

2.5. Statistical analysis

Data were analyzed using appropriate sampling weights and accounted for the unequal probability of selection and oversampling of certain subpopulations. Descriptive statistics were used to compare the characteristics of participants by quintiles of total bilirubin concentration using ANOVA for continuous variables and chi-square test for categorical variables. Multivariable logistic regressions were used to estimate the odds ratios (ORs) with 95% confidence intervals (CIs) for the association between total bilirubin concentration and the risk of NAFLD. Multivariable Cox regressions were used to estimate the hazard ratios (HRs) with 95% CIs for the association between total bilirubin concentration and all-cause mortality among patients with NAFLD. Models were adjusted for age (continuous), sex (male or female), ethnicity (non-Hispanic white, black, Mexican American, Hispanic, and other ethnicity), education status (high school and below, more than high school, or missing), BMI (<18.5 kg/m^2, 18.5 to

<25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2, or missing), smoking status (never, past, current, or missing), alcohol drinking (yes, no, or missing), leisure time physical activity (<500 met/week, 500 to <1000 met/week, ≥1000 met/week, or missing), alkaline phosphatase (ALP) (low, medium, high, or missing), aspartate aminotransferase (AST) (low, medium, high, or missing), alanine aminotransferase (ALT) (low, medium, high, or missing), GGT (low, medium, high, or missing), diabetes (yes, no, or missing), hypertension (yes, no, or missing), and hypercholesterolemia (yes, no, or missing). Restricted cubic splines were used to examine the dose-response associations between total bilirubin concentration and risk of NAFLD and all-cause mortality among patients with NAFLD, and the segmented linear regression was used to calculate the threshold effect. In the exploratory analyses, subgroup and sensitivity analyses were conducted according to the potential confounding variables. All analyses were performed with R software 3.6.2. Statistical significance was indicated by a two-sided *P* value < 0.05.

2.6. Ethical statements

Written informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Ethics Committee of the National Center for Health Statistics Ethics Review Board (NHANES 2005-2010: Protocol #2005-06; NHANES 2011-2016: Protocol #2011-17; NHANES 2017-2018: Protocol #2011-17 and Protocol #2018-01).

3. Results

The weighted demographic characteristics of the study population are summarized in Table 1. The mean age was 46.25 years, and 48.51% were male. Compared with participants without NAFLD, those with NAFLD have lower total bilirubin concentration ($P < 0.001$). Subgroup analysis according to sex showed similar results (Supplementary Table 1). Significant differences in age, sex, ethnicity, education status, BMI, smoking status, alcohol drinking, and history of diabetes, hypertension, and hypercholesterolemia were observed between quintiles of circulating total bilirubin concentration ($P < 0.05$). Compared to participants in total bilirubin concentration quintile 1, those in quintile 5 were more likely to be male, non-Hispanic white, never smokers, and alcohol drinkers, have higher education level, leisure time physical activity, AST, ALT, GGT, and prevalence of hypercholesterolemia, and have lower BMI level, ALP, and prevalence of diabetes and hypertension.

The associations between circulating total bilirubin concentration and risk of NAFLD are shown in Table 2. In multivariable adjusted model, per 1 μmol/L increase in serum bilirubin concentrations were

Table 1
Baseline characteristics of the study populations (*n*=35 912).

Characteristics	Total bilirubin concentration (μmol/L)						*P* value
	Total population	Quintile 1 (≤5.81)	Quintile 2 (5.82-8.55)	Quintile 3 (8.56-10.26)	Quintile 4 (10.27-14.54)	Quintile 5 (≥14.55)	
Age (years)	46.25 ± 0.23	45.81 ± 0.47	45.68 ± 0.29	46.54 ± 0.32	47.00 ± 0.31	46.08 ± 0.33	<0.001
Male, n (%)	48.51	29.70	36.01	45.10	55.13	69.41	<0.001
Ethnicity, n (%)							<0.001
Non-Hispanic white	67.24	61.33	63.03	66.67	70.72	72.10	
Black	10.80	13.30	12.80	11.25	9.47	8.16	
Mexican American	8.76	9.76	9.88	9.29	7.72	7.67	
Other Hispanic	5.58	6.31	6.77	5.48	4.78	4.72	
Others	7.62	9.30	7.53	7.30	7.32	7.34	
Educational status, n (%)							<0.001
High school and below	39.82	41.82	41.79	40.46	39.47	36.17	
More than high school	60.18	58.18	58.21	59.54	60.53	63.83	
Body mass index, n (%)							<0.001
<18.5 kg/m^2	1.69	1.25	1.61	1.69	1.76	1.95	
18.5 to <25 kg/m^2	29.05	21.59	26.97	29.27	30.42	34.12	
25 to <30 kg/m^2	32.83	27.38	30.70	31.95	35.47	36.14	
≥30 kg/m^2	36.43	49.79	40.72	37.09	32.34	27.79	
Smoking status, n (%)							<0.001
Never	55.53	55.65	54.55	53.97	54.82	58.70	
Past	24.34	20.59	23.25	23.87	26.04	26.26	
Current	20.12	23.76	22.20	22.16	19.13	15.04	
Alcohol drinking, n (%)	76.78	69.55	73.69	76.46	78.29	80.76	
Leisure time physical activity, n (%)							<0.001
<500 met/week	57.65	66.27	60.51	59.62	54.96	50.68	
500 to <1000 met/week	12.85	10.79	12.28	14.14	13.07	13.56	
≥1000 met/week	29.50	22.94	27.21	26.24	31.96	35.76	
Alkaline phosphatase, IU/L	68.56 ± 0.24	75.01 ± 0.60	69.40 ± 0.43	68.65 ± 0.35	66.87 ± 0.38	65.75 ± 0.39	<0.001
Aspartate aminotransferase, U/L	25.19 ± 0.10	21.84 ± 0.19	23.84 ± 0.17	24.68 ± 0.21	26.05 ± 0.17	28.13 ±0.31	<0.001
Alanine aminotransferase, U/L	25.17 ± 0.13	21.74 ± 0.30	23.45 ± 0.19	24.55 ± 0.27	26.23 ± 0.24	28.49 ± 0.38	<0.001
Gamma glutamyl transferase, U/L	27.63 ± 0.26	25.23 ± 0.50	25.61 ± 0.39	26.68 ± 0.47	28.37 ± 0.55	31.30 ± 0.890	<0.001
Diabetes, n (%)	9.11	11.36	10.56	9.06	8.23	7.15	<0.001
Hypertension, n (%)	30.35	32.27	30.24	30.58	30.91	28.55	<0.001
Hypercholesterolemia, n (%)	36.05	31.79	34.48	36.41	39.30	36.80	<0.001

Table 2
Association between total bilirubin concentration and risk of non-alcoholic fatty liver disease.

Total bilirubin concentration (μmol/L)	NAFLD	Without NAFLD	Crude model	Age, sex-adjusted model	Multivariable adjusted model
			Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
FLI-NAFLD					
Total bilirubin (continuous)			0.97 (0.96, 0.97)	0.95 (0.94, 0.96)	0.96 (0.95, 0.97)
Total bilirubin					
Quintile 1	2347 (14.16)	1990 (10.29)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Quintile 2	4701 (28.36)	4917 (25.43)	0.75 (0.67, 0.84)	0.71 (0.63, 0.79)	0.82 (0.70, 0.96)
Quintile 3	2763 (16.67)	3047 (15.76)	0.71 (0.62, 0.81)	0.61 (0.54, 0.70)	0.73 (0.60, 0.89)
Quintile 4	3880 (23.41)	4949 (25.59)	0.63 (0.56, 0.72)	0.50 (0.43, 0.57)	0.61 (0.50, 0.73)
Quintile 5	2885 (17.40)	4433 (22.93)	0.54 (0.48, 0.62)	0.39 (0.34, 0.45)	0.48 (0.40, 0.59)
P for trend			<0.001	<0.001	<0.001
USFLI-NAFLD					
Total bilirubin (continuous)			0.97 (0.97, 0.98)	0.95 (0.94, 0.96)	0.96 (0.95, 0.98)
Total bilirubin, quintile					
Quintile 1	611 (10.79)	1059 (9.08)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Quintile 2	1451 (25.62)	2709 (23.22)	0.86 (0.74, 1.00)	0.80 (0.69, 0.93)	0.96 (0.78, 1.19)
Quintile 3	906 (16.00)	1832 (15.70)	0.83 (0.70, 0.97)	0.72 (0.61, 0.85)	0.83 (0.65, 1.07)
Quintile 4	1489 (26.29)	3116 (26.71)	0.75 (0.64, 0.88)	0.60 (0.50, 0.71)	0.73 (0.59, 0.89)
Quintile 5	1206 (21.30)	2950 (25.29)	0.64 (0.54, 0.74)	0.45 (0.38, 0.54)	0.55 (0.43, 0.70)
P for trend			<0.001	<0.001	<0.001

* Adjust for: age (continuous), sex (male or female), ethnicity (non-Hispanic white, black, Mexican American, other Hispanic, other ethnicities), education status (high school and below, more than high school, or missing), body mass index (<18.5 kg/m^2, 18.5 to <25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2, or missing), smoking status (never, past, current, or missing), alcohol drinking (yes, no, or missing), leisure time physical activity (<500 met/week, 500 to <1000 met/week, ≥1000 met/week, or missing), alkaline phosphatase (low, medium, high, or missing), aspartate aminotransferase (low, medium, high, or missing), alanine aminotransferase (low, medium, high, or missing), gamma glutamyl transferase (low, medium, high, or missing), diabetes (yes, no, or missing), hypertension (yes, no, or missing), and hypercholesterolemia (yes, no, or missing).

negatively associated with risk of NAFLD in both of FLI-NAFLD (OR: 0.96, 95% CI: 0.95, 0.97) and USFLI- NAFLD (OR: 0.96, 95% CI: 0.95, 0.98) groups. Furthermore, compared to participants with lowest quintile of total bilirubin concentration, those with highest quintile had lower risk of NAFLD in both FLI-NAFLD (OR: 0.48, 95% CI: 0.40, 0.59) and USFLI-NAFLD (OR: 0.55, 95% CI: 0.43, 0.70) groups. Similar results were also observed in crude and age, sex-adjusted models. The association between total bilirubin concentration and all-cause mortality among patients with NAFLD is shown in Table 3. Compared to participants with lowest quintile of total bilirubin concentration, the association between total bilirubin concentration and all-cause mortality was not significant among those with highest quintile of total bilirubin concentration (HR: 0.89, 95% CI: 0.66, 1.20).

Fig. 1 shows the restricted spline curves for associations between circulating total bilirubin concentration and risk of NAFLD, and the nonlinear U-shaped associations were observed (all $P_{overall}$ < 0.001; all $P_{nonlinearity}$<0.001). The segmented linear regression analysis showed negative associations between total bilirubin concentration and risk of NAFLD in both of FLI-NAFLD (OR: 0.94, 95% CI: 0.93, 0.95) and USFLI-NAFLD (OR: 0.95, 95% CI: 0.93, 0.96) groups when total bilirubin concentration was below the turning point (FLI-NAFLD: 18.81 μmol/L; USFLI-NAFLD: 15.39 μmol/L) and these associations were not significant when total bilirubin concentration was higher than the turning point. The likelihood ratio test results comparing non-segmented models to the segmented regression model were significant (< 0.01) (Table 4). Furthermore, the restricted spline curves also showed a nonlinear U-shaped relationship between total bilirubin concentration and all-cause mortality among participants with NAFLD ($P_{overall}$ <0.001; $P_{nonlinearity}$ <0.001) (Fig. 2). Threshold effect analysis using segmented linear regression analysis showed that all-cause mortality decreased with increased total bilirubin concentration up to the turning point (11.97 μmol/L) (OR: 0.97, 95% CI: 0.95, 1.00), and then all-cause mortality increased with increasing total bilirubin concentration (OR: 1.03, 95% CI: 1.02, 1.04) (Table 4).

The subgroup analysis results showed that participants with the highest quintile of total bilirubin concentration were negatively associated with risk of FLI-NAFLD across strata of age, sex, race, educational status, BMI, smoking status, alcohol drinking, leisure time physical activity, and ALP, AST, ALT, and GGT levels (Table 5). Furthermore, the results for the risk of USFLI-NAFLD were similar in all subgroups (*P* interaction > 0.05) (Supplementary Table 2). Sensitivity analyses were also conducted by excluding participants with

Table 3
Association between total bilirubin concentration and all-cause mortality among patients with non-alcoholic fatty liver disease.

Total bilirubin concentration (μmol/L)	Death	Alive	Crude model	Age, sex-adjusted model	Multivariable adjusted model
			Hazard ratio (95% CI)	Hazard ratio (95% CI)	Hazard ratio (95% CI)
Total bilirubin					
Quintile 1	88 (5.24)	2254 (15.15)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Quintile 2	136 (8.10)	1933 (12.99)	1.08 (0.73, 1.60)	1.04 (0.68, 1.60)	1.12 (0.74, 170)
Quintile 3	522 (31.07)	4865 (32.69)	1.07 (0.78, 1.47)	0.85 (0.62, 1.16)	0.88 (0.65, 1.18)
Quintile 4	273 (16.25)	1944 (13.06)	1.21 (0.88, 1.66)	0.75 (0.54, 1.03)	0.83 (0.61, 1.13)
Quintile 5	661 (39.35)	3884 (26.10)	1.24 (0.91, 1.68)	0.74 (0.54, 1.01)	0.89 (0.66, 1.20)
P for trend			0.048	0.003	0.228

* Adjust for: age (continuous), sex (male or female), ethnicity (non-Hispanic white, black, Mexican American, other Hispanic, other ethnicity), education status (high school and below, more than high school, or missing), body mass index (<18.5 kg/m^2, 18.5 to <25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2, or missing), smoking status (never, past, current, or missing), alcohol drinking (yes, no, or missing), leisure time physical activity (<500 met/week, 500 to <1000 met/week, ≥1000 met/week, or missing), alkaline phosphatase (low, medium, high, or missing), aspartate aminotransferase (low, medium, high, or missing), alanine aminotransferase (low, medium, high, or missing), gamma glutamyl transferase (low, medium, high, or missing), hypertension (yes, no, or missing), and hypercholesterolemia (yes, no, or missing).

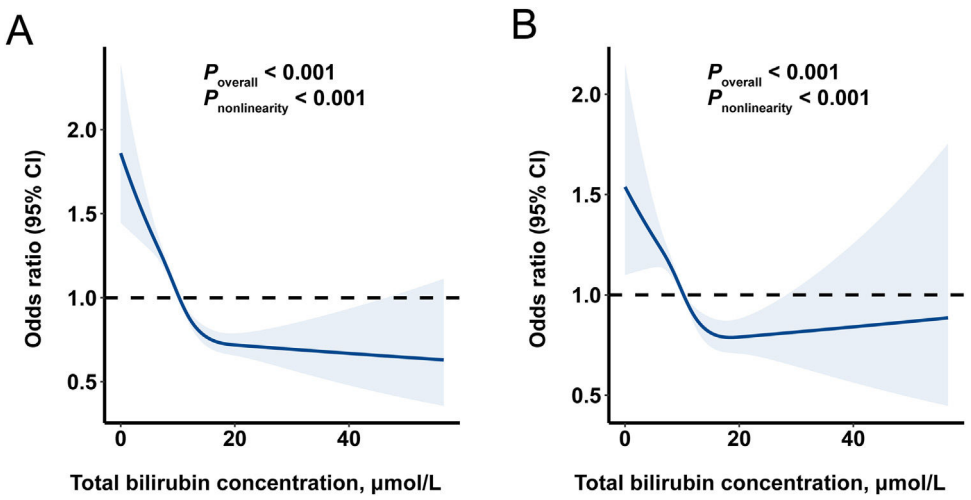

Fig. 1. Dose-response association between circulating total bilirubin concentration and risk of non-alcoholic fatty liver disease. A, FLI-NAFLD; B, USFLI-NAFLD.

Table 4
Threshold effect analysis of total bilirubin concentration and all-cause mortality among patients with non-alcoholic fatty liver disease using segmented linear regression.

	FLI-NAFLD		USFLI-NAFLD		All-cause mortality	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Total bilirubin, μmol/L						
<Turning point [a]	0.94 (0.93, 0.95)	<0.001	0.95 (0.93, 0.96)	<0.001	0.97 (0.95, 1.00)	0.024
≥Turning point [a]	1.01 (0.99, 1.03)	0.239	1.00 (0.98, 1.02)	0.853	1.03 (1.02, 1.04)	<0.001
Log likelihood ratio test [a]	<0.001		<0.001		<0.001	

[a] The turning point for FLI-NAFLD was 18.81 μmol/L, for USFLI-NAFLD was 15.39 μmol/L, and for all-cause mortality was 11.97 μmol/L
[b] Log likelihood ratio test results comparing linear regression model with two segmented linear regression model.

diabetes, hypertension, and hypercholesterolemia, respectively, and the associations did not appreciably change (Supplementary Table 3). Regarding the association between total bilirubin concentration and all-cause mortality among patients with NAFLD, we also conducted a subgroup analysis of age, sex, race, educational status, BMI, smoking status, alcohol drinking, and leisure time physical activity (Table 6), and sensitivity analysis by excluding participants with diabetes, hypertension, and hypercholesterolemia (Supplementary Table 4) and found that the results were largely unchanged.

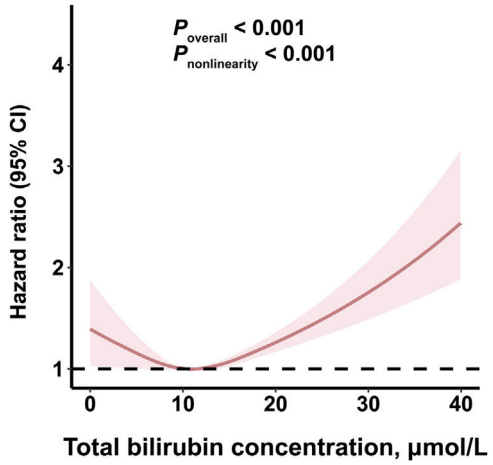

Fig. 2. Restricted spline curves for association between circulating total bilirubin concentration and all-cause mortality among patients with non-alcoholic fatty liver disease.

4. Discussion

In this large cross-sectional analysis of a representative sample of US adults, we found that participants with a higher circulating total bilirubin concentration had a lower risk of NAFLD. The significant negative association was robust across multiple subgroups and sensitivity analyses. In addition, this study is the first to examine the nonlinear association of total bilirubin concentration with the risk of NAFLD and all-cause mortality by fitting a restricted cubic spline, and the results indicated a U-shaped relationship. These findings suggest that mildly elevated circulating total bilirubin concentrations were associated with a decreased risk of NAFLD and all-cause mortality.

Accumulating evidence has revealed negative associations between bilirubin concentrations and multiple metabolic diseases, such as obesity, diabetes, metabolic syndrome, atherosclerosis, and stroke[28–31]. Although the association between circulating bilirubin concentration and the risk of NAFLD has been reported in previous studies, most previous studies were limited to Asian populations and had inconsistent results [13,14,16,17,32]. Two previous studies found no significant association between total or indirect bilirubin concentration and NAFLD [16,17]. By contrast, two studies conducted in European populations have demonstrated that circulating total bilirubin concentration was associated with NAFLD risk [4,33]. The present large-scale study is the first to demonstrate the negative association between total bilirubin concentration and the risk of NAFLD in the general US population.

NAFLD is a multisystem disease that involves dysregulation of several biological pathways. Oxidative stress and inflammation have emerged as critical aspects of the pathogenesis and progression of NAFLD [7]. Fat accumulation in the liver that exceeds the liver's metabolic capacity can lead to an imbalance in lipid metabolism and lipotoxicity, which contributes to cell stress (including oxidative stress,

Table 5
Association between total bilirubin concentration and risk of FLI-non-alcoholic fatty liver disease in different subgroups.

	Total bilirubin concentration (μmol/L)				
	Quintile 1 Odds ratio (95% CI)	Quintile 2 Odds ratio (95% CI)	Quintile 3 Odds ratio (95% CI)	Quintile 4 Odds ratio (95% CI)	Quintile 5 Odds ratio (95% CI)
Age					
<50 years	1.00 (Ref.)	0.77 (0.60, 0.99)	0.72 (0.55, 0.95)	0.57 (0.44, 0.74)	0.48 (0.36, 0.64)
≥50 years	1.00 (Ref.)	0.87 (0.69, 1.10)	0.77 (0.58, 1.01)	0.67 (0.52, 0.86)	0.51 (0.40, 0.65)
Sex					
Male	1.00 (Ref.)	1.09 (0.83, 1.43)	1.00 (0.74, 1.35)	0.83 (0.62, 1.12)	0.66 (0.49, 0.89)
Female	1.00 (Ref.)	0.71 (0.58, 0.88)	0.65 (0.51, 0.82)	0.53 (0.42, 0.67)	0.39 (0.29, 0.53)
Race					
Non-Hispanic white	1.00 (Ref.)	0.89 (0.71, 1.13)	0.84 (0.63, 1.11)	0.67 (0.51, 0.87)	0.52 (0.40, 0.68)
Others	1.00 (Ref.)	0.74 (0.60, 0.90)	0.59 (0.48, 0.72)	0.52 (0.41, 0.65)	0.43 (0.34, 0.54)
Educational status					
High school and below	1.00 (Ref.)	0.81 (0.64, 1.02)	0.73 (0.56, 0.95)	0.59 (0.46, 0.76)	0.45 (0.32, 0.61)
More than high school	1.00 (Ref.)	0.82 (0.65, 1.04)	0.73 (0.55, 0.96)	0.62 (0.48, 0.80)	0.50 (0.39, 0.65)
BMI					
<30 kg/m^2	1.00 (Ref.)	0.85 (0.70, 1.04)	0.75 (0.60, 0.94)	0.67 (0.54, 0.82)	0.54 (0.44, 0.67)
≥30 kg/m^2	1.00 (Ref.)	0.82 (0.62, 1.09)	0.71 (0.51, 1.00)	0.56 (0.41, 0.78)	0.35 (0.24, 0.51)
Smoking status					
Never	1.00 (Ref.)	0.73 (0.57, 0.93)	0.66 (0.49, 0.88)	0.51 (0.40, 0.65)	0.43 (0.33, 0.58)
Past or current	1.00 (Ref.)	0.93 (0.70, 1.25)	0.83 (0.59, 1.15)	0.74 (0.55, 0.99)	0.54 (0.40, 0.74)
Alcohol dinking					
No	1.00 (Ref.)	0.82 (0.62, 1.08)	0.65 (0.48, 0.87)	0.49 (0.36, 0.65)	0.49 (0.36, 0.67)
Yes	1.00 (Ref.)	0.81 (0.66, 0.99)	0.71 (0.57, 0.88)	0.62 (0.50, 0.76)	0.47 (0.38, 0.58)
Leisure time physical activity					
<500 MET/week	1.00 (Ref.)	0.80 (0.68, 0.93)	0.74 (0.62, 0.88)	0.60 (0.51, 0.71)	0.51 (0.43, 0.61)
≥500 MET/week	1.00 (Ref.)	0.83 (0.66, 1.04)	0.65 (0.50, 0.83)	0.55 (0.43, 0.70)	0.44 (0.34, 0.56)
Alkaline phosphatase					
Low/medium	1.00 (Ref.)	0.74 (0.59, 0.91)	0.66 (0.52, 0.84)	0.54 (0.42, 0.69)	0.39 (0.30, 0.51)
High	1.00 (Ref.)	1.04 (0.84, 1.29)	0.92 (0.69, 1.22)	0.78 (0.62, 0.99)	0.69 (0.53, 0.89)
Aspartate aminotransferase					
Low/medium	1.00 (Ref.)	0.81 (0.65, 1.00)	0.70 (0.55, 0.89)	0.54 (0.43, 0.68)	0.38 (0.30, 0.50)
High	1.00 (Ref.)	0.86 (0.64, 1.17)	0.81 (0.59, 1.12)	0.75 (0.55, 1.01)	0.65 (0.47, 0.90)
Alanine aminotransferase					
Low/medium	1.00 (Ref.)	0.91 (0.76, 1.10)	0.78 (0.63, 0.97)	0.69 (0.56, 0.86)	0.53 (0.46, 0.66)
High	1.00 (Ref.)	0.65 (0.47, 0.89)	0.65 (0.46, 0.93)	0.42 (0.30, 0.60)	0.38 (0.26, 0.57)
Gamma glutamyl transferase					
Low/medium	1.00 (Ref.)	0.85 (0.69, 1.05)	0.79 (0.63, 0.99)	0.62 (0.49, 0.78)	0.48 (0.38, 0.61)
High	1.00 (Ref.)	0.81 (0.62, 1.06)	0.71 (0.52, 0.97)	0.64 (0.48, 0.86)	0.56 (0.41, 0.76)

* Adjust for: age (continuous), sex (male or female), ethnicity (non-Hispanic white, black, Mexican American, other Hispanic, other ethnicity), education status (high school and below, more than high school, or missing), body mass index (<18.5 kg/m^2, 18.5 to <25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2, or missing), smoking status (never, past, current, or missing), alcohol drinking (yes, no, or missing), leisure time physical activity (<500 met/week, 500 to <1000 met/week, ≥1000 met/-week, or missing), alkaline phosphatase (low, medium, high, or missing), aspartate aminotransferase (low, medium, high, or missing), alanine aminotransferase (low, medium, high, or missing), gamma glutamyl transferase (low, medium, high, or missing), diabetes (yes, no, or missing), hypertension (yes, no, or missing), and hypercholesterolemia (yes, no, or missing).

and endoplasmic reticulum stress) and the activation of inflammasomes, thereby stimulating tissue regeneration and fibrogenesis [34]. The mechanisms underlying the negative association between bilirubin and NAFLD might be attributed to the antioxidant and anti-inflammatory properties of bilirubin [9,10]. Bilirubin can protect various proteins, lipid membranes, and cells from damage in vitro studies [35]. In addition, bilirubin, as a powerful signaling molecule, can participate in endocrine activities and activate various nuclear and cytoplasmic receptors, such as aryl hydrocarbon, constitutive androstane receptors, or peroxisome proliferator-activated receptors α, which may involve the occurrence and development of NAFLD [12,36,37].

The assessment of total bilirubin concentration is usually performed as part of a routine test of liver function, and its physiological level varies from 3.4 to 17.1 μmol/L [38]. Our study is the first to evidence the nonlinear U-shaped relationship between total bilirubin concentration and the risk of NAFLD. The results indicate that elevated total bilirubin concentration below the turning point (18.81 μmol/L) is associated with a lower risk of NAFLD. These findings suggest that only mildly elevated total bilirubin concentration within the physiological range may play an antioxidant and anti-inflammatory role in NAFLD. Interestingly, similar to our findings, a recent cross-sectional study demonstrated that telomere length increased with increased bilirubin concentration up to 0.5 mg/dL, beyond which the association was nonsignificant, indicating that mildly increased bilirubin concentration is protective against telomere shortening [19]. Furthermore, regarding the association of bilirubin with telomere length, previous study has found that there were longer telomeres in male individuals chronically exposed to increased unconjugated bilirubin, as well as in rat model of unconjugated hyperbilirubinaemia [39]. Similarly, previous studies also reported the nonlinear U-shaped relationship between total bilirubin concentration and the risk of coronary heart disease, ischemic heart disease, and diabetic retinopathy [20,21,40].

The relationship between total bilirubin concentration and mortality in the general population or in patients with pre-existing disease is unclear [23,41-45]. To date, few studies have examined the association between total bilirubin concentration and all-cause mortality among patients with NAFLD, whereas our study demonstrated

Table 6
Association between total bilirubin concentration and all-cause mortality among patients with non-alcoholic fatty liver disease in different subgroups.

	Total bilirubin concentration (μmol/L)				
	Quintile 1 Hazard ratio (95% CI)	Quintile 2 Hazard ratio (95% CI)	Quintile 3 Hazard ratio (95% CI)	Quintile 4 Hazard ratio (95% CI)	Quintile 5 Hazard ratio (95% CI)
Age					
<50 years	1.00 (Ref.)	0.76 (0.30, 1.93)	0.84 (0.40, 1.73)	1.50 (0.65, 3.44)	1.36 (0.64, 2.91)
≥50 years	1.00 (Ref.)	1.31 (0.83, 2.07)	1.02 (0.72, 1.43)	0.91 (0.64, 1.28)	1.04 (0.74, 1.45)
Sex					
Male	1.00 (Ref.)	0.94 (0.49, 1.77)	0.78 (0.49, 1.25)	0.76 (0.47, 1.23)	0.75 (0.48, 1.17)
Female	1.00 (Ref.)	1.28 (0.81, 2.04)	0.93 (0.67, 1.29)	0.78 (0.53, 1.14)	1.05 (0.72, 1.53)
Race					
Non-Hispanic white	1.00 (Ref.)	1.05 (0.72, 1.52)	0.87 (0.64, 1.19)	0.77 (0.56, 1.08)	0.84 (0.61, 1.15)
Others	1.00 (Ref.)	1.12 (0.75, 1.67)	1.11 (0.78, 1.56)	1.09 (0.74, 1.59)	1.29 (0.91, 1.83)
Educational status					
High school and below	1.00 (Ref.)	0.96 (0.56, 1.63)	0.83 (0.54, 1.29)	0.84 (0.54, 1.30)	0.91 (0.57, 1.43)
More than high school	1.00 (Ref.)	1.40 (0.80, 2.44)	0.96 (0.63, 1.45)	0.83 (0.52, 1.33)	0.88 (0.57, 1.35)
BMI					
<30 kg/m^2	1.00 (Ref.)	1.28 (0.61, 2.65)	1.93 (0.48, 1.83)	0.71 (0.36, 1.40)	1.04 (0.51, 2.10)
≥30 kg/m^2	1.00 (Ref.)	1.08 (0.68 1.71)	0.88 (0.65, 1.20)	0.93 (0.67, 1.29)	0.83 (0.60, 1.16)
Smoking status					
Never	1.00 (Ref.)	0.97 (0.51, 1.83)	0.77 (0.48, 1.23)	0.69 (0.41, 1.15)	0.85 (0.52, 1.40)
Past or current	1.00 (Ref.)	1.17 (0.72, 1.91)	0.93 (0.65, 1.35)	0.89 (0.59, 1.36)	0.85 (0.58, 1.25)
Alcohol dinking					
No	1.00 (Ref.)	1.69 (0.98, 2.89)	1.41 (0.88, 2.29)	1.13 (0.67, 1.91)	1.41 (0.86, 2.30)
Yes	1.00 (Ref.)	0.83 (0.58, 1.19)	0.82 (0.61, 1.11)	0.83 (0.61, 1.14)	0.87 (0.64, 1.18)
Leisure time physical activity					
<500 MET/week	1.00 (Ref.)	0.97 (0.61, 1.54)	0.79 (0.56, 1.13)	0.79 (0.54, 1.14)	0.90 (0.63, 1.29)
≥500 MET/week	1.00 (Ref.)	1.67 (0.66, 4.27)	1.11 (0.52,2.37)	0.90 (0.41, 1.97)	0.84 (0.38, 1.86)
Alkaline phosphatase					
Low/medium	1.00 (Ref.)	1.14 (0.72, 1.81)	0.85 (0.59, 1.21)	0.70 (0.47, 1.04)	0.84 (0.57, 1.23)
High	1.00 (Ref.)	1.07 (0.50, 2.28)	0.90 (0.53, 1.51)	1.02 (0.59, 1.78)	0.94 (0.55, 1.62)
Aspartate aminotransferase					
Low/medium	1.00 (Ref.)	1.06 (0.67, 1.68)	0.88 (0.62, 1.24)	0.78 (0.54, 1.14)	0.76 (0.51, 1.12)
High	1.00 (Ref.)	1.20 (0.65, 2.24)	0.82 (0.52, 1.29)	0.81 (0.51, 1.28)	0.98 (0.64, 1.49)
Alanine aminotransferase					
Low/medium	1.00 (Ref.)	0.94 (0.55, 1.61)	0.91 (0.59, 1.41)	0.69 (0.43, 1.12)	0.84 (0.54, 1.29)
High	1.00 (Ref.)	1.41 (0.77, 2.57)	0.80 (0.51, 1.27)	1.09 (0.70, 1.70)	0.97 (0.61, 1.55)
Gamma glutamyl transferase					
Low/medium	1.00 (Ref.)	1.11 (0.66, 1.87)	0.87 (0.55, 1.39)	0.70 (0.42, 1.16)	0.68 (0.43, 1.08)
High	1.00 (Ref.)	1.13 (0.65, 1.97)	0.86 (0.57, 1.29)	0.95 (0.61, 1.47)	1.04 (0.67, 1.61)

* Adjust for: age (continuous), sex (male or female), ethnicity (non-Hispanic white, black, Mexican American, other Hispanic, other ethnicity), education status (high school and below, more than high school, or missing), body mass index (<18.5 kg/m^2, 18.5 to <25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2, or missing), smoking status (never, past, current, or missing), alcohol drinking (yes, no, or missing), leisure time physical activity (<500 met/week, 500 to <1000 met/week, ≥1000 met/-week, or missing), alkaline phosphatase (low, medium, high, or missing), aspartate aminotransferase (low, medium, high, or missing), alanine aminotransferase (low, medium, high, or missing), gamma glutamyl transferase (low, medium, high, or missing), diabetes (yes, no, or missing), hypertension (yes, no, or missing), and hypercholesterolemia (yes, no, or missing).

a nonlinear U-shaped relationship between them. When total bilirubin concentration was less than 11.97 μmol/L, increased total bilirubin concentration was associated with decreased all-cause mortality, and the mortality risk increased as total bilirubin increased above this inflection point. Similarly, a previous study demonstrated a U-shaped relationship between serum total bilirubin and mortality in patients with end-stage renal disease receiving peritoneal dialysis and found that participants with bilirubin concentrations of 0.5-0.6 mg/dL had the lowest 3-year mortality rate [46].

Among the many potential causes of elevated circulating bilirubin concentration is Gilbert's syndrome. Moreover known as benign hyperbilirubinemia, Gilbert's syndrome is characterized by mildly elevated total bilirubin with no laboratory signs of haemolysis or liver damage [47]. A previous study indicated that the plasma antioxidant capacity of patients with Gilbert's syndrome mildly increased due to the increase of unconjugated bilirubin [48]. A case-control study has reported that the serum bilirubin concentration in NAFLD patients shows a decreasing trend, while the prevalence of phenotypic Gilbert syndrome was significantly lower [49]. Moderate unconjugated hyperbilirubinemia may reduce platelet hyperreactivity and prothrombin phenotype by protecting transmembrane proteins and lipids against attack by reactive oxygen species (ROS), which is a related risk factor for cardiovascular disease [50]. Despite the health promoting effects of mild hyperbilirubinemia, we still need to identify the presence of underlying hepatobiliary diseases affecting bilirubin metabolism/excretion in clinical practice.

In recent years, NAFLD has become an increasingly common disease worldwide, resulting in a major health and socioeconomic burden, and there is currently no effective drug treatment. The findings of the present study suggest that bilirubin may be a biomarker for the reduced prevalence of NAFLD and a predictor of all-cause mortality among patients with NAFLD, which is of clinical significance. Furthermore, our study has several notable strengths. First, our study was based on a large representative sample of the US population with standardized data collection procedures and rigorous quality control measures. Second, we provided the first evidence for the nonlinear U-shaped relationships between total bilirubin concentration and NAFLD risk and all-cause mortality by using restricted cubic splines and segmented linear regression. Third, we conducted a comprehensive series of subgroup analyses of age, sex, race, educational

status, BMI, smoking status, alcohol consumption, leisure time physical activity, and ALP, AST, GGT and ALT levels, and sensitivity analyses excluding participants with diabetes, hypertension, and hypercholesterolemia. The association between total bilirubin concentration and NAFLD risk was robust across multiple analyses.

Our study also has several limitations. First, the cross-sectional nature of the study led to difficulties in inferring causal relationships. A subsequent prospective study is essential to confirm our results. Second, NAFLD cases in the present study were not identified using liver biopsies; such an invasive diagnostic method is not suitable for large-scale population-based studies. Despite this, we used both FLI and USFLI as indicators of NAFLD. FLI and USFLI are recognized as rapid, valid, and reliable techniques for identifying NAFLD cases in large populations [25,27]. Third, although we adjusted for many potential confounders in our analyses, residual confounding such as drug use could not be excluded.

5. Conclusions

Our study demonstrated that higher elevated circulating total bilirubin concentration within the physiological range may play an antioxidant and anti-inflammatory role and was associated with a decreased risk of NAFLD and all-cause mortality.

Author contributions

HH, QY and NQ conceptualized the study, analyzed the data, and drafted the manuscript. BS, YM, and ZF critically reviewed and revised the manuscript. ZL and LC supervised the project. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Declaration of interests

None.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.aohep.2023.101177.

References

[1] Powell EE, Wong VW, Rinella M. Non-alcoholic fatty liver disease. Lancet 2021;397(10290):2212–24. https://doi.org/10.1016/S0140-6736(20)32511-3.
[2] Paik JM, Golabi P, Younossi Y, Mishra A, Younossi ZM. Changes in the global burden of chronic liver diseases from 2012 to 2017: the growing impact of NAFLD. Hepatology 2020;72(5):1605–16. https://doi.org/10.1002/hep.31173.
[3] Targher G, Tilg H, Byrne CD. Non-alcoholic fatty liver disease: a multisystem disease requiring a multidisciplinary and holistic approach. Lancet Gastroenterol Hepatol 2021;6(7):578–88. https://doi.org/10.1016/S2468-1253(21)00020-0.
[4] Kunutsor SK, Frysz M, Verweij N, Kieneker LM, Bakker SJL, Dullaart RPF. Circulating total bilirubin and risk of non-alcoholic fatty liver disease in the PREVEND study: observational findings and a Mendelian randomization study. Eur J Epidemiol 2020;35(2):123–37. https://doi.org/10.1007/s10654-019-00589-0.
[5] Barb D, Repetto EM, Stokes ME, Shankar SS, Cusi K. Type 2 diabetes mellitus increases the risk of hepatic fibrosis in individuals with obesity and nonalcoholic fatty liver disease. Obesity (Silver Spring) 2021;29(11):1950–60. https://doi.org/10.1002/oby.23263.
[6] Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States national health and nutrition examination survey. Aliment Pharmacol Ther 2015;41(1):65–76. https://doi.org/10.1111/apt.13012.
[7] Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic steatohepatitis: a review. JAMA 2020;323(12):1175–83. https://doi.org/10.1001/jama.2020.2298.
[8] Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. Proc Natl Acad Sci USA 2002;99(25):16093–8. https://doi.org/10.1073/pnas.252626999.
[9] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235(4792):1043–6. https://doi.org/10.1126/science.3029864.
[10] Ziberna L, Jenko-Praznikar Z, Petelin A. Serum bilirubin levels in overweight and obese individuals: the importance of anti-inflammatory and antioxidant responses. Antioxidants (Basel) 2021;10(9):1352. https://doi.org/10.3390/antiox10091352.
[11] Jangi S, Otterbein L, Robson S. The molecular basis for the immunomodulatory activities of unconjugated bilirubin. Int J Biochem Cell Biol 2013;45(12):2843–51. https://doi.org/10.1016/j.biocel.2013.09.014.
[12] Vitek L, Hinds Jr. TD, Stec DE, Tiribelli C. The physiology of bilirubin: health and disease equilibrium. Trends Mol Med 2023;29(4):315–28. https://doi.org/10.1016/j.molmed.2023.01.007.
[13] Kwak MS, Kim D, Chung GE, Kang SJ, Park MJ, Kim YJ, et al. Serum bilirubin levels are inversely associated with nonalcoholic fatty liver disease. Clin Mol Hepatol 2012;18(4):383–90. https://doi.org/10.3350/cmh.2012.18.4.383.
[14] Shu X, Zheng Y, Chen Z, Guo Q, Wang Y, Ji R, et al. Association between bilirubin and nonalcoholic fatty liver disease in the non-obese Chinese population: a cross-sectional study. Ann Transl Med 2022;10(9):522. https://doi.org/10.21037/atm-22-1187.
[15] Bellarosa C, Bedogni G, Bianco A, Cicolini S, Caroli D, Tiribelli C, et al. Association of serum bilirubin level with metabolic syndrome and non-alcoholic fatty liver disease: a cross-sectional study of 1672 obese children. J Clin Med 2021;10(13):2812. https://doi.org/10.3390/jcm10132812.
[16] Tian J, Zhong R, Liu C, Tang Y, Gong J, Chang J, et al. Association between bilirubin and risk of Non-Alcoholic Fatty Liver Disease based on a prospective cohort study. Sci Rep 2016;6:31006. https://doi.org/10.1038/srep31006.
[17] Chang Y, Ryu S, Zhang Y, Son HJ, Kim JY, Cho J, et al. A cohort study of serum bilirubin levels and incident non-alcoholic fatty liver disease in middle aged Korean workers. PLoS One 2012;7(5):e37241. https://doi.org/10.1371/journal.pone.0037241.
[18] Inoguchi T, Nohara Y, Nojiri C, Nakashima N. Association of serum bilirubin levels with risk of cancer development and total death. Sci Rep 2021;11(1):13224. https://doi.org/10.1038/s41598-021-92442-2.
[19] Hao L, Chen Q, Chen X, Zhou Q. Association of serum total bilirubin concentration with telomere length: the national health and nutrition examination survey. Oxid Med Cell Longev 2021;2021:4688900. https://doi.org/10.1155/2021/4688900.
[20] Lai X, Fang Q, Yang L, Chen X, Wang H, Ma L, et al. Direct, indirect and total bilirubin and risk of incident coronary heart disease in the Dongfeng-Tongji cohort. Ann Med 2018;50(1):16–25. https://doi.org/10.1080/07853890.2017.1377846.
[21] Liu M, Wang J, He Y. The U-shaped association between bilirubin and diabetic retinopathy risk: a five-year cohort based on 5323 male diabetic patients. J Diabetes Res 2018:4603087 2018. https://doi.org/10.1155/2018/4603087.
[22] Gazzin S, Vitek L, Watchko J, Shapiro SM, Tiribelli C. A novel perspective on the biology of bilirubin in health and disease. Trends Mol Med 2016;22(9):758–68. https://doi.org/10.1016/j.molmed.2016.07.004.
[23] Wagner KH, Wallner M, Mölzer C, Gazzin S, Bulmer AC, Tiribelli C, et al. Looking to the horizon: the role of bilirubin in the development and prevention of age-related chronic diseases. Clinical Sci (London, England : 1979) 2015;129(1):1–25. https://doi.org/10.1042/cs20140566.
[24] Vítek L. Bilirubin and atherosclerotic diseases. Physiol Res 2017;66(Suppl 1):S11–20. https://doi.org/10.33549/physiolres.933581.
[25] European Association for the Study of the L. European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Diabetologia 2016;59(6):1121–40. https://doi.org/10.1007/s00125-016-3902-y.
[26] Cardoso BR, Tan SY, Daly RM, Via JD, Georgousopoulou EN, George ES. Intake of nuts and seeds is associated with a lower prevalence of nonalcoholic fatty liver disease in us adults: findings from 2005-2018 NHANES. J Nutr 2021;151(11):3507–15. https://doi.org/10.1093/jn/nxab253.
[27] Golabi P, Paik JM, Harring M, Younossi E, Kabbara K, Younossi ZM. Prevalence of high and moderate risk nonalcoholic fatty liver disease among adults in the United States, 1999-2016. Clin Gastroenterol Hepatol 2021;20(12):2838–2847.e7. https://doi.org/10.1016/j.cgh.2021.12.015.
[28] Su Q, Chen H, Du S, Dai Y, Chen C, He T, et al. Association between serum bilirubin, lipid levels, and prevalence of femoral and carotid atherosclerosis: a population-based cross-sectional study. Arterioscler Thromb Vasc Biol 2023;43(1):136–45. https://doi.org/10.1161/ATVBAHA.122.318086.
[29] Wang J, Zhang X, Zhang Z, Zhang Y, Zhang J, Li H, et al. Baseline serum bilirubin and risk of first stroke in hypertensive patients. J Am Heart Assoc 2020;9(12):e015799. https://doi.org/10.1161/JAHA.119.015799.
[30] Jirásková A, Škrha J, Vítek L. Association of low serum bilirubin concentrations and promoter variations in the UGT1A1 and HMOX1 genes with type 2 diabetes mellitus in the czech population. Int J Mol Sci 2023;24(13):10614. https://doi.org/10.3390/ijms241310614.

[31] Vítek L. The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases. Front Pharmacol 2012;3:55. https://doi.org/10.3389/fphar.2012.00055.
[32] Luo L, An P, Jia X, Yue X, Zheng S, Liu S, et al. Genetically regulated bilirubin and risk of non-alcoholic fatty liver disease: a mendelian randomization study. Front Genet 2018;9:662. https://doi.org/10.3389/fgene.2018.00662.
[33] Puri K, Nobili V, Melville K, Corte CD, Sartorelli MR, Lopez R, et al. Serum bilirubin level is inversely associated with nonalcoholic steatohepatitis in children. J Pediatr Gastroenterol Nutr 2013;57(1):114–8. https://doi.org/10.1097/MPG.0b013e318291fefe.
[34] Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018;24(7):908–22. https://doi.org/10.1038/s41591-018-0104-9.
[35] Ioannou GN, Liou IW, Weiss NS. Serum bilirubin and colorectal cancer risk: a population-based cohort study. Aliment Pharmacol Ther 2006;23(11):1637–42. https://doi.org/10.1111/j.1365-2036.2006.02939.x.
[36] Vítek L. Bilirubin as a signaling molecule. Med Res Rev 2020;40(4):1335–51. https://doi.org/10.1002/med.21660.
[37] Vítek L, Tiribelli C. Bilirubin: The yellow hormone? J Hepatol 2021;75(6):1485–90. https://doi.org/10.1016/j.jhep.2021.06.010.
[38] Gazzin S, Masutti F, Vitek L, Tiribelli C. The molecular basis of jaundice: an old symptom revisited. Liver Int 2017;37(8):1094–102. https://doi.org/10.1111/liv.13351.
[39] Tosevska A, Moelzer C, Wallner M, Janosec M, Schwarz U, Kern C, et al. Longer telomeres in chronic, moderate, unconjugated hyperbilirubinaemia: insights from a human study on Gilbert's syndrome. Sci Rep 2016;6:22300. https://doi.org/10.1038/srep22300.
[40] Breimer LH, Wannamethee G, Ebrahim S, Shaper AG. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. Clin Chem 1995;41(10):1504–8 https://www.ncbi.nlm.nih.gov/pubmed/7586525.
[41] Chen Z, He J, Chen C, Lu Q. Association of total bilirubin with all-cause and cardiovascular mortality in the general population. Front Cardiovasc Med 2021;8:670768. https://doi.org/10.3389/fcvm.2021.670768.
[42] McCallum L, Panniyammakal J, Hastie CE, Hewitt J, Patel R, Jones GC, et al. Longitudinal blood pressure control, long-term mortality, and predictive utility of serum liver enzymes and bilirubin in hypertensive patients. Hypertension 2015;66(1):37–43. https://doi.org/10.1161/HYPERTENSIONAHA.114.04915.
[43] Su HH, Kao CM, Lin YC, Lin YC, Kao CC, Chen HH, et al. Relationship between serum total bilirubin levels and mortality in uremia patients undergoing long-term hemodialysis: a nationwide cohort study. Atherosclerosis 2017;265:155–61. https://doi.org/10.1016/j.atherosclerosis.2017.09.001.
[44] Bunte S, Walz R, Merkel J, Torregroza C, Roth S, Lurati Buse G, et al. Bilirubin-A possible prognostic mortality marker for patients with ECLS. J Clin Med 2020;9(6):1727. https://doi.org/10.3390/jcm9061727.
[45] Vitek L, Hubacek JA, Pajak A, Doryńska A, Kozela M, Eremiasova L, et al. Association between plasma bilirubin and mortality. Ann Hepatol 2019;18(2):379–85. https://doi.org/10.1016/j.aohep.2019.02.001.
[46] Yang TL, Lin YC, Lin YC, Huang CY, Chen HH, Wu MS. Total bilirubin in prognosis for mortality in end-stage renal disease patients on peritoneal dialysis therapy. J Am Heart Assoc 2017;6(12):e007507. https://doi.org/10.1161/JAHA.117.007507.
[47] Vítek L, Tiribelli C. Gilbert's syndrome revisited. J Hepatol 2023;79(4):1049–55. https://doi.org/10.1016/j.jhep.2023.06.004.
[48] Vítek L, Jirsa M, Brodanová M, Kalab M, Marecek Z, Danzig V, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis 2002;160(2):449–56. https://doi.org/10.1016/s0021-9150(01)00601-3.
[49] Petrtýl J, Dvořák K, Stříteský J, Leníček M, Jirásková A, Šmíd V, et al. Association of serum bilirubin and functional variants of heme oxygenase 1 and bilirubin UDP-glucuronosyl transferase genes in czech adult patients with non-alcoholic fatty liver disease. Antioxidants (Basel) 2021;10(12):2000. https://doi.org/10.3390/antiox10122000.
[50] Kundur AR, Singh I, Bulmer AC. Bilirubin, platelet activation and heart disease: a missing link to cardiovascular protection in Gilbert's syndrome? Atherosclerosis 2015;239(1):73–84. https://doi.org/10.1016/j.atherosclerosis.2014.12.042.