Original Article

Association between serum ferritin and non-alcoholic fatty liver disease among middle-aged and elderly Chinese with normal weight

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Background and Objectives: Non-alcoholic fatty liver disease (NAFLD) in normal-weight population is becoming an important health issue. This study aimed to assess the association between serum ferritin (SF) and NAFLD among middle-aged and elderly Chinese with normal weight. **Methods and Study Design:** A total of 2029 Chinese adults aged 35-70 years with normal weight were involved in this cross-sectional study. General information, lifestyle factors and laboratory characteristics were collected. The concentrations of serum alanine aminotransferase (ALT) and SF were recorded. Receiver operating characteristic (ROC) was applied to assess predictive performance of SF for NAFLD. Logistic regression analysis was conducted to evaluate the associations of SF with NAFLD and elevated ALT. **Results:** Compared with controls, subjects with NAFLD had higher SF concentrations (p<0.001). In multivariate logistic regression analyses, the odds ratios (ORs) with 95% confidence intervals (CIs) of NAFLD were 3.19 (2.07-4.92) for the highest versus lowest quartile of SF. ROC analysis revealed a predictive ability of SF for NAFLD with an area under the curve of 0.660 (95% CI, 0.63-0.69). In addition, higher SF was significantly associated with increased risk of elevated ALT (OR=1.84, 95%CI: 1.32-2.55). In stratified analyses by gender and age, the positive associations of SF with the risk of NAFLD and elevated ALT were only observed in women and 35~49y group. **Conclusions:** SF was positively associated with the risk of NAFLD and elevated ALT among normal-weight Chinese adults. ROC analysis suggested that SF may serve as an indicator of predicting NAFLD.

Key Words: non-alcoholic fatty liver disease, ferritin, alanine aminotransferase, Chinese, normal weight

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver injury ranging from benign accumulation of fat in hepatocytes to non-alcoholic steatohepatitis, fibrosis, cirrhosis and liver failure.[1,2] Currently, it is the predominant liver disorder worldwide in parallel with the epidemics of obesity and diabetes mellitus.[3,4] The global prevalence of NAFLD is 25.24% and has been on the rise in recent decades.[5,6] In China, a meta-analysis in 2014 showed that a pooled prevalence of NAFLD was 20.9% (17.95%~22.31%) for general population.[7]

NAFLD is commonly observed in overweight or obese individuals.[8] However, there is emerging evidence of NAFLD in normal-weight individuals, and a reported proportion of normal-weight subjects in NAFLD from China was 15%.[9] NAFLD with normal weight has been recognized as a significant health issue for its easily delayed diagnosis and probably causing higher overall mortality than obese or overweight patients.[10] Studies on this group may promote the awareness and management of NAFLD in normal-weight individuals. Additionally, several studies have indicated that NAFLD patients with normal weight had some different characteristics and metabolic profiles in comparison to obese patients.[11] For example, NAFLD patients with normal weight had less insulin sensitivity and lower fasting glucose,[12,13] which means there are less clinical biochemical indicators at early stages in this group. Besides, studies on risk factors of NAFLD among normal-weight individuals are scant.[10]

Iron metabolism has been linked to the development of NAFLD.[14] And patients with NAFLD are frequently reported to be mild iron overload.[15,16] Serum ferritin (SF) has been used commonly to assess iron status and determine iron overload conditions in clinical and epidemiological studies.[17] SF increases in obesity-related chronic inflammation such as diabetes and metabolic syndrome (MetS).[18] As a possible hepatic manifestation of MetS,[19] NAFLD has been reported to associate with SF, while the roles of SF in the development of NAFLD are still disputed.[20-24] A meta-analysis in 2017 involving 14 prospec-

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tive and case-control studies concluded that SF levels were elevated in NAFLD patients, and were associated with the severity of NAFLD.[25] However, none of the included studies focused on normal-weight population, while the influence of obesity-related inflammatory conditions might be ruled out in normal-weight population.

NAFLD is the main cause of elevated plasma concentration of aminotransferases including alanine aminotransferase (ALT) and aspartate aminotransferase (AST),[26] and ALT is considered as a more specific biomarker of liver injury.[27] In previous studies, elevated serum ALT was positively associated with SF and the degree of ironload.[28,29] However, a national, population-based study in the United States suggested that the elevated ALT was positively associated with the transferrin saturation and serum iron concentration, while the association was not significant in participants with normal BMI (BMI <25 kg/m^2).[30] To our best knowledge, human studies regarding the association between SF and ALT levels among normal-weight population are limited.

To date, there are few known studies on relationship between SF and NAFLD in normal-weight population in China, while SF may serve as one of the clinical biomarkers for diagnosis of NAFLD and has some prognostic significance in liver damage and fibrosis.[14,31,32] In the present study, we conducted a cross-sectional analysis to determine the association between SF and NAFLD in middle-aged and elderly Chinese individuals with BMI-defined normal weight (18.5 kg/m^2 ≤ BMI <24 kg/m^2). Moreover, the relationship between SF and concentration of ALT was also evaluated.

METHODS

Study population

Subjects were recruited from Medical Examination Center of the Affiliated Hospital of Qingdao University from January to December 2016. The present study population consisted of 2466 Han Chinese aged 35~70 years. Anthropometric measurement, interview, laboratory analysis and ultrasound examinations for the diagnosis of NAFLD were performed in all participants. Individuals without information on the liver ultrasound findings (n=33) were excluded. We also excluded subjects with excessive alcohol consumption (>140g/week for men and >70 g/week for women, n=396), those with other unknown causes of elevated liver enzymes or aminotransferases 3 times higher than the upper limit of normal (laboratory normal range: 0–39 U/L) (n=6), and those with positive hepatitis B surface antigen or hepatitis C antibody or with a history of virus hepatitis, liver carcinoma or autoimmune liver disease (n=2). Finally, 2029 adults (639 men and 1390 women) aged 35-70 years with BMI-defined normal weight were included (Figure 1). Normal BMI was defined as a BMI ranging from 18.5 to 23.9 kg/m^2 according to BMI cut-off points for Chinese adults.[33,34] All participants in this study signed their written informed consent before participating in this study, and the study was approved by Ethics Committee of Medical College of Qingdao University (Ethical approval number: [Medical College of Qingdao University 20130304]; Clinical trial registration number: ChiCTR-OCS-14004819).

Data collection and measurements

Standardized questionnaires were used to collect information of age, gender, medical history and lifestyle including current smoking and alcohol consumption. Alcohol consumption was evaluated by the frequency of alco-

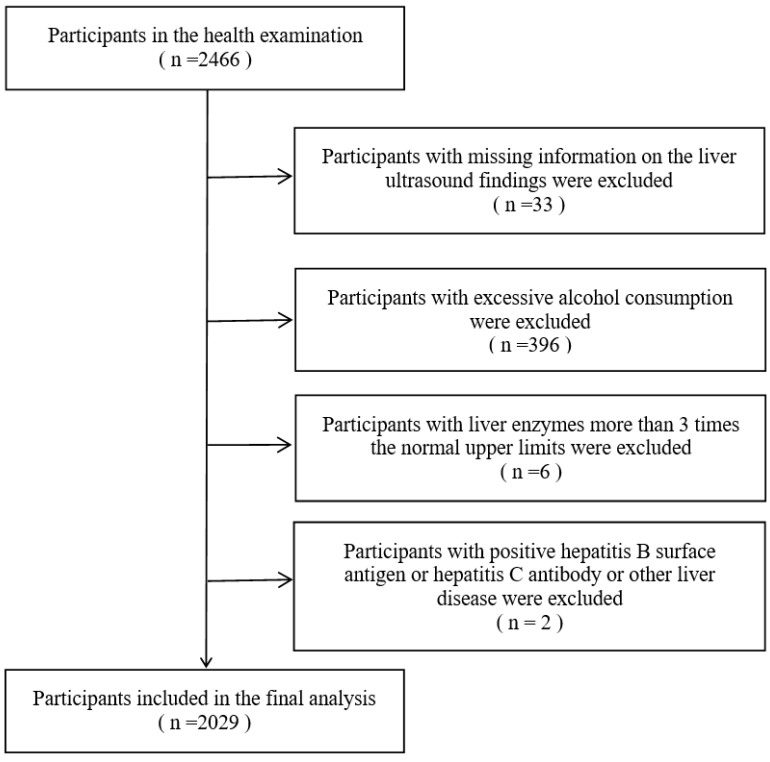

Figure 1. Flow chart of the selection of eligible study population in the final analysis.

hol intake per week and the usual amount consumed per occasion. Weight was measured via a calibrated beam balance to the nearest 0.1 kg without heavy clothes and shoes. The measurement of standing height was taken to the nearest 0.1 cm wearing not shoes. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured 3 times in the sitting position using a standard mercurial sphygmomanometer after a 10-minute rest. All data collection and measurement were performed by trained workers.

Collecting venous blood samples after overnight fasting for 8 to 12 hours, and the blood glucose, ALT, serum uric acid, triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by an automatic analyzer (Beckman CX-7 Biochemical Autoanalyzer, Brea, CA, USA). SF concentrations were measured by radioimmunology (North Institute of Bio-Tech, Beijing, China). The cut points of 30 IU/L for men and 19 IU/L for women were defined as the upper limits of ALT.[35,36]

Definitions

Hepatic steatosis was diagnosed by abdominal ultrasonography and radiologists were blinded to the biochemical examinations of participants. NAFLD was diagnosed by the presence of at least two of the following three abnormal findings of abdominal ultrasonography: (i) diffusely increased echogenicity as often compared to hypoechogenicity of kidney cortex; (ii) ultrasound beam attenuation; and (iii) intrahepatic structure blurring.[37] The presence of diabetes was recorded as fasting blood glucose ≥7.0 mmol/L, or a history of diabetes, or current use of anti-diabetes treatment.[38] In accordance with the current Chinese guidelines for the prevention and treatment of hypertension, presence of hypertension was diagnosed by any of the following: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, a history of hypertension, or current treatment for hypertension. Hyperlipidemia was defined as presence of at least one of the following: TG ≥1.69 mmol/L, TC ≥5.20 mmol/L, LDL-C ≥3.37 mmol/L, and HDL-C ≤1.04 mmol/L,[39] or a history of hyperlipidemia or current treatment for dyslipidemia. Hyperuricemia was diagnosed as serum uric acid ≥7.0 mg/dL in men, and ≥6.0 mg/dL in women.[40]

Statistical analysis

Statistical analyses were carried out with the Statistical Package for the Social Sciences (SPSS), version 18 (SPSS Inc, Chicago, IL, U.S.A.). Main characteristics of the subjects were presented as mean ± standard deviation (SD) or median (interquartile ranges) for continuous variables, and frequencies (percentage) for categorical variables. Student's t-tests (for continuous variables with a normal distribution) or Mann-Whitney U tests (for non-normally distributed data) were used to evaluate the differences between participants with and without NAFLD. Differences in categorical variables were assessed by Chi-squared tests. The concentration of SF was categorised based on quartiles (quartile 1: <25th percentile, quartile 2: ≥25th to 50th percentile, quartile 3: ≥50th to 75th percentile, quartile 4: ≥75th percentile). The odds ratio (OR) with 95% confidence intervals (CIs) were calculated from univariate and multivariate logistic regression analyses to determine the associations of SF with the risk of NAFLD and elevated ALT. In multivariate logistic regression analyses, model 1 was adjusted for age, gender and BMI. Model 2 was further adjusted for current smoking status, diabetes, hypertension, hyperlipidemia, and hyperuricemia. Stratified analyses were performed based on gender (men and women) and age (35~49y and ≥50y) to evaluate the associations of SF with NAFLD and ALT. The predicting values of SF for NAFLD were evaluated by plotting the area under the receiver operating characteristic (ROC) curve (AUC) and calculating the sensitivity and specificity. For all above, a two-tailed *p*-value <0.05 indicated statistically significant.

RESULTS

Characteristics of study subjects

Among 2029 normal-weight adults, approximately 16.7% (n=338) were diagnosed as NAFLD. Baseline characteristics of subjects are shown in Table 1. Compared with the controls, those with NAFLD were more likely to be older, male, current smokers, and have diabetes, hypertension, hyperlipidemia, and hyperuricemia. Levels of BMI, ALT and SF were higher in participants with NAFLD than the controls (all *p*-value <0.05).

Association between SF and NAFLD

As presented in Table 2, concentrations of SF (OR=4.64, 95% CI: 3.21-6.71) was associated with an increased risk of NAFLD for highest versus lowest quartile in unadjusted model. After adjustment for age, gender and BMI (model 1), the results (OR=3.51, 95% CI: 2.31-5.34) remained similar to the crude ORs. After further adjustment for more potential confounders, including current smoking status, hypertension, diabetes, hyperlipidemia, and hyperuricemia (model 2), SF was still significantly positively associated with the risk of NAFLD. The corresponding OR (95% CIs) was 3.19 (2.07–4.92).

The associations between SF and NAFLD of stratified analyses were evaluated (Supplementary table 1). In stratified analyses by gender, SF was positively associated with the risk of NAFLD in women. The corresponding OR (95% CIs) was 2.29 (1.31-3.99) in model 2 for highest versus lowest quartile. For men, SF was positively associated with NAFLD in unadjusted model (OR=2.10, 95% CI: 1.24-3.57) and model 1 (OR=1.97, 95% CI: 1.14-3.41). After further adjustment for potential confounders (model 2), the association between SF and NAFLD was not significant (OR=1.67, 95% CI: 0.94-2.95). In stratified analyses by age, for participants younger than 50 years old (n=1088), the multivariate-adjusted ORs (95% CIs) was 5.99 (2.51-14.30) for highest versus lowest quartile. No significant association was observed between SF (OR=1.41, 95% CI: 0.90-2.23) and the risk of NAFLD in participants aged ≥50 years old (n=941) after fully adjustment (model 2). Additionally, ROC analysis of diagnostic value for NAFLD is summarized in Table 3. AUC was 0.660 (0.63, 0.69) for total participants. Specifically, AUC values were 0.60 (0.54, 0.65) for men and 0.66 (0.62, 0.70) for women.

Table 1. Characteristics of participants by NAFLD.

Characteristics	NAFLD (n=338)	Non-NAFLD (n=1691)	Total (n=2029)	*p* value
Age, years	52 (46, 58)	47 (43, 55)	48 (44, 56)	<0.001
BMI, kg/m²	22.9 (22.2, 23.4)	22.1 (20.9, 23.0)	22.3 (21.1, 23.1)	<0.001
ALT, U/L	22.0 (17.0, 30.0)	17.0 (13.0, 21.0)	17.0 (13.0, 23.0)	<0.001
Hemoglobin, g/L	145 (136, 156)	137 (130, 147)	138 (130, 149)	<0.001
SF, μg/L	77.4 (38.7, 122)	44.9 (18.9, 81.4)	48.6 (21.0, 88.5)	<0.001
Sex				<0.001
Men	150/338 (44.4%)	489/1691 (28.9%)	639/2029 (31.5%)	
Women	188/338 (55.6%)	1202/1691 (71.08%)	1390/2029 (68.5%)	
Current smoking				<0.01
Yes	39/338 (11.5%)	123/1691 (7.27%)	162/2029 (7.98%)	
No	299/338 (88.5%)	1568/1691 (92.7%)	1867/202 (92.0%)	
Diabetes				<0.001
Yes	40/338 (11.8%)	58/1691 (3.4%)	98/2029 (4.83%)	
No	298/338 (88.2%)	1633/1691 (96.6%)	1931/202 (95.2%)	
Hypertension				<0.001
Yes	166/338 (49.1%)	536/1691 (31.7%)	702/2029 (34.6%)	
No	172/338 (50.9%)	1155/1691 (68.3%)	1327/2029 (65.4%)	
Hyperlipidemia				<0.001
Yes	256/338 (75.7%)	955/1691 (56.5%)	1211/2029 (59.7%)	
No	82/338 (24.3%)	736/1691 (43.5%)	818/2029 (40.3%)	
Hyperuricaemia				<0.001
Yes	61/338 (18.1%)	57/1691 (3.37%)	118/2029 (5.82%)	
No	277/338 (81.9%)	1634/1691 (96.6%)	1911/2029 (94.2%)	

BMI: body mass index; ALT: alanine aminotransferase; Hb: hemoglobin; SF: serum ferritin; NAFLD: non-alcoholic fatty liver disease.
Data are presented as means±standard deviations for normally distributed variables and medians (interquartile ranges) for the non-normally distributed variables.
Comparisons between participants with and without NAFLD were performed by using Student's t-tests or Mann-Whitney U tests for continuous variables, and Chi-square tests for categorical variables

Table 2. ORs and 95% CIs for NAFLD according to quartiles of SF in the study population

	Cutoff levels (μg/L)	OR (95% CI)		
		Crude	Model1	Model2
Q1	<21.0	1 (Ref.)	1 (Ref.)	1 (Ref.)
Q2	21.0-48.6	1.71 (1.14, 2.56)*	1.54 (1.01, 2.35)*	1.57 (1.03, 2.42)*
Q3	48.6-88.5	2.00 (1.35, 2.98)***	1.52 (0.99, 2.32)	1.47 (0.95, 2.27)
Q4	≥88.5	4.64 (3.21, 6.71)***	3.51 (2.31, 5.34)***	3.19 (2.07, 4.92)***
	p-trend	<0.001	<0.001	<0.001

SF, serum ferritin; NAFLD, non-alcoholic fatty liver disease.
Crude OR: Odds ratios (95% CIs) without any adjustment. Model 1: Odds ratios (95% CIs) adjusted for BMI, age, and sex; Model 2: Odds ratios (95% CIs) adjusted for BMI, age, sex, current smoking status, hypertension, diabetes, hyperlipidemia and hyperuricaemia.
*p<0.05; **p<0.01, ***p<0.001.

Association between SF and elevated ALT

As shown in Table 4, SF was not significantly associated with elevated ALT in unadjusted model (OR=1.19, 95% CI: 0.90-1.56). After adjustment for age, gender and BMI (model 1), SF was positively associated with elevated ALT. After further adjustment for potential confounders (model 2), SF was still significantly associated with elevated ALT. The corresponding ORs (95% CIs) of elevated ALT were 1. 90 (1.37-2.63) and 1.84 (1.32, 2.55) for highest versus lowest quartile in model 1 and model 2, respectively.

The associations between SF and elevated ALT in stratified analyses were also evaluated (Supplementary table 2). In stratified analyses by gender, the multivariate-adjusted ORs (95% CIs) of elevated ALT for SF was 1.53 (1.07-2.19) for the highest versus lowest quartile for women. However, no significant associations were found between SF and elevated ALT across quartiles 2 to 4 compared with quartile 1 for men. Stratified analyses by age indicated that significantly positive association between SF and elevated ALT was only observed in participants younger than 50 years (OR=1.90, 95% CI: 1.17, 3.09).

DISCUSSION

This study demonstrated that elevated SF was positively associated with the risk of NAFLD and elevated ALT after adjustment for potential confounders in middle-aged and elderly Chinese population with normal weight. According to the analyses stratified by age and gender, the significantly positive associations of SF with the risk of NAFLD and elevated ALT were only observed in women and 35~49y group in multivariate-adjusted model. In addition, adults with high SF levels of 93.6 μg/L for men, 35.6 μg/L for women are at the greatest risk for developing NAFLD. SF might be considered as a predictor for NAFLD in individuals with normal weight.

Table 3. Receiver operating characteristics (ROC) curve analysis for SF as a predictor of NAFLD stratified by gender

	SF cut-point (μg/L)	sensitivity (%)	specificity (%)	AUC (95%CI)	*p*
Total	72.5	54.1	70.3	0.66 (0.63, 0.69)	<0.001
Male	93.6	64.7	70.3	0.60 (0.54, 0.65)	<0.001
Female	35.6	69.1	55.4	0.66 (0.62, 0.70)	<0.001

AUC: area under curve; SF: serum ferritin; NAFLD: non-alcoholic fatty liver disease.

Table 4. ORs and 95% CIs for elevated ALT levels according to quartiles of SF in total participants

	Cutoff levels (μg/L)	OR (95% CI)		
		Crude	Model1	Model2
Q1	<21.0	1 (Ref.)	1 (Ref.)	1 (Ref.)
Q2	21.0-48.6	1.09 (0.83, 1.43)	1.11 (0.84, 1.48)	1.12 (0.84, 1.49)
Q3	48.6-88.5	1.35 (1.03, 1.76)*	1.47 (1.10, 1.97)*	1.48 (1.10, 1.99)**
Q4	≥88.5	1.19 (0.90, 1.56)	1. 90 (1.37, 2.63)***	1.84 (1.32, 2.55)***
	p-trend	0.22	<0.001	<0.001

SF: serum ferritin.
Crude OR: Odds ratios (95% CIs) without any adjustment. Model 1: Odds ratios (95%CIs) adjusted for BMI, age, and sex; Model 2: Odds ratios (95% CIs) adjusted for BMI, age, sex, current smoking status, hypertension, diabetes, hyperlipidemia, and hyperuricaemia.
*p<0.05; **p<0.01, ***p<0.001.

Previous studies on general[41,42] or obese individuals[22,43] have shown that iron metabolism disorders played a significant role in the development of NAFLD. Canbakan et al[32] conducted a study on patients with NAFLD in US, and found that SF level was one of the major risk factors predicting NAFLD. Jiang et al[31] revealed that SF was an independent parameter associated with NAFLD in Chinese adults. One case-control study[44] on Japanese population suggested that high SF concentration was a distinguishing feature of progressing NAFLD patients independent of HFE gene mutations. However, a case-control study[45] in Italy found that hepatic iron, ferritin and transferrin were not independent predictors of NAFLD. And findings of this study challenged the hypothesis that iron metabolism played a primary role in development of NAFLD. Abdominal adiposity is a major risk factor for the development of NAFLD.[46] Notably, Chinese people have different abdominal fat distributions and genetic characteristics compared with Europeans.[47] However, evidence on relationship between SF and NAFLD development among normal-weight population in China remains limited.

The underlying mechanism of the positive association of SF with NAFLD has not been completely understood. Several studies demonstrated that the hepatic iron accumulation could lead to the metabolic disorders and liver injury.[48] Some researches believed that elevated deposition of hepatic iron may potentiate the progression of NAFLD by catalyzing reactive oxygen species generation.[16,49] And oxidative stress mediated by iron plays an essential role in ferritin-induced cell death.[50] In vitro, ferritin released from hepatocytes has been shown to mediate apoptosis involving Fas (CD95) signalling,[51] the upregulation of p53, and increased mitochondrial membrane permeability.[50] Additionally, ferritin could also activate hepatic stellate cells through induction of signaling cascade and potentially drive hepatic fibrogenesis in an iron-independent manner.[52]

As a sensitive biomarker of liver injury, elevated ALT was found positively associated with SF among participants with normal BMI in our study. Previously, a study[28] in 1985 reported a significant correlation between serum ALT and serum ferritin concentration. And in this study, it was inferred that excess iron could damage hepatocyte membrane resulting in leakage of aminotransferases into plasma or increased synthesis of aminotransferases. A cohort study in France based on 197 children with severe obesity supported that higher ferritin concentrations are significantly associated with the elevation of ALT.[29] Hsiao et al[22] also noticed that SF was associated with elevated ALT in apparently obese patients. A study among 13,605 U.S adult participants reported that elevated ALT was positively associated with the transferrin saturation and serum iron concentration in general participants, while the association was not significant in participants with normal BMI (BMI <25 kg/m^2).[30]

Our study has several strengths. First, to our best knowledge, this is the first study to evaluate the associations of SF with the risk of NAFLD and elevated ALT in normal-weight Chinese. The results might promote greater awareness among normal-weight individuals about the risk of NAFLD. Second, the positive associations of SF with the risk of NAFLD and elevated ALT were statistically significant after adjustment for potential confounders, which confirmed the associations. Additionally, we also conducted the analyses stratified by age and gender to further investigate the aforementioned association. On the other hand, there are several limitations in our study. First, although ultrasound scan has a good sensitivity and specificity in identifying liver steatosis,[53] it's not the gold standard for the diagnosis of NAFLD. Second, the cross-sectional design makes it difficult to infer the causality. Moreover, we cannot rule out the possibility of residual confusion caused by other confounding factors.

Conclusion

In conclusion, SF was positively associated with the risk of NAFLD and elevated ALT in normal-weight Chinese. And SF may serve as an indicator of predicting NAFLD. Further large prospective studies are needed to validate these findings.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURES

Conflict of interest: None.

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