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ORIGINAL ARTICLE

# Association of glycated hemoglobin with the risk of advanced fibrosis in non-alcoholic fatty liver disease patients without diabetes

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**KEYWORDS**
Advanced fibrosis;
Glycated hemoglobin A1c;
Non-alcoholic fatty liver disease;
Obesity

**Summary**
*Background:* Association of diabetes with non-alcoholic steatohepatitis has been well documented. However, it remains unclear whether there is an association between levels of glycated hemoglobin ($HbA_{1c}$) with severity of non-alcoholic fatty liver disease (NAFLD). This study was aimed to explore the relationship between levels of $HbA_{1c}$ and the risk of advanced fibrosis in patients with NAFLD.
*Methods:* A cross-sectional study was performed on 4826 apparently healthy Chinese, who underwent a health check between January 2015 and December 2016. NAFLD was defined as hepatic steatosis on ultrasonography in the absence of excessive alcohol use or other identifiable causes. The risk of advanced fibrosis was assessed by NAFLD fibrosis Score.
*Results:* Among 4826 individuals studied, 1630 were diagnosed with NAFLD. In a multivariable-adjusted model, high $HbA_{1c}$ levels were associated independently with increased prevalence of NAFLD. The adjusted odds ratio [95% confidence interval (95% CI)] for NAFLD, when compared with the highest $HbA_{1c}$ quartile and the lowest $HbA_{1c}$ quartile, was 2.72 (2.07—3.58; *P* for trend < 0.001). A strong association was also observed between $HbA_{1c}$ level and the risk of fibrosis in patients with NAFLD in multivariable analyses, with the extreme-quartile odds ratio of 2.69 (95% CI: 1.60—4.53; *P* for trend < 0.001). This association remained significant even in subjects without diabetes.

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*Conclusions:* We concluded that high $HbA_{1c}$ level was associated strongly and independently with increased risk of advanced fibrosis in NAFLD patients without diabetes.


| | |
|---|---|
| NAFLD | non-alcoholic fatty liver disease |
| NASH | non-alcoholic steatohepatitis |
| T2DM | type-2 diabetes mellitus |
| $HbA_{1c}$ | glycated hemoglobin |
| NFS | Non-Alcoholic Fatty Liver Disease Fibrosis Score |
| BMI | body mass index |
| HDL | high-density lipoprotein |
| HOMA-IR | homeostasis model assessment of insulin resistance |
| OR | odds ratio |
| CI | confidence interval |

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder in industrialized western countries, and is becoming highly prevalent in Asia [1,2]. NAFLD encompasses a broad spectrum of liver diseases, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) with different degrees of fibrosis. The appearance of fibrosis is the most important feature of NAFLD associated with liver-related events and overall mortality [3].

In addition to its potential to progress to cirrhosis and other end-stage liver diseases, NAFLD is also considered as the hepatic component of metabolic syndrome, and is strongly associated with other metabolic comorbidities such as obesity and diabetes. A bidirectional association between NAFLD and type-2 diabetes mellitus (T2DM) has been strongly established [3]. It has been suggested that patients with T2DM show a high prevalence of NAFLD, and even a high prevalence of advanced fibrosis [4]. Thus, diabetes is considered as a predictor for advanced stages of fibrosis among NAFLD patients.

As an emerging diagnostic tool for diabetes, glycated hemoglobin ($HbA_{1c}$) has been increasingly accepted as an index of mean glycemia, and a measure of risk for the development of diabetic complications [5,6]. Compared with conventional diagnostic tool for diabetes, $HbA_{1c}$ has the advantages of readily assessable for measurement, biologically stable, and availability of standardized assays. A recent study reported a strong relationship between $HbA_{1c}$ levels and prevalence of NAFLD [7], suggesting $HbA_{1c}$ is a novel measure of risk for NAFLD. Available evidence also demonstrated that patients with advanced stages of NAFLD show an increased risk of diabetes. Based on the bidirectional causal relationship between NAFLD and T2DM mentioned above, we hypothesized that $HbA_{1c}$ levels may be a predictor for severity of NAFLD. Nevertheless, there is no association study on the relationship of $HbA_{1c}$ level and advanced fibrosis in patients with NAFLD. Therefore, we aimed to investigate the association of $HbA_{1c}$ levels with the prevalence and severity of NAFLD in a population in southern regions of China.

## Methods

### Study population

This study was performed in the 3rd Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong Province, China, from January 2015 to December 2016. The samples included 4726 employees and their spouses, aged 20—65 years old, from three large companies, and 2052 inhabitants aged over 50 years old from a large community in Guangzhou. We enrolled 5679 people who underwent a comprehensive annual or biennial examination at the Center for Health Examination and completed a standardized questionnaire. All participants were ethnically Han Chinese. Among these subjects, 853 were excluded for one or more of the following criteria: missing data on an abdominal ultrasonography or other covariates; positive serologic markers for hepatitis B or C virus; alcohol intake of greater than 140 g/week (male) or 70 g/week (female); history of malignancy; type 1 diabetes or anemia; use of agents associated with NAFLD within the past year such as valproate, amiodarone, tamoxifen, methotrexate, or corticosteroids. Patients with other known liver diseases, such as Wilson's disease, autoimmune hepatitis or liver transplantation, were also excluded. Thus, the final samples comprised 4826 participants, and all subsequent analyses were restricted to this group. Written informed consent was obtained from each participant before the study began. All procedures in this study were approved by the Ethic Committee of Sun Yat-sen University.

### Measurements

Liver ultrasonography (Siemens ACUSON sequoia 512, Acuson, CA, USA) was performed to evaluate hepatic steatosis. Hepatic ultrasonography examinations were performed by four experienced ultrasonographists who were blinded to the clinical data. The ultrasonographic diagnosis of fatty liver was defined by conventional criteria, such as evidence of diffuse hyperechogenicity of liver in relation to the kidney or spleen, ultrasound beam attenuation, and poor visualization of intra-hepatic structures [8].

NAFLD was defined as the presence of fatty liver following exclusion of excessive alcohol consumption

(a threshold of > 140 g/week for male and > 70 g/week for female) or other identifiable causes such as viral or autoimmune liver diseases [9]. In patients with NAFLD, serum markers for fibrosis were used to assess risk of advanced fibrosis. NAFLD Fibrosis Score (NFS) was calculated according to the published formula: NFS = −1.675 + 0.037 × age (years) + 0.094 × body mass index (BMI, kg/m$^2$) + 1.13 × impaired fasting glycemia or diabetes (yes = 1, no = 0) + 0.99 × aspartate aminotransferase/alanine aminotrasferase ratio − 0.013 × platelet (× 10$^9$/L) − 0.66 × albumin (g/dL) [10]. The NFS had an area under the receiver operating curve of 0.85 for predicting advanced fibrosis. Two cutoff points were used to categorize patients with NAFLD into three groups: high probability (> 0.676), intermediate probability (0.676 to −1.455), and low probability (< −1.455) for advanced fibrosis. A Score below −1.455 had 90% sensitivity of exclusion of advanced fibrosis, whereas a Score > 0.676 had 97% specificity for the presence of advanced fibrosis [11].

A standardized questionnaire on the general information that includes examination date, birth date, sex, physical activity at leisure-time, smoking habit, alcohol consumption, and medication history were conducted through a face-to-face interview. Smoking status was defined as at least one cigarette a day and lasting more than a year. Alcohol drinking status was defined as drinking any type of alcoholic beverage at least once a week and lasting more than half a year. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, an existing medical diagnosis, or use of anti-hypertensive agents. BMI (kg/m$^2$) was calculated as weight in kilograms divided by height in meters squared and obesity was defined as BMI ≥ 25 kg/m$^2$ [12]. Diabetes was defined either as fasting serum glucose ≥ 126 mg/dL, or $HbA_{1c}$ ≥ 6.5%, or use of anti-diabetic agents. Metabolic syndrome was diagnosed according to National Cholesterol Education Program Adult Treatment Plan III. Hypertriglyceridemia was defined as an existing diagnosis in medical record or serum triglycerides > 150 mg/dL. Low high-density lipoprotein (HDL) cholesterol was defined as serum HDL cholesterol < 40 mg/dL in men and < 50 mg/dL in women. Insulin resistance was assessed based on the calculation of homeostasis model assessment of insulin resistance (HOMA-IR).

Fast blood samples were drawn from antecubital vein after an overnight or at least 10 hours fasting. The blood samples were analyzed within 4 hours after collection. Plasma glucose and $HbA_{1c}$ levels were measured by glucose oxidase method and high-performance liquid chromatography (D-10, Bio-Rad, Hercules, CA, USA, reference range was 4.0−6.0%), respectively. The $HbA_{1c}$ assay has been certified by the National Glycohemoglobin Standardization Program as having documented traceability to the Diabetes Control and Complications Trial reference method. The intra and inter assay variation coefficients were 0.46% and 0.53%, respectively.

### Statistical analyses

The clinical characteristics were shown as means ± standard deviation or medians with interquartile ranges in cases of continuous data. Categorical data were presented as number and percentages of total subjects. The variables with skewed distributions were log-transformed before analysis. Comparisons of categorical variables were performed using the Chi$^2$-test. Numeric variables were compared by ANOVA or Kruskal−Wallis non-parametric tests depending on distribution of variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by logistic regression models to determine the independent association between $HbA_{1c}$ quartiles and the presence of NAFLD. Generalized linear models were used to associate $HbA_{1c}$ level with the risk of advanced fibrosis based on NFS. Age, sex, smoke status, alcohol intake, leisure-time physical activity, obesity, hypertension, diabetes, hypertriglyceridemia, low HDL cholesterol, and HOMA-IR were considered as potential confounders and were included in the models.

NFS was used to assess severity among patients with NAFLD. As only less than 1% of NAFLD patients were identified with high probability for advanced fibrosis, we combined the intermediate and high probability of advanced fibrosis before analysis. To control for the effects of sex or obesity, the patients with NAFLD were stratified into two groups depending on the presence of either sex or obesity. Stratified analyses were also conducted to explore whether the association of $HbA_{1c}$ with the presence and severity of NAFLD differed by the presence of diabetes. In addition, we performed sensitivity analyses using other established formula for advanced fibrosis in NAFLD, such as fibrosis 4 Score (Supplemental Methods) [13].

Statistical analyses were performed using SPSS 19.0 (IBM SPSS Inc., Chicago, IL, USA) for Windows. All *P*-values were two-tailed, and statistical significance was set at $P < 0.05$.

## Results

### Participant characteristics

The clinical characteristics of participants involved in this study were summarized in Table 1. Within the 4826 participants, the average age was 49 ± 14 years; of whom 54.8% were male, 38.6% with obesity, and 8.5% with diabetes. Patients with NAFLD were largely older in age, with higher prevalence in male, and in those with obesity, diabetes, hypertension and metabolic syndrome. They showed higher serum triglycerides level and HOMA-IR, and lower HDL cholesterol level when compared with those without NAFLD. In particular, patients with NAFLD had significantly higher fasting glucose and $HbA_{1c}$ levels than those without NAFLD.

### $HbA_{1c}$ and risk factors of NAFLD

The relationship between $HbA_{1c}$ levels with conventional NAFLD risk factors was shown in Table 2. $HbA_{1c}$ level was associated significantly with sex, obesity, hypertension, diabetes, metabolic syndrome, hypertriglyceridemia, and low HDL cholesterol in the univariate analysis. In the multivariate model, association of sex, obesity, diabetes, hypertriglyceridemia, and low HDL cholesterol with the level of $HbA_{1c}$ remained significant.

**Table 1** Clinical characteristics of participants with or without non-alcoholic fatty liver disease (NAFLD), as reflected by the measurement of NAFLD Fibrosis Score (NFS).

| Characteristics | All | No. NAFLD | NAFLD, low NFS | NAFLD, intermediate or high NFS | *P*-value |
|---|---|---|---|---|---|
| No. of participants | 4826 | 3196 | 1129 | 501 | |
| Age, y | 48.8 ± 13.5 | 48.7 ± 13.9 | 44.5 ± 10.4 | 59.5 ± 10.9 | < 0.001 |
| Male, % | 54.8 | 48.8 | 77.3 | 42.5 | < 0.001 |
| Body mass index, kg/m$^2$ | 24.2 ± 3.6 | 22.9 ± 3.0 | 26.5 ± 3.0 | 27.2 ± 3.6 | < 0.001 |
| Obesity, % | 38.6 | 22.7 | 69.7 | 70.1 | < 0.001 |
| Hypertension, % | 19.1 | 14.5 | 29 | 26.1 | < 0.001 |
| Diabetes, % | 8.5 | 5.1 | 3.7 | 40.9 | < 0.001 |
| Metabolic syndrome, % | 11 | 4.4 | 15.8 | 42.1 | < 0.001 |
| Current smoker, % | 12.6 | 12 | 13.7 | 14.2 | 0.171 |
| Alcohol drinking, % | 9.2 | 8.9 | 10 | 9.4 | 0.527 |
| Leisure-time physical activity, % | | | | | < 0.001 |
| None | 23.2 | 15.3 | 39.7 | 36.9 | |
| < 30 min/day | 18.7 | 17.5 | 19.8 | 24.4 | |
| ≥ 30 min/day | 58 | 67.2 | 40.6 | 38.7 | |
| Systolic blood pressure, mmHg | 121.5 ± 18.6 | 118.2 ± 17.4 | 128.7 ± 19.6 | 126.7 ± 18.5 | < 0.001 |
| Diastolic blood pressure, mmHg | 73.2 ± 10.5 | 71.2 ± 9.7 | 77.4 ± 10.8 | 76.4 ± 11.3 | < 0.001 |
| Aspartate aminotransferase, U/L | 22 (19–27) | 22 (19–26) | 24 (20–30) | 23 (19–29) | < 0.001 |
| Alanine aminotransferase, U/L | 23 (16–34) | 20 (15–28) | 33 (24–48) | 24 (18–35) | < 0.001 |
| Gamma-glutamytransferase, U/L | 32 (21–48) | 26 (18–41) | 44 (31–56) | 47 (36–56) | < 0.001 |
| Total cholesterol, mg/dL | 205.7 ± 40.7 | 201.6 ± 39.8 | 209.1 ± 40.0 | 224.1 ± 42.6 | < 0.001 |
| Triglycerides, mg/dL | 109.9 (77.1–163.0) | 94.8 (69.1–132.0) | 149.7 (110.8–224.6) | 155.9 (114.3–233.1) | < 0.001 |
| HDL cholesterol, mg/dL | 52.0 ± 13.2 | 54.8 ± 13.4 | 45.2 ± 10.2 | 48.8 ± 11.1 | < 0.001 |
| LDL cholesterol, mg/dL | 127.1 ± 35.9 | 123.6 ± 35.8 | 131.7 ± 34.2 | 139.1 ± 36.3 | < 0.001 |
| Albumin, g/dL | 4.50 (4.46–4.67) | 4.50 (4.49–4.62) | 4.56 (4.39–4.77) | 4.49 (4.12–4.67) | < 0.001 |
| Platelets, × 10$^9$/L | 204.0 (193.6–216.6) | 198.7 (192.0–210.7) | 214.9 (204.0–221.9) | 212.9 (200.3–220.2) | < 0.001 |
| HOMA-IR | 1.61 (1.20–2.67) | 1.36 (1.11–1.87) | 2.54 (1.77–3.60) | 3.39 (2.33–5.19) | < 0.001 |
| Glucose, mg/dL | 95.9 (89.6–103.9) | 94.3 (88.6–100.9) | 96.5 (90.5–102.2) | 116.1 (105.5–134.2) | < 0.001 |
| $HbA_{1c}$, % | 5.5 (5.3–5.8) | 5.4 (5.2–5.7) | 5.6 (5.3–5.8) | 6.1 (5.8–6.9) | < 0.001 |

HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-IR: homeostasis model assessment of insulin resistance; $HbA_{1c}$: glycated hemoglobin.

**Table 2** Associations of glycated hemoglobin levels with risk factors of non-alcoholic fatty liver disease (NAFLD).

| Risk factors of NAFLD | Univariate | | Multivariate[a] | |
|---|---|---|---|---|
| | β | *P*-value | β | *P*-value |
| Age | 0.377 | < 0.001 | 0.236 | < 0.001 |
| Male | 0.032 | 0.026 | 0.021 | 0.045 |
| Obesity | 0.158 | < 0.001 | 0.043 | < 0.001 |
| Hypertension | 0.088 | < 0.001 | 0.013 | 0.245 |
| Diabetes | 0.693 | < 0.001 | 0.629 | < 0.001 |
| Hypertriglyceridemia | 0.169 | < 0.001 | 0.06 | < 0.001 |
| Low HDL cholesterol | 0.043 | 0.003 | 0.026 | 0.01 |
| Metabolic syndrome | 0.271 | < 0.001 | 0.002 | 0.876 |

HDL: high-density lipoprotein.
[a] Multivariate model indicates the independent determinants of glycated hemoglobin using linear regression with the risk factors of NAFLD entered into the similar model.

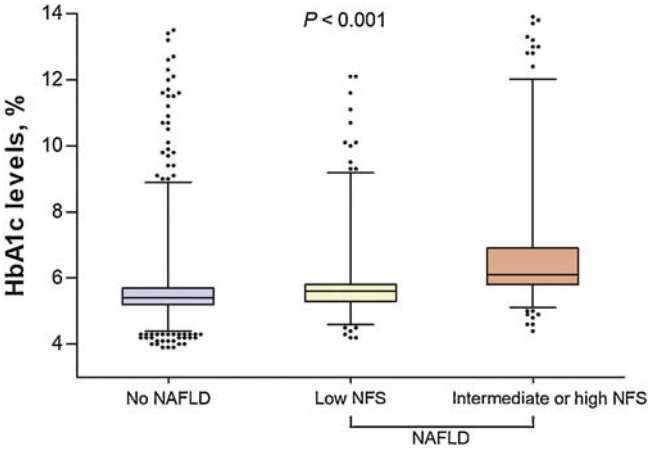


**Figure 1** Association of glycated hemoglobin ($HbA_{1c}$) levels with the severity of non-alcoholic fatty liver disease (NAFLD) based on NAFLD Fibrosis Score (NFS).

## $HbA_{1c}$ and NAFLD

In the population studied, 33.8% had NAFLD, of whom 30.7% had a high or intermediate probability to develop advanced fibrosis as suggested by the NFS. Division of all subjects into quartiles according to $HbA_{1c}$ level showed an independently association of $HbA_{1c}$ level with the presence of NAFLD in a dose-response manner (Table 3). In logistic regression analysis, subjects in the highest quartile of $HbA_{1c}$ level, when compared with the lowest quartile, showed a significantly higher risk for NAFLD (OR: 6.71; 95% CI: 5.37—8.37; *P* for trend < 0.001) after adjustment for age and sex. These associations remained significant after further adjustment with multivariates, including smoke status, alcohol intake, leisure-time physical activity, obesity, hypertension, diabetes, hypertriglyceridemia, low HDL cholesterol, and HOMA-IR.

## $HbA_{1c}$ and the risk of advanced fibrosis

Strong association was observed between $HbA_{1c}$ level and the severity of NAFLD based on NFS (Fig. 1, *P* for trend < 0.001). Box-plot data as categorized by sex or obesity of individual participants also showed significant associations of $HbA_{1c}$ levels with severity of NAFLD, which were not altered by controlling for the confounders (Fig. 2A—D).

The association between $HbA_{1c}$ level and risk of advanced fibrosis was further examined in patients with NAFLD. $HbA_{1c}$ levels were positively correlated with NFS as continuous variables (Fig. 3, *P*-value < 0.001). Division of the NAFLD patients into quartiles according to $HbA_{1c}$ level showed a significant association of $HbA_{1c}$ with risk of advanced fibrosis as suggested by the NFS. In logistic regression analysis, the age-sex-adjusted and fully-adjusted ORs (95% CI) for the highest $HbA_{1c}$ quartile were 10.68 (6.92—16.48) and 2.69 (1.60—4.53), respectively (Figure S1A, *P* for trend < 0.001).

## $HbA_{1c}$ and NAFLD in subjects with or without diabetes

Associations of $HbA_{1c}$ levels with the presence and severity of NAFLD remained significant in subjects without diabetes, in a dose-response manner (Table 4 and Fig. 2F, *P* for trend < 0.001). But $HbA_{1c}$ levels did not correlate with the presence of NAFLD (Table 4; extreme-quartile OR: 1.33; 95% CI: 0.63—2.81; *P* for trend = 0.511), or the risk of advanced fibrosis (Fig. 2E, *P* for trend = 0.993) in diabetics.

**Table 3** Odds ratios[a] (ORs) of non-alcoholic fatty liver disease by glycated hemoglobin ($HbA_{1c}$) quartiles.

| $HbA_{1c}$ levels, % | Number | Cases | Age-sex-adjusted OR (95% CI) | Multivariate-adjusted OR (95% CI) | |
|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 |
| Quartile 1 (≤ 5.2) | 1197 | 219 | 1 | 1 | 1 |
| Quartile 2 (5.3—5.5) | 1402 | 408 | 1.94 (1.60—2.35) | 1.86 (1.53—2.27) | 1.54 (1.24—1.93) |
| Quartile 3 (5.6—5.8) | 1098 | 448 | 3.46 (2.82—4.24) | 3.19 (2.59—3.94) | 2.39 (1.89—3.02) |
| Quartile 4 (≥ 5.9) | 1129 | 555 | 6.71 (5.37—8.37) | 6.03 (4.80—7.57) | 2.72 (2.07—3.58) |
| *P* for trend | | | < 0.001 | < 0.001 | < 0.001 |

CI: confidence interval.
Model 1: adjustment for age, sex, smoke status, alcohol intake, leisure-time physical activity; model 2: model 1 plus adjustment for obesity, hypertension, diabetes, hypertriglyceridemia, low high-density lipoprotein cholesterol, and HOMA-IR.
[a] Estimated from logistic regression models.

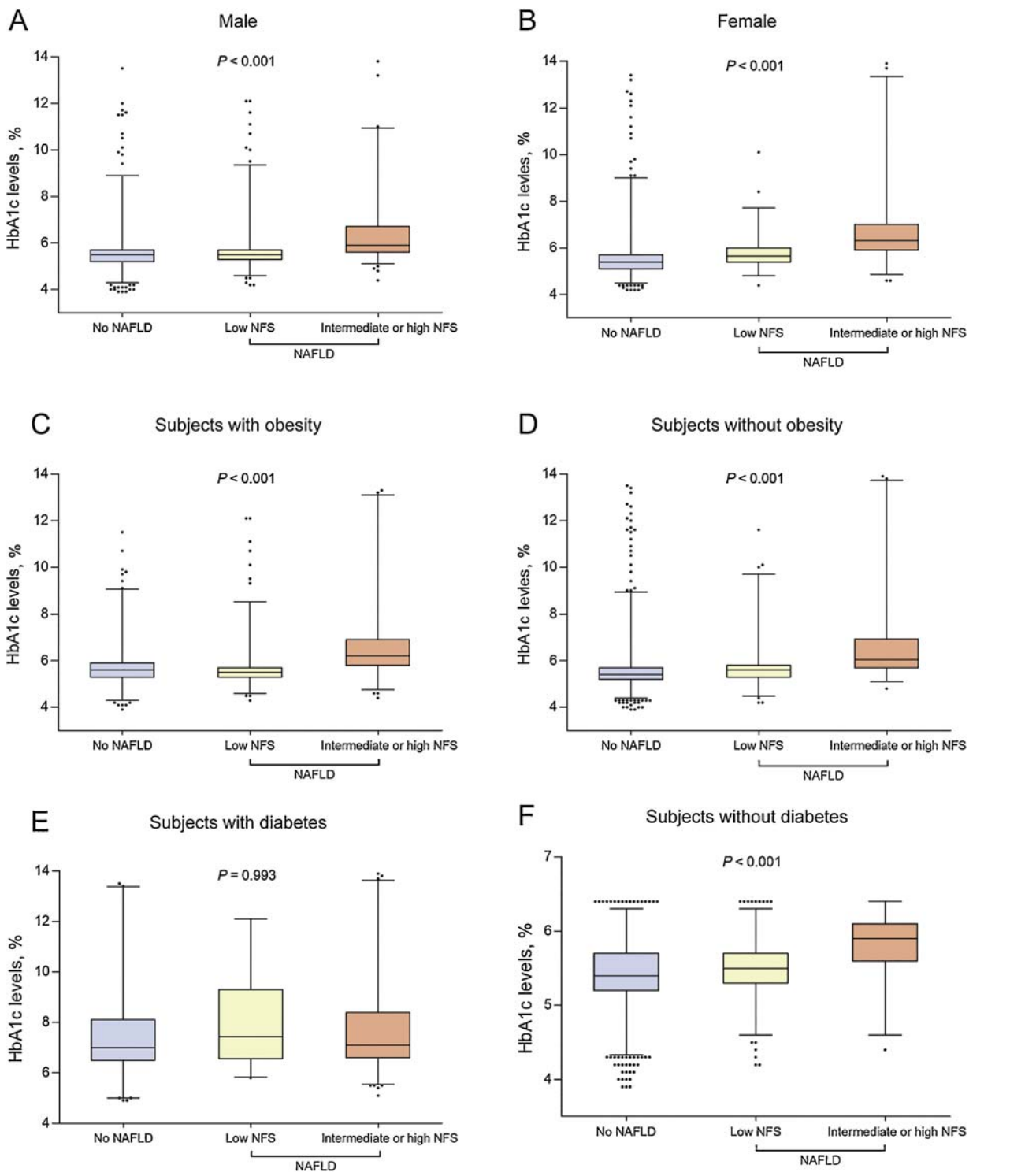


**Figure 2** Changes of glycated hemoglobin ($HbA_{1c}$) levels in patients with non-alcoholic fatty liver disease (NAFLD) based on NAFLD Fibrosis Score (NFS) in men (A), women (B), subjects with obesity (C) or without obesity (D), and subjects with diabetes (E) or without diabetes (F). *P*-value represents comparisons among the three groups.

## Sensitivity analyses

In sensitivity analyses with severity of NAFLD based on the fibrosis 4 Score, there was no significant change in either direction or magnitude of estimates (Figures S1B, S2, and S3).

## Discussion

In this population-based study, we demonstrate that $HbA_{1c}$ level is associated with the presence and severity of NAFLD, independent of potential confounders. $HbA_{1c}$ level is increased with increasing NFS among patients with NAFLD,

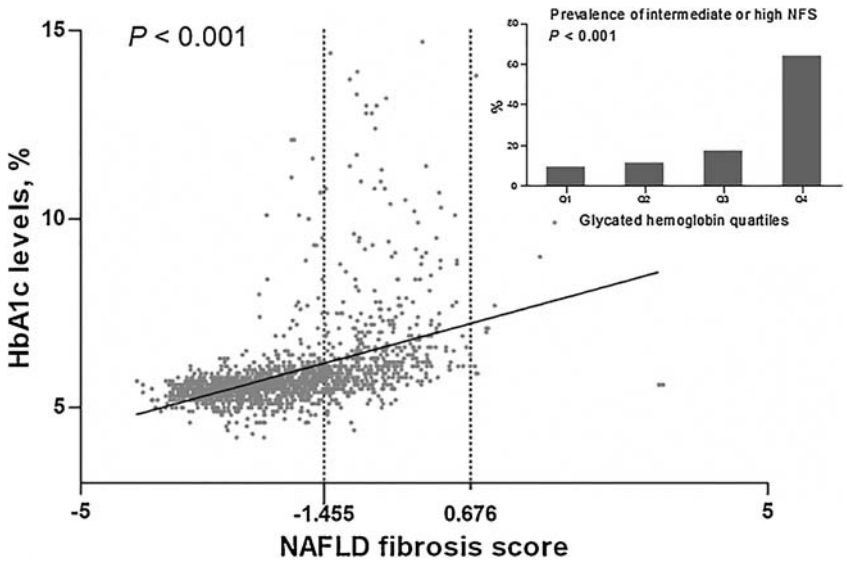


**Figure 3** Relationship between glycated hemoglobin ($HbA_{1c}$) level and non-alcoholic fatty liver disease (NAFLD) Fibrosis Score (NFS) in patients with NAFLD. The insert summarized the presence of intermediate or high NFS in patients with different $HbA_{1c}$ quartiles.

**Table 4** Odds ratios[a] (ORs) of non-alcoholic fatty liver disease by glycated hemoglobin ($HbA_{1c}$) quartiles in subjects with or without diabetes.

| $HbA_{1c}$ levels, % | Number | Cases | Age-sex-adjusted OR (95% CI) | Multivariate-adjusted OR (95% CI) | |
|---|---|---|---|---|---|
| | | | | Model 1 | Model 2 |
| Subjects with diabetes | | | | | |
| Quartile 1 (≤ 6.5) | 97 | 50 | 1 | 1 | 1 |
| Quartile 2 (6.6—7.0) | 102 | 61 | 1.43 (0.80—2.54) | 1.46 (0.81—2.65) | 1.15 (0.58—2.28) |
| Quartile 3 (7.1—8.2) | 106 | 67 | 1.66 (0.93—2.95) | 1.71 (0.94—3.09) | 1.41 (0.70—2.81) |
| Quartile 4 (≥ 8.3) | 106 | 69 | 1.87 (1.05—3.34) | 1.94 (1.07—3.53) | 1.33 (0.63—2.81) |
| *P* for trend | | | 0.067 | 0.058 | 0.511 |
| Subjects without diabetes | | | | | |
| Quartile 1 (≤ 5.2) | 1190 | 218 | 1 | 1 | 1 |
| Quartile 2 (5.3—5.4) | 884 | 225 | 1.54 (1.24—1.91) | 1.54 (1.23—1.92) | 1.30 (1.01—1.68) |
| Quartile 3 (5.5—5.7) | 1345 | 512 | 3.00 (2.46—3.65) | 2.75 (2.25—3.37) | 2.22 (1.77—2.79) |
| Quartile 4 (≥ 5.8) | 996 | 428 | 4.89 (3.90—6.13) | 4.39 (3.48—5.53) | 2.56 (1.97—3.34) |
| *P* for trend | | | < 0.001 | < 0.001 | < 0.001 |

CI: confidence interval.
Model 1: adjustment for age, sex, smoke status, alcohol intake, leisure-time physical activity; model 2: model 1 plus adjustment for obesity, hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol, and HOMA-IR.
[a] Estimated from logistic regression models.

and NAFLD patients with intermediate or high NFS show the highest $HbA_{1c}$ levels, suggesting that $HbA_{1c}$ level is an independent predictor of advanced fibrosis.

Our findings gain supports from a prior study that reports a similar relationship between $HbA_{1c}$ level and the presence of NAFLD in an elder population [7]. Here, we extend the evidence of the association between $HbA_{1c}$ level and the risk of NAFLD and advanced fibrosis to a general population. As mentioned previously, fibrosis stage is the most important feature of NAFLD associated with adverse outcomes. However, to distinguish advanced fibrosis from simple steatosis, patients are required to undergo a liver biopsy, which cannot be performed on general healthy individuals. Therefore, a series of non-invasive tools are needed to assess the severity of liver disease.

As a measure of average glycemia, $HbA_{1c}$, are currently used to monitor glycemic control in patients with diabetes [14]. Although there were few studies focused on the relationship between $HbA_{1c}$ and fibrosis in NAFLD patients, some small studies have demonstrated that oral glucose tolerance may be useful for assessment of fibrosis in NALFD patients [15]. A mini clinical study (39 patients with biopsy-proven NAFLD) found that a decrease in $HbA_{1c}$ level was associated with improvement in liver fibrosis [16], suggesting that $HbA_{1c}$ may be a potential marker for severity of NAFLD. In the present study, we consistently found the association

between $HbA_{1c}$ level and the risk of NAFLD and its severity in a large population. Along with monitoring the liver function status, our study highlights the necessity for active assessment of $HbA_{1c}$ in NAFLD patients and provides evidence that high $HbA_{1c}$ level may serve as a reference for physicians in addressing surveillance strategies for high risk patients and defining the appropriate time for clinical interventions.

The association between $HbA_{1c}$ level and the risk of NAFLD and its severity is mainly explained by the bidirectional relationship between T2DM and NAFLD. Diabetes is suggested to promote development of NASH, and increase risk of progression to cirrhosis and hepatocellular carcinoma [17]. Moreover, patients with coexistence of NAFLD and prediabetes or T2DM are common to develop higher risks of severe complications and worse metabolic profiles. In the present study, we also found that the association between $HbA_{1c}$ level and the risk of NAFLD and its severity persisted even after excluding patients with diabetes, indicating that $HbA_{1c}$ level is a potential index for management of non-diabetic NAFLD patients. A better understanding of the pathogenic mechanisms of fatty liver, together with its relationship with glycometabolism, have given great insight into a paradigm shift in the management of NAFLD.

However, it is vital to notice that these associations were not present among diabetic patients. The absence of association between $HbA_{1c}$ level and NAFLD can be explained in two ways. First, the samples of our study derived from a population who underwent regular medical check-up and management. Thus, patients with newly diagnosed diabetes in the study population were scarce, and most established diabetes were on anti-diabetic treatments (oral hypoglycemic agents or insulin). In this case, mean glycemia assessed by $HbA_{1c}$ level could not evaluate the ''natural'' glucose fluctuations. Second, it was recently suggested that non-invasive scoring systems are less accurate at predicting fibrosis in NAFLD patients with diabetes. In a longitudinal study of 284 cases of NAFLD where liver biopsy was undertaken, the accuracy of three serum fibrosis algorithms, including fibrosis 4 Score, was significantly diminished in diabetics compared to non-diabetics [18]. As shown in that study, NFS was likely to be a more reliable prognostic tool than other non-invasive serum fibrosis models. Nevertheless, NFS of utilizing the presence of impaired fasting glucose as a covariate may also overestimate the fibrosis stage in diabetics, leading an information bias. So, the problem with relationship between $HbA_{1c}$ level and fibrosis stages using serum Score systems in patients with NAFLD and diabetes is of concern, and further studies are warranted to explore such issue.

There are several limitations to the present study. Assessments of the presence and severity of NAFLD are mainly based on ultrasonic diagnosis and NFS. It has been demonstrated that sensitivity of ultrasonic measurement is relatively inaccurate when steatosis is less than 30% [19], and there is a limitation of using NFS to assess the severity of NAFLD as it relies on single measurement of serum biomarkers without histological evidence. However, the technical and ethical concerns for taking liver biopsy from asymptomatic subjects are significant obstacles for the study design. In addition, NFS have been validated in NAFLD patients of varying ethnicity, and is widely used for identifying patients with risk for advanced fibrosis [3,11]. Based on these points, ultrasound and NFS are more feasible for population-based studies, because of their non-invasive nature and safer to perform. Another limitation is that definition of fibrosis stages using NFS may be complicated by the inclusion of hyperglycemia in the formula, and the combination intermediate with high NFS overestimates the severity of NAFLD in patients with diabetes, which leads to a selection bias in the present study. For example, the prevalence of diabetes in NAFLD patients with intermediate or high NFS was relatively high (40.2%). To minimize this complication, we examine the association of $HbA_{1c}$ level with sensitivity analyses of NAFLD as indicated by the fibrosis 4 Score that does not include glucose status, and we also perform additional analyses with the diabetics excluded. We find that these associations do not show obvious change. Lastly, we have studied a population of middle-aged and old Chinese with a high prevalence of obesity, so present results should be taken with caution when generalized to other populations.

In summary, our results indicate that $HbA_{1c}$ level is associated with the presence of NAFLD in Chinese adults, and high $HbA_{1c}$ level is also independently associated with increased risks of advanced fibrosis in NAFLD patients without diabetes, supporting a proactive approach in assessment of $HbA_{1c}$ in the management of patients with NAFLD.

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## Disclosure of interest

The authors declare that they have no competing interest.

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## Appendix A Supplementary data

Supplementary data related to this article can be found, in the online version, at https://doi.org/10.1016/j.clinre.2018.08.007.

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