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ORIGINAL ARTICLE

Increased risks of different grades of non-alcoholic fatty liver disease in prediabetic subjects with impaired fasting glucose and glucose tolerance, including the isolated glycosylated hemoglobin levels of 5.7–6.4% in a Chinese population

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ABSTRACT

Aims/Introduction: Contrary to the results of the majority of studies on diabetes, there are some conflicting results regarding the relationship between non-alcoholic fatty liver disease (NAFLD) and prediabetes. No study has investigated the relationship between isolated glycated hemoglobin (HbA1c) in the range of 5.7–6.4% (HbA1c 5.7–6.4%) and NAFLD. Our aim was to investigate the effect of different glycemic statuses on NAFLD concomitantly categorized by fasting plasma glucose, 2-h plasma glucose and HbA1c levels.

Materials and Methods: NAFLD was classified into three groups by ultrasonographic examination results: normal, mild and moderate-to-severe. Glycemic status was divided into five groups: normoglycemia, isolated HbA1c 5.7–6.4%, impaired fasting glucose without impaired glucose tolerance (IGT), IGT and newly diagnosed diabetes. For multivariable logistic regression analyses, the outcome variable was the classified three grades of fatty changes in the liver after adjusting for other potential risk covariables.

Results: In this cross-sectional research, a total of 8,571 eligible individuals were enrolled and divided into three groups: 5,499 without fatty liver, 2,113 with mild NAFLD and 959 with moderate-to-severe NAFLD. Multivariable logistic regression analysis showed that IGT, impaired fasting glucose without IGT and isolated HbA1c 5.7–6.4% were associated with a higher risk of NAFLD in addition to newly diagnosed diabetes. Other positively predictive variables were male sex, obesity, overweight, central obesity, increased triglyceride and C-reactive protein >1 mg/L. Negatively associated factors were elevated high-density lipoprotein cholesterol levels.

Conclusions: Besides diabetes, the increased risks of different grades of NAFLD were found for prediabetic individuals categorized by impaired fasting glucose without IGT, IGT and isolated HbA1c 5.7–6.4%.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by an abnormal fat accumulation of ≥5%, which is referred to as hepatic steatosis, without excessive alcohol consumption and competing liver disease etiologies[1,2]. NAFLD is the most common chronic liver disease in Western countries, with a global prevalence of 25.24%[2]. NAFLD comprises a wide spectrum of histological abnormalities ranging from simple hepatic steatosis, non-alcoholic steatohepatitis, liver fibrosis, cirrhosis and eventually hepatocellular carcinoma[3]. Many metabolic abnormalities have been found to be associated with NAFLD[1,2], and its main mechanism has been linked with insulin resistance[4]. NAFLD has been found to coexist with diabetes mellitus[5], with a prevalence rate ranging between 49.6 and 74% in patients with diabetes mellitus[6]. Additionally, diabetes mellitus has been considered to be a predictor of advanced stages of NAFLD[6,7]. However, independent of obesity or other metabolic factors, NAFLD per se can also be a cause of insulin resistance, and has a direct role in the pathogenesis of diabetes mellitus[5,7].

Since 2009, in addition to pre-existing diabetes based on fasting plasma glucose (FPG) and 2-h plasma glucose (2-h PG) after an oral glucose tolerance test (OGTT), glycated hemoglobin (HbA1c) has been suggested to be a diagnostic criterion for the detection of diabetes and prediabetes[8]. However, many reports have pointed out diagnostic discrepancies among the three criteria used to categorize hyperglycemia, which could indicate distinct pathophysiological processes and aspects of glucose metabolism different from impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)[8–10]. When compared with use of the OGTT, there are several advantages of using FPG and HbA1c values, including greater convenience and fewer day-to-day perturbations (or greater stability), respectively[11]. The detection rate of diabetes mellitus did not decrease when HbA1c and FPG were combined relative to detection by 2-h PG after OGTT[12]. Therefore, the 2-h PG values after OGTT have gradually become less frequently used, because it is more time-consuming, relatively expensive and requires that patients fast. These strategies might lead to loss of early interventions for some patients with undiagnosed diabetes, and loss of early prevention for NAFLD progression in the role of exacerbating the diabetes[7].

In contrast to the results of the majority of studies on diabetes mellitus, although the prevalence of NAFLD is higher in prediabetes individuals than in normoglycemic individuals[13], there are some conflicting results from studies that have explored the relationship between NAFLD and prediabetes, including IFG and IGT results[14–16]. Yamada *et al.*[16] found that fatty liver predicted IFG and diabetes mellitus on the basis of FPG level alone. In contrast, Mohan *et al.*[15] concluded that NAFLD was only associated with diabetes mellitus, but not with IFG or IGT, a finding that was also discordant with the finding of Shiga *et al.*[14] of NAFLD by 2-h PG levels. In addition to IFG and IGT, the status of NAFLD associated with the newly categorized isolated high HbA1c group, which might have been categorized into the normal glycemic population in the past, had not been previously understood. Nevertheless, the prevalence of prediabetes with NAFLD has been estimated to range from 44 to 62% in the USA, from 45 to 78% in Germany and 60% in Korea, which suggests a future epidemic of liver complications[17]and the development of diabetes[18]. The disappearance of fatty liver could also be a prognostic factor for regression from IGT to normal glucose regulation in individuals with NAFLD[19]. Therefore, the aim of the present study was to investigate the relationship between NAFLD and different glycemic statuses concomitantly categorized by FPG, 2-h PG and HbA1c in a Chinese population.

METHODS

Study population

All individuals examined at the Health Examination Center of National Cheng Kung University Hospital, Tainan, Taiwan, from June 2001 to December 2010 were initially included. This retrospective study used a cross-sectional design without any personally identifiable information. None of the women participants were pregnant. Individuals of both sexes with significant alcohol drinking >140 g/week were first excluded according to the definitions of the American College of Gastroenterology and American Association for the Study of Liver Disease[20]. The other exclusion criteria were as follows: (i) age <18 years; (ii) presence of hepatitis B, hepatitis C or other liver diseases, such as autoimmune hepatitis, drug-related liver disease, biliary hepatic injury, liver cirrhosis or any other self-reported liver disease; (iii) anemia (hemoglobin <13.0 g/dL in men and <12.0 g/dL in women) or hemolytic anemia and other self-reported hemoglobinopathies[21]; (iv) serum creatinine >1.5 mg/dL; (v) congestive heart failure; (vi) diabetes and hypertension history; (vii) inflammation/infection disease, or white blood cell count<12/L and C-reactive protein (CRP) <7 mg/L; (viii) use of medications, such as oral hypoglycemic agents, antihypertensive agents, lipid-lowering agents, Chinese herbs, estrogen and steroids; (ix) obstructive sleep apnea; and (x) history of cancer. Habitual exercise was defined as vigorous exercise of at least three times per week[22]. Finally, a total of 8,571 eligible individuals (64% men and 36% women) were included in the final analysis. The study protocol was approved by the Ethics Committee for Human Research at National Cheng Kung University Hospital, Taiwan (Approval No. ER-108-257).

Clinical parameter assessment

All participants completed a structured questionnaire containing questions regarding medical history, medication use and lifestyle habits, including alcohol consumption and regular exercise. While fasting and wearing only light indoor clothes without shoes, each participant had their bodyweight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) measured by a well-trained nurse, and the body mass index (BMI) was calculated.

Following the recommendations of the Health Promotion Administration in Taiwan, overweight and obesity were respectively defined as a $27 > \text{BMI} \geq 24$ and BMI ≥ 27 kg/m^2[23]. Waist circumference (WC) was measured from the midpoint between the lower rib margin and the iliac crest while the participants were standing with normal expiration at the end. WC ≥90 cm in men or ≥80 cm in women were defined as central obesity[24].Blood pressure was measured in the right arm with a DINAMAP vital sign monitor (Model 1846SX DINAMAP Monitor; Critikon Inc., Tampa, FL, USA) in the sitting position after 10 min of rest, and the mean systolic and diastolic blood pressure (SBP and DBP) values of the two measurements were recorded. Newly categorized hypertension was defined as SBP >140 or DBP >90 mmHg without a history of hypertension[25]. After 10 h of fasting, a venous blood sample was drawn for the measurement of complete blood cell counts, FPG, HbA1c, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and CRP. The Fibrosis-4 score was calculated as (age [years] × AST) / (platelet count × ALT$^{1/2}$), and a lower cut-off value of Fibrosis-4 <1.30 was used for indicating no hepatic advanced fibrosis[26].Then, 75 g of anhydrous glucose in 250 mL of water was administered over the course of 5 min. A blood sample to measure 2-h PG was collected 2-h after the participant began to drink the glucose solution. FPG and 2-h PG levels were determined by using the hexokinase method (Roche Diagnostic GmbH, Mannheim, Germany). HbA1c levels were measured by using ion-exchange high-performance liquid chromatography (HbA1c, BIO-RAD V-II TURBO Hemoglobin HbA1c program; Bio-Rad Laboratories, Inc., Kent, UK). For our study purpose of the effect of glycemic statuses on the risk of NAFLD, FPG, 2-h PG and HbA1c values were concomitantly used for categorizing hyperglycemic status into five categories according to the 2009 American Diabetes Association diagnostic criteria[8], which were: (i) normoglycemic: FPG <5.6 mmol/L, 2-h PG <7.8 mmol/L and HbA1c <5.7%; (ii) isolated HbA1c 5.7–6.4%: FPG <5.6 mmol/L, 2-h PG <7.8 mmol/L and HbA1c 5.7–6.4%; (iii) IGT: 2-h PG of 7.8–11.0 mmol/L, FPG <7.0 mmol/L and HbA1c < 6.5%; (iv) IFG without IGT: FPG of 5.7–7.0 mmol/L, 2-h PG <7.8 mmol/L and HbA1c < 6.5%; and (v) newly diagnosed diabetes (NDD): FPG ≥7.0 mmol/L, 2-h PG ≥11.1 mmol/L or HbA1c ≥6.5%. The prediabetic status included groups 2, 3 and 4.

Ultrasonography of the liver

Abdominal ultrasonographic examination was carried out by two experienced radiologists who used high-resolution ultrasonography plus convex-type real-time electronic scanners (Xario SSA-660A; Toshiba, Nasu, Japan) and a 3.5-MHz linear transducer. Mild NAFLD was defined as a slight increase in liver echogenicity, mild attenuation in penetration by the ultrasound signal, and a slight decrease in the lucidity of the borders of the intrahepatic vessel walls and diaphragm. Moderate NAFLD was defined as a diffuse increase in liver echogenicity, greater attenuation in penetration by the ultrasound signal and a decrease in the visualization of the intrahepatic vessel walls, particularly the peripheral branches. Severe NAFLD was defined as a gross increase in liver echogenicity, greater attenuation in penetration by the ultrasound signal, and poor or no visualization of the hepatic vessels and diaphragm[27]. Considering that <1% of our study population was identified to be having severe NAFLD (just 72 participants), we merged the moderate and severe groups. Therefore, the extent of fatty changes in the liver on ultrasonography of all the recruited participants was categorized into the following three grades: none, mild and moderate-to-severe.

Statistical analysis

All data analyses were carried out using SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA). The comparisons of significant differences in unadjusted independent continuous variables were carried out using ANOVA with Scheffé's *post-hoc* test, and the χ^2-test was used for categorical variables. Although there was a specific order, but no exact spacing that existed between the three stages of our dependent variables based on ultrasonography, ordinal logistic regression analysis was not adapted because of the significant proportional odds ($P < 0.001$). Instead, multivariable logistic regression models were further carried out to identify the classified three grades of fatty changes in the liver from the effect of different glycemic statuses, including isolated HbA1c ranging from 5.7 to 6.4% (HbA1c 5.7–6.4%), IGT, IFG without IGT and NDD, and other potential risk or metabolic covariables, such as age, sex, obesity, overweight, central obesity, hypertension, triglyceride, HDL-C, CRP and habitual exercise. Three kinds of models were shown in the adjustment of overweight, obesity and central obesity, either separately or simultaneously. *P*-values <0.05 were considered to be indicative of statistical significance.

RESULTS

Table 1 presents a comparison of the clinical characteristics of the participants who were divided into three groups by the different degree of fatty change in the liver based on abdominal ultrasonographic findings, including 5,499 participants without fatty liver, 2,113 participants with mild NAFLD and 959 participants with moderate-to-severe NAFLD. The participants with NAFLD had significantly different age, sex, BMI, SBP, DBP, WC, cholesterol, TG, HDL-C, CRP, AST, ALT, platelet, FGP, 2-h PG, HbA1c and habitual exercise. Scheffé's *post-hoc* test showed that in the moderate-to-severe NAFLD group had higher values of BMI, SBP, DBP, WC, TG, CRP, AST, ALT, FGP, 2-h PG, HbA1c and lower values of HDL-C than the mild group. In this recruited study population, the proportion without an advanced degree of fibrosis predicted by Fibrosis-4 scores among these three groups was not significantly different. In addition, to clarify the effect of the metabolic categories, especially regarding the five glycemic statuses (the distribution

is shown in Figure 1), there were also significant differences between these three groups, as seen in Table 1.

In Table 2, multivariable logistic regression analysis adjusted in addition to glycemic parameters for other reported potential factors subsequently showed that each prediabetes status and diabetes status was associated with a higher risk of NAFLD. Although obesity and overweight were controlled for in model 1, and central obesity was controlled for in model 2, each prediabetes status was significantly persisted and associated with an increased risk of NAFLD after adjustment, including male participants, and increased triglyceride and CRP levels. In contrast, elevated HDL-C levels were associated with lower risks of NAFLD. In model 3, when obesity, overweight and central obesity were considered concomitantly, we still found a consistent tendency of the odds ratios of the remaining variables toward prediction of developing NAFLD.

DISCUSSION

This is the first study to show a relationship between different grades of NAFLD and prediabetes categorized concomitantly by HbA1c, FPG and 2-h PG levels. Independent of other metabolic risk and lifestyle factors, in addition to participants with NDD, we found that participants with IFG and IGT had higher risks of NAFLD. Furthermore, the present study showed that NAFLD was significantly associated with individuals suffering from chronic hyperglycemic disease with isolated HbA1c 5.7–6.4%, which was previously categorized into the normal glycemic status and is therefore being ignored or not being studied.

Previously, the association between fatty liver and prediabetes with IFG or IGT was inconsistent, not to mention that there was a lack of evidence with isolated HbA1c 5.7–6.4%[13–16]. NAFLD was more prevalent in individuals with prediabetes than in normoglycemic individuals[13], However, Rajput *et al.*[13]

Table 1 | Comparison of clinical characteristics among participants with the different ultrasound-based grading of the extent of fatty change in the liver

Variables	NAFLD			*P*-value*	*Post-hoc* test**
	1. None (n = 5,499)	2. Mild (n = 2,113)	3. Moderate-to-severe (n = 959)		
Age (years)	45.65 ± 12.61	49.17 ± 11.15	48.23 ± 11.11	<0.001	a, b
Sex, male (*n*/ratio)	3,114/56.6	1,623/76.8	749/78.1	<0.001	NA
BMI (kg/m^2)	22.76 ± 2.71	26.13 ± 2.66	28.17 ± 3.45	<0.001	a, b, c
WC (cm)	79.01 ± 8.54	89.03 ± 7.36	94.26 ± 9.03	<0.001	a, b, c
SBP (mmHg)	113.98 ± 15.26	122.10 ± 15.62	126.17 ± 16.08	<0.001	a, b, c
DBP (mmHg)	67.36 ± 9.86	73.35 ± 9.98	75.66 ± 10.64	<0.001	a, b, c
Cholesterol (mmol/L)	5.04 ± 0.93	5.32 ± 0.95	5.41 ± 0.92	<0.001	a, b
Triglyceride (mmol/L)	1.22 ± 0.71	1.86 ± 1.10	2.23 ± 1.23	<0.001	a, b, c
HDL-C (mmol/L)	1.37 ± 0.36	1.13 ± 0.26	1.06 ± 0.25	<0.001	a, b, c
CRP (mg/L)	1.24 ± 1.55	1.97 ± 1.77	2.34 ± 1.83	<0.001	a, b, c
AST (U/L)	22.73 ± 8.33	26.78 ± 14.67	33.50 ± 18.94	<0.001	a, b, c
ALT (U/L)	23.52 ± 14.27	36.17 ± 23.97	54.00 ± 36.18	<0.001	a, b, c
Platelet ($10^3/\mu$L)	248.37 ± 54.87	253.86 ± 57.23	257.38 ± 56.07	<0.001	a, b
FIB-4 index score†, ≥1.3 (*n*/ratio)	1,003/18.2	344/16.3	150/15.6	0.038	NA
FPG (mmol/L)	4.87 ± 0.76	5.25 ± 1.15	5.50 ± 1.25	<0.001	a, b, c
2h-PG (mmol/L)	6.14 ± 2.32	7.37 ± 3.16	8.28 ± 3.72	<0.001	a, b, c
HbA1c (%)	5.51 ± 0.54	5.77 ± 0.82	5.91 ± 0.91	<0.001	a, b, c
Different glycemic status					
Normoglycemic (*n*/ratio)	3,551/60.9	776/36.7	258/26.9	<0.001	NA
Isolated HbA1c 5.7–6.4%‡ (*n*/ratio)	977/17.8	490/21.3	172/28.3		
IGT‡ (*n*/ratio)	727/13.2	450/23.2	271/17.9		
IFG without IGT‡ (*n*/ratio)	224/4.1	160/7.6	80/8.3		
NDD (*n*/ratio)	220/4.0	237/11.2	178/18.6		
Habitual exercise§ (*n*/ratio)	452/8.2	140/6.6	60/6.3	0.016	NA

Data are presented as the mean ± standard deviation or numbers/ratio. *$P < 0.05$, for ANOVA or χ^2-tests. **$P < 0.05$, analysis of continuous variables with Scheffé's *post-hoc* tests: a–1 versus 2; b–1 versus 3; c–2 versus 3. †Fibrosis-4 (FIB-4) was calculated using the equation: (age × aspartate transaminase) / (platelet counts × alanine aminotransferase$^{1/2}$). ‡Prediabetes: total distributions as shown in Figure 1. §Defined as exercise at least three times per week. 2h-PG, 2-h post-load glucose; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; FIB-4, fibrosis-4; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NA, not applicable; NAFLD, non-alcoholic fatty liver disease; NDD, newly diagnosed diabetes; SBP, systolic blood pressure; WC, waist circumference.

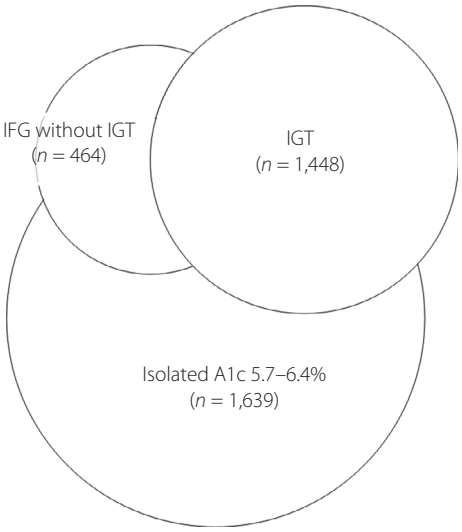

Figure 1 | Except for normoglycemic (*n* = 4,385) and newly diagnosed diabetes (*n* = 635) individuals, the Venn diagram represents different domains and agreements of prediabetes. A1c, glycated hemoglobin; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

did not show the respective effect of IFG or IGT. Yamada *et al.*[16] concluded that fatty liver was significantly associated with IFG patients; however, the study only had fasting glucose data, but no 2-h PG data. In contrast, Shiga *et al.*[14] showed that NAFLD was clearly related to 2-h PG, but not to FPG. Mohan *et al.*[15] found that NAFLD was only associated with diabetes mellitus, but not with IFG or IGT, which was explained by the small sample size of their prediabetes individuals. Although Shiga *et al.*[14] pointed out the important relationship of 2-h PG, but not of FPG, with NAFLD, they should have noted if there were confounding effects, because the significant adjusted findings of calculated insulin resistance to NAFLD by Shiga *et al.*[14] and by Mohan *et al.*[15] were determined by FPG.

Academically, individuals with isolated IFG predominantly showed hepatic insulin resistance, whereas those with isolated IGT showed moderate-to-severe muscle insulin resistance[28]. In addition to skeletal muscle insulin resistance accompanying insulin resistance at other sites, accumulated fat in the liver is frequently accompanied by hepatic insulin resistance, but there is considerable debate regarding whether or not this correlation is causal[29].

A major underlying mechanism of NAFLD is insulin resistance[4] because of the findings of increased gluconeogenesis and glycogenolysis in the liver, as well as reduced glucose disposal in peripheral muscles, which exacerbates hyperglycemia and consequently hyperinsulinemia, and the findings of upregulated lipogenic factors and promotion of hepatic lipogenesis, which contribute to development of NAFLD[7]. HbA1c is a marker representing the mean glucose concentration reflecting the cumulative glycemic history over the past 2–3 months[30]. Among individuals with normal oral glucose tolerance, but isolated high HbA1c, Fu *et al.*[10] found that they showed impaired early-phase β-cell dysfunction and increased generalized insulin resistance. Additionally, underlying systemic insulin resistance resulting in hyperinsulinemia[31] might reasonably explain our finding that prediabetes with HbA1c 5.7–6.4% significantly increased the risk for NAFLD, which is similar to the finding of Giulio *et al.* of NAFLD in lean individuals with normal glucose tolerance[4]. Therefore, irrespective of IFG or IGT, prediabetes classified according to HbA1c 5.7–6.4% not only represents a chronic overt hyperglycemic state with fewer day-to-day fluctuations[11], but also contributes to the development of NAFLD in a pathophysiological manner.

Taken together, our work shows for the first time that NAFLD groups presented with both significant IFG and IGT manifestation after adjusting for other metabolic risk factors. Additionally, although the sample size of the IFG without IGT group in the present study was smaller than that of the IGT group, the odds ratio for NFALD was somewhat greater in the IFG without IGT group than that in the IGT group, which highlighted the importance of investigating NAFLD with hepatic insulin resistance in future research.

Despite conflicting evidence for sex as a risk factor for NAFLD[32], the present study showed that male sex was associated with a higher risk of developing NAFLD. When compared with premenopausal women (data not shown) who were understood to have a protective effect of estrogen against development of NAFLD, male sex was still associated with an increased risk of developing NAFLD[32].

Obesity is an important underlying cause of NAFLD, and BMI is believed to be correlated with NAFLD[33]. WC is another anthropometric index that is a strong predictor of the presence of NAFLD[13,34]. As neither BMI nor WC could replace visceral fat adiposity as the most vital predictor of NAFLD[34], we concomitantly used these two predictors for final adjustment and still found that they were highly independent predictors of NAFLD. Otherwise, obesity-related adipose tissue dysfunction or insulin resistance could contribute to a pro-inflammatory state with increased cytokines[35]. As we had initially excluded individuals with inflammation or signs of infection, the present study showed a positive association of CRP ≥1 mg/L, which was consistent with the finding of a previous study that showed increased levels of tumor necrosis factor-alpha and interleukin-6 in NAFLD patients[35,36].

The non-significant difference of the proportion of advanced fibrosis among the three grades of fatty changes in the liver indicates that after our strict exclusion criteria, the present study population was relatively healthy, thereby minimizing the effect of confounders and improving our research for exploring the relationship between hyperglycemic and NAFLD parameters. Our findings related to the role of lipid profiles in NAFLD were consistent with those of other studies in which increased triglyceride levels increased the risk of NAFLD and increased HDL levels decreased the risk[37,38]. Habitual exercise will cause

Table 2 | Adjusted odds ratio (OR) and 95% confidence interval (CI) of variables for the risk of mild and moderate-to-severe fatty liver based on multivariable logistic regression analysis.

Variables	Model 1				Model 2				Model 3			
	Mild vs no NAFLD		Moderate-to-severe vs no NAFLD		Mild vs no NAFLD		Moderate-to-severe vs no NAFLD		Mild vs no NAFLD		Moderate-to-severe vs no NAFLD	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Age	1.01	(1.00–1.01)*	1.00	(0.99–1.00)	1.00	(1.00–1.01)	0.98	(0.97–0.99) *	1.01	(1.00–1.01)	0.99	(0.98–1.00) *
Sex (male vs female)	1.65	(1.44–1.90)**	1.57	(1.28–1.92)**	2.26	(1.97–2.58)**	2.26	(1.85–2.75) **	1.82	(1.58–2.09) **	1.84	(1.50–2.27) **
Overweight (yes vs no)†	4.28	(3.74–4.90) **	6.61	(5.10–8.56) **		–		–	3.39	(2.93–3.92) **	4.00	(3.03–5.28) **
Obesity (yes vs no)†	12.16	(10.23–14.45) **	43.85	(33.35–57.33) **		–		–	7.13	(5.80–8.76) **	16.16	(11.80–22.13) **
Central Obesity (yes vs no)‡		–		–	4.85	(4.29–5.48) **	13.33	(11.06–16.06) **	1.99	(1.71–2.31) **	3.68	(2.94–4.62) **
Hypertension (yes vs no)§	1.19	(0.99–1.44)	1.26	(1.00–1.61)	1.19	(0.99–1.42)	1.31	(1.04–1.65)	1.15	(0.95–1.38)	1.19	(0.93–1.51)
Triglycemide (mmol/L, ≥1.7 vs <1.7)	2.33	(2.04–2.66) **	3.44	(2.88–4.12) **	2.41	(2.12–2.74) **	3.52	(2.95–4.19) **	2.30	(2.01–2.63) **	3.39	(2.83–4.07) **
HDL-C (mmol/L, ≥1 vs <1)	0.66	(0.58–0.76) **	0.55	(0.45–0.66) **	0.63	(0.55–0.72) **	0.51	(0.43–0.62) **	0.68	(0.59–0.78) **	0.57	(0.47–0.69) **
CRP (mg/L, ≥1 vs <1)	1.43	(1.26–1.62) **	1.54	(1.28–1.86) **	1.56	(1.38–1.76) **	1.77	(1.48–2.13) **	1.39	(1.23–1.58) **	1.47	(1.21–1.78) **
Prediabetes		–		–		–		–		–		–
Isolated HbA1c 5.7%–6.4% vs normoglycemic	1.53	(1.31–1.79) **	1.58	(1.24–2.01) **	1.62	(1.39–1.89) **	1.69	(1.34–2.14) **	1.51	(1.29–1.77) **	1.53	(1.20–1.95) *
IGT vs normoglycemic	1.65	(1.40–1.96) **	2.80	(2.22–3.52) **	1.69	(1.43–1.99) **	2.90	(2.32–3.64) **	1.62	(1.36–1.92) **	2.70	(2.14–3.41) **
IFG without IGT vs normoglycemic	1.97	(1.53–2.52) **	3.10	(2.22–4.33) **	2.14	(1.67–2.73) **	3.31	(2.38–4.60) **	1.92	(1.50–2.47) **	3.03	(2.16–4.24) **
NDD vs normoglycemic	2.49	(1.96–3.16) **	5.23	(3.88–7.05) **	2.37	(1.88–2.99) **	4.97	(3.73–6.64) **	2.37	(1.86–3.01) **	4.80	(3.55–6.49) **
Habitual exercise¶, ≥3 vs <3 times/week	0.73	(0.58–0.92)	0.73	(0.53–1.02)	0.83	(0.67–1.04)	0.88	(0.64–1.22)	0.76	(0.60–0.95)*	0.76	(0.54–1.06)

*$P < 0.05$, **$P < 0.001$. †Overweight was defined as 27 > BMI ≥ 24; Obesity was defined as BMI ≥ 27. ‡Central obesity was defined as waist circumference ≥90 cm for male and ≥80 cm for female. §Hypertension was defined as mean systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. ¶Habitual exercise was defined as exercise at least three times per week. CRP, C-reactive protein; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease; NDD, newly diagnosed diabetes.

increased levels of HDL[39]; however, both of which were inconsistent with the finding of the decreased risk of NAFLD. This insignificant result of habitual exercise on NAFLD might be related to the small sample size of that in each group (shown in Table 1). In addition, when we changed the classification of fatty liver to with versus without, the effect of habitual exercise on decreasing fatty liver was significant (adjusted odds 0.76, 95% confidence interval 0.61–0.95, $P = 0.014$), which was in concordance with the results of a previous study[40].

The difference between age and different grades of NAFLD was not significant before adjustment by Scheffé's *post-hoc* test initially shown in Table 1. In Table 2, although the effects of age on NAFLD between the three models under adjustments in the present study were not consistent with the findings of another study[41], inclusion of age might lead to collinearity with highly associated variables, which might be mediated by intermediate parameters.

This large study cohort had some limitations. The first was the cross-sectional design, which meant that it was not possible to determine if associations of factors with NAFLD were causal. Second, the participants were limited to a Chinese population, so the findings might not be applicable to other population groups; however, it complemented the research between different ethnic groups. Third, the present data were extracted from a health management center, and although we applied many exclusion criteria to minimize confounding effects, the results should be interpreted carefully when trying to apply them to the general population. Finally, although abdominal ultrasonographic examination might not be the gold standard for NAFLD diagnosis, it is the most commonly used tool, with 60–94% sensitivity and 66–95% specificity[7]. Our data collection and interpretation were also limited to two experienced radiologists to minimize potential operator-dependent biases.

In conclusion, the present study results showed that prediabetes status characterized by HbA1c 5.7–6.4%, in addition to characterization by NDD, IFG without IGT and IGT, was also associated with an increased risk of NAFLD. In addition to the traditionally used FPG or 2-h PG, individuals who have been diagnosed as having NAFLD should also have their HbA1c status checked, which might provide additional evidence that prediabetes status can be established on the basis of isolated HbA1c 5.7–6.4%.

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DISCLOSURE

The authors declare no conflict of interest.

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