

Lean Nonalcoholic Fatty Liver Disease and Risk of Incident Diabetes in a Euglycemic Population Receiving Health Checkups: A Cohort Study

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Original investigation

Keywords: Lean non alcoholic fatty liver disease, Type 2 diabetes, BAAT scores, Obesity

Posted Date: June 10th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-34083/v1>

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Abstract

Background: Although recent evidence suggests that nonalcoholic fatty liver disease (NAFLD) is associated with insulin resistance and an increased risk of diabetes, the association between lean NAFLD and incident diabetes is unclear. This study aimed to investigate whether lean NAFLD and overweight/obese NAFLD have similar or dissimilar effects on the risk of new-onset diabetes.

Methods: A longitudinal study was performed in 14,482 euglycemic adults who participated in a health check-up program. Fatty liver was diagnosed by abdominal ultrasonography. The outcome of interest was incident diabetes. Cox proportional hazards regression models were applied to calculate HRs with 95% CIs for future diabetes risk.

Results: During the median 6.0 years of follow-up, 356 cases of diabetes occurred. Despite a low probability of hepatic fibrosis indicated by the BAAT score, lean NAFLD was positively associated with an increased risk of diabetes. Moreover, after adjusting for sociodemographic and potential confounders, the fully adjusted HRs (95% CIs) for incident diabetes between lean NAFLD and overweight/obese NAFLD to the reference (lean without NAFLD) were 2.58 (95% CI 1.68 to 3.97) and 2.52 (95% CI 1.79 to 3.55), respectively. In post hoc analysis, the HR (95% CI) for diabetes comparing lean NAFLD to obese/overweight NAFLD was 1.02 (95% CI 0.68 to 1.54, $p = 0.909$). The results were robust to challenges in multiple subgroup analyses and appeared to be more pronounced for female participants (p for interaction = 0.005).

Conclusions: In this cohort study, lean patients with NAFLD had a risk of incident type 2 diabetes similar to that of overweight/obese ones with NAFLD. These findings suggest that lean NAFLD is not a benign condition. Further investigations are needed to gain a better understanding of the pathogenesis and natural history of NAFLD in lean subjects.

Background

Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming a serious public health issue, as it is associated with elevated liver-related and -unrelated morbidity and mortality.[1] The overall prevalence of NAFLD is estimated to be 25% in the general population worldwide.[2] NAFLD is typically linked to obesity, though it can also occur in a substantial proportion of individuals with a normal or low body mass index (BMI), which is termed lean NAFLD and frequently overlooked in clinical practice.[3-5]

Although the mechanisms involved in the development and progression of NAFLD in individuals with a lean status are not entirely understood, current evidence suggests that they may differ from those occurring in individuals with obesity. Nonetheless, it is well established that genetic predisposition, such as polymorphisms in PNPLA3,[6, 7] SREBP,[8] CETP,[9] and TM6SF2[7], appears to be more important in the pathophysiology of lean NAFLD. Moreover, limited studies of prognosis have found that in the long term, lean subjects with NAFLD are at a higher risk for severe liver disease[10] and mortality[11] than obese ones with NAFLD. All these factors deliver a key message that lean NAFLD is a distinctive phenotype rather than a byproduct of BMI.[12]

The liver is a key organ that plays critical roles in the regulation of systemic glucose and lipid metabolism.[13] A recent meta-analysis involving 19 observational studies with 296,439 participants convincingly demonstrated that NAFLD is significantly associated with a twofold increased risk of new-onset diabetes (random-effects hazard ratio 2.22, 95% CI 1.84–2.60; $I^2 = 79.2\%$). [14] However, data describing the association between lean NAFLD and type 2 diabetes (T2DM) risk are scarce. Therefore, we conducted a longitudinal cohort study to investigate the effect of lean NAFLD and its severity on the risk of incident diabetes.

Methods

Date sources and study population

This is an observational cohort study performed by using participants' data from the DRYAD public database (<https://datadryad.org>). Initially, the records of 20,944 participants who attended a comprehensive medical examination program at Murakami Memorial Hospital between 2004 and 2015 were extracted by Okamura T et al.[15, 16] The details of the medical health check-up programme were described previously.[15, 17] Individuals were excluded at baseline for the following reasons: 1) no available records for abdominal ultrasonography and important variables including age, sex, BMI, waist circumference (WC),

blood pressure, or fasting plasma glucose (FPG), triglycerides (TG), and glycated hemoglobin (HbA1c) levels; 2) other known chronic liver diseases, such as liver cirrhosis (history or findings on ultrasound), viral hepatitis (defined by serum positive serological markers for HBV or HCV), or alcoholic fatty liver disease (mean alcohol consumption at least 60 g for males and 40 g for females per day); 3) use of any medication; or 4) diagnosed with diabetes or impaired fasting glucose (IFG) at baseline. To avoid reverse association, individuals with a follow-up period < 1 year were excluded. Moreover, participants with an undefined diabetes status at the follow-up visit were also excluded. Some participants met more than one exclusion criterion. Ultimately, 14,482 subjects were selected for further analysis in the present study (Figure 1).

This study conformed to the Declaration of Helsinki. Given that our data were obtained from the public database, no prior ethical approval was required. The requirement for informed consent was also waived as the data were anonymous.

Data acquisition

As described in the previous study,[15] at each visit to the health check center, the participants' demographic data, including age, sex, smoking status, drinking status, exercise habits and medication history, were acquired from a standardized questionnaire by the same trained team of interviewers. Smoking status was categorized as non, ex-, or current smoker.[18] Individuals who performed any type of physical activity at least once a week on a regular basis were considered regular exercisers.[19] Average alcohol consumption per week was calculated by asking the frequency and amount of alcoholic beverage during the prior month. Grade of alcohol consumption was defined as follows: no or minimal (< 40 g/wk); light (40 - 140 g/wk); moderate (>140 g/wk). [20] Physical parameters, including height, weight, waist circumference, and blood pressure, were measured by trained investigators under standardized conditions according to a standard protocol. BMI was calculated as weight in kilograms divided by height in meters squared, and the result is expressed in units of kg/m².

Venous blood specimens drawn from the antecubital vein were obtained after an overnight fast of at least 8 h. Laboratory analyses, including TGs, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), gamma glutamyltransferase (GGT), HbA1c, FPG, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels, were carried out in accordance with relevant guidelines and regulations using a Modular Analytics system (Hitachi High-Technologies Corp., Ltd., Tokyo, Japan), which is widely applied for biochemical analysis in Japan.

Abdominal ultrasonographic examinations were performed by trained sonographers using an Aloka SSD-650CL ultrasound machine (Aloka Co., Ltd., Tokyo, Japan) at baseline, and all ultrasonographic images were stored as photocopies.[18] Fatty liver was diagnosed by gastroenterologists according to the following four known criteria: hepatorenal echo contrast, liver brightness, gradual attenuation of far-field, and vascular blurring.[21] The clinicians were all blinded to the clinical data of the participants.

Endpoint and definitions

The endpoint was the occurrence of incident diabetes during the follow-up period. According to the diagnostic criteria of the American Diabetes Association,[22] diabetes was defined as FPG level ≥ 7.00 mmol/L and/or HbA1c $\geq 6.5\%$ and/or self-reported diabetes that was previously diagnosed by a physician and/or current use of anti-hyperglycemic agents.

NAFLD was defined as the presence of fatty liver in the absence of excessive alcohol consumption. There is no standard definition of lean NAFLD; however, numerous studies recommended a cut-off point of BMI 23 kg/m² for Asian populations,[23-25] and the following four groups were assessed: lean without NAFLD, lean with NAFLD, overweight/obese without NAFLD, and overweight/obese with NAFLD. For further NAFLD categorization, a representative noninvasive score was used to assess the severity of fibrosis. BAAT scores consist of the sum of the following categorical variables: BMI (≥ 28 kg/m² = 1), age (≥ 50 years = 1), ALT [≥ 2 UNL (male ≥ 60 IU/L; female ≥ 40 IU/L) = 1], and triglycerides (≥ 1.7 mmol/L = 1). Patients with NAFLD were further categorized into two groups: low (BAAT < 2) and high (BAAT ≥ 2) probability of advanced fibrosis.[26]

Visceral-fat obesity was defined as a waist circumference ≥ 90 cm in males or ≥ 80 cm in females.[27]

Statistical analysis

Categorical variables are presented as counts (percentages), and continuous data are expressed as means (standard deviations, SD). The characteristics of the study participants at baseline are summarized in Table 1, and significant differences among the four groups were analyzed by one-way analysis of variance (ANOVA) followed by the LSD post hoc test and Chi-square test for continuous variables and categorical variables, respectively.

Table 1
Baseline characteristics of all participants.

Characteristics	Total	Lean without NAFLD	Overweight/obese without NAFLD	Lean with NAFLD	Overweight/obese with NAFLD	p value [†]	p value [‡]
Number	14482 (100)	8900 (61.5)	3008 (20.8)	514 (3.5)	2060 (14.2)	-	-
Age (yrs)	43.7 (8.8)	43.0 (8.9)	44.7 (8.8)	45.6 (8.3)	44.6 (8.2)	< 0.001	0.014
Male	7898 (54.5)	3746 (42.1)	2031 (67.5)	408 (79.4)	1713 (83.2)	< 0.001	0.044
Body weight (kg)	60.7(11.6)	54.5(7.8)	68.9(8.3)	61.3(6.7)	75.1(10.5)	< 0.001	< 0.001
BMI (kg/m ²)	22.1(3.1)	20.2 (1.7)	24.8 (1.7)	21.7 (1.1)	26.4 (2.7)	< 0.001	< 0.001
Waist circ. (cm)	76.5 (9.1)	71.5(6.3)	83.0 (5.9)	78.3 (4.8)	88.1 (7.1)	< 0.001	< 0.001
Smoking status						< 0.001	0.066
Never	8456 (58.4)	5847 (65.7)	1463 (48.6)	252 (49.0)	894 (43.4)		
Former	2752 (19.0)	1344 (15.1)	722 (24.0)	129 (25.1)	557 (27.0)		
Current	3274 (22.6)	1709 (19.2)	823 (27.4)	133 (25.9)	609 (29.6)		
Alcohol intake (g/wk)	47.3 (82.0)	41.3 (76.0)	62.9 (93.1)	40.3 (76.7)	52.6 (87.8)	< 0.001	0.002
Non-drinker	11085 (76.5)	7039 (79.1)	2078 (69.1)	413 (80.4)	1555 (75.5)	< 0.001	0.038
Regular exerciser	2522 (17.4)	1614 (18.1)	537 (17.9)	82 (16.0)	289 (14.0)	< 0.001	0.267
SystolicBP (mmHg)	115 (15.0)	110 (13.4)	120 (14.2)	117 (13.5)	126 (14.7)	< 0.001	< 0.001
DiastolicBP (mmHg)	72 (10.5)	69 (9.4)	75 (9.9)	74 (9.4)	79 (10.1)	< 0.001	< 0.001
TC (mg/dl)	198.1(33.3)	193.2 (32.3)	202.2 (33.2)	207.7 (33.5)	211.0 (33.2)	< 0.001	0.040
Triglycerides (mg/dl)	80.9 (58.0)	63.4 (41.4)	91.7 (58.5)	111.0 (70.196)	133.3 (74.8)	< 0.001	< 0.001
HDL-c (mg/dl)	56.3 (15.5)	61 (15.3)	51.4 (12.9)	49.6 (13.5)	45 (10.5)	< 0.001	< 0.001
FPG (mg/dl)	92.9(7.5)	91.0 (7.2)	94.7 (6.9)	96.2 (7.0)	97.5 (6.4)	< 0.001	< 0.001
HbA1c (%)	5.2 (0.3)	5.1 (0.3)	5.2 (0.3)	5.3 (0.3)	5.3 (0.3)	< 0.001	0.054
HbA1c (mmol/mol)	33.0 (3.5)	32.6 (3.4)	33.0 (3.5)	34.0 (3.7)	34.4 (3.6)	< 0.001	0.054
AST (IU/L)	18.4 (8.7)	17.2 (8.5)	18.4 (6.8)	20.4 (7.1)	23.2 (10.4)	< 0.001	< 0.001
ALT (IU/L)	20.1 (14.5)	16.2 (11.6)	20.7 (10.5)	26.7 (13.6)	34.1 (20.3)	< 0.001	< 0.001

GGT (IU/L)	20.3 (18.2)	16.6 (14.2)	23.1 (19.4)	24.8 (18.1)	31.2 (25.2)	< 0.001	< 0.001
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† p values using ANOVA for comparisons among four groups; ‡ p value using LSD-t as the post hoc analysis for comparing lean NAFLD group and overweight /obese with NAFLD group.

Data are means (s.d.) and counts (percentages); ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase, HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; TC, total cholesterol.

Cox proportional hazards regression analyses were performed to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for incident diabetes according to different phenotypes, with patients with a lean status and without NAFLD defined as the reference group. We used three models with progressive adjustments: model 1 was adjusted for age and sex; model 2 was further adjusted for smoking status (non-, ex-, or current), grade of alcohol consumption (no/minimal, light, or moderate), and regular exerciser; model 3 was further adjusted for variables associated with metabolic syndrome, including visceral-fat obesity (presence or absence), blood pressure (SBP and DBP), triglycerides, total cholesterol, HDL-c, and HbA1c. Potential confounders in multivariable models were selected based on their associations with the outcome or a change in effect estimate of more than 10%.

Stratified analyses were conducted in various subgroups, and their interactions were also tested. Each stratification adjusted for all the factors (age, sex, smoking status, grade of alcohol consumption, regular exerciser, visceral-fat obesity, blood pressure, triglycerides, total cholesterol, HDL-c, and HbA1c), except for the stratification factor itself. Moreover, we evaluated the effect of the severity of NAFLD on incident diabetes using Cox proportional hazards modeling, in which models were not adjusted for age or triglycerides, as these factors were included in the calculation of BAAT scores. Statistical analyses were conducted in IBM Statistical Package for the Social Sciences (SPSS) 21 (version 21.0, Armonk, NY). A two-tailed p-value < 0.05 was considered statistically significant.

Results

The basic characteristics and lifestyle of the participants are summarized in Table 1. The present study enrolled 14,482 individuals with a mean age of 43.7 years and a mean BMI of 22.1 kg/m²; 54.5% was male (n = 7898). The proportions of lean without NAFLD, overweight/obese without NAFLD, lean with NAFLD, and overweight/obese with NAFLD were 61.5% (8900), 20.8% (3008), 3.5% (514), and 14.2% (2060), respectively. In comparison with NAFLD patients with obesity/overweight, NAFLD patients with a lean status appeared to have a better biochemical profile. In detail, patients with NAFLD and obesity had higher levels of blood lipids (triglycerides and total cholesterol), elevated blood pressure (SBP and DBP) and increased waist circumference and liver enzymes (ALT, AST, and GGT) (all *p* < 0.05, Table 1).

During the median 6.0 years of follow-up, there were 356 cases of T2DM (271 men and 85 women). The average follow-up period did not differ significantly among the four groups (*p* = 0.351, data not shown). The cumulative incidence of diabetes in patients with NAFLD was almost 7.5 times higher than that in subjects without NAFLD (13.31 vs 1.82 per 1000 person-years, *p* for log rank test < 0.001, data not shown). Of the 2574 patients with NAFLD at baseline, the incidence densities of new-onset diabetes were 9.61 and 14.26 per 1000 person-years in individuals with a lean and obese/overweight status, respectively (*p* for log rank test = 0.032, Table 2). Additionally, we found that the overall incidence of diabetes in men was higher than in women (5.24 vs 2.08 per 1000 person-years, *p* for log rank test < 0.001, data not shown).

Table 2

Hazard ratios (HRs) of incidence of diabetes in relation to non - alcoholic fatty liver disease (NAFLD) and body mass index (BMI) status in overall participants.

Phenotype	No. of participates	Person - Years	No. of events	Incidence density*	Crude HR (95 % CI)	Multivariate - adjusted HR (95 %CI)		
						Model 1	Model 2	Model 3
Lean without NAFLD	8900	56862.1	86	1.51	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Overweight/obese without NAFLD	3008	19481.6	53	2.72	1.77 (1.25 - 2.49)	1.58 (1.12 - 2.23)	1.59 (1.12 - 2.24)	0.95 (0.66 - 1.37)
Lean with NAFLD	514	3329.8	32	9.61	6.31 (4.20 - 9.46)	5.42 (3.58 - 8.21)	5.61 (3.70 - 8.52)	2.58 (1.68 - 3.97)
Overweight/obese with NAFLD	2060	12971.3	185	14.26	9.49 (7.35 - 12.26)	8.340 (6.40 - 11.03)	8.54 (6.50 - 11.23)	2.52 (1.79 - 3.55)

CI, confidence interval; NAFLD non-alcoholic fatty liver disease.

* Incidence density per 1000 person-years.

The reference group was lean without NAFLD; a 95% CI that does not include 1.00 were considered statistically significant.

Crude model was unadjusted; Model 1 was adjusted for age and sex; Model 2 was adjusted for model 1 plus smoking status, grade of alcohol consumption, and regular exercise; Model 3 was further adjusted for model 2 plus various variables associated with metabolic syndrome including presence or absence visceral fat obesity, blood pressure levels, triglycerides, total cholesterol, HDL-c, and HbA1c at baseline.

Visceral fat obesity was defined as waist circumference >90 cm in male and >80 cm in female.

The hazard ratios of incident T2DM in relation to the four phenotypes are provided in Table 2. In the crude model, the HRs for incident diabetes compared to lean without NAFLD were 1.77 (95% CI: 1.25 - 2.49), 6.31 (95% CI: 4.20 - 9.46), and 9.49 (95% CI: 7.35 - 12.26) for the overweight/obese without NAFLD, lean NAFLD, and overweight/obese NAFLD groups, respectively. After progressive adjustment for age, sex, smoking status, grade of alcohol consumption, regular exerciser and confounders associated with metabolic syndrome, the overweight/obese without NAFLD group did not show a significant risk of diabetes compared with the lean without NAFLD group, whereas the lean NAFLD and obese/overweight NAFLD groups all had a significantly higher risk of incident diabetes (all $p < 0.001$). More importantly, the fully adjusted HRs for new-onset diabetes risk were similar in the lean NAFLD and overweight/obese NAFLD groups (2.58 vs 2.52, Table 2). In post hoc analysis, the HR (95% CI) for incident diabetes comparing lean NAFLD to overweight/obese NAFLD was 1.02 (95% CI 0.68 to 1.54, $p = 0.909$, date not shown). In addition, the results of sensitivity analysis using FPG levels as baseline glycemic status were similar to those of analyses using HbA1c as a confounder (see Additional file 1, supplementary table 1).

Subsequently, stratified analyses by subgroup defined by age, sex, smoking status, and regular exerciser were carried out, and the positive association between lean NAFLD and future risk of diabetes persisted in all subgroups. Interestingly, this relationship appeared to be more substantial in females (p for interaction = 0.005, Figure 2 and supplementary table 2). For female participants, the multivariable-adjusted HRs (95% CIs) for incident diabetes comparing lean with NAFLD and overweight/obese with NAFLD to the reference category were 5.53 (95% CI 2.30 to 13.30) and 4.36 (95% CI 2.13 to 8.93); for male participants, the corresponding HRs (95% CIs) were 2.02 (95% CI 1.24 to 3.31) and 2.04 (95% CI 1.39 to 2.98), respectively.

To evaluate the effect of the severity of NAFLD on the development of diabetes, all participants were further reclassified into six subgroups based on combinations of BMI status, NAFLD status, and BAAT scores. Among those with overweight/obese NAFLD,

the fullyadjusted HRs (95% CI) for diabetes comparing low BAAT and high BAAT vs. lean without NAFLD groups were 2.463 (1.747 - 3.473) and 2.891 (1.860 - 4.494), respectively (Table 3). Among lean NAFLD subjects, lower BAAT scores had a significant positive effect on incident diabetes risk (adjusted HR: 2.618, 1.701 - 4.029, $p < 0.001$, Table3), though higher BAAT scores did not confer an increased risk of incident diabetes (adjusted HR: 2.241, 0.692 - 7.266, $p = 0.18$, Table3). As our study included only 37 NAFLD participants with a lean status and a high probability of advanced fibrosis (BAAT score ≥ 2), these results should be interpreted cautiously.

Table 3

Hazard ratios (HRs) of incidence of diabetes in relation to the severity of non-alcoholic fatty liver disease (NAFLD) and body mass index (BMI) status.

	Multivariate-adjusted* HR (95 %CI)		
	No NAFLD	NAFLD with BAAT < 2	NAFLD with high BAAT ≥ 2
Lean	1.000 (reference)	2.671(1.713 - 4.163)	2.241(0.692 - 7.266)
Overweight/obese	0.97(0.67 - 1.404)	2.463(1.747 - 3.473)	2.891(1.860 - 4.494)

CI, confidence interval; NAFLD, non-alcoholic fatty liver disease.

The reference group was lean without NAFLD; a 95% CI that does not include 1.00 were considered statistically significant.

*Adjusted for sex, smoking status, grade of alcohol consumption, regular exercise, presence or absence visceral fat obesity, blood pressure levels, total cholesterol, HDL-c, and HbA1c at baseline.

Discussion

In this longitudinal study of 14,482 euglycemic adults without IFG and diabetes at baseline, subjects with a lean status and NAFLD diagnosed using ultrasound had a significantly higher risk of new-onset type 2 diabetes. Notably, among our study participants, those with NAFLD who were lean had approximately the same risk of eventually developing diabetes type 2 as individuals with NAFLD who were overweight/obese, even though the former exhibited lower levels of metabolic syndrome risk factors. This positive effect was evident in all subgroups considered and more pronounced in female participants. Moreover, lean subjects with a low probability of hepatic fibrosis indicated by a BAAT score also had a higher risk of developing diabetes after adjusting for sociodemographic and key confounding factors.

Convincing evidence has shown that NAFLD is involved in the early pathogenesis of type 2 diabetes, as it contributes substantially to the development of insulin resistance.[13, 28, 29] Our data also confirm that the incidence rate of T2DM in NAFLD patients compared to those without NAFLD was more than sevenfold. Regardless, there are limited studies investigating the impact of lean NAFLD on diabetes incidence. For example, a cohort study of 4629 Japanese adults conducted by Takuya et al. first indicated that the diabetes risk in NAFLD individuals who were not overweight (BMI < 23.0 kg/m²) was higher than that in subjects without NAFLD who were overweight; however, the sample size was relatively small, and there was a lack of adjustment for metabolic factors.[30] Recently, another large cohort study from Korea (n = 51,463) reported that the presence and severity of NAFLD in adults of normal weight (BMI of 23 - 24.9 kg/m²) had a close association with increased diabetes risk.[31] Unfortunately, the influence of waist circumference on the association of lean NAFLD with diabetes risk was not fully considered in these studies, and in fact, waist circumference is closely linked to diabetes, regardless of BMI.[32] In our study, after adjustment for waist circumference and other known confounders, we further confirmed that individuals with NAFLD who were lean had a significant risk of incident T2DM.

Moreover, our study found that lean NAFLD carries a risk of T2DM that rivals the risk in overweight/obese NAFLD, even though the overall changes in metabolic risk factors were more slight in the lean NAFLD group, which might indicate that subjects with NAFLD who are lean have a severe liver disease similar to that of subjects with NAFLD who are overweight/obese. Such speculation is supported by a recent study with 664 biopsy-proven NAFLD Asian patients showing that the severity of hepatic histology in nonobese NAFLD is comparable to that in obese NAFLD.[33] Consistently, a study of 466 Caucasian subjects with biopsy-proven NAFLD demonstrated that lean NAFLD is characterized by a severe histological picture similar to that of obese NAFLD and the former cases are more progressed compared to overweight cases.[34] In our study, we attempted to evaluate the

association between lean NAFLD severity and future diabetes risk. Numerous noninvasive scoring systems, including the NAFLD fibrosis score, BAAT score, and BARD score, have been proposed to stage NAFLD, though there is no consensus to date.[35] To eliminate potential confounding, the BAAT score was selected to define NAFLD with advanced liver fibrosis, as it does not involve information about glycemic status.[26] It has been reported that the sensitivity of BAAT scores for the identification of advanced fibrosis is 94.9% with a cutoff value of 2.[35] Unfortunately, as very few subjects who were lean had evidence of advanced fibrosis based on BAAT score in our study (n = 37), there was limited power to evaluate associations between liver fibrosis and future diabetes risk in lean NAFLD. Nevertheless, we also noted that NAFLD subjects who were lean, even those with a low BAAT score, had a significantly elevated incidence of diabetes.

In addition, the positive association between NAFLD and incident diabetes was more prominent in female participants. Similarly, a recent study conducted by Alina M. Allen et al. showed that females with NAFLD might lose the cardiovascular disease protection conferred by sex.[36] Although the exact reason for this sexual difference remains unclear, there are several possible explanations. First, NAFLD refers to a spectrum of lesions ranging from pure steatosis to nonalcoholic steatohepatitis (NASH) and advanced fibrosis. Most studies note a sex difference in this process, though conflicting data exist. A study conducted by Bambha et al. pointed out that women are already twice as likely as men to develop from NASH after adjusting for demographic and metabolic factors.[37] In agreement, a recent systematic review involving 54 studies showed that once NAFLD is established, females are at a higher risk for advanced fibrosis than are men, especially after the age of 50 years.[38] It has been proven that patients with NASH have more severe adipose tissue insulin resistance and hyperinsulinemia than do patients with simple steatosis, which might promote the pathogenesis of diabetes.[39] Additionally, differences in skeletal muscle mass according to sex is another potential mechanism.[40]

Several limitations should be considered in interpreting the results of our study. First, fatty liver was defined by abdominal ultrasound, which might be inaccurate when fat infiltration upon liver biopsy is < 30%. Liver biopsy is the gold standard for diagnosing and staging NAFLD; however, it is not applicable and realistic for large-sample surveys due to its invasive nature and high cost. Regardless, a meta-analysis containing 46 studies revealed that ultrasound had high sensitivity and specificity for the evaluation of hepatic steatosis when compared to histological data (73.3% and 69.6%, respectively).[41] Second, the diagnosis of diabetes in our study was mainly based on self-reporting or single measurements of HbA1c or FPG, without repeated confirmation on at least two separated days; nevertheless, this is an intrinsic limitation of all large observational surveys. Third, selection bias existed, in that our study participants were recruited from a health promotion center, and thus they might be more concerned about their health than the general population; hence, the generalizability of our study to other populations is uncertain. Finally, we adjusted as many important variables as possible (e.g., age, waist circumference, lifestyle factors). Given the nature of observational studies, residual confounding by unmeasured factors (e.g., insulin resistance and genetic variability) is unavoidable. Despite these limitations, the large sample size, long follow-up time, and use of a comprehensive and standardized database should provide reliable support for the relationship between lean NAFLD and future diabetes risk and pave the way for future prospective and histologically based studies.

Conclusions

In summary, this analysis of a large community cohort supports the existence of a significant association between lean NAFLD and an increased risk of incident diabetes. More importantly, our study adds novel evidence that lean NAFLD has a similar diabetes risk as overweight/obese NAFLD. Considering that patients with lean NAFLD are typically asymptomatic and always fail to seek medical advice, individuals at high risk for lean NAFLD should be followed up and assessed regularly to prevent associated complications through appropriate intervention in clinical settings. Currently, the optimal management for subjects with lean NAFLD is unclear, and lifestyle interventions, including diet and physical activity, might be effective in this population[42] and should be further explored.

List Of Abbreviations:

NAFLD Nonalcoholic fatty liver disease

T2DM Type 2 diabetes

BMI Body mass index

WC Waist circumference

IFG Impaired fasting glucose

IR Insulin resistance

TC Total cholesterol

TGs Triglycerides

HDL-c High-density lipoprotein cholesterol

LDL-c Low-density lipoprotein cholesterol

FPG Fasting plasma glucose

AST Aspartate aminotransferase

ALT Alanine aminotransferase

HR Hazard ratio

Declarations:

Ethics approval and consent to participate:

This study conformed to the Declaration of Helsinki. Given that our data were obtained from the public database, no prior ethical approval was required. The requirement for informed consent was also waived as the data were anonymous.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets generated during and/or analysed during the current study are available in the DRYAD repository (<https://datadryad.org>).^[16]

Competing interests:

The authors have declared no conflict of interest.

Funding:

This study was supported by the National Natural Science Foundation of China (No.81870507 and No.81900675) and Key Research and development Programme of Shannxi (2019 KW-32). None of the funding sources were involved in the study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit the manuscript for publication.

Authors' contributions:

All authors contributed significantly. LMW and XC: researched data, conducted the analysis, drafted and revised the manuscript. HLJ and LC: contributed to study design and method, analysed and interpreted the data. YLL, ZTL and RXY: helped with the statistical analyses and reviewing the database. All authors read and approved the final version of this manuscript.

Acknowledgments:

We appreciate Takuro Okamura¹ and Michiaki Fukui et al. for sharing their scientific knowledge.

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Figures

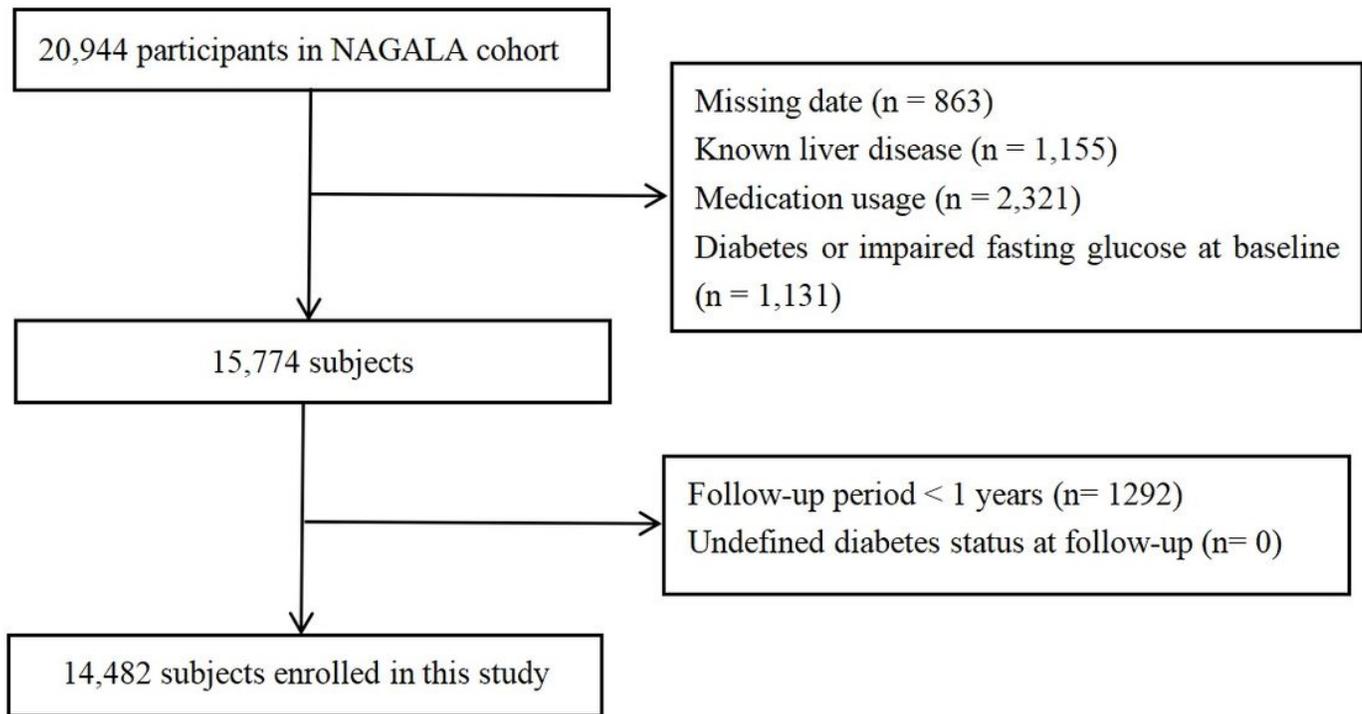


Figure 1

Flow diagram for cohort recruitment.

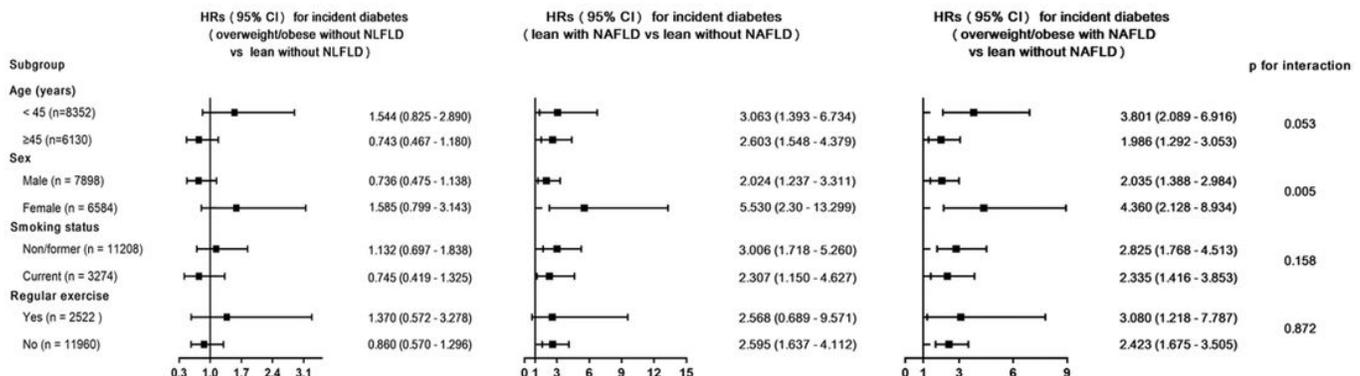


Figure 2

HRs of incidence of diabetes in relation to NAFLD and BMI status in various subgroups. The reference group was lean without NAFLD; a 95% CI that does not include 1.00 were considered statistically significant. When analysing a subgroup, age, sex, smoking status, grade of alcohol consumption, regular exercise, presence or absence visceral fat obesity, blood pressure levels, triglycerides, total cholesterol, HDL-c, and HbA1c were all adjusted, except the stratification factor itself.

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