Nonalcoholic Fatty Liver Disease Without overlapping Metabolic Associated Fatty Liver Disease and the risk of incident type 2 diabetes

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Author contributions

All authors planned, designed, and implemented the study, including quality assurance and control. S Ryu analyzed the data and developed the analytical strategy. Y Chang and S Ryu supervised the field activities. Y Cho and Y Chang drafted the manuscript with additional writing input from C Byrne and S Wild. All authors interpreted the results and contributed to critical revisions of the manuscript.

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Abbreviations

BMI: Body mass index

BP: Blood pressure

CI: Confidence intervals

CVD: Cardiovascular disease

HbA1c: Glycated hemoglobin

HEPA: Health-enhancing physical activity

HOMA-IR: homeostatic model assessment of insulin resistance

HR: Hazard ratios

hs-CRP: High-sensitivity C-reactive protein

MAFLD: Metabolic dysfunction-associated fatty liver disease

NAFLD: Nonalcoholic fatty liver disease

T2D: Type 2 diabetes

Conflict of interest: No conflict of interest to declare

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Data Availability Statement

The data are not publicly available outside the hospital because of institutional review board restrictions (the data were not collected in a manner that could be widely distributed). However, the analytical methods are available from the corresponding author upon request.

ABSTRACT

Background & Aims: Re-classifying NAFLD as metabolic-associated fatty liver (MAFLD) has been proposed. While some people fulfill criteria for NAFLD, they do not have MAFLD; and whether NAFLD-only subjects have increased risk of type 2 diabetes remains unknown. We compared risk of incident T2D in individuals with: a) NAFLD-only; and b) MAFLD, to individuals without fatty liver, considering effect-modification by sex.

Methods: 246,424 Koreans without diabetes or a secondary cause of ultrasound-diagnosed hepatic steatosis were studied. Subjects were stratified into: (a) NAFLD-only status, and (b) NAFLD that overlapped with MAFLD (MAFLD). Cox proportional hazards models with incident T2D as the outcome were used to estimate hazard ratios (HRs) for: (a) and (b). Models were adjusted for time-dependent covariates and effect-modification by sex was analysed in sub-groups.

Results: 5,439 participants had NAFLD-only status and 56,839 met MAFLD criteria. During a median follow-up of 5.5 years, 8,402 incident cases of T2D occurred. Multivariable-adjusted HRs (95% CI) for incident T2D comparing NAFLD-only and MAFLD to the reference (neither condition) were 2.39 (1.63-3.51) and 5.75 (5.17-6.36) (women), and 1.53 (1.25-1.88) and 2.60 (2.44-2.76) (men), respectively. The increased risk of T2D in the NAFLD-only group was higher in women than in men (p–interaction by sex <0.001) and consistently observed across all subgroups. Risk of T2D was increased in lean participants regardless of metabolic dysregulation (including prediabetes).

Conclusions: NAFLD-only participants without metabolic dysregulation and the criteria for MAFLD, are at increased risk of developing T2D. This association was consistently stronger

in women than in men.

Keywords: nonalcoholic fatty liver disease; metabolic dysfunction-associated fatty liver disease; type 2 diabetes; cohort study

Lay summary: Whether nonalcoholic fatty liver disease (NAFLD) in the absence of metabolic-dysfunction associated fatty liver disease (MAFLD) remains a risk factor for developing type 2 diabetes is not known. In a large study, we show that NAFLD in the absence of MAFLD is a risk factor for developing type 2 diabetes. This association was much stronger in women than men, even when restricted to lean, healthy individuals. Our findings suggest that people with NAFLD but without MAFLD need help to attenuate their risk of developing diabetes.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is becoming an emerging pandemic in recent decades, accounting for 25% of the adult global population [1]. Since nonalcoholic steatohepatitis was first introduced by Ludwig and colleagues in 1980, the term NAFLD has been widely used to define this condition in the absence of secondary causes of steatogenic liver disease [2]. More recently the term metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed to represent an inclusive definition that allows for the coexistence of hepatic steatosis, moderate alcohol consumption, and the presence of metabolic risk factors. Criteria for MAFLD are hepatic steatosis with one of: overweight or obesity, type 2 diabetes (T2D), or manifestations of metabolic disorders [3].

Despite the substantial overlap between NAFLD and MAFLD, 8% to 25% of patients with NAFLD do not fulfill the criteria for MAFLD [4,5]. The new MAFLD criteria may lead to some individuals meeting the diagnostic criteria for NAFLD but not MAFLD, implying that NAFLD without MAFLD is a “benign” condition. MAFLD and NAFLD are not synonymous diagnoses, have some important differences, and are not fully interchangeable [6]. Individuals with fatty liver and metabolic dysregulation, as well as those with other liver disease causes such as alcohol, viruses, or medication are included in the MAFLD criteria, and those with fatty liver but no metabolic dysregulation who are lean, (who were previously considered as having NAFLD), are excluded from the new MAFLD criteria.

NAFLD has been shown to independently predict the development of incident T2D, metabolic syndromes, and cardiovascular disease [7]; specifically, ultrasonography-diagnosed NAFLD significantly increases the risk of incident T2D by 1.5- 2 fold [8]. Irrespective of

metabolic abnormalities, lean NAFLD is a stronger risk factor for incident diabetes than the presence of overweight/obesity without NAFLD [9], and NAFLD increased the risk of developing T2D independent of insulin resistance or overweight/obesity [10]. Since there are subjects with NAFLD but without MAFLD who are lean and do not have metabolic dysregulation, it is unclear whether this group of individuals is at increased risk of future diseases such as T2D.

It is important to understand how subtypes of fatty liver disease affect the risk of established extrahepatic complications. As differential sex-specific effects have previously been identified, it is also important to elucidate whether there are sex-specific differences in the associations between NAFLD-only status, MAFLD and extrahepatic complications. Evidence suggests that sexual dimorphism in NAFLD, primarily due to sex hormones such as estradiol, plays an essential role in the regulation of metabolic genes with sex-biased expression [11]; specifically, estradiol has been shown to have a protective effect on female livers, as demonstrated in de novo [11]. A real-world data supports the notion, suggesting that NAFLD improves risk prediction of T2D with sex-specific effects [12]. Thus, we aimed to compare the risk of incident T2D in individuals with (a) NAFLD-only status and (b) NAFLD status that overlapped with MAFLD, compared to participants without fatty liver disease, and evaluated whether the effect of either type of fatty liver disease was modified by sex.

Materials and Methods

Study populationAs part of the Kangbuk Samsung Health Study, the current cohort comprised Korean adults who underwent annual or biennial health screenings at the Kangbuk Samsung Hospital Total Healthcare Centers in Seoul and Suwon, South Korea [13]. Our study

was limited to men and women who had undergone a comprehensive health examination (including abdominal ultrasound) between 2011 and 2019 and had at least one follow-up visit before December 31, 2020 (n=374,496). We excluded 128,072 participants who met the following criteria: T2D at baseline, history of malignancy, known liver disease, excessive alcohol consumption (defined as ≥30 g/day for men and ≥20 g/day for women [14]), positive serologic markers for hepatitis B or C, or use of steatogenic mediations. Participants were also excluded if they had missing information on liver ultrasound, alcohol intake, body mass index (BMI), waist circumference, prevalent diabetes, and laboratory data, including blood glucose, glycated hemoglobin (HbA1c), homeostatic model assessment of insulin resistance (HOMA-IR), and high-sensitivity C-reactive protein (hs-CRP) levels. Ultimately, 246,424 eligible participants (126,287 men and 120,137 women) were included (see **Figure 1** for a flow diagram of the study design).

This study was approved by the institutional review board of Kangbuk Samsung Hospital (IRB No. KBSMC 2022-10-037) and was exempted from the requirement of informed consent owing to the use of anonymized retrospective data that were routinely collected during health examinations.

Data collection

Based on a standardized, structured, and self-administered questionnaire, the cohort dataset included information on sociodemographic health-related behaviors, medical history, and anthropometric and laboratory measurements [13]. The mean alcohol intake per day was calculated as the amount of alcohol consumed per drinking day in standard units and the frequency of alcohol consumption was likewise determined. Smoking status was categorized

as “never,” “former,” or “current.” Using the validated Korean version of the International Physical Activity Questionnaire short form, physical activity was converted to metabolic equivalents (min/week) and classified as inactive, minimally active, or health-enhancing physical activity (HEPA) [15]. Hypertension was defined as blood pressure (BP; systolic/diastolic) ≥140/90 mmHg or the current use of BP-lowering medication. Obesity was defined as a BMI ≥25 kg/m^2 and lean as a BMI <23 kg/m^2 according to Asian-specific criteria [16].

After at least 10 hours of fasting, blood samples were obtained to measure laboratory glycemic parameters, including levels of fasting glucose and HbA1c, fasting serum lipid profiles, liver enzymes, insulin, and hs-CRP. HOMA-IR was calculated using the following equation: fasting blood insulin (mU/mL) × fasting blood glucose (mmol/L) / 22.5. The cutoff value of 2.5 was used to define insulin resistance [17]. HbA1c levels were measured using the Cobas Integra 800 (Roche Diagnostics, Rotkreuz, Switzerland) with a turbidimetric inhibition immunoassay for hemolyzed whole blood. The intra- and inter-assay coefficients of variation were 2.3% and 2.4 %, respectively.

T2D, the primary outcome of this study, was defined as a fasting serum glucose level ≥126 mg/dL (7 mmol/L), HbA1c ≥6.5% (48 mmol/mol), or current use of insulin or glucose-lowering medications. Prediabetes was defined as a fasting glucose level of 100-125 mg/dL (5.6-6.9 mmol/L), HbA1c 5.7%-6.4% (39-46 mmol/mol), and no history of diabetes mellitus or glucose-lowering medication use.

Diagnosis of NAFLD and MAFLD

Abdominal ultrasonography was performed by experienced radiologists who were

unaware of the study objectives, and hepatic steatosis was diagnosed using the following standard criteria: a diffuse increase in fine echoes in the liver parenchyma compared with those in the kidney or spleen parenchyma, deep beam attenuation, and bright vessel walls [18]. The inter- and intra-observer reliability values for fatty liver diagnosis were substantial (kappa statistic = 0.74) and excellent (kappa statistic = 0.94), respectively [13]. Since secondary causes of steatosis, such as excessive alcohol use (defined as ≥30 g/day for men and ≥20 g/day for women), have already been excluded (see the flow chart in **Figure 1**), we considered ultrasound-defined hepatic steatosis as a diagnosis of NAFLD. We used the Fibrosis-4 (FIB-4), a validated non-invasive indices of advanced fibrosis, to evaluate HS severity [19,20]. The FIB-4 cut-off points were defined as ≥2.67 (high risk) for predicting probability of advanced fibrosis [19,20].

People with fatty liver were then divided into two groups: NAFLD-only (i.e. fatty liver in the absence of MAFLD, defined below) and MAFLD. MAFLD was defined as the presence of both hepatic steatosis based on ultrasound and metabolic criteria [3] and meeting overweight/obesity criteria (defined as BMI ≥23.0 kg/m^2 for Asians) or having metabolic dysregulation which was defined having at least two metabolic abnormalities, including (a) waist circumference ≥90 cm in men and ≥80 cm in women, (b) BP ≥130/85 mmHg or receiving BP-lowering drug, (c) serum triglycerides ≥150 mg/dL or receiving specific drug treatment, (d) serum high-density lipoprotein <40 mg/dL for men and <50 mg/dL for women, (e) prediabetes (i.e., fasting glucose levels of 100-125 mg/dL [5.6-6.9 mmol/L] or HbA1c of 5.7%-6.4% [39-46 mmol/mol]), (f) HOMA-IR score ≥2.5, or (g) hs-CRP level >2 mg/dL. As T2D events were the primary endpoint of our study and participants with diabetes have already been excluded at baseline, T2D was not used as a criterion for diagnosing MAFLD.

222 ***Statistical analysis***

223 We summarized the baseline characteristics of men and women according to the
224 following groupings: (a) neither NAFLD nor MAFLD; (b) NAFLD-only; and (c) NAFLD
225 overlapping with MAFLD (MAFLD group). Incidence was described as the number of cases
226 per 1,000 person-years. Follow-up started from the baseline visit and was terminated at the
227 endpoint or the last health screening examination (December 31, 2020), whichever occurred
228 first. Cox proportional hazard models were used to estimate hazard ratios (HRs) with 95%
229 confidence intervals (CIs) for incident T2D, comparing the risk of incident T2D in those with
230 NAFLD-only status or MAFLD with those having neither condition (i.e., NAFLD or
231 MAFLD) (the reference group). The proportional hazard assumptions were examined with
232 the log–log plot of survival estimate, with no evidence of violation of the assumption.
233 The multivariable-adjusted model included age, center (Seoul or Suwon),
234 examination year, alcohol consumption (<10 or ≥10 g/day), smoking status (never, former,
235 current, or unknown), physical activity level (inactive, minimally active, HEPA, or unknown),
236 education level (below college graduate, college graduate or higher, or unknown), family
237 history of diabetes, history of hypertension, presence of prediabetes, and use of lipid-
238 lowering medications. We then conducted time-dependent analyses in which the updated
239 status of NAFLD, MAFLD, and other covariates (smoking status, alcohol consumption,
240 physical activity, hyperlipidemia medication, presence of prediabetes, and history of
241 hypertension) during the follow-up period, which were treated as time-varying covariates,
242 whereas baseline age, center, year of screening examination, family history of diabetes, and
243 education level were treated as time-fixed variables.
244 The effect of modification by sex on the association between NAFLD category and

incident diabetes was assessed using likelihood ratio tests, comparing models with and without multiplicative interaction terms.

We performed a sensitivity analysis to assess the association between NAFLD-only status and T2D development in men and women under the following conditions: 1) restricted to lean participants with BMI <23 kg/m^2, 2) restricted to participants without metabolic dysregulation, 3) lean participants also without metabolic dysregulation, and 4) participants without prediabetes. We also conducted an additional analysis to examine whether there exists a dose-response relationship between NAFLD or MAFLD with advanced fibrosis, as indicated by high FIB-4 scores, and the risk of developing T2D.

All estimated *p*-values were two-sided, and significance was defined as a *p*-value < 0.05. We used STATA version 17.0 (Stata Corp LP, College Station, TX, USA) for statistical analyses.

RESULTS

The baseline characteristics of the women and men included in our study were described according to the NAFLD and MAFLD criteria. The three groups were: neither NAFLD nor MAFLD, NAFLD-only status, and MAFLD (**Table 1 for the total population, Supplementary Tables 1 and 2 for men and women, respectively)**. The mean age of the study participants was 37.2 (SD, 7.8) years. Of those who met the NAFLD-only status, 84.7% among women and 91.7% among men also met the criteria for MAFLD. By contrast, 15.3% of women and 8.3% of men with NAFLD did not meet the criteria for MAFLD and were classified as NAFLD-only.

Compared to individuals with neither NAFLD nor MAFLD, those with NAFLD-only

status were older, less physically active, and had higher levels of lipids, liver enzymes, and HOMA-IR. Compared to participants with MAFLD, those with NAFLD-only status were younger and had more favorable metabolic profiles, but were less likely to be physically active and drink alcohol.

During 1,318,784 person-years of follow-up (median, 5.5 years; interquartile range, 3.0-7.9 years; maximum, 9.8 years), 8,402 cases of incident T2D were identified. The incidence rates per 10^3 person-years were 2.9 with neither NAFLD nor MAFLD, 5.1 with NAFLD-only status, and 18.2 in participants with MAFLD. Notably, the highest incidence rate of 22.1 was present among women with MAFLD (**Table 2**). After adjusting for potential confounders, such as age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, hyperlipidemia medication, family history of diabetes, history of hypertension, and presence of prediabetes, the multivariable-adjusted HRs (95% CIs) for incident T2D were 1.79 (1.49-2.14) for NAFLD-only status and 3.16 (2.99-3.34) for MAFLD, compared to neither condition. The associations were stronger in women than in men (p for interaction by sex <0.001). The HRs were 2.39 (1.63-3.51) for NAFLD-only status and 5.75 (5.17-6.36) for MAFLD in women, and 1.53 (1.25-1.88) for NAFLD-only status and 2.60 (2.44-2.76) for MAFLD in men.

In a time-dependent model that included the updated (and potentially changing) status of NAFLD or MAFLD and other confounders, such as smoking status, alcohol consumption, physical activity, lipid-lowering medication, history of hypertension, and presence of prediabetes as time-varying covariates, aHRs (95% CIs) became even stronger for participants with MAFLD, but were slightly attenuated for those with NAFLD-only status; nevertheless, they remained statistically significant in men and women combined. During the

follow-up, 57% of the 5,439 participants with NAFLD-only status transitioned to MAFLD (40.5% for women and 63.5% for men).

The risk of developing T2D in individuals with NAFLD-only status compared to the reference group (those with neither NAFLD nor MAFLD) was further evaluated in sensitivity analyses restricted to lean participants, those without metabolic dysregulation, lean participants without metabolic dysregulation, and those without prediabetes (**Table 3**). Among lean participants, the multivariable-adjusted HRs (95% CIs) for incident T2D comparing NAFLD-only status to the reference group were 2.91 (1.96-4.31) for women and 1.83 (1.47-2.27) for men (*p* for the interaction by sex <0.001). A similar pattern, even with stronger associations, was observed in other subgroups. In participants without metabolic dysregulation, the multivariable-adjusted HRs (95% CIs) for incident T2D, comparing NAFLD-only status to the reference group, were 3.39 (2.29-5.03) for women and 1.69 (1.37-2.09) for men (p for interaction by sex = 0.002). Similarly, in lean without metabolic dysregulation, 3.50 (2.35-5.23) for women and 1.95 (1.56-2.44) for men (p for interaction by sex = 0.012). In participants without prediabetes, the HRs were 3.49 (1.95-6.23) for women and 1.31 (0.95-1.80) for men (p for interaction by sex <0.001).

Although we performed an additional analysis for risk of incident T2D according to fatty liver disease categories with advanced fibrosis defined as high FIB-4 scores, the cases of prevalent NAFLD-only or MAFLD with advanced fibrosis were too small to evaluate a dose response relationship with incident T2D (**Supplementary Table 3**).

DISCUSSION

In this cohort study of 246,424 Korean adults (mean age, 37.2 years) with over 1.3 million person-years of follow-up, fatty liver was associated with an increased risk of

incident T2D, either as NAFLD-only, or classified as MAFLD. The increased risk of incident diabetes in individuals with NAFLD-only status compared to those with neither NAFLD nor MAFLD was stronger in women than in men, and this association was even stronger in lean women without metabolic dysregulation. These associations remained significant even after adjusting for the updated fatty liver status and potential confounders between baseline and follow-up as time-varying covariates. Furthermore, a significantly stronger association between NAFLD-only status and incident diabetes risk in women was consistently observed in all subgroups that included lean participants only, those without metabolic dysregulation, lean participants without metabolic dysregulation, and those without prediabetes. Consequently, these data show that NAFLD is an independent risk factor for the development of subsequent T2D, regardless of BMI or metabolic dysregulation.

In our study, most people with fatty liver fall into the MAFLD group, as described in other populations [21,22]. As expected, given that the MAFLD criteria better reflect metabolically driven liver disease, participants meeting the MAFLD criteria exhibited the highest risk of developing T2D. In a study of 7,761 US adults during a 23-year median follow-up, MAFLD was associated with an increased risk of all-cause mortality; whilst the association for NAFLD was not statistically significant (multivariable HR 1.05; 95% CI, 0.95–1.17)[21]. Another study of 765 Japanese individuals with fatty liver disease demonstrated that the MAFLD criteria identified patients with significant hepatic fibrosis [23], with further similar evidence from a current meta-analysis [24]. MAFLD is also more likely to be associated with metabolic dysregulation and chronic kidney disease than NAFLD [25]. Although MAFLD seems to be a more potent contributor to the development of various health outcomes than NAFLD-only status, our data show that the clinical implications of developing extra-hepatic

complications in NAFLD-only individuals should be considered.

A retrospective cohort of 6,873 Chinese participants, with a 4.6-year of follow-up found that both NAFLD and MAFLD was associated with higher risks of incident diabetes (risk ratio [RR] 2.01; 95% CI, 1.65-2.46 and 2.08; 95% CI, 1.72-2.52, respectively). Particularly, individuals with MAFLD with excessive alcohol consumption (RR 2.49; 95% CI, 1.64-3.78) and HBV infection (RR 1.98; 95% CI, 1.11-3.52) had higher risks of incident diabetes, suggesting that alcohol consumption and viral hepatitis do not add to the risk of steatosis/steatohepatitis per se[26]. However, the previous study did not specifically focus on the population discordant for NAFLD/MAFLD, i.e. on the specific patient population with, NAFLD but without MAFLD (NAFLD only), that was investigated in our study. Our study highlights the clinical significance of monitoring for incident T2D in a specific population that could be overlooked during the transition from NAFLD to MAFLD.

In a nationwide study of 9 million Korean adults using the fatty liver index score as a proxy for the presence of fatty liver, the risk of CVD in people with NAFLD-only status was significantly increased, compared to the risk in those with neither NAFLD nor MAFLD [27]. In another retrospective cohort study of 913 Korean adults, with NAFLD-only status, there was a higher risk of developing metabolic syndrome compared to subjects with neither NAFLD nor MAFLD [22], which is in line with our findings. Few previous studies have assessed T2D risk among individuals with NAFLD but without MAFLD. In a 7-year follow-up of a prospective cohort study conducted in Sri Lanka, approximately 30 participants with NAFLD-only status had a higher risk of incident diabetes compared to those in the control group (neither NAFLD nor MAFLD) [28]. However, this was a very small study; there was also no assessment of a sex-specific interaction, and there was no consideration of changes in

NAFLD or MAFLD status between baseline and follow-up. Notably, in our study of 250,000 people, these associations remained significant even after adjusting for change in status between baseline and follow-up of NAFLD or MAFLD in a time-dependent model. Furthermore, this association between NAFLD-only status and incident T2D was consistently observed in all subgroups (i.e., restricted to lean participants, those without metabolic dysregulation, lean participants without metabolic dysregulation, and those without prediabetes).

We found that the risk of incident T2D in participants with either NAFLD-only status or MAFLD was higher in women than in men, implying that sex modifies this association. Accumulating evidence suggests that NAFLD and T2D both exhibit sexually dimorphic features [29-32]. In our study, the mean age of the women at baseline was 36.6 (SD, 7.7) years. The women were mostly premenopausal, whereas after menopause, there is a loss of estrogen protection and an unfavorable alteration in body composition [33,34]. In premenopausal women, the presence of NAFLD may attenuate the protective effects of premenopausal status on CVD [29,30,35]. Although the exact mechanism underlying the sex-modification effect on both NAFLD and T2D remains unclear, hepatic fat may be a potential determinant of metabolic dysregulation in premenopausal women, which could negate the benefit of estrogen on cardiometabolic risk. Further studies are needed to compare the risk of T2D in women with NAFLD-only according to menopausal status or different reproductive hormone levels. When the study participants were restricted to lean individuals, those without metabolic dysregulation, or both, and those without prediabetes, the risk of incident T2D was higher among those with NAFLD-only status than in those with neither NAFLD nor MAFLD. Although NAFLD is strongly associated with obesity, approximately 40% of the global

NAFLD population is classified as non-obese [36]. Thus, the association between NAFLD and T2D cannot be fully explained by excessive adiposity measured by BMI or waist circumference. Intrahepatic di-acylglycerol and triglyceride content is more strongly related to systemic and peripheral insulin resistance than visceral or subcutaneous fat content and intramyocellular lipid [37-39] and increased hepatic lipid content may play a more significant role in developing insulin resistance, ultimately affecting risk of T2D.

In NAFLD, ectopic fat accumulation occurs in the liver that is independent of general and abdominal obesity [40,41]. Insulin resistance is considered a primary factor in the development of NAFLD, as demonstrated by reduced glucose disposal during the euglycemic-hyper-insulinemic clamp studies in NAFLD patients, including those of normal weight [40]. Likewise, NAFLD-only individuals without obesity or metabolic dysregulation are characterized by hepatic and systemic insulin resistance and are at risk of developing T2D [42]. In patients with NAFLD, the fatty liver may produce various proteins called hepatokines and release them into circulation.[43] Although the role of each hepatokine in relation to T2D risk remains not fully understood, fetuin-A is among the most extensively studied.[43] Fetuin-A is the most well-known hepatokine primarily produced and released by the liver and elevated serum levels of fetuin-A have been observed in individuals with NAFLD [43]. Hepatic expression of fetuin-A is upregulated by free fatty acids through nuclear factor kappa (NF-κB) signaling and by glucose through extracellular signal-regulated kinase (ERK)1/2 signaling [44,45]. Fetuin-A is strongly linked to NAFLD and insulin resistance [43] and has been shown to inhibitor the insulin receptor tyrosine kinase in liver and skeletal muscle, thereby interrupting insulin signaling in these insulin sensitive tissues responsible for insulin-mediated glucose uptake [46]. Consequently, elevated levels of fetuin-A in patients with NAFLD may contribute

405 to an increased risk of developing type 2 diabetes.

406 Since our study involved relatively young adults and there was an insufficient
407 sample size of individuals with advanced fibrosis in either NAFLD or MAFLD (11
408 participants fell in NAFLD-only plus high FIB-4 category and among them, only one person
409 developed diabetes), we could not establish a significant dose-response relationship with FIB-
410 4 score in our further analysis of incident T2D. Furthermore, we excluded all who had
411 diabetes at the study baseline. It is important to note that NAFLD and T2D form part of a
412 vicious spiral of worsening diseases, where one condition affects the other and vice versa [47].
413 Given that diabetes markedly increases the risk of liver fibrosis [47,48], excluding individuals
414 with T2D to define the diabetes-free at baseline might result in specific selection of those
415 with fibrosis but not related to T2D. However, due to the limited number of participants with
416 NAFLD or MAFLD and high fibrosis scores, further cohort studies are needed to determine
417 the role of NAFLD or MAFLD with different degree of liver fibrosis in the development of
418 diabetes, while considering their interrelationship and longitudinal trajectory in appropriate
419 population settings. The current study has some limitations. First, ultrasonography was used
420 instead of liver magnetic resonance imaging, computed tomography, or liver biopsy to
421 identify fatty liver. However, liver biopsy is neither feasible nor ethical for healthy
422 participants, and imaging modalities such as computed tomography or magnetic resonance
423 imaging are not practical or cost-effective for routine healthcare check-ups in this large
424 population. Importantly, according to a meta-analysis of observational studies, conventional
425 ultrasonography is able to detect hepatic steatosis (HS) with a sensitivity of 82% and
426 specificity of 80% for histologically defined HS of 5% or more. It is worth noting that the
427 majority of subjects in this meta-analysis had mild HS (i.e., less than 30% steatotic

hepatocytes on histology) [49]. Second, T2D was defined using fasting glucose and HbA1c measurements with no data from a 2-hour post-challenge glucose test. However, HbA1c is now widely accepted as a diagnostic test for T2D diagnosis and monitoring in clinical practice around the world. HbA1c measurement is also useful in large cohort studies because it is not affected by acute perturbations (i.e. induced by exercise or dietary change), and measurement of HbA1c is robust and reproducible [50]. Third, in the present study, NAFLD plus concomitant metabolic dysregulations meeting the MAFLD criteria were evaluated instead of the original MAFLD definition, which does not exclude secondary liver disease or excessive alcohol consumption. Thus, our findings, obtained in a very large cohort study should be reproduced in other cohorts. Finally, although studying relatively young subjects has the advantage that subjects have relatively few co-morbidities that may affect key exposures and outcomes, the findings in our study need to be further evaluated in other older populations.

In conclusion, individuals with NAFLD-only status were at a higher risk of developing T2D than those with neither NAFLD nor MAFLD. There was powerful effect modification by sex and stronger associations were noted in women. The association between NAFLD-only status and incident T2D was consistent across all subgroups, including lean participants and/or those without metabolic dysregulation, and those without prediabetes, indicating that NAFLD without MAFLD is not a benign condition. Thus, we suggest that individuals with NAFLD-only status, even those that are lean and with normal metabolic parameters, also need regular monitoring and potential intervention. We suggest that further studies are now needed to investigate whether people with NAFLD but with no evidence of MAFLD are likely to benefit from prevention strategies and treatments to attenuate and

ameliorate their increased risk of T2D.

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Table 1. Estimated[*] mean values (95% CI) and adjusted[*] proportions (95% CI) of the baseline characteristics of study participants with respect to fatty liver disease category (n = 246,424)

Characteristics	Neither NAFLD nor MAFLD	NAFLD-only	MAFLD
Number of participants	184,146	5,439	56,839
Age (years)	36.6 (36.6-36.7)	38.3 (38.1-38.5)	39.1 (39.1-39.2)
Men (%)	41.2 (41.0-41.4)	71.5 (70.3-72.7)	82.1 (81.7-82.4)
Alcohol intake (%) [†]	29.1 (28.9-29.3)	23.9 (22.9-24.8)	30.1 (29.7-30.4)
Current smoker (%)	16.7 (16.5-16.9)	16.5 (15.7-17.3)	18.6 (18.4-18.9)
Education level (%) [‡]	85.7 (85.6-85.9)	88.5 (87.6-89.4)	83.3 (82.9-83.7)
HEPA (%) [§]	16.2 (16.0-16.4)	11.3 (10.5-12.1)	12.9 (12.6-13.1)
History of hypertension (%)	4.2 (4.1-4.3)	2.2 (1.9-2.6)	8.9 (8.7-9.1)
History of CVD (%)	0.7 (0.7-0.8)	0.8 (0.5-1.0)	0.7 (0.7-0.8)
Lipid-lowering medication use (%)	1.0 (1.0-1.1)	1.4 (1.1-1.7)	2.3 (2.2-2.4)
Family history of diabetes (%)	13.5 (13.3-13.6)	16.5 (15.5-17.5)	18.1 (17.8-18.5)
Obesity (%) [‖]	13.8 (13.6-13.9)	-	58.8 (58.4-59.2)
Body mass index (kg/m^2)	22.1 (22.1-22.1)	21.3 (21.3-21.4)	25.9 (25.9-25.9)
Waist circumference (cm)	78.2 (78.2-78.2)	77.5 (77.4-77.7)	87.8 (87.7-87.9)
SBP (mmHg)	106.8 (106.7-106.8)	105.3 (105.0-105.5)	112.4 (112.3-112.5)
DBP (mmHg)	68.0 (68.0-68.1)	67.4 (67.2-67.6)	72.1 (72.0-72.1)
Fasting glucose (mg/dL)	91.9 (91.9-92.0)	91.7 (91.5-92.0)	95.4 (95.3-95.5)
HbA1c (%)	5.5 (5.5-5.5)	5.5 (5.5-5.5)	5.6 (5.6-5.6)
Total cholesterol (mg/dL)	188.1 (188.0-188.3)	194.4 (193.5-195.2)	202.4 (202.1-202.6)
LDL-C (mg/dL)	115.6 (115.5-115.8)	123.5 (122.7-124.3)	131.7 (131.4-131.9)
HDL-C (mg/dL)	62.0 (62.0-62.1)	59.4 (59.1-59.8)	51.7 (51.6-51.8)
Triglycerides (mg/dL)	90.4 (90.1-90.7)	94.8 (93.2-96.4)	148.6 (148.0-149.1)
GTP (U/L)	22.5 (22.4-22.7)	24.9 (24.2-25.6)	38.7 (38.4-38.9)
ALT (U/L)	18.4 (18.3-18.5)	22.8 (22.3-23.2)	34.9 (34.8-35.1)
AST (U/L)	20.0 (20.0-20.1)	20.9 (20.6-21.2)	25.3 (25.2-25.4)
hs-CRP (mg/L)	8.66 (8.53-8.80)	7.01 (6.24-7.78)	14.59 (14.34-14.84)
HOMA-IR	1.17 (1.16-1.17)	1.24 (1.21-1.27)	2.16 (2.15-2.16)

[*]Adjusted for age; [†] ≥10 g/day; [‡] ≥college graduate; [§] health-enhancing physical activity; [‖] BMI ≥25 kg/m^2
Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; GTP, glutamyl transpeptidase; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure

Table 2. Absolute and relative estimates of diabetes incidence with respect to fatty liver disease category

Fatty liver disease category	PY	Incident cases	Incidence rate (/10^3 PY)	Age-adjusted HR (95% CI)	Multivariable-adjusted HR* (95% CI)	HR (95% CI)† in model 2 with time-dependent variables
Total						
Neither NAFLD nor MAFLD	993,873	2,885	2.9	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD-only	29,943	152	5.1	1.62 (1.38-1.91)	1.79 (1.49-2.14)	1.57 (1.27-1.93)
MAFLD	294,968	5,365	18.2	5.69 (5.43-5.95)	3.16 (2.99-3.34)	3.30 (3.11-3.50)
Women						
Neither NAFLD nor MAFLD	576,185	1,001	1.7	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD-only	7,926	34	4.3	2.19 (1.56-3.09)	2.39 (1.63-3.51)	1.46 (0.90-2.37)
MAFLD	47,614	1,050	22.1	10.59 (9.70-11.57)	5.75 (5.17-6.36)	5.46 (4.92-6.06)
Men						
Neither NAFLD nor MAFLD	417,688	1,884	4.5	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD-only	22,017	118	5.4	1.16 (0.96-1.40)	1.53 (1.25-1.88)	1.45 (1.15-1.83)
MAFLD	247,354	4,315	17.4	3.71 (3.51-3.92)	2.60 (2.44-2.76)	2.66 (2.49-2.84)

The *p*-value for the interaction of sex and fatty liver disease category with the risk of diabetes was <0.001 (multivariable model).

* Estimated from Cox proportional hazards models; the multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, use of lipid-lowering medication, family history of diabetes, prediabetes and history of hypertension.

† Estimated from Cox proportional hazard models with group status according to the changes in NAFLD or MAFLD status, smoking status, alcohol consumption, physical activity, hyperlipidemia medication, prediabetes and history of hypertension as time-dependent categorical variables; baseline age, center, year of screening examination, family history of diabetes, and education level as time-fixed variables

Abbreviations: CI, confidence interval; HR, hazard ratio; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, nonalcoholic fatty liver disease; PY, person-years

Table 3. Development of diabetes in nonalcoholic fatty liver disease among restricted subgroups.

Fatty liver disease category	Lean participants (n=132,529)	Participants without metabolic dysregulation (n=166,356)	Lean participants without metabolic dysregulation (n=115,171)	Participants without prediabetes (n=152,563)
	Total			
Neither NAFLD nor MAFLD	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD-only	2.06 (1.70-2.49)	1.92 (1.59-2.31)	2.19 (1.79-2.67)	1.61 (1.21-2.13)
	Women			
Neither NAFLD nor MAFLD	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD-only	2.91 (1.96-4.31)	3.39 (2.29-5.03)	3.50 (2.35-5.23)	3.49 (1.95-6.23)
	Men			
Neither NAFLD nor MAFLD	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
NAFLD-only	1.83 (1.47-2.27)	1.69 (1.37-2.09)	1.95 (1.56-2.44)	1.31 (0.95-1.80)
p for interaction	<0.001	0.002	0.012	<0.001

* Estimated from Cox proportional hazards models; the multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking status, physical activity, education level, medication for hyperlipidemia, family history of diabetes, prediabetes and history of hypertension.
Abbreviations: MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, nonalcoholic fatty liver disease

Figure legend

Fig. 1. Flow chart of the study population