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

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

Yoosun Cho, MD, PhD [1]; Yoosoo Chang, MD, PhD [2,3,4*]; Seungho Ryu, MD, PhD [2,3,4*]; Sarah H. Wild, MB, BChir, PhD [5]; Christopher D. Byrne, MB BCh, PhD [6,7]

[1]Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[2]Center for Cohort Studies, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[3]Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[4]Department of Clinical Research Design & Evaluation, Samsung Advanced Institute for Health Sciences & Technology, Sungkyunkwan University, Seoul, Republic of Korea

[5]Usher Institute, University of Edinburgh, Edinburgh, U.K.

[6]Nutrition and Metabolism, Faculty of Medicine, University of Southampton, Southampton, U.K.

[7]National Institute for Health and Care Research Southampton Biomedical Research Centre, University Hospital Southampton, Southampton, U.K.

[*] **Co-Correspondence:** S Ryu and Y Chang should be considered joint corresponding author

Seungho Ryu, MD, PhD, Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building

B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

Email: sh703.yoo@gmail.com

Yoosoo Chang, MD, PhD, Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

Tel: +82-2-2001-5139; Fax: +82-2-757-0436; Email: yoosoo.chang@gmail.com

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.

Word count: abstract 250 words, **text** 4,949 words (including title page, abstract, lay summary and main text)

Number of figures and tables: Tables, 3; Figures, 1

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

Acknowledgments

We thank our staff members at the Kangbuk Samsung Health Study for their hard work, dedication, and support. This study was supported by the SKKU Excellence in Research Award Research Fund, Sungkyunkwan University (2020), and the National Research Foundation of Korea funded by the Ministry of Science, ICT, and Future Planning (NRF-2021R1A2C1012626). CDB was supported in part by the Southampton National Institute for Health Research Biomedical Research Centre (IS-BRC-20004), U.K.

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 population As 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

177 Physical Activity Questionnaire short form, physical activity was converted to metabolic
178 equivalents (min/week) and classified as inactive, minimally active, or health-enhancing
179 physical activity (HEPA) [15]. Hypertension was defined as blood pressure (BP; systolic/diastolic)
180 ≥140/90 mmHg or the current use of BP-lowering medication. Obesity was defined as a BMI
181 ≥25 kg/m^2 and lean as a BMI <23 kg/m^2 according to Asian-specific criteria [16].

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

190 T2D, the primary outcome of this study, was defined as a fasting serum glucose level
191 ≥126 mg/dL (7 mmol/L), HbA1c ≥6.5% (48 mmol/mol), or current use of insulin or glucose-
192 lowering medications. Prediabetes was defined as a fasting glucose level of 100-125 mg/dL
193 (5.6-6.9 mmol/L), HbA1c 5.7%-6.4% (39-46 mmol/mol), and no history of diabetes mellitus
194 or glucose-lowering medication use.

195 ***Diagnosis of NAFLD and MAFLD***

196 Abdominal ultrasonography was performed by experienced radiologists who were
197 unaware of the study objectives, and hepatic steatosis was diagnosed using the following
198 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.

Statistical analysis

We summarized the baseline characteristics of men and women according to the following groupings: (a) neither NAFLD nor MAFLD; (b) NAFLD-only; and (c) NAFLD overlapping with MAFLD (MAFLD group). Incidence was described as the number of cases per 1,000 person-years. Follow-up started from the baseline visit and was terminated at the endpoint or the last health screening examination (December 31, 2020), whichever occurred first. Cox proportional hazard models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for incident T2D, comparing the risk of incident T2D in those with NAFLD-only status or MAFLD with those having neither condition (i.e., NAFLD or MAFLD) (the reference group). The proportional hazard assumptions were examined with the log–log plot of survival estimate, with no evidence of violation of the assumption.

The multivariable-adjusted model included age, center (Seoul or Suwon), examination year, alcohol consumption (<10 or ≥10 g/day), smoking status (never, former, current, or unknown), physical activity level (inactive, minimally active, HEPA, or unknown), education level (below college graduate, college graduate or higher, or unknown), family history of diabetes, history of hypertension, presence of prediabetes, and use of lipid-lowering medications. We then conducted time-dependent analyses in which the updated status of NAFLD, MAFLD, and other covariates (smoking status, alcohol consumption, physical activity, hyperlipidemia medication, presence of prediabetes, and history of hypertension) during the follow-up period, which were treated as time-varying covariates, whereas baseline age, center, year of screening examination, family history of diabetes, and education level were treated as time-fixed variables.

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

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

322 In our study, most people with fatty liver fall into the MAFLD group, as described in
323 other populations [21,22]. As expected, given that the MAFLD criteria better reflect metabolically
324 driven liver disease, participants meeting the MAFLD criteria exhibited the highest risk of
325 developing T2D. In a study of 7,761 US adults during a 23-year median follow-up, MAFLD
326 was associated with an increased risk of all-cause mortality; whilst the association for NAFLD
327 was not statistically significant (multivariable HR 1.05; 95% CI, 0.95–1.17)[21]. Another study
328 of 765 Japanese individuals with fatty liver disease demonstrated that the MAFLD criteria
329 identified patients with significant hepatic fibrosis [23], with further similar evidence from a
330 current meta-analysis [24]. MAFLD is also more likely to be associated with metabolic
331 dysregulation and chronic kidney disease than NAFLD [25]. Although MAFLD seems to be a
332 more potent contributor to the development of various health outcomes than NAFLD-only
333 status, our data show that the clinical implications of developing extra-hepatic complications
334 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 to an increased risk of developing type 2 diabetes.

Since our study involved relatively young adults and there was an insufficient sample

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

450

451

452

453

454

455

456

457

458

459

460

461

REFERENCES

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.* 2016;64(1):73-84.
2. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc.* 1980;55(7):434-438.
3. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol.* 2020;73(1):202-209.
4. Lim GEH, Tang A, Ng CH, et al. An Observational Data Meta-analysis on the Differences in Prevalence and Risk Factors Between MAFLD vs NAFLD. *Clin Gastroenterol Hepatol.* 2021.
5. Wong VW, Wong GL, Woo J, et al. Impact of the New Definition of Metabolic Associated Fatty Liver Disease on the Epidemiology of the Disease. *Clin Gastroenterol Hepatol.* 2021;19(10):2161-2171 e2165.
6. Targher G. From nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease: more than a single-letter change in an acronym. *Hepatoma Res.* 2021;7(47).
7. Lonardo A, Ballestri S, Guaraldi G, et al. Fatty liver is associated with an increased risk of diabetes and cardiovascular disease - Evidence from three different disease models: NAFLD, HCV and HIV. *World J Gastroenterol.* 2016;22(44):9674-9693.
8. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol.* 2016;31(5):936-944.
9. Sinn DH, Kang D, Cho SJ, et al. Lean non-alcoholic fatty liver disease and development of diabetes: a cohort study. *Eur J Endocrinol.* 2019;181(2):185-192.
10. Sung KC, Jeong WS, Wild SH, Byrne CD. Combined influence of insulin resistance, overweight/obesity, and fatty liver as risk factors for type 2 diabetes. *Diabetes Care.* 2012;35(4):717-722.
11. Lefebvre P, Staels B. Hepatic sexual dimorphism - implications for non-alcoholic fatty liver disease. *Nat Rev Endocrinol.* 2021;17(11):662-670.
12. Kim Y, Chang Y, Ryu S, Wild SH, Byrne CD. NAFLD improves risk prediction of type 2 diabetes: with effect modification by sex and menopausal status. *Hepatology.* 2022;76(6):1755-1765.
13. Chang Y, Ryu S, Sung KC, et al. Alcoholic and non-alcoholic fatty liver disease and associations with coronary artery calcification: evidence from the Kangbuk Samsung Health Study. *Gut.* 2019;68(9):1667-1675.
14. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology.* 2012;142(7):1592-1609.
15. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. *Medicine and science in sports and exercise.* 2003;35(8):1381-1395.
16. World Health Organization, Regional Office for the Western Pacific. *The Asia-Pacific*

perspective: redefining obesity and its treatment. Sydney: Health Communications Australia; 2000.
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-419.
18. Mathiesen UL, Franzen LE, Aselius H, et al. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. *Dig Liver Dis.* 2002;34(7):516-522.
19. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology.* 2007;45(4):846-854.
20. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* 2009;7(10):1104-1112.
21. Kim D, Konyn P, Sandhu KK, Dennis BB, Cheung AC, Ahmed A. Metabolic dysfunction-associated fatty liver disease is associated with increased all-cause mortality in the United States. *J Hepatol.* 2021;75(6):1284-1291.
22. Sinn DH, Kang D, Choi SC, et al. Non-Alcoholic Fatty Liver Disease Without Metabolic Associated Fatty Liver Disease and the Risk of Metabolic Syndrome. *Clin Gastroenterol Hepatol.* 2022.
23. Yamamura S, Eslam M, Kawaguchi T, et al. MAFLD identifies patients with significant hepatic fibrosis better than NAFLD. *Liver Int.* 2020;40(12):3018-3030.
24. Ayada I, van Kleef LA, Alferink LJM, Li P, de Knegt RJ, Pan Q. Systematically comparing epidemiological and clinical features of MAFLD and NAFLD by meta-analysis: Focusing on the non-overlap groups. *Liver Int.* 2022;42(2):277-287.
25. Kang SH, Cho Y, Jeong SW, Kim SU, Lee JW, Korean NSG. From nonalcoholic fatty liver disease to metabolic-associated fatty liver disease: Big wave or ripple? *Clin Mol Hepatol.* 2021;27(2):257-269.
26. Liang Y, Chen H, Liu Y, et al. Association of MAFLD With Diabetes, Chronic Kidney Disease, and Cardiovascular Disease: A 4.6-Year Cohort Study in China. *J Clin Endocrinol Metab.* 2022;107(1):88-97.
27. Lee H, Lee YH, Kim SU, Kim HC. Metabolic Dysfunction-Associated Fatty Liver Disease and Incident Cardiovascular Disease Risk: A Nationwide Cohort Study. *Clin Gastroenterol Hepatol.* 2021;19(10):2138-2147 e2110.
28. Niriella MA, Ediriweera DS, Kasturiratne A, et al. Outcomes of NAFLD and MAFLD: Results from a community-based, prospective cohort study. *PLoS One.* 2021;16(2):e0245762.
29. Lonardo A, Carani C, Carulli N, Loria P. 'Endocrine NAFLD' a hormonocentric perspective of nonalcoholic fatty liver disease pathogenesis. *J Hepatol.* 2006;44(6):1196-1207.
30. Goossens GH, Jocken JWE, Blaak EE. Sexual dimorphism in cardiometabolic health: the role of adipose tissue, muscle and liver. *Nat Rev Endocrinol.* 2021;17(1):47-66.
31. Lonardo A, Suzuki A. Sexual Dimorphism of NAFLD in Adults. Focus on Clinical Aspects and Implications for Practice and Translational Research. *J Clin Med.* 2020;9(5).

32. Balakrishnan M, Patel P, Dunn-Valadez S, et al. Women Have a Lower Risk of Nonalcoholic Fatty Liver Disease but a Higher Risk of Progression vs Men: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol.* 2021;19(1):61-71 e15.
33. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. *Dig Liver Dis.* 2015;47(3):181-190.
34. Mantovani A, Petracca G, Beatrice G, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. *Gut.* 2021;70(5):962-969.
35. Kim Y, Chang Y, Ryu S, Wild SH, Byrne CD. NAFLD improves risk prediction of type 2 diabetes: with effect modification by sex and menopausal status. *Hepatology.* 2022.
36. Ye Q, Zou B, Yeo YH, et al. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2020;5(8):739-752.
37. Stefan N, Kantartzis K, Machann J, et al. Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med.* 2008;168(15):1609-1616.
38. Hwang JH, Stein DT, Barzilai N, et al. Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies. *Am J Physiol Endocrinol Metab.* 2007;293(6):E1663-1669.
39. Non-alcoholic Fatty Liver Disease Study G, Lonardo A, Bellentani S, et al. Epidemiological modifiers of non-alcoholic fatty liver disease: Focus on high-risk groups. *Dig Liver Dis.* 2015;47(12):997-1006.
40. Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes.* 2001;50(8):1844-1850.
41. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab.* 2002;87(7):3023-3028.
42. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol.* 2017;14(1):32-42.
43. Stefan N, Schick F, Birkenfeld AL, Haring HU, White MF. The role of hepatokines in NAFLD. *Cell Metab.* 2023;35(2):236-252.
44. Takata H, Ikeda Y, Suehiro T, et al. High glucose induces transactivation of the alpha2-HS glycoprotein gene through the ERK1/2 signaling pathway. *J Atheroscler Thromb.* 2009;16(4):448-456.
45. Dasgupta S, Bhattacharya S, Biswas A, et al. NF-kappaB mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte function effecting insulin resistance. *Biochem J.* 2010;429(3):451-462.
46. Auberger P, Falquerho L, Contreres JO, et al. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. *Cell.* 1989;58(4):631-640.
47. Byrne CD. Banting memorial lecture 2022: 'Type 2 diabetes and nonalcoholic fatty liver disease: Partners in crime'. *Diabet Med.* 2022;39(10):e14912.
48. Vuorinen M, Mannisto VT, Salomaa V, et al. Attribution of diabetes to the development of severe liver disease in the general population. *Liver Int.* 2022;42(10):2186-2194.
49. Ballestri S, Mantovani A, Byrne CD, Lonardo A, Targher G. Diagnostic accuracy of

ultrasonography for the detection of hepatic steatosis: an updated meta-analysis of observational studies. *Metabolism and Target Organ Damage.* 2021;1(1):7.
50. Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes with A1C. *Diabetes Care.* 2011;34 Suppl 2:S184-190.

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

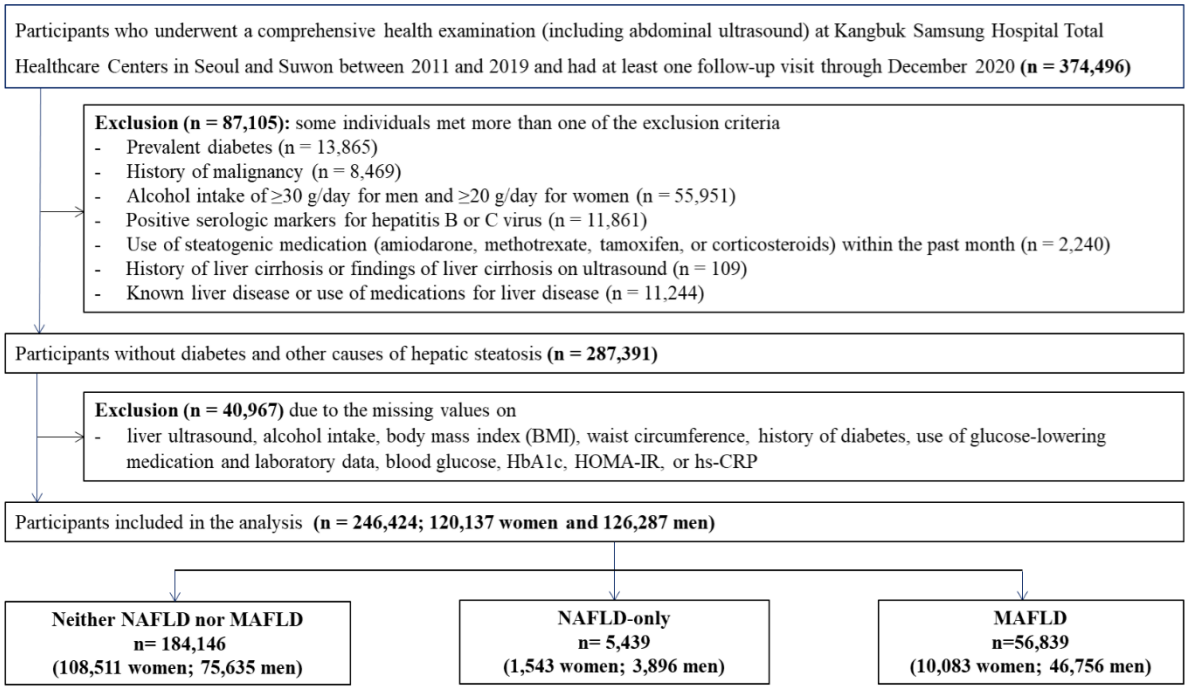

Table 1. Estimated[*] mean values (95% CI) and adjusted[*] proportions (95% CI) of the baseline characteristics of study participants with respect to fatty liver disease category (n = 246,424)

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

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

Yoosun Cho, MD, PhD[1]; Yoosoo Chang, MD, PhD [2,3,4*]; Seungho Ryu, MD, PhD [2,3,4*]; Sarah H. Wild, MB, BChir, PhD [5]; Christopher D. Byrne, MB BCh, PhD [6,7]

[1]Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[2]Center for Cohort Studies, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[3]Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[4]Department of Clinical Research Design & Evaluation, Samsung Advanced Institute for Health Sciences & Technology, Sungkyunkwan University, Seoul, Republic of Korea

[5]Usher Institute, University of Edinburgh, Edinburgh, U.K.

[6]Nutrition and Metabolism, Faculty of Medicine, University of Southampton, Southampton, U.K.

[7]National Institute for Health and Care Research Southampton Biomedical Research Centre, University Hospital Southampton, Southampton, U.K.

[*] **Correspondence:**

Seungho Ryu, MD, PhD, Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

Email: sh703.yoo@gmail.com

Yoosoo Chang, MD, PhD, Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

Tel: +82-2-2001-5139; Fax: +82-2-757-0436; Email: yoosoo.chang@gmail.com

Table of contents:

Supplementary 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 among women (n = 120,137)

Characteristics	Neither NAFLD nor MAFLD	NAFLD-only	MAFLD
Number of participants	108,511	1,543	10,083
Age (years)	36.6 (36.6-36.7)	38.3 (38.1-38.5)	39.1 (39.1-39.2)
Alcohol intake (%) †	11.0 (10.8-11.1)	10.5 (8.9-12.1)	12.2 (11.5-12.9)
Current smoker (%)	1.8 (1.7-1.8)	2.4 (1.6-3.2)	2.3 (2.0-2.6)
Education level (%) ‡	80.9 (80.7-81.2)	82.4 (80.5-84.3)	70.2 (69.3-71.1)
HEPA (%) §	13.4 (13.2-13.6)	11.4 (9.9-12.9)	12.4 (11.8-13.0)
History of hypertension (%)	2.1 (2.0-2.2)	1.2 (0.8-1.6)	5.4 (5.1-5.7)
History of CVD (%)	0.5 (0.5-0.5)	0.3 (0.1-0.5)	0.6 (0.4-0.7)
Lipid-lowering medication use (%)	0.8 (0.8-0.9)	1.6 (1.2-2.1)	1.7 (1.5-1.8)
Family history of diabetes (%)	15.4 (15.2-15.6)	18.7 (16.8-20.6)	20.8 (20.0-21.6)
Obesity (%) ‖	6.8 (6.6-6.9)	0	59.2 (58.2-60.2)
Body mass index (kg/m^2)	21.1 (21.0-21.1)	21.1 (21.0-21.2)	26.2 (26.2-26.3)
Waist circumference (cm)	74.0 (74.0-74.1)	74.7 (74.3-75.0)	86.5 (86.3-86.6)
SBP (mmHg)	101.1 (101-101.2)	100.1 (99.6-100.6)	109.2 (109.0-109.4)
DBP (mmHg)	64.5 (64.5-64.6)	64.1 (63.7-64.5)	69.2 (69.0-69.4)
Fasting glucose (mg/dL)	90.1 (90.1-90.2)	90.8 (90.4-91.2)	95.1 (94.9-95.2)
HbA1c (%)	5.5 (5.5-5.5)	5.5 (5.5-5.5)	5.5 (5.5-5.5)
Total cholesterol (mg/dL)	183.9 (183.7-184.0)	190.7 (189.1-192.2)	197.8 (197.2-198.4)
LDL-C (mg/dL)	107.8 (107.7-108.0)	115.9 (114.5-117.2)	127.6 (127.0-128.1)
HDL-C (mg/dL)	67.6 (67.5-67.6)	65.0 (64.3-65.7)	53.9 (53.7-54.2)
Triglycerides (mg/dL)	75.1 (74.8-75.3)	81.4 (79.4-83.4)	127.8 (127.0-128.6)
GTP (U/L)	14.9 (14.9-15.0)	17.3 (16.6-18.0)	25.1 (24.8-25.4)
ALT (U/L)	14.2 (14.1-14.3)	16.7 (16.1-17.3)	24.4 (24.2-24.7)
AST (U/L)	18.1 (18.0-18.2)	18.7 (18.2-19.1)	21.6 (21.4-21.8)
hs-CRP (mg/L)	0.74 (0.72-0.75)	0.63 (0.51-0.75)	1.80 (1.75-18.47)
HOMA-IR	1.17 (1.16-1.18)	1.31 (1.26-1.36)	2.45 (2.43-2.47)

*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, non-alcoholic fatty liver disease; SBP, systolic blood pressure

Supplementary Table 2. Estimated* mean values (95% CI) and adjusted* proportions (95% CI) of the baseline characteristics of study participants with respect to fatty liver disease category among men (n = 126,287)

Characteristics	Neither NAFLD nor MAFLD	NAFLD-only	MAFLD
Number of participants	75,635	3,896	46,756
Age (years)	36.6 (36.6-36.7)	38.3 (38.1-38.5)	39.1 (39.1-39.2)
Alcohol intake (%) †	46.4 (46.1-46.8)	38.2 (36.7-39.7)	47.6 (47.1-48.0)
Current smoker (%)	29.9 (29.5-30.2)	29.3 (27.8-30.7)	33.2 (32.8-33.6)
Education level (%) ‡	90.1 (89.9-90.3)	93.4 (92.6-94.2)	90.8 (90.5-91.0)
HEPA (%) §	19.0 (18.7-19.3)	12.1 (11.1-13.1)	14.5 (14.1-14.8)
History of hypertension (%)	6.4 (6.2-6.5)	3.1 (2.6-3.7)	12.2 (11.9-12.5)
History of CVD (%)	0.9 (0.9-1.0)	1.1 (0.8-1.4)	0.9 (0.8-1.0)
Lipid-lowering medication use (%)	1.3 (1.2-1.3)	1.2 (0.9-1.6)	2.6 (2.5-2.8)
Family history of diabetes (%)	11.6 (11.4-11.8)	14.3 (13.2-15.4)	15.7 (15.4-16.0)
Obesity (%) ‖	21.1 (20.8-21.4)	0	67.7 (67.3-68.1)
Body mass index (kg/m^2)	23.2 (23.2-23.2)	21.9 (21.9-22.0)	26.5 (26.5-26.5)
Waist circumference (cm)	82.3 (82.3-82.4)	80.8 (80.5-81.0)	91.0 (90.9-91.0)
SBP (mmHg)	112.3 (112.2-112.3)	110.1 (109.8-110.5)	117.1 (117.0-117.2)
DBP (mmHg)	71.4 (71.4-71.5)	70.6 (70.4-70.9)	75.2 (75.2-75.3)
Fasting glucose (mg/dL)	93.7 (93.7-93.8)	93.1 (92.8-93.3)	96.7 (96.6-96.8)
HbA1c (%)	5.5 (5.5-5.5)	5.5 (5.5-5.5)	5.6 (5.6-5.6)
Total cholesterol (mg/dL)	192.2 (192.0-192.4)	197.9 (196.9-199.0)	206.5 (206.2-206.8)
LDL-C (mg/dL)	123.2 (123.0-123.5)	130.4 (129.4-131.3)	138.1 (137.8-138.4)
HDL-C (mg/dL)	56.6 (56.5-56.7)	54.3 (53.9-54.7)	47.4 (47.3-47.5)
Triglycerides (mg/dL)	104.7 (104.2-105.3)	108.8 (106.5-111.2)	164.6 (163.9-165.3)
GTP (U/L)	29.5 (29.3-29.8)	32.5 (31.4-33.5)	47.5 (47.2-47.8)
ALT (U/L)	22.2 (22.1-22.4)	27.6 (26.9-28.2)	40.6 (40.5-40.8)
AST (U/L)	21.8 (21.7-21.9)	22.8 (22.4-23.2)	27.7 (27.5-27.8)
hs-CRP (mg/L)	1.00 (0.98-1.03)	0.78 (0.68-0.89)	1.45 (1.42-1.48)
HOMA-IR	1.17 (1.17-1.18)	1.20 (1.17-1.23)	2.07 (2.06-2.08)

*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, non-alcoholic fatty liver disease; SBP, systolic blood pressure

Supplementary Table 3. 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)	Multivariable-adjusted HR* (95% CI)
Total				
Neither NAFLD nor MAFLD	993,873	2,885	2.9	1.00 (reference)
NAFLD-only	29888	152	5.1	1.77 (1.48-2.13)
NAFLD-only plus high FIB-4	45	1	22.1	8.57 (1.21-60.91)
MAFLD	294453	5,350	17.7	3.16 (2.99-3.34)
MAFLD plus high FIB-4	469	15	19.3	2.75 (1.47-5.12)
Women				
Neither NAFLD nor MAFLD	576,185	1,001	1.7	1.00 (reference)
NAFLD-only	7,911	33	4.2	2.31 (1.56-3.41)
NAFLD-only plus high FIB-4	10	1	103.2	57.17 (8.02-407.5)
MAFLD	47,513	1,046	22.0	5.76 (5.18-6.40)
MAFLD plus high FIB-4	79	4	50.3	3.63 (0.90-14.59)
Men				
Neither NAFLD nor MAFLD	417,688	1,884	4.5	1.00 (reference)
NAFLD-only	21,976	118	5.4	1.53 (1.25-1.88)
NAFLD-only plus high FIB-4	36	0	0	-
MAFLD	246,940	4,304	17.4	2.60 (2.44-2.76)
MAFLD plus high FIB-4	389	11	28.3	2.50 (1.24-5.01)

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.