NAFLD improves risk prediction of type 2 diabetes: with effect modification by sex and menopausal status

Running title: Sex dimorphism, NAFLD and diabetes risk

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Abbreviation list

APRI, AST to platelet ratio index; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; FIB-4, Fibrosis-4 index for liver fibrosis; HFS, Hepamet fibrosis score; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IDI, integrated discrimination improvement; NFS, NAFLD fibrosis score; NRI, net reclassification improvement; SBP, systolic blood pressure; T2D, type 2 diabetes

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ABSTRACT

Background & Aims: The effects of sex and menopausal status on the association between NAFLD and incident type 2 diabetes (T2D) remain unclear. We investigated the effect modification by sex and menopause in the association between NAFLD and T2D; also, added predictive ability of NAFLD for the risk of T2D was assessed. **Approach & Results:** This cohort study comprised 245,054 adults without diabetes (109,810 premenopausal women; 4,958 postmenopausal women; 130,286 men). Cox proportional hazard models were used to estimate hazard ratios (HRs; 95% confidence intervals [CIs]) for incident T2D according to NAFLD status. The incremental predictive role of NAFLD for incident T2D was assessed using the area under the receiver operating characteristic curve, net reclassification improvement, and integrated discrimination improvement. A total of 8,381 participants developed T2D (crude incidence rate/10^3 person-years: 2.9 premenopausal women; 12.2 postmenopausal women; 9.3 men) during median follow-up of 5.3 years. NAFLD was positively associated with incident T2D in all groups. After adjustment for potential confounders, the multivariable-adjusted HRs (95% CIs) for incident T2D comparing NAFLD to no NAFLD were 4.63 (4.17–5.14), 2.65 (2.02–3.48), and 2.16 (2.04–2.29) in premenopausal women, postmenopausal women and men, respectively. The risks of T2D increased with NAFLD severity as assessed by serum fibrosis markers, and the highest relative excess risks were observed in premenopausal women. The addition of NAFLD to conventional risk factors improved risk prediction for incident T2D in both sexes, with a greater improvement in women than men. **Conclusions:** NAFLD, including more severe NAFLD, is a stronger risk factor for incident T2D in premenopausal women than in post-menopausal women or men; protection against T2D is lost in pre-menopausal women with NAFLD.

Nonalcoholic fatty liver disease (NAFLD) is a multisystem disease that increases the risk of cardiovascular disease (CVD), CVD mortality, and all-cause mortality.(1) Systemic metabolic dysfunction occurs with NAFLD, which is strongly associated with type 2 diabetes (T2D) and obesity.(1) Epidemiologic evidence suggests a strong bidirectional relationship between T2D and NAFLD; NAFLD increases the risk of T2D, and the development of T2D increases the risk of NAFLD progression to liver fibrosis.(1, 2)

It is well documented that sexual dimorphism is a feature that exists in both NAFLD and T2D with women generally being at a lower risk for these conditions than men.(3-7) The differences in susceptibility to cardiometabolic conditions in women of reproductive age are largely attributable to the role of female sex hormones in energy metabolism, body composition, vascular function, and inflammatory responses.(8) The protective role of endogenous estrogen has been demonstrated by an increased risk of unfavorable cardiometabolic traits in post-menopausal women.(4) Indeed, emerging data have indicated that men and postmenopausal women are at increased risk of NAFLD (9-13) and T2D (8) compared to premenopausal women, suggesting that menopause is an important modulator in the pathogenesis of both conditions. While consideration of sex and age is critical in determining risk, disease prevention, and personalized therapeutic approaches, currently available data do not adequately address the impact of sex and reproductive status on relevant complications associated with NAFLD. Furthermore, it has not yet been investigated whether sex and menopause prospectively modulate the relationship between NAFLD and the risk of T2D.

This study aimed to evaluate the role of menopausal status and sex as effect modifiers in the association between NAFLD and incident T2D risk. In addition, we assessed whether diagnosis of NAFLD improves risk prediction for incident T2D.

METHODS

Study participants

This study was conducted as part of the Kangbuk Samsung Health Study, a cohort study of Korean men and women aged ≥18 years who underwent comprehensive annual or biennial examinations at the Kangbuk Samsung Hospital Total Healthcare Center in Seoul and Suwon, South Korea, as previously described.(14) The present cohort study included participants who underwent a comprehensive health examination between January 2011 and December 2018 and had at least one follow-up visit before December 31, 2020 (n = 365,686) (**Figure 1**). Ultimately, 245,054 participants without diabetes at baseline, consisting of 109,810 premenopausal women, 4,958 postmenopausal women and 130,286 men were included (see **Supplementary Material**).

This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (IRB No. KBSMC 2021-10-043), which waived the need for informed consent owing to the use of de-identified retrospective data from routine health screening. All procedures performed in the study were in accordance with the Declaration of Helsinki regarding ethical standards for research involving human subjects.

Data collection

Standardized, self-administered questionnaires, physical measurements, abdominal ultrasonography results, and serum biochemical measurements were collected at each visit during the basic health checkup program.(14) (see **Supplementary Material**)

Postmenopausal status was defined as the absence of menstruation for ≥1 year.(15)

Sitting blood pressure (BP), height, weight, and waist circumference were measured by trained nurses. Obesity was defined according to the Asian-specific criteria (16): obese I, body mass index (BMI) of 25 to 29.9 kg/m^2; and obese II, BMI ≥30 kg/m^2. Hypertension was defined as

a systolic BP (SBP) ≥140 mmHg, diastolic BP ≥90 mmHg, or current use of antihypertensive medications.

Blood specimens were collected after at least 10 h of fasting. Levels of lipid profiles, liver enzymes, albumin, glucose, high sensitivity C-reactive protein (hsCRP), and platelet count were measured. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as follows: 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) Glycated hemoglobin (HbA1c) levels were measured using a Cobas Integra 800 (Roche Diagnostics, Rotkreuz, Switzerland) with a turbidimetric inhibition immunoassay for hemolyzed whole blood. The intra- and interassay coefficients of variation were 2.3% and 2.4 %, respectively.

T2D was defined as a fasting serum glucose level ≥126 mg/dL, HbA1c ≥6.5% (48 mmol/mol), or current use of insulin or antidiabetic medications; and prediabetes was defined as a fasting glucose level of 100–125 mg/dL, HbA1c 5.8–6.4%, and no history of diabetes mellitus or anti-diabetic medication use.

Diagnosis of hepatic steatosis and fibrosis

Fatty liver was diagnosed on the basis of abdominal ultrasonography performed by experienced radiologists using standard criteria (see **Supplementary Material**). NAFLD was defined as the presence of fatty liver in the absence of excessive alcohol use (<20 g/day and <30 g/day for women and men, respectively) or any other identifiable cause.(18) Four noninvasive fibrosis indices were used to assess NAFLD severity: the Fibrosis-4 Index for Liver Fibrosis (FIB-4) (main analysis), NAFLD fibrosis score (NFS), AST to platelet ratio index (APRI), and Hepamet fibrosis score (HFS) (for supplemental analyses)(19, 20) (see **Supplementary Material**).

Statistical analyses

Descriptive statistics were used to summarize the participants' characteristics according to the presence of NAFLD separately for pre- and postmenopausal women and men.

We examined the association between NAFLD and its severity as per FIB-4 (main), NFS, APRI, and HFS with the development of T2D. The primary endpoint was incident T2D during follow-up; and the follow-up duration for each participant was extended from the baseline examination until the development of the endpoint, the time of menopause (for premenopausal women), or the last health examination conducted prior to December 31, 2020, whichever came first. Women who transitioned from premenopausal status to menopausal status during follow-up were treated as being censored at the time of transition. Incidence rates were calculated as the number of incident cases divided by follow-up person-years. Cox proportional hazard models were used to estimate the HRs with 95% CIs for the development of incident T2D. Initially, we adjusted for age. Model 1 was further adjusted for the study center (Seoul, Suwon), year of the screening examination, alcohol consumption, smoking status, physical activity, education level, hyperlipidemia medication, family history of diabetes, history of hypertension, and BMI (continuous). Model 2 was further adjusted for SBP, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels, HOMA-IR, and hs-CRP. To evaluate the effects of NAFLD status changes and change in other covariates during the follow-up period, we performed additional analyses by introducing NAFLD status and other factors as time-varying covariates in the models (time-dependent models). The proportional hazards assumption was assessed via estimated log (-log) survival curves, and no violation of the assumption was found. The effect modification by menopausal status and sex on the association between NAFLD and incident T2D was assessed by including terms for interaction with NAFLD in multivariable models (see **Supplementary Material**).

Harrell's C-index (the area under the receiver operating characteristic curve [AUROC]), an estimate of the concordance probability adapted for survival analysis, was used to assess whether NAFLD, and the addition of NAFLD to conventional risk factors for T2D, predicted T2D. The concordance probability is the most commonly applied global measure of discrimination when the outcome is survival time.(21) In addition to the AUROC, we further calculated the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to quantify the incremental predictive ability by adding NAFLD to conventional risk factors (see **Supplementary Material**).

Statistical analyses were performed using STATA version 16.0 (StataCorp LP, College Station, TX, USA). Statistical significance was set at $P < 0.05$.

RESULTS

The participants' baseline characteristics are presented for the six strata of the study population by NAFLD status for pre- and post-menopausal women and for men (**Table 1**). The mean (SD) age of pre- and postmenopausal women and men was 35.5 (6.2), 58.5 (6.3) and 37.9 (7.9) years, respectively. Age, alcohol intake, smoking status, hypertension, lipid-lowering medication usage, family history of diabetes, adiposity parameters, glycemic parameters, total cholesterol, triglycerides, liver enzymes, and hs-CRP levels were higher in the NAFLD groups than in the non-NAFLD group, while physical activity and HDL-C were higher in the non-NAFLD group than in all NAFLD groups.

Table 2 presents the risk of incident T2D according to NAFLD status, sex, and menopausal status. Within 1,294,034.8 follow-up person-years (median, 5.3 years; interquartile range, 2.9–7.8 years; maximum 10 years), 8,381 subjects developed incident T2D (incidence rates per 10^3 person-years were 6.5 overall; 2.9 for premenopausal women; 12.2 for postmenopausal women

and 9.3 for men). NAFLD was positively and independently associated with incident T2D in all groups; however, the relative excess risk was significantly stronger in premenopausal women than in the other groups (*P* for interaction <0.001). The incidence rate for T2D was lowest in premenopausal women without NAFLD. After adjustments for age, sex, center, year of screening, alcohol consumption, smoking status, physical activity, education level, medication for hyperlipidemia, family history of T2D, and history of hypertension (Model 1), the multivariable-adjusted HRs (95% CIs) for incident T2D in premenopausal women, postmenopausal women, and men comparing NAFLD to no NAFLD were 4.63 (4.17–5.14), 2.65 (2.02–3.48), and 2.16 (2.04–2.29), respectively. These associations were slightly attenuated after further adjustment for SBP; total cholesterol, HDL-C, triglyceride, and hsCRP; and HOMA-IR, although remained significant. In time-dependent models, the association was slightly attenuated but remained highly significant, indicating that change in status of NAFLD or other covariates during the follow-up period did not affect the association between baseline NAFLD and development of incident T2D.

In a multivariate model, in which waist circumference instead of BMI was adjusted for as a continuous variable, the HRs (95% CI) in premenopausal women, postmenopausal women, and men were 3.33 (2.99–3.70), 2.17 (1.65–2.85), and 1.58 (1.48–1.68), respectively, when comparing NAFLD with no NAFLD (**Supplementary Table 1**). When both waist circumference and BMI were included together in the model as continuous variables were included together in the model, similar associations were observed compared to the inclusion of either covariate alone.

Table 3 presents the incremental predictive ability for incident T2D after adding NAFLD to the base models that included conventional T2D risk factors used in risk prediction models.(22, 23) The addition of NAFLD to the base model improved the AUROC for predicting incident

T2D to a greater extent in women than in men. The NRIs of the NAFLD were 0.170 ($P < 0.001$), 0.264 ($P < 0.001$), and 0.079 ($P < 0.001$) for premenopausal women, postmenopausal women, and men, respectively. Similarly, greater improvement in the IDI with the addition of the NAFLD was found in women compared to men. When triglycerides were also added to the base model (**Supplementary Table 2**), the addition of NAFLD still significantly improved the AUROC across all categories as in the original base model; the discrimination power was highest in premenopausal women compared with men or postmenopausal women. Importantly, improvements in NRI and IDI were observed across all groups when NAFLD was added to the model even when serum triglyceride level was added to the base model; however, it was higher in both groups of women than in men. Likewise, significant improvements of discriminatory powers based on AUROC, NRI, and IDI were observed when NAFLD status was added to the American Diabetes Association diabetes risk score (**Supplementary Table 3**) and Leicester Diabetes Risk Score. (**Supplementary Table 4**)

Incident T2D risk according to NAFLD and according to more severe NAFLD status (assessed by the FIB-4 score) were also investigated (by sex and menopausal status) (**Supplementary Table 5**). Overall, low to intermediate or high FIB-4 scores were positively associated with an increased risk of incident T2D in all three groups. Multivariable-adjusted HRs (95% CIs) for incident T2D comparing NAFLD with low and intermediate or high FIB-4 scores (to no NAFLD as reference group) were 4.60 (4.14–5.11) and 5.34 (3.48–8.19), in premenopausal women; 2.98 (2.22–4.00) and 2.16 (1.51-3.10) in postmenopausal women; and 2.17 (2.05–2.30) and 1.96 (1.70-2.25) for men, respectively. These associations remained significant, after further adjustments for SBP, total cholesterol, HDL-C, triglyceride, and hsCRP levels; and HOMA-IR, and when the variables were treated as time-dependent covariates. To verify the results, the analyses were repeated using two other liver fibrosis scores as additional markers

of severe NAFLD: the NFS (**Supplementary Table 6**), APRI (**Supplementary Table 7**) and HFS (**Supplementary Table 8**). In these analyses, the risk of T2D increased with NAFLD severity in a dose-response manner in premenopausal women, postmenopausal women, and men; the relative excess risk was greater in premenopausal women than in men or postmenopausal women. These findings were similar when the incident T2D risks based on NAFLD severity as measured by FIB-4, NFS, and APRI were assessed after adjusting for either waist circumference instead of BMI, or both (**Supplementary Table 9**).

After excluding individuals with pre-diabetes or insulin resistance, defined as HOMA-IR ≥2.5 (17), the results were similar to those of the original analyses (**Supplementary Tables 10 and 11**). When individuals with BMI ≥ 30 kg/m^2 were excluded to minimize the potential influence of morbid obesity which might have led to misclassification of some women as being menopausal, the association between NAFLD and incident diabetes became slightly stronger in premenopausal women with an HR (95% CI) of 3.29 (2.93-3.69), whereas the association remained similar in postmenopausal women or in men (**Supplementary Table 10**). The associations with NAFLD severity were also similar to the original findings without exclusion of those with morbid obesity (**Supplementary Tables 12**). In addition, when analysis was performed with data from the women using oral contraceptives, the associations remained virtually unchanged for both NAFLD and its severity (**Supplementary Table 10** and **Supplementary Table 13)**.

Among premenopausal women with available pelvic ultrasonography, which was performed by experienced gynecologists (n = 30,591), premenopausal women with both NAFLD and polycystic ovaries had the highest risk for incident diabetes with corresponding HR of 8.33 (95% CI, 3.95-17.56). However, a significantly higher risk of incident diabetes was still observed in premenopausal women with NAFLD and without polycystic ovaries [HR (95% CI)

= 2.95 (2.30-3.79)] (**Supplementary Table 14**).

DISCUSSION

In this large cohort of 245,054 Korean men and premenopausal and postmenopausal women, with approximately 1.3 million person-years of follow-up, our novel results show that the relative excess risk of T2D associated with NAFLD was considerably higher in premenopausal women than in postmenopausal women and men. In all groups, NAFLD and biomarkers of severe NAFLD were significantly associated with an even higher risk of incident T2D, and in all groups of women and men, NAFLD status improved risk prediction for incident T2D, over and above conventional T2D risk factors. These associations between NAFLD and incident T2D were independent of age; physical activity; family history of diabetes; BMI; waist circumference; total cholesterol and triglyceride levels; HOMA-IR; and hsCRP level.

There has been growing recognition that NAFLD and T2D exhibit sexually dimorphic characteristics.(3-5, 7, 24) While it is well established that NAFLD plays a “catalytic role” in the development of T2D,(2, 25) which is also consistent with our study, sex-differences in the association between NAFLD and incident T2D remain unclear. Accumulating evidence has suggested that premenopausal women are protected from cardiometabolic risk due to sex- and sex-related factors, with an increase in the risk after menopause due to the changes in fat distribution and energy balance related to the changes in reproductive hormone levels.(26, 27) Although the role of sex and menopausal status has been actively investigated in the setting of NAFLD,(5, 10-12) whether menopausal status differentially affects the association between NAFLD and the risk of T2D remain unknown. A previous cross-sectional study has specifically explored the sex-specific relationship between NAFLD and T2D, and the results suggested a stronger association with T2D in men compared to that in women.(28) However, this study was

limited by the cross-sectional study design, inclusion of mostly middle-aged or older women, and no differentiation between pre- and postmenopausal women. A recent meta-analysis of over 500,000 individuals has not found significant effect modification by sex in the association between NAFLD and incident T2D in a meta-regression analysis.(2) However, none of the studies included in this meta-analysis performed a separate analysis between men and women, which makes it difficult to draw a definite conclusion on the effect of sex; also, menopausal status or sex hormones were not adequately taken into consideration in the eligible studies.(2) Our study, for the first time, has demonstrated that associations between NAFLD, including more severe NAFLD, and T2D risk are differentially modulated by both sex and menopausal status.

The mechanisms linking NAFLD and its severity with incident T2D have been previously reviewed in detail.(29) NAFLD with varying degrees of hepatic fibrosis may aggravate hepatic insulin resistance, causing the release of proinflammatory mediators and prodiabetogenic hepatokines such as fetuin-A/B and fibroblast growth factor 21 may increase the risk of developing T2D.(30) Particularly, fetuin-A acts as a ligand of TLR4 receptors through which lipids induce insulin resistance.(31) A previous Mendelian randomization study also supports the notion that genetic polymorphisms associated with severe NAFLD are also associated with an increased risk of developing T2D.(32) However, the underlying causes of the effect modification of sex and menopausal status in the association between NAFLD and incident T2D risk are unclear. Estradiol, the dominant form of estrogen, has a strong antioxidant property that suppresses liver fibrosis and reduces hepatocyte apoptosis and stellate cell activation by impeding reactive oxygen species production in cultures.(6) Moreover, hepatocellular fatty acid β-oxidation is impaired in aromatase-deficient mice that cannot produce estrogen, and this was restored after estradiol replacement.(33) Furthermore, estrogen

promotes accumulation of gluteal-femoral adipose tissue in premenopausal women; and premenopausal women have a greater proportion of lower-body fat than men or postmenopausal women.(7) Gluteofemoral fat is associated with higher insulin sensitivity(34) and insulin sensitivity aids in lipid re-distribution into subcutaneous rather than visceral adipose depot, supporting a potential role of estrogen in the sex- and menopause-related modifications observed in NAFLD-T2D relationship.(7) Given the evidence suggesting the biological plausibility of our findings, sex- and menopausal-status-specific approaches should be considered in diabetes risk management, particularly in patients with NAFLD.

Our findings have revealed that the incidence of T2D in premenopausal women with NAFLD was similar to, or even higher, than that observed in men, suggesting that protection against incident T2D in premenopausal women essentially disappears with NAFLD development. Although, to the best of our knowledge, there is no prior work reporting comparable findings, our results align with previous studies that have consistently observed the inexistence of the female advantage among premenopausal women in the presence of abnormal metabolic profiles.(11, 35-37) A prior systematic review found no differences in susceptibility to NAFLD between male and female patients with T2D, contrary to the general population in which men are more frequently affected than women.(35) Other recent studies also showed that the presence of NAFLD attenuated protection against CVD in premenopausal women, which, together with our findings, may point to an effect of NAFLD on shared risk factors for both CVD and T2D.(36, 37) Although exact mechanisms remain to be elucidated, based on our findings, hepatic fat may represent increased metabolic stress in premenopausal women, which could offset the protective effect of estrogen in lowering the risk of developing T2D. Further investigations are needed to confirm our findings regarding the loss of protection against cardiometabolic diseases in premenopausal women with NAFLD.

Another important finding of our study is that the addition of NAFLD to known T2D risk factors used in the existing T2D prediction models improves the predictive ability of T2D. Several non-invasive risk scores for predicting T2D have been validated.(38) According to a validation study of 12 non-invasive prediction models analyzed by sex, the *C* statistics ranged from 0.76 to 0.81 overall, from 0.73 to 0.79 in men, and from 0.78 to 0.81 in women.(38) In our study, NAFLD significantly improved the discrimination for T2D risk overall for men and both groups of women, compared with the base model. Discrimination was improved regardless of the inclusion (or not) of serum triglyceride levels in the model. Importantly, the discrimination ability of our model with NAFLD is on par with previously reported ranges(38) in men (*C* statistics ~0.76) and is higher in women, especially premenopausal women (*C* statistic ~0.84). The improvement was observed both in men and women, although the discrimination was greatest in premenopausal women compared with that among postmenopausal women or men. Our finding of the added value of NAFLD in T2D prediction may be clinically important, suggesting that a NAFLD diagnosis combined with traditional risk factors for T2D (that are readily available in routine clinical practice) can provide valuable extra information regarding who should be identified as at-risk for T2D. Notably, pre-menopausal women are normally considered at low risk of T2D, and a diagnosis of NAFLD markedly improves risk prediction for T2D in this group. That said, the performance and applicability of a prediction model is likely to be setting- or country-specific; thus, the generalizability of the model with NAFLD should be further tested and validated in different populations and/or countries.

In our study, the association between NAFLD and incident T2D did not materially change after adjusting for BMI, waist circumference, or both. Although obesity is closely correlated with both NAFLD and T2D, the precise contribution of obesity to pathogenesis of T2D in the setting

of NAFLD is unclear. In our study population, obese individuals represented approximately half the population with NAFLD at baseline, indicating that NAFLD is much more prevalent in obese individuals. However, the association between NAFLD and incident T2D was not fully explained by the excessive adiposity as measured by BMI or waist circumference, indicating an independent role of NAFLD in T2D development. This notion is partly supported by previous literature suggesting that intrahepatic triglyceride content is more strongly associated with systemic and peripheral insulin resistance than with intramyocellular lipid, visceral fat content, or subcutaneous fat content.(35, 39, 40) Thus, there may be a greater role of increased hepatic fat content in the development of peripheral insulin resistance to affect risk of T2D.

Our study has some limitations. First, the diagnosis of NAFLD, including the diagnosis of more severe NAFLD was based on ultrasound and liver fibrosis markers instead of liver biopsy. However, liver biopsy would be unethical and not feasible in this large-scale cohort study. Liver ultrasound and noninvasive liver fibrosis indices (FIB-4, NFS, APRI, HFS) have all been widely used in epidemiologic studies and have also been validated by liver biopsy.(19, 20) Although NFS includes impaired fasting glucose, a strong risk factor for T2D, we obtained remarkably similar results using the FIB-4 and APRI which do not include glucose status. Second, the diagnosis of T2D was established based on a single fasting glucose and HbA1c measurements due to the lack of information on the 2-h glucose test, whereas clinical diagnosis should be confirmed based on repeat measurements. That said, HbA1c concentration and not an oral glucose tolerance test is used in clinical practice to diagnose T2DM. Also, to define the incidence of T2D, we included HbA1c which has good preanalytical stability and is not affected by acute perturbations (e.g., stress, exercise, or smoking).(41) In the group of pre-menopausal women, the mean age was <40 years. Therefore, it is plausible that a small

384 percentage of women who developed diabetes in this group, developed type 1 diabetes.
385 However, this percentage would have been very small in this age group and, importantly, any
386 misclassification of diabetes-type would have attenuated the strength of the association
387 between NAFLD and T2D in this group. Third, for the data obtained before 2018, it was not
388 feasible to differentiate between oral contraceptive and intrauterine devices (hormone-
389 containing devices) due to the way the question was worded (only one question was used to
390 collect this data). In addition, detailed information on oral contraceptives or intrauterine
391 devices, such as the type, dose, duration of oral contraceptive use, or type of intrauterine device
392 (hormonal vs. non-hormonal), was not obtained, limiting our ability to analyze the detailed
393 patterns of use. Furthermore, information on other categories of hormonal contraception, such
394 as depo injection or subcutaneous implants, which could affect menstrual bleeding patterns,
395 was unavailable. Lastly, our cohort of relatively young and middle-aged Koreans may limit the
396 generalizability of our findings to other populations of different ages, ethnicities, or with
397 different comorbidities.
398 Our study has several notable strengths, including the longitudinal, prospective design that
399 enabled us to observe the temporal associations between NAFLD (and more severe NAFLD
400 based on serum fibrosis markers), with the risk of incident T2D. Furthermore, the large sample
401 size, the use of carefully standardized clinical, imaging, and laboratory procedures, and the
402 inclusion of lifestyle factors, and the repeated measurements allowed us to account for possible
403 confounders as time-varying covariates. Lastly, the inclusion of relatively healthy, younger
404 individuals reduced the potential for survivor bias caused by selecting subjects with severe
405 diseases as well as comorbidity-related bias, which is a common limitation of previous studies
406 involving patients with biopsy-proven advanced stage NAFLD.
407 In conclusion, our results show that in both men and women, a diagnosis of NAFLD improves

the prediction of risk of developing T2D, over and above classical diabetes-risk factors. In the presence of NAFLD, the relative increase in the risk of T2D risk (compared to those without NAFLD) was greatest in premenopausal women than in men or postmenopausal women. Importantly, our time-dependent analyses take account of any change in NAFLD status or change in other key covariates between baseline and follow up, and even in these analyses the presence of baseline NAFLD was associated with incident T2D at follow up, in both groups of women and in men. We suggest that premenopausal women diagnosed with NAFLD should be considered at high risk of T2D, and this group should be targeted for diabetes prevention. Our study also underscores the need for age-and sex-specific approaches for diabetes risk assessment and management.

Conflict of interest

All authors declare that they have no conflict of interests.

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Author Contributions

Yejin Kim: interpretation of data, drafting and critical revision of the manuscript.

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Seungho Ryu: study concept and design, acquisition of data, analysis and interpretation of data, and critical revision of the manuscript

Sarah H. Wild: interpretation of data and critical revision of the manuscript

Christopher D Byrne: study concept and design, interpretation of data and critical revision of the manuscript

All authors confirm that they had full access to all the data.

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Figure legend

Figure 1. Flow chart describing the selection of the study participants

NAFLD, nonalcoholic fatty liver disease