History of gestational diabetes and incident nonalcoholic fatty liver disease

History of gestational diabetes and incident nonalcoholic fatty liver disease: The Kangbuk Samsung Health Study

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Conflict of interest

None declared.

Study Highlights

WHAT IS KNOWN

- Gestational diabetes mellitus (GDM) is a risk factor for type 2 diabetes and NAFLD.
- It is inconsistent whether insulin resistance or diabetes mediate the association between GDM and NAFLD

WHAT IS NEW HERE

- GDM is a strong risk factor for moderate-to-severe liver steatosis, irrespective of diabetes development or insulin resistance.
- Diabetes development and insulin resistance each mediate <10% of the association between GDM and NAFLD.

ABSTRACT

Objectives: We examined the relationship between a prior history of gestational diabetes mellitus (pGDM) and risk of incident nonalcoholic fatty liver disease (NAFLD) and investigated the effect of insulin resistance or development of diabetes as mediators of any association.

Methods: We performed a retrospective cohort study of 64,397 Korean parous women without NAFLD. The presence of, and the severity of NAFLD at baseline and follow-up were assessed using liver ultrasonography. Cox proportional hazards models were used to determine adjusted hazard ratios (aHRs) for incident NAFLD according to a self-reported GDM history, adjusting for confounders as time-dependent variables. Mediation analyses were performed to examine whether diabetes or insulin resistance may mediate the association between pGDM and incident NAFLD.

Results: During a median follow-up of 3.7 years, 6,032 women developed incident NAFLD (of whom 343 had moderate-to-severe NAFLD). Multivariable aHRs (95% confidence intervals) comparing women with time-dependent pGDM to the reference group (no pGDM) was 1.46 (1.33–1.59) and 1.75 (1.25–2.44) for incident overall NAFLD and moderate-to-severe NAFLD, respectively. These associations remained significant in analyses restricted to women with normal fasting glucose <100 mg/dl or that excluded women with prevalent diabetes at baseline or incident diabetes during follow-up. Diabetes and insulin resistance (HOMA-IR) each mediated <10% of the association between pGDM and overall NAFLD development.

Conclusions: A prior history of GDM is an independent risk factor for NAFLD development. Insulin resistance, measured by HOMA-IR, and development of diabetes each explained only <10% of the association between GDM and incident NAFLD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has emerged as a global public health burden alongside the epidemics of obesity and type 2 diabetes. The estimated global prevalence of NAFLD is 25%–30% in adults (1). NAFLD increases the risk of both liver-specific complications and extrahepatic diseases (2, 3). However, the lack of approved pharmacological treatments for NAFLD (4) means that it is important to identify modifiable risk factors and apply effective interventions to prevent NAFLD.

Gestational diabetes mellitus (GDM), defined as impaired glucose metabolism during pregnancy (5), is becoming increasingly common and affects between 1 in 8 and and 1 in 25 pregnancies (6, 7). GDM increases the risk of adverse outcomes for both mother and offspring, that include subsequent type 2 diabetes (8) and cardiovascular disease (CVD) for both mother and child in later life (9). GDM is closely associated with obesity, insulin resistance (IR), and dyslipidemia (10) and cross-sectional and cohort studies have investigated the association between a history of GDM and subsequent risk of NAFLD (11-15). The association of GDM with NAFLD is well described, but whether the association is independent of type 2 diabetes or IR is inconsistent and limited (13-15).

We investigated the association between prior history of GDM (pGDM) and the development of NAFLD while accounting for changes in risk factors and potential confounders during the follow-up period in a large cohort of healthy middle-aged parous Korean women. We also evaluated the role of IR and diabetes as potential mediators of this association.

RESEARCH DESIGN AND METHODS

The present cohort study of parous women was performed as part of the Kangbuk Samsung Health Study, a large-scale cohort study of Korean adults who underwent annual or biennial health screening examinations at Kangbuk Samsung Hospital Total Healthcare Centers in Seoul and Suwon, South Korea (17). Out of all parous women attending screening visits between 2015 and 2019, the overall proportion of follow-up before December 2020 was 79.2% (**Supplementary Tables 1 and 2, Supplemental Digital Content 1)**. Our study was restricted to premenopausal women aged < 50 years who had one or more births, underwent a comprehensive health examination between 2015 and 2019, and had at least one follow-up visit before December 2020 (n = 90,679). We excluded women with ultrasound-defined fatty liver at baseline and then those with potential secondary cause of fatty liver (**Figure 1**). Then, we excluded women with missing information on pGDM, fatty liver, alcohol consumption, and covariates, resulting in the final sample of 64,397.

This study adhered to both the Declarations of Helsinki and Istanbul and was approved by the Institutional Review Board of Kangbuk Samsung Hospital (IRB No. KBSMC 2022-06-007), which waived the requirement for informed consent owing to the use of anonymized retrospective data that were routinely collected during health examinations.

Data collection

The dataset included socio-demographic factors, health-related behaviors, medical and pregnancy history, parity, and other reproductive characteristics provided by participants in self-report questionnaires, along with anthropometric and laboratory measurements (17)**.** Information via questionnaire, liver ultrasound, glycemic parameters and other covariates were measured at baseline and subsequent visits. The age at first birth was available in a subsample of the participants (n=55,407 our of 64,397) as this question was not a basic part

of questionnaire but assessed as a part of a separate 'health risk assessment' that not all the participants received. Smoking status was categorized as never, former, or current. The average alcohol consumption per day was estimated using the recorded frequency and amount of alcohol consumed per drinking day in standard units. Physical activity levels were measured using the validated Korean version of the International Physical Activity Questionnaire short form and classified as inactive, minimally active, or health-enhancing physical activity (HEPA) based on metabolic equivalents (min/week)(18).

Obesity was defined as a BMI of ≥25 kg/m^2 according to Asian-specific criteria (19). Metabolic syndrome (MetS) was determined by having three or more components among five components (20): triglyceride (TG) ≥150 mg/dl; high-density lipoprotein (HDL) <50 mg/dl; blood pressure (BP) ≥130/85 mmHg or use of BP-lowering medication; fasting glucose ≥100 mg/dl or use of glucose-lowering medication; and abdominal obesity defined as WC of ≥85 cm (21).

Hypertension was defined as BP of ≥140/90 mmHg or the use of BP-lowering medication. Blood samples collected after at least 10 hours of fasting were used to measure serum lipid profiles, glycemic parameters, liver enzyme levels, and high-sensitivity C-reactive protein levels. HOMA-IR was estimated and IR was defined by a HOMA-IR ≥ 2.5 (22).

Type 2 diabetes was defined as fasting serum glucose level ≥126 mg/dL, HbA1c ≥6·5% (48 mmol/mol), a history of diabetes, or the current use of glucose-lowering medications.

Definition of GDM history

During the health screening examination, a self-report questionnaire was used to assess

pGDM, with the question “Have you ever been diagnosed with gestational diabetes by physicians?” and two response options (yes or no). Women who answered “yes” were considered to have a pGDM. Importantly, in South Korea, all pregnant women are recommended to undergo GDM screening at 24–28 weeks, regardless of the underlying GDM risk (23). GDM screening is performed by a two-step approach or one step approach according to the standard guidelines (see Text, **Supplemental Digital Content 2**, which demonstrates the screening approaches of GDM).

Liver ultrasound measures and definition of NAFLD

Abdominal ultrasonography was performed by experienced radiologists who were unaware of the objectives of the study. Any fatty liver was diagnosed according to the following standard criteria: a diffuse increase in fine echoes in the liver parenchyma compared with those in the kidney or spleen parenchyma, deep beam attenuation, and bright vessel walls. As we had excluded other potential causes of fatty liver (see exclusion criteria), fatty liver was considered NAFLD. Furthermore, moderate-to-severe NAFLD was diagnosed as follows: 1) slightly impaired visualization of the intrahepatic vessels and diaphragm, and increased liver echogenicity or 2) poor penetration of the posterior segment of the right lobe, poor or no visualization of the hepatic vessels and diaphragm, and a significant increase in hepatic echogenicity (26). The inter-observer and intra-observer reliability values for fatty liver diagnosis were substantial (kappa statistic = 0.74) and excellent (kappa statistic = 0.94), respectively (17).

Statistical analysis

The primary endpoints were a) overall incident NAFLD and b) incident moderate-to-severe NAFLD. Each outcome was analyzed independently and considered as a separate

endpoint. Incidence was expressed as the number of cases per 1000 person-years with follow-up from baseline visit until the date of the primary endpoint or the last health screening exam (December 31, 2020), whichever occurred first.

Cox proportional hazard models were used to estimate adjusted hazard ratios (aHRs) with 95% confidence intervals (CIs) for each primary endpoint, to compare women with and without (reference) pGDM. The multivariable-adjusted model was progressively adjusted for age; center (Seoul or Suwon), examination year, alcohol consumption (<10 or ≥10 g/day), age at first birth, smoking status (never, former, current smoker, or unknown), physical activity level (inactive, minimally active, HEPA, or unknown), education level (below college graduate, college graduate or higher, or unknown), hyperlipidemia medication use, history of hypertension, history of CVD, and BMI. To take account of changes in pGDM and other covariates during the follow-up period, we conducted time-dependent analyses, in which the updated pGDM and other covariates were treated as time-varying covariates.

We also used mediation analysis to evaluate potential mediators of the association between pGDM and incident NAFLD. We used the Stata command med4way (27) (see Text, **Supplemental Digital Content 2**, which describes the mediation analysis used). The outcome was studied using a Cox proportional model as med4way is fully integrated with Stata's way of handling survival data. The regression model for the potential mediators were a logistic regression model for diabetes and a linear regression model for HOMA-IR, which was log-transformed to normalize the data before the analyses. The controlled direct effects (CDE) were estimated at a fixed level of the mediator: at non-diabetes status or at the mean level of HOMA-IR. Indirect effects were estimated from the relative risk due to mediated interaction and pure indirect effect. The proportion mediated provides an estimate of the proportion of the total GDM effect that acts through its association with the potential

mediator. Furthermore, we evaluated other potential mediators, including BMI, waist circumference, eGFR, hs-CRP, lipid profiles, and MetS.

We performed sensitivity analyses to explore any associations between pGDM and incident NAFLD by: 1) restricting the sample to women with normal fasting glucose <100 mg/dl, 2) excluding women who developed diabetes during the follow-up and those with prevalent diabetes. Subgroup analyses were also conducted based on adiposity measures, HOMA-IR, hs-CRP level, and MetS and its components. Since our study is retrospective, we used the current values of metabolic risk factors at baseline health examination, as pre-pregnancy or pregnancy measurements were unavailable.

We performed additional analysis considering the 3-year and 5-year look-back periods to ascertain prevalent NAFLD and prevalence of comorbidities (25, 26). Comorbid conditions including history of hypertension, history of diabetes, history of CVD and NAFLD were considered as prevalent if these conditions were observed during the 3-year and 5-year look-back period including time at baseline.

STATA version 17.0 (Stata Corp LP, College Station, TX, USA) was used to perform statistical analyses. A two-sided P-value of <0.05 was considered statistically significant.

RESULTS

After excluding participants who met the exclusion criteria, 64,397 women were included in the study (**Figure 1**). The prevalence of pGDM at baseline was 7% (**Table 1**). Women with a pGDM tended to be younger and more highly educated, with an unfavorable lipid profile and higher waist circumference, diastolic BP, and fasting glucose, alanine

aminotransferase, gamma-glutamyl transferase, and HOMA-IR levels compared to women without a pGDM. Women with pGDM were more likely to be older at first live birth, compared to those without pGDM (59.4% and 50.1% of ≥30 years at first birth, respectively) (**Table 1**).

The median follow-up duration was 3.7 years (interquartile range: 2.0–4.4 years; maximum: 6.0 years). During 213,135 person-years of follow-up, 6,032 cases of incident NAFLD (28.3 cases per 10^3 person-years) and 343 cases of incident moderate-to-severe NAFLD were identified (1.5 cases per 10^3 person-years) (**Table 2**). The multivariable aHRs (95% CIs) comparing pGDM to the reference was 1.39 (1.27–1.51) for all incident NAFLD and 1.86 (1.35–2.55) for moderate-to-severe NAFLD. After further adjustment for waist circumference, lipid profiles, eGFR, and hs-CRP, the significant associations persisted (**Supplementary Table 3, Supplemental Digital Content 1**). In a time-dependent model including the updated status of pGDM and changes in BMI and other confounders as time-dependent covariates, aHRs (95% CIs) comparing pGDM to the reference were 1.46 (1.33-1.59) for incident all NAFLD and 1.75 (1.25-2.44) for moderate-to-severe NAFLD **(Table 2)**.

The results of the med4way mediation analysis for the association between pGDM and all NAFLD and its severe form, by diabetes or HOMA-IR are presented in **Table 3.** The association between pGDM and incident NAFLD was mediated by IR (assessed by HOMA-IR) or development of diabetes with less than 10%. IR and diabetes contributes to neither interaction nor mediated interaction (**Table 3**). Additionally, the association between pGDM and incident NAFLD was also mediated by waist circumference, eGFR, hs-CRP, and lipid profiles, with the highest proportion of mediation observed for triglycerides (10%) (**Supplementary Tables 4 and 5, Supplemental Digital Content 1**). BMI only contributes

to interaction but not mediation. Mediated interactions for waist circumference and triglycerides were significant; however, these interactions only minimally contribute to the incidence of NAFLD (1%). MetS also only negligibly mediated the association between pGDM and NAFLD without significant mediation proportion for both all NAFLD and its severe form.

Sensitivity analyses (**Supplementary Table 6, Supplemental Digital Content 1**) consistenly showed an increased risk of incident NAFLD in women with normal fasting glucose or women after excluding those with prevalent or incident diabetes. The association did not significantly differ by subgroups **(see Supplementary Figure, Supplemental Digital Content 1).**

Subgroup analyses stratified by age group (<35 years, 35-39 years, and ≥40 years) with additional adjustment for age at first birth, yielded consistent results across the age subgroups, consistent with the original findings, and with no significant interaction by age **(see Text, Supplemental Digital Content 1; Supplementary Table 7, Supplemental Digital Content 2).**

Considering the look-back periods, the increased risk of NAFLD among women with pGDM remained robust with stronger association for moderate-to-severe NAFLD (**see Text, Supplemental Digital Content 1; Supplementary Table 8, Supplemental Digital Content 2).**

DISCUSSION

Our study found that women with a pGDM had approximately a 2-fold increased risk of developing moderate-to-severe NAFLD after about 4 years of follow-up, independent of

measured potential confounders or prevalent or incident diabetes. Mediation analyses showed that IR (assessed by HOMA-IR) and development of diabetes partially mediated the associations between pGDM and incident NAFLD, explaining less than 10% of the association, suggesting that other factor(s) associated with pGDM may be responsible for the increased risk of incident NAFLD.

Previous cross-sectional (11, 12, 30) and cohort studies (13-15, 31, 32) have investigated the association between GDM and NAFLD risk. Women with pGDM (vs. without pGDM) have a 7–12-fold higher risk of developing incident type 2 diabetes (33, 34), which is closely associated with NAFLD (35). Thus, the interrelationships between these conditions must be considered when investigating whether pGDM *per se* is an independent risk factor for NAFLD. Previous cohort studies have reported mixed results on whether type 2 diabetes is a mediator or confounder in the association between GDM and NAFLD (13-15). In a cohort study from the Coronary Artery Risk Development in Young Adults study, comprising Black and White Americans, a positive association between GDM history and NAFLD at year 25 was found; however, this association was fully attenuated by adjusting for incident diabetes (14).

A cross-sectional study in the U.S. population found no increased prevalence of steatosis or fibrosis about 20-25 years after pregnancy among women with pGDM but without type 2 diabetes (31); but there may be several explanations for the discrepancy between their results and ours. Our study was characterized by a large sample size of younger age group (~63% of women aged <40 years), lower prevalence of comorbidities, a focus on NAFLD, a cohort study design and mediation analyses. In our study, the association between pGDM and NAFLD tended to be robust and stronger in the younger group aged <40 years (vs. older group), possibly due to lower recall bias and less residual confounding by

comorbidities. On the contrary, the cross-sectional study by Ciardullo S et al. included a low proportion of young women aged <40 years (less than 30%), women with a higher prevalence of comorbidities and no exclusion of secondary cause of steatosis such as HCV and excessive alcohol consumption. Given the differences in various features of the study design, the two studies are not directly comparable.

In line with our study, a prospective study including 607 women with GDM and 619 women without GDM from the Danish National Birth Cohort reported a positive association between GDM and the subsequent higher fatty liver biomarker scores, irrespective of the subsequent development of prediabetes or type 2 diabetes (13). It is important to note that previous cohort studies have been limited by the use of clinical NAFLD diagnoses based on electronic medical records (15), which were likely to markedly underestimate the proportion with NAFLD; use of proxy measures for diagnosing NAFLD, such as biomarker scores (rather than liver imaging or liver biopsy) (13); or participants with unknown status of NAFLD at baseline (14, 15, 30-32). The strengths of our study include the large sample size of 64,397 parous Korean women without ultrasound-defined NAFLD at baseline and repeated measurements during follow up including liver ultrasonography, glycemic status, and other confounders, enabling us to take account of a change in the status of risk factors between baseline and follow up.

Our study used several different approaches, including mediation analyses, sensitivity analyses that restricted women with normoglycemia or without incident diabetes during follow-up, and analyses by clinically relevant subgroups. These approaches consistenly demonstrated an independent role for GDM in NAFLD development, highlighting that pGDM in parous women may help identify women at high risk of developing NAFLD who may benefit from lifestyle-change measures to mitigate their risk of

developing NAFLD and associated multisystem complications (2, 37).

Despite obesity being a known risk factor for NAFLD, our study found a significant association between pGDM and increased risk of incident NAFLD, even after adjusting for or stratifying by BMI or waist circumference. In Asia, where up to 19% of the NAFLD population is classified as non-obese (38), lean NAFLD shares an altered metabolic and cardiovascular profile with obese NAFLD, possible due to an altered fat distribution; excessive visceral adiposity and/or decreased protective fat tissues (39). Further research using detailed adiposity measures is needed to elaborate the differential effect of various body composition phenotypes on the risk of incident NAFLD in women with pGDM.

The refined mediation analysis used in the present study helps disentangle the pathways between GDM and NAFLD. These data provides clinically relevant information on the proportion of subjects with NAFLD due to pGDM alone, and the proportion due to interaction and mediation, by plausible pathophysiological factors, e.g. abdominal ectopic fat accumulation (40, 41), renal dysfunction (2, 42), inflammation (43, 44), metabolic syndrome (40, 41) and dyslipidemia (40, 41) in addition to diabetes or insulin resistance. Most of the metabolic abnormalities above except for BMI partially mediate the pGDM-NAFLD association by less than 10 %. Waist circumference and triglyceride, particularly, contributed to interaction, mediation, and mediated interaction together, indicating that the relationship between GDM and the development of NAFLD is complex and involves intricate biological interactions and mediations of abnormal metabolic features, visceral fat accumulation and triglyceride infiltration into hepatocytes. MetS negligibly mediated the association between pGDM and NAFLD without significant mediation proportion for NAFLD. In our study, prevalence of MetS was only 2.3% in women with pGDM, which limited to estimate the mediation effect of MetS.

The mechanism of the association between pGDM and NAFLD could not be explained by two potential key mediators, i.e. prevalent and incident type 2 diabetes, and insulin resistance assessed by HOMA-IR. Women with GDM predisposed to pancreatic β-cell dysfunction have insufficient insulin secretion to meet the extra gestational demands on glucose metabolism (51). For women with pGDM who have decreased insulin sensitivity and increased insulin secretion, compared to women with no previous history of GDM (12), compensatory hyperinsulinemia could play a role in NAFLD development since insulin stimulates hepatic lipogenesis (52, 53). Impaired insulin sensitivity reduces suppression of hepatic glucose production and insulin-stimulated glucose uptake in skeletal muscle, and increases fatty acids produced from adipose tissue (51), leading to an increased influx of fatty acids to the liver, consequently resulting in the development of NAFLD. Accumulation of lipid in hepatocytes in the form of hepatic di-acyl glycerols (DAGs) potential leads to increased hepatic inflammation and subsequent oxidative stress (53). Altered glucose metabolism, as seen in pGDM, may also influence development of liver fibrosis in NAFLD potentially via GDF-15 signaling via hepatic TGF-beta receptors (55). Furthermore, lower levels of adiponectin or other adipocytokines in women with pGDM might contribute to other pathophysiological pathways linking GDM and NAFLD (56).

Although insulin resistance seems to be a key pathophysiological factor in mediating the association between pGDM and NAFLD development, its mediation effect on the association was less than 10% in our study. In our study, we used Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), one of the insulin resistance indices proposed by Matthews et al. (22). This index has been shown to significantly correlate with a measure of whole body insulin sensitivity as determined by hyperinsulinaemic euglycaemic glycemic clamp in non-diabetic and diabetic subjects (46, 47). Although HOMA-IR is

accepted as a good measure for assessment of whole body insulin sensitivity, the correlation between HOMA-IR and glucose disposal rate, a measure of peripheral insulin resistance, can vary depending on the characteristics of study population and these insulin sensitivity measures are not free of measurement errors (48-50). Therefore, in our study, we cannot rule out the potential mediation effect of residual IR or skeletal muscle and adipose tissue IR, on the association between GDM and NAFLD risk.

The present study has some inherent limitations imposed by the study design. First, pGDM was identified based on self-report using a self-administered, structured questionnaire, which may have led to misclassification of GDM and attenuated the strength of the observed association towards the null. Even so, a self-reported diagnosis of GDM has been found to be accurate, compared with medical records as the reference standard, with a sensitivity of 93% and specificity of 100% (13, 25). Second, ultrasonography was performed to identify all NAFLD (and moderate-to-severe NAFLD in the sub-group), rather than liver biopsy, liver magnetic resonance, or computed tomography imaging. Therefore, there is a possibility of misclassification of NAFLD. Third, to define diabetes, we used single fasting glucose and HbA1c measurements only, since data from a 2-hour glucose tolerance test were not available. However, HbA1c is a practical test for diagnosing hyperglycaemia in large populations due to greater pre-analytical stability than blood glucose and there is little effect from acute perturbations such as diet, exercise, and smoking (57). Fourth, information on pre-pregnancy risk factors, such as BMI and fasting glucose levels, history of polycystic ovarian syndrome as well as GDM severity, was not available. Fifth, since our study participants were healthy middle-aged Korean adults with good access to health care facilities, the generalizability of our findings to other ethnic or demographic groups needs to be confirmed. We could not examine the association between pGDM and incident NAFLD, while taking into the exact

timing of pGDM onset and NAFLD onset, such as whether it occurred pre-pregnancy, during pregnancy, postpartum or at subsequent follow-up. Similarly, potential mediators at single point time of each visit were assessed 1–2 years apart, thereby limiting exact estimations of pGDM, NAFLD onset time and duration and comprehensive evaluation of mediators throughout the follow-up period. Therefore, there may be some residual misclassification of potential mediators or residual measurement errors due to inherent limitation of measured mediators (e.g., HOMA-IR is not perfect measure of IR). Also, the possibility of unmeasured or residual confounders cannot be excluded from our findings. Future cohort studies with further consideration of prepregnancy metabolic profiles, timing of GDM and NAFLD onset and more accurate measures of IR are needed to support our findings.

Despite these limitations, our cohort study demonstrates that the pGDM is a strong and independent risk factor for developing ultrasound-diagnosed NAFLD, and we show that IR, the development of diabetes and other metabolic factors may play a role in mediating this association. pGDM may help identify a sub-group of women at high risk of developing NAFLD and who are particularly likely to benefit from lifestyle measures known to attenuate the risk of developing NAFLD. We suggest that follow-up for women with pGDM should provide support for lifestyle changes and that screening for NAFLD should be considered in addition to screening for type 2 diabetes.

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Authors' contributions

All authors planned, designed and implemented the study, including quality assurance and control. SR analyzed the data and designed the analytic strategy. CK contributed to the additional analyses, data interpretation, and critical revisions. YChang and SR supervised field activities. YCho and YChang drafted the manuscript with contributions from SW and CB. All authors interpreted the results and contributed to critical revisions of the manuscript. All authors approved the final version of this manuscript.

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Data Availability Statement

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

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605

606 **Table 1. Age-adjusted means and proportions (95% CI) of baseline characteristics by**
607 **the history of gestational diabetes mellitus (n = 64,397)**

Characteristics	History of gestational diabetes mellitus		*p*-value
	No	Yes	
Number	59,714	4,683	
Age (years)	38.4 (38.3-38.4)	37.7 (37.6-37.8)	<0.001
Seoul center (%)	45.1 (44.8-45.5)	41.3 (39.9-42.7)	<0.001
Current smoker (%)	0.9 (0.8-1.0)	1.1 (0.8-1.4)	0.215
Alcohol intake (%)*	8.5 (8.3-8.8)	8.3 (7.5-9.1)	0.591
HEPA (%)	11.5 (11.3-11.8)	10.9 (10.0-11.8)	0.205
High education level (%)†	84.9 (84.6-85.2)	87.5 (86.6-88.5)	<0.001
Diabetes (%)	0.3 (0.2-0.3)	3.3 (2.7-3.8)	<0.001
Hypertension (%)	1.9 (1.8-2.0)	2.5 (2.0-3.0)	0.004
History of CVD (%)	0.5 (0.4-0.5)	0.5 (0.3-0.6)	0.998
Lipid-lowering drug use (%)	0.4 (0.3-0.4)	0.8 (0.5-1.1)	<0.001
Early menarche (%)	6.4 (6.2-6.6)	7.1 (6.4-7.8)	0.055
Age at first live birth (years)			<0.001
<25	3.0 (2.8-3.1)	1.6 (1.2-2.0)	
25-29	46.9 (46.5-47.3)	39.0 (37.5-40.4)	
≥30	50.1 (49.7-50.6)	59.4 (57.9-60.9)	
Metabolic syndrome (%)	1.1 (1.1-1.2)	2.3 (1.8-2.7)	<0.001
Obesity (%)‡	7.5 (7.3-7.7)	8.2 (7.4-9)	0.078
Body mass index (kg/m^2)	21.3 (21.3-21.3)	21.4 (21.3-21.4)	0.088

Waist circumference (cm)	73.8 (73.8-73.9)	74.3 (74.1-74.5)	<0.001
SBP (mmHg)	101.3 (101.2-101.4)	101.6 (101.3-101.8)	0.080
DBP (mmHg)	64.4 (64.4-64.5)	64.7 (64.5-65)	0.006
Glucose (mg/dl)	90.6 (90.5-90.6)	93.7 (93.5-93.9)	<0.001
Glycated hemoglobin (%)	5.4 (5.4-5.4)	5.5 (5.5-5.5)	<0.001
Total cholesterol level (mg/dl)	181.9 (181.7-182.1)	184.7 (183.9-185.6)	<0.001
LDL-C level (mg/dl)	110.8 (110.6-111)	113.8 (113-114.5)	<0.001
HDL-C level (mg/dl)	68.2 (68.0-68.3)	67.5 (67.0-67.9)	0.002
Triglyceride level (mg/dl)	76.4 (76.1-76.7)	79.6 (78.5-80.6)	<0.001
AST (U/l)	17.7 (17.6-17.7)	17.8 (17.6-18)	0.198
ALT (U/l)	14.2 (14.1-14.3)	14.7 (14.4-15)	<0.001
GGT (U/l)	14.9 (14.8-15.0)	15.7 (15.3-16.0)	<0.001
hs-CRP (mg/l)	0.76 (0.74-0.79)	0.78 (0.70-0.86)	0.001
HOMA-IR	1.30 (1.29-1.31)	1.42 (1.39-1.45)	<0.001

608 Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CI, confidence interval;
609 CVD, cardiovascular disease; DBP, diastolic blood pressure; GGT, gamma-glutamyl transferase;
610 HEPA, health-enhancing physical activity; HDL-C, high-density lipoprotein cholesterol; HOMA-IR,
611 homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein;
612 LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.
613 * ≥10 g of ethanol per day; † ≥college graduate; ‡body mass index ≥ 25 kg/m^2
614 Number of participants with missing on age at first live birth-8,990 (14.0%)
615

Table 2. Development of nonalcoholic fatty liver disease by history of gestational diabetes mellitus at baseline (n = 64,397)

Gestational diabetes mellitus	Person-years	Incident cases	Incidence rate (/10^3 PY)	Age-adjusted HR (95% CI)	Multivariable-adjusted HR* (95% CI)	HR (95% CI)† in a model with time-dependent variables
All NAFLD						
No	197705.0	5465	27.6	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	15429.9	567	36.7	1.39 (1.28-1.52)	1.39 (1.27-1.51)	1.46 (1.33-1.59)
Moderate-to-severe NAFLD						
No	207805.3	298	1.4	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	16508.2	45	2.7	1.94 (1.42-2.66)	1.86 (1.35-2.55)	1.75 (1.25-2.44)

* Estimated from Cox proportional hazards models. Multivariable model was adjusted for age, center, examination year, alcohol consumption, smoking status, physical activity level, education level, BMI, history of hypertension, history of CVD, lipid-lowering drug use and age at first birth

† Estimated from Cox proportional hazard models with a history of gestational diabetes, smoking status, alcohol consumption, physical activity level, BMI, history of hypertension, history of CVD and lipid-lowering drug use, as time-dependent variables and baseline age, center, examination year, education level and age at first pregnancy as time-fixed variables.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; PY, person-years.

‡Please note that current BMI rather than pre-pregnancy BMI was considered a potential mediator.

Table 3. Mediation analysis of the association between history of gestational diabetes mellitus at baseline and development of nonalcoholic fatty liver disease (n =64,397)

Gestational diabetes mellitus	Excess relative risk * (95% CI)	
	Diabetes as potential mediator	HOMA-IR as potential mediator
All NAFLD		
Controlled direct effect (CDE)†	0.35 (0.23-0.47)	0.35 (0.22-0.47)
Reference interaction	0.001 (▼0.001-0.003)	▼0.003 (▼0.021-0.014)
Mediated interaction	0.01 (▼0.01-0.03)	0.005 (▼0.003-0.012)
Pure indirect effect	0.01 (0.003-0.026)	0.03 (0.02-0.04)
Total effect	0.37 (0.25-0.49)	0.38 (0.25-0.50)
Proportion mediated ‡	0.07 (0.02-0.12)	0.09 (0.04-0.13)
Moderate-to-severe NAFLD		
Controlled direct effect (CDE)†	0.84 (0.24-1.43)	0.67 (▼0.01-1.36)
Reference interaction	▼0.001 (▼0.006-0.005)	0.03 (▼0.10-0.16)
Mediated interaction	▼0.01 (▼0.08-0.06)	0.02 (▼0.01-0.06)
Pure indirect effect	0.02 (▼0.02-0.06)	0.05 (0.03-0.07)
Total effect	0.85 (0.26-1.44)	0.77 (0.12-1.42)
Proportion mediated ‡	0.02 (▼0.06-0.09)	0.09 (0.01-0.17)

* Estimated from Stata command *med4way*. The regression model for the outcome was a Cox proportional hazard model. The regression model for the mediator were logistic regression model for diabetes and linear regression for HOMA-IR. The following potential confounders were included in models: age, center, examination year, alcohol consumption, smoking status, physical activity level, education level, BMI, history of hypertension, history of CVD, lipid-lowering drug use and age at first birth

† The CDE was estimated at a fixed level of the mediator (at non-diabetes status or at the mean level of HOMA-IR)

‡ Proportion mediated provides an estimate of the proportion of the total GDM effect that acts through its association with the potential mediator.

Indirect effect was the relative risk due to mediated interaction and pure indirect effect.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; PY, person-years.

▼negative

644 **Figure legend**

645 **Figure 1.** Flow chart of study population

646 **Table legends**

647 **Table 1.** Age-adjusted means and proportions (95% CI) of baseline characteristics by the
648 history of gestational diabetes mellitus (n = 64,397)

649 **Table 2.** Development of nonalcoholic fatty liver disease by history of gestational diabetes
650 mellitus at baseline (n = 64,397)

651 **Table 3.** Mediation analysis of the association between history of gestational diabetes
652 mellitus at baseline and development of nonalcoholic fatty liver disease (n = 64,397)

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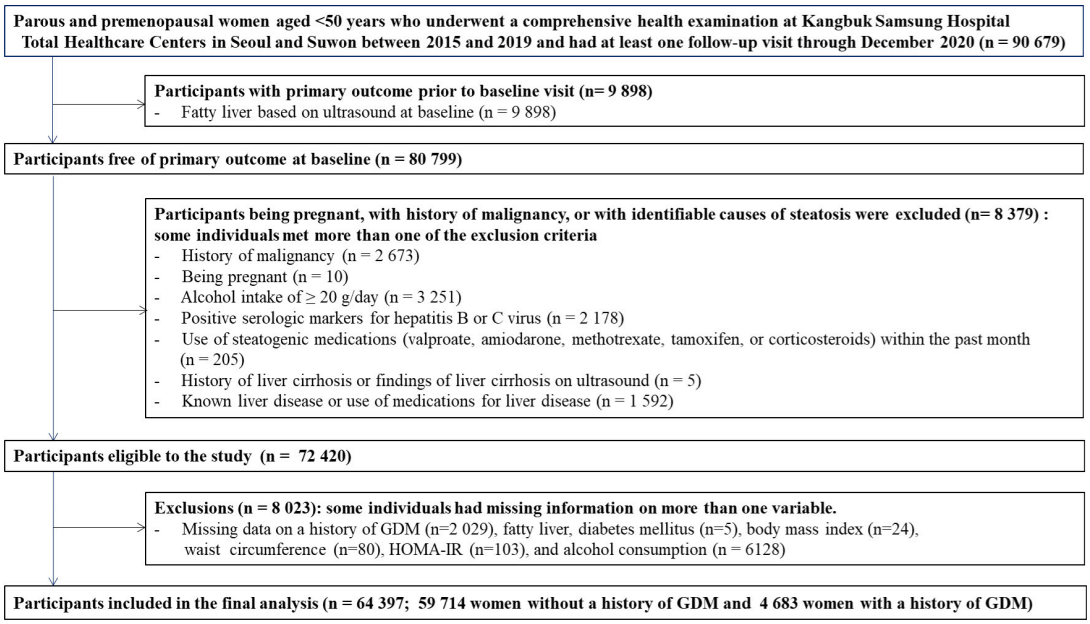
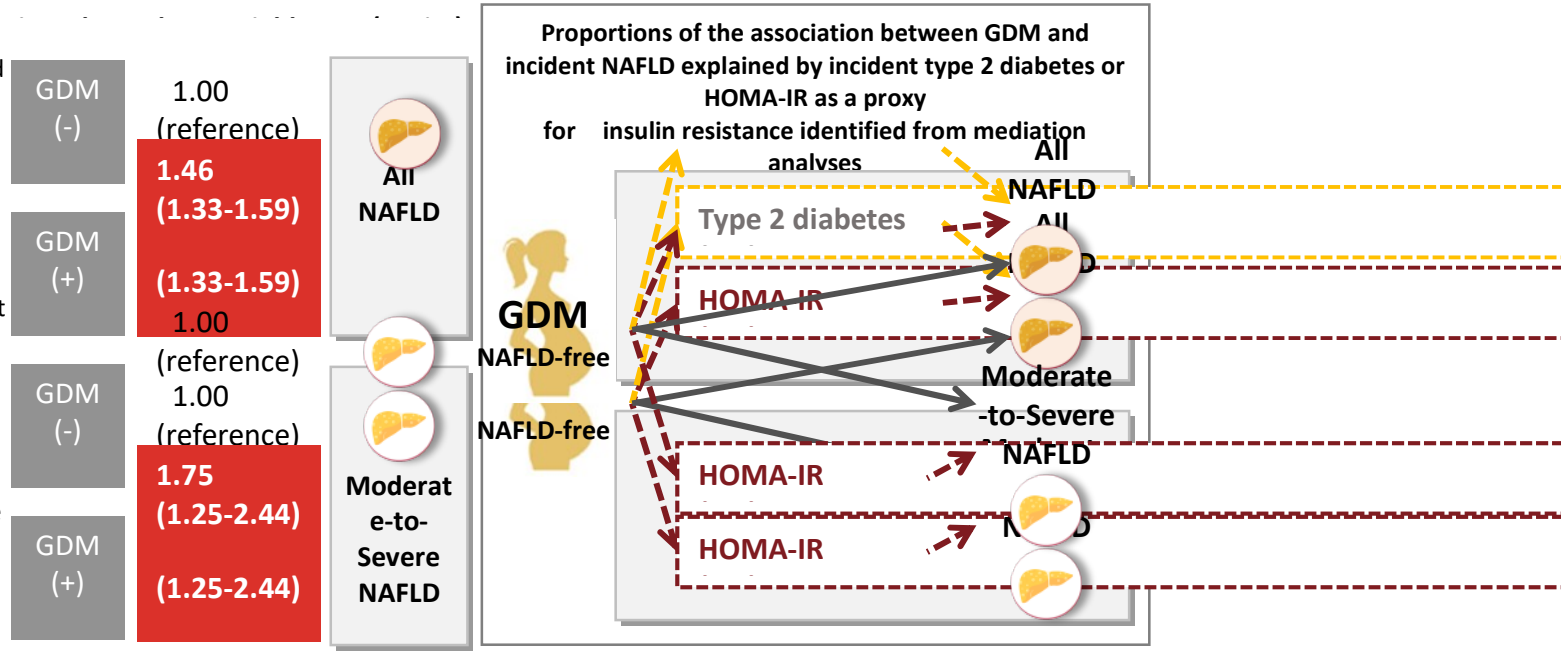

Parous and premenopausal women aged <50 years who underwent a comprehensive health examination at Kangbuk Samsung Hospital Total Healthcare Centers in Seoul and Suwon between 2015 and 2019 and had at least one follow-up visit through December 2020 (n = 90 679)
Participants with primary outcome prior to baseline visit (n= 9 898)
- Fatty liver based on ultrasound at baseline (n = 9 898)
Participants free of primary outcome at baseline (n = 80 799)
Participants being pregnant, with history of malignancy, or with identifiable causes of steatosis were excluded (n= 8 379) : some individuals met more than one of the exclusion criteria
- History of malignancy (n = 2 673)
- Being pregnant (n = 10)
- Alcohol intake of ≥ 20 g/day (n = 3 251)
- Positive serologic markers for hepatitis B or C virus (n = 2 178)
- Use of steatogenic medications (valproate, amiodarone, methotrexate, tamoxifen, or corticosteroids) within the past month (n = 205)
- History of liver cirrhosis or findings of liver cirrhosis on ultrasound (n = 5)
- Known liver disease or use of medications for liver disease (n = 1 592)
Participants eligible to the study (n = 72 420)
Exclusions (n = 8 023): some individuals had missing information on more than one variable.
- Missing data on a history of GDM (n=2 029), fatty liver, diabetes mellitus (n=5), body mass index (n=24), waist circumference (n=80), HOMA-IR (n=103), and alcohol consumption (n = 6128)
Participants included in the final analysis (n = 64 397; 59 714 women without a history of GDM and 4 683 women with a history of GDM)

654

History of gestational diabetes and incident nonalcoholic fatty liver disease: The Kangbuk Samsung Health Study

64,397 eligible participants without NAFLD at baseline were followed up for a median of 3.7 years

- Cox proportional hazards used to investigate the association between prior history of GDM and incident NAFLD
- All NAFLD represents all cases of incident hepatic steatosis at follow up and the sub-group with moderate to severe NAFLD represents moderate
- Change during follow-up were accounted for by using time-varying variables in the



[Cho] et al. *Am J Gastroenterol.* [2023]. [doi]
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February 18th, 2023

Jasmohan S. Bajaj, Richmond, VA
Millie D. Long, Chapel Hill, NC
Editors-in-Chief

The American Journal of Gastroenterology

Dear Drs. Juan Fernando Gallegos-Orozco, Jasmohan S. Bajaj and Millie D. Long

Thank you for your constructive suggestions regarding our manuscript titled, "**History of gestational diabetes and incident nonalcoholic fatty liver disease: The Kangbuk Samsung Health Study**" (AJG-22-2193) and for giving us an opportunity to revise and improve the manuscript.

We have revised the manuscript according to the reviewers' recommendations and comments. Furthermore, we have conducted additional analyses, which have strengthened the validity of our study considerably; and importantly, our original conclusions remain unchanged.

According to the Editor's recommendation we have moved the supplemental methods to the main manuscript to improve the clarity.

In addition we have uploaded a version with the changes yellow highlighted, reflecting the modifications to the manuscript, as well as our point-by-point responses to the reviewers' comments, which detail the changes made in response to these comments.

We believe that our manuscript has been improved substantially through this process and are pleased to submit the revised version of the manuscript for publication in the *American Journal of Gastroenterology*.

During the revision process, an additional author, Prof. Kim (Chanmin Kim, PhD), was added to the title page to reflect his significant contribution to the additional analyses, data interpretation, and revisions. All other authors have approved the addition of Prof. Kim as a new co-author.

There are no conflicts of interest to declare, and all authors have participated in this work and have read and approved this manuscript. This paper is an original article that has not been published, and has not been submitted for publication elsewhere.

Thank you for your consideration. We look forward to hearing from you.

Sincerely,

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