Supplementary materials

Fasting ketonuria and risk of incident non-alcoholic fatty liver disease with and without liver fibrosis in non-diabetic adults

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S1. Study participants and exclusion criteria

The present study was conducted as part of the Kangbuk Samsung Health Study which is a cohort study of Korean men and women aged ≥18 years who underwent comprehensive annual or biennial examinations at Kangbuk Samsung Hospital Total Healthcare Center in Seoul and Suwon, South Korea.

Participants who underwent a comprehensive health examination between January 2011 and December 2017 and had at least one follow-up visit before December 31, 2019 (n = 336,594) were included in the study. A total of 183,518 subjects were excluded based on the following criteria: missing information on urinary ketone levels, alcohol consumption, or components of the NAFLD fibrosis score (NFS) or fibrosis-4 (FIB-4), including body mass index (BMI), aspartate aminotransferase (AST), or alanine transaminase (ALT) levels; presence of diabetes (defined as fasting serum glucose ≥126 mg/dL (7.0 mmol/L), glycated hemoglobin ≥6.5% (48mmol/mol), and self-reported use of insulin, or antidiabetic medications); a history of malignancy; alcohol intake ≥30 g/day for men and ≥20 g/day for women(1); positive serologic markers for hepatitis B or C virus; use of steatogenic medications such as amiodarone, methotrexate, tamoxifen, or corticosteroids within the past year; history of liver cirrhosis or ultrasound findings of fatty liver or liver cirrhosis; known liver disease or use of medications for liver disease; ultrasound findings of HS; and intermediate or high probability of fibrosis based on NFS or FIB-4 at baseline. Because some participants met more than one exclusion criteria, a total of 153,076 participants with no NAFLD and low probability of advanced fibrosis at baseline were included in the first part of the main analysis.

For the second part of the analysis in which the effects of ketonuria change status on the development of HS and its severity were evaluated, those with missing data on ketonuria status either for baseline or the 2nd visit were further excluded; the remaining total of 99,896 subjects being included in the analysis.

The study was approved by the Kangbuk Samsung Hospital Institutional Review Board (IRB No. KBSMC 2020-12-040) which waived requirement for informed consent since de-identified retrospective data from routine health screening were used.

S2-1. Measurement

Data on physical measurements, abdominal ultrasonography, and serum biochemical measurements were collected at each visit as part of the basic health check-up program. Information on demographic characteristics, lifestyle factors, medical history, and medication use were collected via standardized, self-administered questionnaires as previously described (2). Current average alcohol use per day was assessed using the frequency of alcohol drinking per week and the amount of alcohol consumed per drinking day.

Physical activity levels were assessed using the validated Korean version of the International Physical Activity Questionnaire Short Form (3, 4). Physical activity levels were classified into three categories: inactive, minimally active, and health-enhancing physical activity (HEPA) (5). Health-enhancing physical activity (HEPA) was defined as follows: (1) vigorous activity ≥3 days/week with ≥1,500 accumulated metabolic equivalent (MET)-minutes/week, or (2) a combination of walking, moderate- or vigorous-intensity activities for 7 days accumulating to ≥3,000 MET-min/week.

Usual diet consumption during the past year was assessed using a 106-item self-administered food frequency questionnaire (FFQ) which had been designed and validated for use in Korea.(6) Participants were asked how often, on average, they consumed each type of food or beverage during the past year. The FFQ had three predefined categories of portion size, ranging from small to large, and nine predefined categories of frequency, ranging from never or seldom to ≥ three times per day for foods and from never or seldom to ≥ five times per day for beverages. Participants were also asked to report the consumption period (i.e., 3, 6, 9, or 12 months) for seasonal consumption of fruits. A recipe, portion size, and nutrient database was constructed with a food composition table from the Korean Nutrition Society

(7).

Sitting blood pressure (BP), height, weight, and waist circumference were measured by trained nurses. Hypertension was defined as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or current use of antihypertensive medication.

Blood and urine specimens were collected after at least 10 h of fasting. Fasting blood sample measurements included total cholesterol, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides, AST, ALT, gamma-glutamyl transferase (GGT), glucose, uric acid, hsCRP, albumin, and platelet count. 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.

In our laboratory, quality control (QC) for urine analysis was performed according to the manufacturer's instructions using negative and positive QC materials. Reviews on the QC data and analysis of QC materials were performed on a daily basis before reporting patient results. We ensured the results of urine ketone analyses for patients were reported only when QC data were within the acceptable range. The concordance rates were 100% for both negative and positive QC materials (comprising 75% of +2 positive and 25% of +3 positive QC materials) during the study period. The Laboratory Medicine Department at Kangbuk Samsung Hospital has been accredited by the Korean Society of Laboratory Medicine (KSLM) and the Korean Association of Quality Assurance for Clinical Laboratories (KAQACL). The laboratory also participates in the survey proficiency testing provided by the College of American Pathologists (CAP).

S2-2. Ketonuria change status categorization

To evaluate the effect of the change of ketonuria status over time on NAFLD risk, we further classified the subjects according to their ketonuria status at baseline and at the subsequent visit (2^{nd} visit) as follows: a) no ketonuria at baseline and no ketouria at 2^{nd} visit (reference group); b) ketonuria at baseline and no ketonuria at 2^{nd} visit (ketonuria

regressed);c) no ketonuria at baseline and ketouria at 2nd visit (ketonuria developed); and d) ketonuria at baseline and ketouria at 2nd visit (persistent ketonuria).

S2-3. Assessment of hepatic steatosis and its severity

Abdominal ultrasound (US) was performed with a 3.5-MHz transducer by experienced radiologists who were blinded to the aim of the study. Inter- and intra-observer reliabilities for fatty liver diagnosis were shown to be substantial (kappa value = 0.74) and excellent (kappa value = 0.94), respectively.(8)

The FIB-4 index was calculated using the following formula: FIB-4 = [age (years) × AST (U/L)] / [platelet count ($\times 10^9$/L) × ALT (U/L)$^{1/2}$]. The subjects were classified into three groups, reflecting the probability of advanced fibrosis based on the FIB-4 score: low (FIB-4 <1.30), intermediate (FIB-4 1.30-2.66), and high (FIB-4 ≥2.67).(9) NFS was calculated according to the following published formula: NFS = -1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glycemia or diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet ($\times 10^9$/L) - 0.66 × albumin (g/dL).(10) Subjects were also categorized into three groups reflecting the probability of advanced fibrosis based on the NFS: high (NFS >0.676), intermediate (NFS: 0.676 to -1.455), and low (NFS < -1.455).(10)

S3-1. Supplemental statistical analyses

For analysis of the association between ketonuria change status and the incidence of HS, if HS was identified during follow-up, subsequent observations were not included in the analysis and visit 2 was the start of follow-up. Person-years of follow-up were calculated from the date of visit 2 until the date of the primary endpoint (HS or HS with intermediate or high fibrosis scores, separately) or the last screening exam (31 December 2019), whichever came first.

Predefined subgroup analyses were performed based on age (<50 vs. ≥50 years), sex (men vs. women), center (Seoul vs. Suwon), smoking status (non-current smokers vs. current

smokers), alcohol intake (<10 vs. ≥10 g/day), vigorous exercise (< 3 vs. ≥ 3 time per week), BMI (<25 vs. ≥25 kg/m^2), HOMA-IR (<2.5 vs. ≥2.5), and hsCRP (<1.0 vs. ≥1.0 mg/L).

S3-2. Supplemental results

In the pre-specified subgroup analyses (**Supplementary Table 1**), the association between ketonuria and incident HS significantly differed between the BMI subgroups (<25 vs. ≥25 kg/m^2) (*P for interaction* <0.003), with prominent associations in the non-obese group (<25 kg/m^2) but not in the smaller sub-group of overweight subjects (N=18,679).

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