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

Yejin Kim, MHS[1], Yoosoo Chang, MD, PhD[1,2,3], Min-Jung Kwon, MD, PhD[1,4], Yun Soo Hong, MD, MHS[5], Mi Kyung Kim, PhD [6], Won Sohn, MD, PhD[7], Yong Kyun Cho, MD,[7] Hocheol Shin, MD, PhD[1,8], Sarah H. Wild, MB, BChir, PhD[9]; Christopher D Byrne, MB, BCh, PhD [10,11] Seungho Ryu, MD, PhD[1,2,3]

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

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

[3]Department of Clinical Research Design & Evaluation, SAIHST, Sungkyunkwan University, Seoul, Republic of Korea

[4]Department of Laboratory Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[5] Department of Epidemiology and Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA.

[6] Department of Preventive Medicine, College of Medicine, Hanyang University.

[7] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[8] Department of Family Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[9] Usher Institute of Population Health Sciences and informatics, University of Edinburgh, Edinburgh, U.K.

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

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

Address for correspondence: Seungho Ryu, MD, PhD, Kangbuk Samsung Hospital, Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul, South Korea 04514

E-mail: sh703.yoo@gmail.com. Telephone: 82-2-2001-5137. Fax: 82-2-757-0436.

Co-corresponding author: Yoosoo Chang, MD, PhD, Kangbuk Samsung Hospital, Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul, South Korea 04514

E-mail: yoosoo.chang@gmail.com. Telephone: 82-2-2001-5139. Fax: 82-2-757-0436.

Word count: Abstract, 245; Manuscript, 2,996 (Text only)

Number of figures and tables: 1 Figure, 3 Tables

Guarantor of the article: Seungho Ryu, MD, PhD and Yoosoo Chang, MD, PhD

Financial support statement: None to declare.

Conflict of interest statement: The authors have no conflicts of interest to disclose.

Author Contributions:

Yejin Kim: drafting and critical revision of the manuscript

Yoosoo Chang: study concept and design, acquisition of data, interpretation of data, and drafting and critical revision of the manuscript

Min-Jung Kwon: acquisition of data, interpretation of data, and critical revision of the manuscript

Yun Soo Hong: analysis and interpretation of data, and critical revision of the manuscript

Mi Kyung Kim: interpretation of data, and critical revision of the manuscript

Won Sohn: acquisition of data, interpretation of data, and critical revision of the manuscript

Yong Kyun Cho: technical, or material support, and study supervision

Hocheol Shin : technical, or material support, and study supervision

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

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

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

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; CI, confidence interval; CVD, cardiovascular disease; FIB-4, Fibrosis-4 Index; GGT, gamma-glutamyltransferase; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; LCD, low-carbohydrate diet; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score;

STUDY HIGHLIGHTS

1) WHAT IS KNOWN

- Ketone bodies are produced by the liver in response to prolonged fasting, carbohydrate-restricted diet, or intense exercise.

- Spontaneous hyperketonemia has been associated with improved metabolic and inflammatory markers.

- The effects of hyperketonemia on the risk of non-alcoholic fatty liver disease (NAFLD) are not known.

2) WHAT IS NEW HERE

- Fasting ketonuria is associated with a reduced risk of incident NAFLD.
- A decreased risk of worsening fibrosis score was also observed in individuals with fasting ketonuria.
- The effects of ketonuria were stronger in non-obese individuals than in obese individuals.
- The role of increased ketosis in the prevention of NAFLD requires further exploration.

ABSTRACT

Objectives

Dietary carbohydrate restriction or ketogenic diets are known to be beneficial in preventing liver fat accumulation. However, the effect of ketonemia on the risk of non-alcoholic fatty liver disease (NAFLD) in non-diabetic population is largely unknown. We investigated the association between fasting ketonuria and risk of incident NAFLD in healthy adults.

Methods

A cohort of 153,076 nondiabetic Koreans with no hepatic steatosis and low probability of fibrosis at baseline was followed-up for a median of 4.1 years. The outcome was incident hepatic steatosis with or without liver fibrosis, and it was assessed by liver ultrasound and noninvasive fibrosis indices, including fibrosis-4 (FIB-4) and the NAFLD fibrosis score (NFS). Parametric proportional hazard models were used to estimate hazard ratios (HRs) for outcome according to ketonuria status.

Results

Within 677,702.1 person-years of follow-up, 31,079 subjects developed hepatic steatosis. Compared to no ketonuria (reference), fasting ketonuria was significantly associated with a decreased risk of incident hepatic steatosis, with multivariable-adjusted HRs (95% CI) of 0.81 (0.78-0.84). The corresponding HRs for incident hepatic steatosis with intermediate-to-high NFS were 0.79 (0.69-0.90). Similar associations were observed replacing NFS with FIB-4. Additionally, the presence of persistent ketonuria at both baseline and subsequent visit was associated with greatest decrease in the aHR for incident NAFLD.

Conclusions

Ketonuria was associated with a reduced risk of developing incident hepatic steatosis with and without intermediate-to-high probability of advanced fibrosis in a large cohort of nondiabetic healthy individuals. The role of hyperketonemia in the prevention of NAFLD

requires further exploration.

Keywords: Non-alcoholic fatty liver disease; Hyperketonemia; Ketonuria; Liver fibrosis; Cohort study.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the commonest liver diseases and represents a spectrum extending from simple steatosis to non-alcoholic steatohepatitis (NASH) with or without fibrosis (1, 2). Besides its potential of liver-related complications (1), NAFLD is considered a multisystem disease that is positively associated with cardiovascular disease (CVD) risk factors, CVD mortality and all-cause mortality (3, 4). The first-line management of NAFLD management therefore consists of diet and lifestyle modifications, which are effective in ameliorating the early stages of liver disease and improving the associated cardio-metabolic risk factors (5).

Ketone bodies such as acetoacetate, beta-hydroxybutyrate, and acetone, are derived from hepatic beta-oxidation of fatty acids and are used as an alternative energy source under low glucose availability. Mild and controlled production of ketone bodies induced by prolonged fasting or very low carbohydrate intake, known as nutritional ketosis, is distinguishable from pathological ketosis observed in patients with uncontrolled diabetes with hyperglycemia and insulin deficiency (6). In nondiabetic individuals, ketone body levels may increase to >1 mM during periods of extreme fasting, or when on a ketogenic diet (7-9). Non-pathologic or diet-induced hyperketonemia has been associated with improved metabolic and inflammatory markers, including lipids, glycated hemoglobin (HbA1c), high-sensitivity C-reactive protein (hsCRP), and fasting glucose (8, 10-12).

Several clinical studies have suggested a benefit of nutritional ketosis in NAFLD. In a small pilot study of five obese patients with biopsy-proven fatty liver, significant weight loss and histologic improvement were achieved after six months of a low-carbohydrate (<20 g/d) ketogenic diet (10). Another non-randomized trial reported that one year of intervention through inducing nutritional ketosis improved the surrogates of both NAFLD and advanced fibrosis in T2DM patients (13). However, alongside the limited sample size and follow-up duration, most studies to date have only examined the effects of diet-induced ketosis on

NAFLD in patients already presenting with NAFLD at baseline. Until now, the influence of ketonemia on NAFLD development has not been elucidated.

The present study aimed to examine the association between fasting ketonuria and hepatic steatosis with or without an intermediate-to-high probability of advanced liver fibrosis, in a large cohort of Korean nondiabetic men and women.

METHODS

Study participants

The present cohort study included 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). After applying exclusion criteria, the final sample included 153,076 participants. (**Figure 1** and further details in Supplementary materials).

Measurement

Standardized, self-administered questionnaires including a validated food frequency questionnaire (FFQ) (14), physical measurements, abdominal ultrasonography, and serum biochemical measurements were collected at each visit as part of the basic health check-up program (further details in Supplementary materials). Obesity was defined as body mass index (BMI) ≥25 kg/m^2, the proposed cutoff for diagnosis of obesity in Asians (15). Urinary ketones levels were measured semi-quantitatively by urine dipsticks (URiSCAN urine test strips; YD Diagnostics, Yongin-si, Republic of Korea), and were categorized as absent, trace (50 mg/L), 1+ (100 mg/L), 2+ (500 mg/L), and 3+ (1000 mg/L). Ketonuria status was cagetorized as 0 (absent or trace), or 1 (presence, ≥1+) (8).

Diagnosis of hepatic steatosis and its severity

Ultrasonographic diagnosis of fatty liver was made based on an abdominal ultrasound performed by an experienced radiologist using standard criteria, including a diffuse increase in fine echoes in the liver parenchyma in comparison with the kidney or spleen, deep beam attenuation, and bright vessel walls (16). NAFLD was defined as the presence of fatty liver in the absence of excessive alcohol use (<20 and <30 g/day for women and men, respectively) or any other identifiable cause (17). To assess the NAFLD severity, two noninvasive fibrosis indices, NFS and FIB-4, were used (18, 19) (further details in Supplementary Materials).

Statistical analysis

Descriptive statistics were used to summarize participant characteristics according to ketonuria status (0, ≥1). The primary outcomes were the development of incident hepatic steatosis and hepatic steatosis with an intermediate-to-high probability of advanced fibrosis, which were treated as separate endpoints in each model. Person-years of follow-up accrued from baseline until either the occurrence of primary endpoint, or the final examination conducted prior to December 31, 2019, whichever came first. Incidence rates were calculated as the number of incident cases divided by person-years of follow-up.

Parametric proportional hazard models were used to estimate the adjusted hazard ratios (HRs) and the 95% CIs. Model 1 was adjusted for age, center (Seoul or Suwon), year of screening, smoking status (never, past, current, or unknown), alcohol intake (0, <10, ≥10 g/day, or unknown), physical activity (inactive, minimally active, health enhancing physical activity, or unknown), total energy intake, education level (< community college graduate, ≥ community college graduate, or unknown), history of diabetes (only for FIB4 as diabetes is a component of the NFS), history of hypertension, and history of CVD. Previous studies suggest that BMI changes related to ketogenic intervention can be responsible for the health benefits of ketosis, while obesity can be associated with increased insulin levels, which inhibits ketosis (20-22). Thus, we presented a separate model for further adjustment for BMI,

in addition to potential confounders (Model 2). For analysis of NAFLD severity, the BMI adjustment was only applied to hepatic steatosis with intermediate-to-high FIB-4 scores since BMI is a component of the NFS. The proportional hazards assumption was assessed via estimated log (-log) survival curves, and no violation of the assumption was found.

Predefined subgroup analyses were performed (further details in Supplementary materials); Statistical interactions between ketonuria status and subgroup characteristics were assessed using the likelihood ratio test comparing models with and without the multiplicative interaction terms. All statistical analyses were performed using STATA version 16.0 (StataCorp LP, College Station, TX, USA), and p-values < 0.05 were considered statistically significant.

RESULTS

At baseline (**Table 1)**, ketonuria status was positively associated with physical activity, education level, HDL-C, and was inversely associated with male sex, alcohol intake, current smoking, hypertension, history of CVD, obesity, BP, glucose, LDL-C, triglycerides, GGT, and HOMA-IR. Ketonuria status was also associated with a lower level of total energy intake and carbohydrate proportion and a slightly higher proportion of fat intake compared with non-ketonuria status.

Table 2 shows the risk of incident hepatic steatosis and hepatic steatosis with intermediate-to-high probability of advanced fibrosis according to ketonuria status. During follow-up, 31,079 subjects developed hepatic steatosis (incidence rate, 45.9 per 1000 person-years). Median follow-up period was 4.4 years (interquartile range, 2.2-6.3 years, maximum 8.9 years) and the median number of visits per participant was 4 (interquartile range, 3-5). Overall, ketonuria was significantly associated with decreased risk of incident hepatic steatosis and hepatic steatosis plus intermediate/high fibrosis score. After adjusting for age, sex, center, year of screening, alcohol consumption, smoking, physical activity, total energy intake, education level, history of diabetes, history of hypertension, and history of CVD

(Model 1), the HR (95% CI) for incident hepatic steatosis comparing ketonuria group to the reference was 0.81 (0.78-0.84). The associations remained significant after further adjustment for BMI (Model 2; HR, 0.89; 95% CI, 0.86-0.92), as well as when ketonuria and potential confounders were treated as time-varying covariates. The association between ketonuria and incident hepatic steatosis was stronger in non-obese individuals than those with obesity defined by BMI ≥25 kg/m^2 (*P for interaction*=0.001) (Supplementary Table 1).

The multivariable-adjusted HR (95% CI) for hepatic steatosis with intermediate-to-high NFS scores comparing ketornuria to no ketonuria group was 0.79 (0.69-0.90) (Model 1, Table 2). Similarly, in the analysis based on FIB-4 scores, the HR was 0.69 (0.58-0.83). These associations were slightly attenuated after further adjustment for BMI, but remained significant (Model 2; HR, 0.76; 95% CI, 0.64-0.90; *P*=0.002). In the time-dependent model, similar associations were observed (HR, 0.75; 95% CI, 0.63-0.90).

When we compared ketonuria status between first and second visits, 91.7 % of participants with no ketonuria at baseline consistently showed no ketonuria at the subsequent visit. Conversely, only 14.5% of those with ketonuria at baseline had ketonuria at the subsequent visit. **Table 3** presents the association between ketonuria change status from baseline to 2nd visit and the development of hepatic steatosis (further baseline characteristics in Supplementary Table 2 and anthropometric details in Supplementary table 3). The multivariable-adjusted HRs (95% CI) for incident hepatic steatosis comparing 'ketonuria regressed', 'ketonuria developed', and 'persistent ketonuria' groups to the reference group were 0.83 (0.79-0.88), 0.79 (0.74-0.84) and 0.64 (0.55-0.74), respectively (Model 1). The association remained significant after BMI adjustment (Model 2) and in the time-dependent model (Table 3). Ketonuria change status was also associated with the risk of hepatic steatosis + intermediate/high fibrosis scores. In the analysis using FIB-4 (Table 3), the same trend was observed, but the association was attenuated and no longer significant after adjustment for BMI as well as after adjustment for the time-varying covariates in the time-dependent model.

DISCUSSION

Our study showed for the first time that ketonuria was associated with a reduced risk of incident hepatic steatosis, both with/without increased probability of advanced fibrosis, in a large cohort of nondiabetic healthy Korean men and women. None of the 153,076 participants had T2DM or NAFLD at baseline, and the significant associations persisted after adjusting for potential confounders including BMI and time-dependent covariates.

Mildly or moderately elevated ketone bodies in the serum and urine in response to fasting, ketogenic diets, and prolonged exercise constitute the non-pathological form of ketosis (9). Although blood tests provide better diagnostic accuracy for estimating circulating keto-acid levels compared to urine tests (9), urinary keto-acid levels correlate well with serum concentrations measured quantitatively and therefore are considered useful surrogate biomarkers for hepatic ketone body production (23). While the clinical significance of mild ketonuria has not been established, a cross-sectional study demonstrated that fasting ketonuria was associated with a decreased prevalence of obesity, central obesity, and metabolic syndrome (compared to no ketonuria) (24). Another cohort study also showed that fasting ketonuria was associated with reduced risk of incident diabetes independent of other metabolic factors (8). However, no studies have to date investigated the role of ketonuria in NAFLD development in non-diabetic populations. To the best of our knowledge, our study is the first study on the potential benefit of ketonuria in reducing NAFLD risk and its severity in non-diabetic individuals.

While the benefits of ketogenic or severe carbohydrate-restricting diets on hepatic fat content have been well-characterized in several clinical trials with fairly consistent results (20, 21, 25, 26), limited evidence exists regarding its effects on the severity of NAFLD with conflicting results. In a meta-analysis of 10 clinical trials regarding the effects of low-carbohydrate diets (LCD) on liver function, LCDs reduced intrahepatic fat content but did not improve liver enzymes (27), whereas a small interventional study on T2DM patients

demonstrated that liver fibrosis, as assessed by NFS, and liver enzyme levels significantly improved after 1 year of ketosis-inducing dietary interventions (13). In our study, carbohydrate intake was slightly lower, with fat intake being slightly higher in the ketonuria group than in the non-ketonuria group. However, information on habitual diet was collected via a 103-item self-administered FFQ reflective of usual food intake over the past year which was designed for use in South Korea (14). Thus, the FFQ may not reflect the most recent diet characteristics. The South Korean diet is also typically consumed as pre-seasoned dishes that includes various kinds of seasonings including oils. However, seasonings and oils are not included in this FFQ, and nutrient intake estimated by the FFQ, especially for fat and cholesterol, was noted to be lower than that of the dietary records, which is the reference standard (14), possibly limiting the ability to accurately evaluate the composition of macronutrients in the participants' diets (14). Although the benefits of ketonuria observed in our study cannot be directly linked to certain dietary regimens, our findings clearly indicate that mild ketosis, as reflected in urine test, is associated with a reduced risk of NAFLD and its severity in non-diabetic individuals.

In our subgroup analysis, the protective effects of ketonuria against incident NAFLD were significant only in the non-obese group (BMI <25 kg/m^2). Such finding may reflect the close association of obesity with hyperinsulinemia, which promotes the activation of acetyl Co-A carboxylase and consequently increases fatty acid synthesis, directing acetyl Co-A away from ketone body production (22, 28, 29). In light of this, our study provides the first evidence that mild fasting ketonuria, a semi-quantitative indicator of increased ketosis, is associated with reduced risk of incident NAFLD and advanced fibrosis, especially non-obese individuals.

In many previous studies, weight reduction was reported as an intended outcome or a significant consequence of the ketogenic diet (20, 21, 25, 27). Moreover, the magnitude of weight reduction was shown to be positively associated with a decrease in fibrosis severity

(13). It is therefore plausible that the metabolic benefits of induced ketosis on fatty liver shown in these studies may have been mediated by the effects of weight reduction. Although weight-independent benefits of ketogenic diet in NAFLD has been reported in few studies (30, 31), these studies were conducted only for a short-term and have focused on the effects of specific dietary regimen, not on physiological ketosis *per se*. Our findings, using urinary ketones as surrogate marker for physiological ketosis, showed a significant inverse association between ketonuria and the risk of incident NAFLD both with and without advanced fibrosis, even after adjusting for BMI and BMI change as a time-dependent variable. Thus, and importantly, changes in BMI did not seem to fully explain the association between ketonuria and NAFLD in our study.

Furthermore, our findings suggest that the greatest benefit may be obtained from the presence of ketonuria at both baseline and follow-up. The presence of ketonuria at both baseline and follow up was associated with greatest decrease in the aHR for incident NAFLD. Therefore, we can speculate that persistent ketosis produces a 'cumulative' benefit in decreasing NAFLD risk. There is little data on the long-term effects of persistent ketosis with most of the existing studies being conducted in studies of <1 year. However, the long-term continuation of ketogenic diets is not generally recommended due to potential health risks of prolonged ketosis (32). These concerns, however, are mostly applicable to the cases of strict ketogenic diets with extreme carbohydrate restriction which might not be applicable to subjects in our study (in which there was no specific dietary intervention). Further studies are needed in order to confirm our findings and investigate the long-term health effects of mild ketosis in non-diabetic individuals.

The mechanisms by which mild ketonuria reduces the risks of liver steatosis and fibrosis are unclear. Ketogenesis has been associated with protecting against liver injury in mice by up-regulation of the levels of the antioxidant enzymes such as SOD2, Gpx1 (33-35), as well as producing favorable changes in liver metabolism and hepatocyte regeneration, via

stimulating liver autophagy which is normally deregulated in NAFLD (36). Ketone production was also linked to a rapid increase in mitochondrial beta-oxidation leads to decreased hepatic *de novo* lipogenesis and decreased hepatic fat accumulation (30). Also, peroxisome proliferator-activated receptor (PPAR)-alpha (29) is strongly induced in ketogenesis (37), stimulating FGF21 expression, which is known to suppress hepatic lipogenesis and redirects fatty acids to beta-oxidation (29, 38). Other potential mechanisms may involve ketosis-induced reduction of TGF-beta 1, which plays a critical role in the pathogenesis of liver fibrosis and hepatocellular carcinoma, and anti-inflammatory role of ketone bodies (37, 39, 40). In our study, the risk of incident NAFLD was also decreased in the groups where ketonuria was detected (at either baseline, or 2nd visit). These results are potentially clinically relevant because they suggest that any ketonuria in the fasted state (in subjects who do not have diabetes), is associated with decreased risk of developing NAFLD. Hepatic ketogenesis has been associated with total fat oxidation (41), and ketonuria might be an indication of high fat oxidation ability. Whether this finding reflects increased levels of fat oxidation and subsequent increased ketogenesis in subjects at reduced risk of NAFLD cannot be addressed by our study. Further mechanistic research is needed, specifically to address the relationships between fat oxidation, ketogenesis and development of NAFLD.

Several limitations of our study should be considered. First, ultrasonography and liver fibrosis index (NFS and FIB-4) in our analyses was used in lieu of liver biopsy, which is the reference standard for the diagnosis of NAFLD but was considered unfeasible in this large-scale cohort study involving repeat measurements over time. Although liver ultrasound, NFS and FIB-4 have been widely used and well validated by liver biopsy (42, 43), there are other reliable, non-invasive methods to assess liver fibrosis such as transient elastography (44-46). In our study, data on transient elastography were not available because our study was based on de-identified, retrospective cohort data of individuals who participated in a routine health check-up program in which transient elastography was not included. Second, although

semi-quantitative ketosis was assessed by ketonuria measures, urine ketone body levels correlate well with serum ketone body concentration. Furthermore, the urine test is widely used as a less expensive alternative to blood testing, rendering it better suited to large epidemiological studies (23, 47). Third, information on fasting duration, recent dietary characteristics and intermittent fasting, which can affect ketosis, was not available in our study. Additionally, we cannot exclude the possibility of potentially unmeasured or other residual confounding in our study. Lastly, our findings from relatively young and middle-aged Koreans used may limit the generalizability to other age groups, populations with a higher prevalence of comorbidities, or other ethnic groups. In addition, the mean BMI of our cohort (21.9 kg/m^2) is considerably lower than that in some Western countries (e.g., the United States), which further limits the applicability of the findings to the general population in other countries, especially given our findings that the effects of ketonemia may differ by overweight status.

In conclusion, the present study showed that fasting ketonuria was associated with reduced risk of both developing NAFLD and advanced fibrosis in nondiabetic individuals. Our findings suggest potential benefits of hyperketonemia in the prevention of NAFLD and its progression, which warrants further investigation.

Acknowledgements

This study was supported by SKKU Excellence in Research Award Research Fund, Sungkyunkwan University, 2020 and by the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2017R1A2B2008401). CDB is supported in part by the Southampton NIHR Biomedical Research Centre (IS-BRC-20004), UK.

References

1. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2015;149:389-97 e10.

2. Leoni S, Tovoli F, Napoli L, et al. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World J Gastroenterol 2018;24:3361-3373.

3. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2015;62:S47-64.

4. Armstrong MJ, Adams LA, Canbay A, et al. Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology 2014;59:1174-97.

5. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to treatment. Frontline Gastroenterol 2014;5:277-286.

6. Manninen AH. Metabolic effects of the very-low-carbohydrate diets: misunderstood "villains" of human metabolism. J Int Soc Sports Nutr 2004;1:7-11.

7. Gershuni VM, Yan SL, Medici V. Nutritional Ketosis for Weight Management and Reversal of Metabolic Syndrome. Curr Nutr Rep 2018;7:97-106.

8. Kim G, Lee SG, Lee BW, et al. Spontaneous ketonuria and risk of incident diabetes: a 12 year prospective study. Diabetologia 2019;62:779-788.

9. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 1999;15:412-26.

10. Tendler D, Lin S, Yancy WS, Jr., et al. The effect of a low-carbohydrate, ketogenic diet on nonalcoholic fatty liver disease: a pilot study. Dig Dis Sci 2007;52:589-93.

11. Yancy WS, Jr., Olsen MK, Guyton JR, et al. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. Ann Intern Med 2004;140:769-77.

12. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 2003;348:2082-90.

13. Vilar-Gomez E, Athinarayanan SJ, Adams RN, et al. Post hoc analyses of surrogate markers of non-alcoholic fatty liver disease (NAFLD) and liver fibrosis in patients with type 2 diabetes in a digitally supported continuous care intervention: an open-label, non-randomised controlled study. BMJ Open 2019;9:e023597.

14. Ahn Y, Kwon E, Shim JE, et al. Validation and reproducibility of food frequency questionnaire for Korean genome epidemiologic study. Eur J Clin Nutr 2007;61:1435-41.

15. WHO Western Pacific Region, IASO and IOTF. The Asia-Pacific Perspective: Redefining Obesity and Its Treatment. Health Communications Australia Pty Limit: Sdyney, Australia. 2000.

16. 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;34:516-22.

17. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005-23.

18. 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;45:846-54.

19. McPherson S, Stewart SF, Henderson E, et al. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 2010;59:1265-9.

20. Browning JD, Baker JA, Rogers T, et al. Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate

restriction. Am J Clin Nutr 2011;93:1048-52.

21. Volynets V, Machann J, Kuper MA, et al. A moderate weight reduction through dietary intervention decreases hepatic fat content in patients with non-alcoholic fatty liver disease (NAFLD): a pilot study. Eur J Nutr 2013;52:527-35.

22. Soeters MR, Sauerwein HP, Faas L, et al. Effects of insulin on ketogenesis following fasting in lean and obese men. Obesity (Silver Spring) 2009;17:1326-31.

23. Coleman MD, Nickols-Richardson SM. Urinary ketones reflect serum ketone concentration but do not relate to weight loss in overweight premenopausal women following a low-carbohydrate/high-protein diet. J Am Diet Assoc 2005;105:608-11.

24. Joo NS, Lee DJ, Kim KM, et al. Ketonuria after fasting may be related to the metabolic superiority. J Korean Med Sci 2010;25:1771-6.

25. Kirk E, Reeds DN, Finck BN, et al. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. Gastroenterology 2009;136:1552-60.

26. Perez-Guisado J, Munoz-Serrano A. The effect of the Spanish Ketogenic Mediterranean Diet on nonalcoholic fatty liver disease: a pilot study. J Med Food 2011;14:677-80.

27. Haghighatdoost F, Salehi-Abargouei A, Surkan PJ, et al. The effects of low carbohydrate diets on liver function tests in nonalcoholic fatty liver disease: A systematic review and meta-analysis of clinical trials. J Res Med Sci 2016;21:53.

28. Kim HJ, Joo NS, Kim KM, et al. Different response of body weight change according to ketonuria after fasting in the healthy obese. J Korean Med Sci 2012;27:250-4.

29. Tucker B, Li H, Long X, et al. Fibroblast growth factor 21 in non-alcoholic fatty liver disease. Metabolism 2019;101:153994.

30. Mardinoglu A, Wu H, Bjornson E, et al. An Integrated Understanding of the Rapid Metabolic Benefits of a Carbohydrate-Restricted Diet on Hepatic Steatosis in Humans. Cell Metab 2018;27:559-571 e5.

31. Ryan MC, Abbasi F, Lamendola C, et al. Serum alanine aminotransferase levels decrease further with carbohydrate than fat restriction in insulin-resistant adults. Diabetes Care 2007;30:1075-80.

32. Li Z, Heber D. Ketogenic Diets. JAMA 2020;323:386-386.

33. Greco T, Glenn TC, Hovda DA, et al. Ketogenic diet decreases oxidative stress and improves mitochondrial respiratory complex activity. J Cereb Blood Flow Metab 2016;36:1603-13.

34. Brandhorst S, Choi IY, Wei M, et al. A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. Cell Metab 2015;22:86-99.

35. Verweij M, van Ginhoven TM, Mitchell JR, et al. Preoperative fasting protects mice against hepatic ischemia/reperfusion injury: mechanisms and effects on liver regeneration. Liver Transpl 2011;17:695-704.

36. Khambu B, Yan S, Huda N, et al. Autophagy in non-alcoholic fatty liver disease and alcoholic liver disease. Liver Res 2018;2:112-119.

37. Piccinin E, Moschetta A. Hepatic-specific PPARalpha-FGF21 action in NAFLD. Gut 2016;65:1075-6.

38. Zhang Y, Lei T, Huang JF, et al. The link between fibroblast growth factor 21 and sterol regulatory element binding protein 1c during lipogenesis in hepatocytes. Mol Cell Endocrinol 2011;342:41-7.

39. Shimazu T, Hirschey MD, Newman J, et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science 2013;339:211-4.

40. Youm YH, Nguyen KY, Grant RW, et al. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med 2015;21:263-9.

41. Puchalska P, Crawford PA. Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. Cell Metab 2017;25:262-284.

42. Hernaez R, Lazo M, Bonekamp S, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082-90.

43. 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;7:1104-12.

44. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835-47.

45. Petta S, Di Marco V, Pipitone RM, et al. Prevalence and severity of nonalcoholic fatty liver disease by transient elastography: Genetic and metabolic risk factors in a general population. Liver Int 2018;38:2060-2068.

46. Roulot D, Costes JL, Buyck JF, et al. Transient elastography as a screening tool for liver fibrosis and cirrhosis in a community-based population aged over 45 years. Gut 2011;60:977-84.

47. Nakajima K, Oda E. Ketonuria may be associated with low serum amylase independent of body weight and glucose metabolism. Arch Physiol Biochem 2017;123:293-296.

Table 1. Baseline characteristics according ketonuria status among 153,076 participants without nonalcoholic fatty liver disease (NAFLD)

Characteristics	Overall	Ketonuria status		*P value*
		No	Yes	
Number of participants	153,076	133,623	19,453	
Age (years)	36.0 (6.6)	36.1 (6.7)	35.2 (6.0)	<0.001
Men (%)	41.8	42.5	36.8	<0.001
Seoul center (%)	56.6	55.9	61.2	<0.001
Alcohol intake[a] (%)	26.1	26.4	23.7	<0.001
Current smoker (%)	14.6	15.1	10.6	<0.001
HEPA (%)	14.7	14.6	15.7	<0.001
Education level[b] (%)	86.2	86.1	86.9	0.006
Hypertension (%)	4.3	4.5	3.3	<0.001
History of CVD (%)	0.5	0.5	0.4	0.046
Medication for hyperlipidemia (%)	0.7	0.8	0.5	<0.001
Obesity (%)	12.2	12.6	9.2	<0.001
Body mass index (kg/m^2)	21.9 (2.7)	22.0 (2.7)	21.4 (2.6)	<0.001
SBP (mmHg)	105.4 (11.7)	105.5 (11.8)	104.4 (11.5)	<0.001
DBP (mmHg)	67.1 (8.8)	67.3 (8.8)	66.1 (8.5)	<0.001
Glucose (mg/dl)	91.1 (7.5)	91.8 (7.1)	85.7 (8.4)	<0.001
Total cholesterol (mg/dl)	187.3 (31.4)	187.4 (31.3)	187.0 (31.9)	0.084
LDL-C (mg/dl)	113.5 (29.3)	113.6 (29.2)	112.3 (30.3)	<0.001
HDL-C (mg/dl)	62.9 (14.8)	62.4 (14.8)	66.3 (15.0)	<0.001
Triglycerides (mg/dl)	75 (57-103)	78 (59-107)	58 (48-73)	<0.001
ALT (U/L)	15 (11-20)	15 (11-20)	15 (11-20)	0.003
AST (U/L)	18 (15-21)	18 (15-21)	19 (16-22)	<0.001
GGT (U/L)	15 (11-23)	16 (11-23)	14 (11-20)	<0.001
hsCRP (mg/L)	0.3 (0.2-0.6)	0.3 (0.2-0.6)	0.3 (0.2-0.7)	<0.001
HOMA-IR	1.04 (0.70-1.48)	1.09 (0.76-1.53)	0.64 (0.41-0.95)	<0.001
Total energy intake (kcal/d)[c]	1465.2 (1102.5-1860.0)	1469.8 (1106.2-1863.0)	1438.0 (1078.0-1838.4)	<0.001
Carbohyrate proportion (%)	67.9 (61.9-73.1)	67.9 (62.0-73.2)	67.4 (61.2-72.6)	<0.001
Fat proportion (%)	18.4 (14.4-23.1)	18.4 (14.3-23.0)	18.9 (14.8-23.7)	<0.001
Protein proportion (%)	13.5 (12.1-15.3)	13.5 (12.1-15.3)	13.6 (12.1-15.4)	<0.001
Carbohydrate <50 g/day (%)	4.0	3.9	4.4	0.016

Data are expressed as mean (standard deviation), median (interquartile range), or percentage.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; HEPA, health-enhancing physical activity; HDL-C, high-density lipoprotein-cholesterol; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein-cholesterol

[a] ≥ 10 g of ethanol per day.

[b] ≥ College graduate.

[c] Among 110,150 participants with plausible estimated energy intake (within three standard deviations of the log-transformed mean energy intake).

Tables 2. Development of nonalcoholic fatty liver disease (NAFLD) and NAFLD with intermediate-to-high probability of advanced fibrosis by ketonuria category in nondiabetic individuals among 153,076 subjects.

Category of ketonuria status	Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Crude HR (95% CI)	Multivariable-adjusted HR[a] (95% CI)		HR (95% CI)[b] in a model with time-dependent variables
					Model 1	Model 2	
NAFLD							
No ketonuria	587,143.4	27,836	47.4	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ketonuria	90,558.7	3,243	36.8	0.75 (0.72-0.77)	0.81 (0.78-0.84)	0.89 (0.86-0.92)	0.90 (0.86-0.94)
NAFLD + Intermediate/high NFS							
No ketonuria	648,207.5	2,189	3.4	1.00 (reference)	1.00 (reference)		1.00 (reference)
Ketonuria	97,781.8	236	2.4	0.71 (0.62-0.81)	0.79 (0.69-0.90)		0.75 (0.63-0.90)
NAFLD + Intermediate/high FIB-4							
No ketonuria	650,165.4	1,439	2.2	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ketonuria	98,042.4	138	1.4	0.63 (0.53-0.75)	0.69 (0.58-0.83)	0.76 (0.64-0.90)	0.54 (0.41-0.71)

[a]Estimated from parametric proportional hazard models. Multivariable Model 1 was adjusted for sex, center, year of screening, alcohol intake, smoking, physical activity, total energy intake, education level, history of diabetes (only for FIB-4), history of hypertension, and history of cardiovascular disease; Model 2: Model 1 plus adjustment for BMI.

[b]Estimated from parametric proportional hazard models with ketonuria status, smoking, alcohol consumption, physical activity, BMI (only for FIB-4) and total energy intake as time-dependent categorical variables and baseline sex, center, year of screening, education level, history of diabetes (only for FIB-4), history of hypertension, and history of cardiovascular disease as time-fixed variables.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio.

Table 3. Development of nonalcoholic fatty liver disease (NAFLD) and NAFLD with intermediate-to-high probability of advanced fibrosis by ketonuria change category at baseline among NAFLD-free non-diabetic participants with low probability of advanced fibrosis at baseline among 99,869 subjects.

Ketonuria change category	Ketonuria status at 1st and 2nd visits		Person-years (PY)	Incident cases	Incidence density (/ 10^3 PY)	Crude HR (95% CI)	Multivariable-adjusted HR[a] (95% CI)		HR (95% CI)[b] in a model with time-dependent variables
	1st test	2nd test					Model 1	Model 2	
NAFLD									
None (G1)	None	None	288,182.9	13,794	47.2	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Regressed (G2)	Ketonuria	None	42,503.5	1,596	37.0	0.78 (0.74-0.82)	0.83 (0.79-0.88)	0.91 (0.87-0.96)	0.86 (0.82-0.91)
Developed (G3)	None	Ketonuria	29,544.3	966	32.3	0.67 (0.63-0.72)	0.79 (0.74-0.84)	0.81 (0.76-0.87)	0.82 (0.77-0.88)
Persistent (G4)	Ketonuria	Ketonuria	7,808.1	192	24.1	0.50 (0.44-0.58)	0.64 (0.55-0.74)	0.68 (0.59-0.79)	0.66 (0.57-0.76)
NAFLD + intermediate/high NFS scores									
None (G1)	None	None	311,854.0	1,096	3.5	1.00 (reference)	1.00 (reference)		1.00 (reference)
Regressed (G2)	Ketonuria	None	45,294.8	110	2.4	0.69 (0.57-0.84)	0.75 (0.61-0.91)		0.82 (0.66-1.02)
Developed (G3)	None	Ketonuria	31,230.9	72	2.3	0.65 (0.51-0.83)	0.79 (0.62-1.01)		0.87 (0.67-1.13)
Persistent (G4)	Ketonuria	Ketonuria	8,089.1	8	1.0	0.28 (0.14-0.57)	0.39 (0.19-0.78)		0.30 (0.13-0.73)
NAFLD + intermediate/high FIB-4 scores									
None (G1)	None	None	312,601.9	735	2.3	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Regressed (G2)	Ketonuria	None	45,376.7	73	1.6	0.69 (0.54-0.88)	0.74 (0.58-0.94)	0.81 (0.64-1.03)	0.78 (0.58-1.03)
Developed (G3)	None	Ketonuria	31,284.7	45	1.4	0.60 (0.45-0.82)	0.73 (0.54-0.98)	0.75 (0.55-1.01)	0.74 (0.51-1.05)
Persistent (G4)	Ketonuria	Ketonuria	8,090.7	7	0.9	0.37 (0.18-0.78)	0.49 (0.23-1.03)	0.53 (0.25-1.11)	0.49 (0.20-1.19)

[a]Estimated from parametric proportional hazard models. Multivariable Model 1 was adjusted for sex, center, year of screening, alcohol intake, smoking, physical activity, total energy intake, education level, history of diabetes (only for FIB-4), history of hypertension, and history of cardiovascular disease; Model 2: Model 1 plus adjustment for BMI.
[b]Estimated from parametric proportional hazard models with ketonuria change category, smoking, alcohol consumption, physical activity, BMI (only for FIB-4) and total energy intake as time-dependent categorical variables and baseline sex, center, year of screening, education level, history of diabetes (only for FIB-4), history of hypertension, and history of cardiovascular disease as time-fixed variables.
Abbreviations: BMI, body mass index; CI, confidence interval; G1, no ketonuria at baseline and no ketouria at 2nd visit (reference group); G2, ketonuria at baseline and no ketonuria at 2nd visit (ketonuria regressed);G3, no ketonuria at baseline and ketouria at 2nd visit (ketonuria developed); G4, ketonuria at baseline and ketouria at 2nd visit (persistent ketonuria); HR, hazard ratio.

Figure 1. Flow chart of study participants