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Original article

Association of ketone bodies with incident CKD and death: A UK Biobank study

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ABSTRACT

Aims: Although cellular and animal models have suggested a protective effect of ketone bodies (KBs), clinical data are still lacking to support these findings. This study aimed to investigate the association of KB levels with incident chronic kidney disease (CKD) and death.

Methods: This was a prospective cohort study of 87,899 UK Biobank participants without baseline CKD who had plasma levels of β-hydroxybutyrate, acetoacetate, and acetone levels measured at the time of enrollment. The main predictor was plasma total KB, which was the sum of the aforementioned three KBs. The primary outcome was a composite of incident CKD, or all-cause mortality. Secondary outcomes included the individual components of the primary outcome.

Results: During a median follow-up of 11.9 years, a total of 8,145 primary outcome events occurred (incidence rate 8.0/1,000 person-years). In the multivariable Cox model, a 1-standard deviation increase in log total KB was associated with a 7 % [adjusted hazard ratio (aHR), 1.07; 95 % confidence interval (CI), 1.05–1.10] higher risk of the primary outcome. When stratified into quartiles, the aHR (95 % CI) for Q4 versus Q1 was 1.18 (1.11–1.27). This association was consistent for incident CKD (aHR, 1.04; 95 % CI, 1.01–1.07), and all-cause mortality (aHR, 1.10; 95 % CI, 1.07–1.13). Compared with Q1, Q4 was associated with a 12 % (aHR 1.12; 95 % CI 1.02–1.24) and 26 % (aHR 1.26; 95 % CI 1.15–1.37) higher risk of incident CKD and all-cause mortality, respectively.

Conclusions: Higher KB levels were independently associated with higher risk of incident CKD and death.

Introduction

Ketone bodies (KB) serve as an important alternative metabolic energy source in the fasting state [1]. Ketogenesis predominantly occurs in the liver, where free fatty acids are converted to two major KBs: β-hydroxybutyrate and acetoacetate. Circulating levels of serum KBs are determined by the balance of their rates of production and utilization. The KBs can be utilized by extrahepatic tissues through the process of ketolysis, which produces energy [1], and provides an alternative energy source during periods of glucose deficiency.

Until recently, KBs carried a negative clinical stigma as they are key modulators in pathological conditions such as diabetic and alcoholic ketoacidosis [2]. However, accumulating evidence from both cellular and animal models has suggested a protective role of KBs in the development of kidney diseases and cardiovascular diseases (CVDs) [3,4]. For example, daily subcutaneous administration of β-hydroxybutyrate for one month reduced pro-inflammatory cytokine levels including interleukin 1-β and tumor necrosis factor-α, as well as reactive oxygen and nitrogen species levels in the kidney of aged mice [3]. Similarly, in mice with ischemia/reperfusion injury, β-hydroxybutyrate infusion reduced mitochondrial stress in the myocardium [4].

On the other hand, data from clinical studies have been conflicting. While higher levels of KBs have been linked to a higher risk of CVDs and all-cause mortality [5–7], β-hydroxybutyrate infusion was reported to have beneficial hemodynamic effects in patients with heart failure and reduced ejection fraction [8]. Moreover, in patients with chronic kidney disease (CKD) who are at high risk of developing CVD, sodium glucose co-transporter 2 inhibitors (SGLT2is) have been postulated to provide

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cardiorenal benefits to patients with CKD by inducing nutritional ketosis [9]. However, the association between KB levels and adverse kidney outcomes has not been previously investigated in clinical studies.

Hence, this study aimed to investigate whether plasma total KB levels were associated with adverse kidney outcomes and death.

Materials and methods

Study cohort

The UK Biobank is a large-scale, prospective, observational, population-based cohort consisting of 502,536 participants aged 37–73 years from England, Scotland, and Wales between 2006 and 2010. The rationale, design, and protocol summary are described elsewhere [10]. Exclusion criteria were as follows: 1) missing data on sex or race; 2) missing data of baseline estimated glomerular filtration rate (eGFR) or proteinuria; 3) missing data on baseline β-hydroxybutyrate or acetoacetate; 4) baseline eGFR $<$ 60 mL/min/1.73 m^2 or a urine albumin-to-creatinine ratio (UACR) $>$ 30 mg/g; 5) previous organ transplantation; and 6) a history of or current malignancy. We also excluded those with outlier KB levels at baseline, defined as $>$ 99th percentile of observed values. The UK Biobank received ethics approval from the Northwest Multi-centre Research Ethics Committee. Written informed consent was obtained from all study participants.

Data collection and measurements

Baseline demographic and anthropometric data, comorbidities, and medication history were recorded at the time of enrollment. Comorbidities were defined using self-reported medical conditions or International Classification of Diseases, 10th Revision (ICD-10) codes in any primary care data and hospital inpatient data (Table S1; see supplementary materials associated with this article on line). Blood and urine samples were collected and analyzed at a central laboratory. Serum and urine creatinine was measured using an isotope dilution mass spectrometry-traceable method, and the eGFR was calculated using the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [11]. A detailed description of other data collection and laboratory measurement can be found in the Supplementary Methods (see supplementary materials associated with this article on line). Dietary data were collected in 37,743 participants who completed a dietary survey. In the UK Biobank study, the Oxford WebQ, a web-based 24-hour recall questionnaire, was used to collect dietary information [12]. Dietary information was collected between April 2009 and June 2012. Detailed methods on the estimation of total energy and nutrient intakes are described in the Supplementary Methods (see supplementary materials associated with this article on line).

Ketone body quantification by NMR

EDTA plasma samples (118,000 in total) were collected at the time of study enrollment. For NMR analysis, samples were shipped on dry ice to Nightingale Health's laboratories in Finland and KBs that included β-hydroxybutyrate, acetoacetate, and acetone were measured between June 2019 and April 2020 using six NMR spectrometers. Accredited quality control was performed to eliminate systemic and technical variance. Only samples and biomarkers that underwent this quality control process were stored in the UK Biobank database. Detailed protocols on sample collection and metabolomic quantification are described elsewhere [13–16]. Plasma levels of the three KB were summed as plasma total KB.

Study predictors and outcomes

The primary predictor was plasma total KB level, and participants were classified into quartile groups according to plasma total KB level. Additionally, we used this variable as a continuous variable. Similarly, serum β-hydroxybutyrate and acetoacetate levels were used as secondary predictors. The primary outcome was a composite of incident CKD or all-cause mortality. Secondary outcomes included the individual outcomes of incident CKD and all-cause mortality. The definition of incident CKD was based on ICD-10 codes in any primary care data, hospital inpatient data, and death register records or Office of Population Censuses and Surveys Classification of Interventions and Procedures-version 4 (OPCS-4) codes in hospital inpatient data. All-cause mortality was ascertained from any cause determined by linkage to the National Health Service Central Register (for participants in England and Wales) and the National Records of Scotland (for participants in Scotland). Participants were followed from enrollment until the development of incident CKD, all-cause mortality, loss to follow-up, or end of the study. Loss to follow-up and the end of the study period were censoring events. The last follow-up date was February 28, 2021 for participants in England and Scotland, and February 28, 2018 for participants in Wales.

Statistical analyses

Baseline characteristics of the study cohort are described using means with standard deviation (SD) for normally distributed continuous variables or medians with interquartile ranges for skewed data. Categorical variables are presented as numbers and percentages. Incidence rates of study outcomes were calculated as the number of cases per 1000 person-years. For trend analyses, linear regression and the Mann-Kendall trend test were used for normally distributed and skewed data, respectively. Cumulative incidences of the study outcomes according to plasma total KB quartiles were estimated using Kaplan-Meier analyses, and compared using the log-rank test. For the incident CKD outcome, the cumulative incidence function curve was derived using the cumulative incidence function and the statistical difference was compared using Gray's test [17]. The association between log-transformed plasma total KB, β-hydroxybutyrate, acetoacetate, and incident CKD and death was also assessed using the Cox proportional hazards model, and the assumptions were confirmed using Schoenfeld residuals. Cox proportional hazards models were constructed as follows: model 1 was unadjusted; model 2 was adjusted for age, sex, race, Townsend deprivation index, income level, physical activity, alcohol intake, smoking status, body mass index (BMI), comorbidities of hypertension, diabetes, and CVD; and model 3 was further adjusted for baseline eGFR, UACR, low-density lipoprotein cholesterol, triglycerides, high-sensitivity C-reactive protein (hs-CRP), use of angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers, statins, total daily dietary carbohydrate, protein, fat intake, and total daily energy intake. For the analysis of incident CKD, a cause-specific competing risk model was used, where death before CKD development was treated as a competing risk and appropriately censored. The results are presented as hazard ratios (HRs) and 95 % confidence intervals (CIs).

The MICE (multivariate imputation by chained equations) method of multiple multivariate imputations in STATA was used for missing data imputations implying the missing at random assumptions (Table S2; see supplementary materials associated with this article on line). Predictive mean matching and logistic regression was used for missing continuous and categorical variables, respectively. Five complete data sets were created to account for missing values. To achieve maximum accuracy, each set with missing values was imputed in the multivariate Cox proportional hazards models. The estimates of the variables were averaged to give a single mean estimate, with standard errors adjusted according to Rubin's rules [18].

Sensitivity analyses were performed to confirm the robustness of the study findings. We constructed an additional dataset that incorporated the GP data with follow-up measurements of serum creatinine levels in 33,011 participants, where a stricter definition of CKD was used, based on ICD-10 codes, OPCS-4 codes, or two consecutive measurements of

Table 1
Baseline characteristics of participants according to quartile of plasma total KB level.

	Overall (n = 87,899) (10.5–370.3 μmol/L)	Quartile of log-transformed plasma total KB level Q1 (n = 21,975) (< 46.7 μmol/L)	Q2 (n = 21,975) (46.7–62.2 μmol/L)	Q3 (n = 21,975) (62.2–91.7 μmol/L)	Q4 (n = 21,974) (> 91.7 μmol/L)	P for trend
Plasma total KB, μmol/L	62 (47, 92)	38 (32, 43)	54 (50, 58)	73 (67, 81)	134 (108, 183)	
Plasma β-hydroxybutyrate, μmol/L	41 (28, 64)	21 (15, 25)	34 (30, 38)	49 (44, 55)	97 (76, 136)	
Plasma acetoacetate, μmol/L	9 (6, 15)	6 (4, 8)	8 (5, 10)	11 (8, 14)	21 (15, 29)	
Plasma acetone, μmol/L	13 (11, 15)	11 (10, 12)	12 (11, 13)	13 (12, 15)	18 (15, 21)	
Age, years	56.0 (8.1)	54.4 (8.1)	55.8 (8.1)	56.8 (8.0)	57.1 (7.9)	< 0.001
Male	45,902 (52)	13,251 (60)	10,429 (48)	9842 (45)	12,380 (56)	< 0.001
Ethnicity						< 0.001
White	82,938 (94)	20,669 (94)	20,634 (94)	20,684 (94)	20,951 (95)	
Black	1339 (1.5)	388 (1.8)	360 (1.6)	355 (1.6)	236 (1.1)	
Others	3622 (4.1)	918 (4.2)	981 (4.5)	936 (4.3)	787 (3.6)	
Townsend deprivation index	−2.1 (−3.6, 0.7)	−2.2 (−3.7, 0.6)	−2.1 (−3.6, 0.7)	−2.0 (−3.6, 0.8)	−2.1 (−3.6, 0.7)	< 0.001
Income						< 0.001
<18,000 EUR	16,267 (22)	3535 (19)	4010 (21)	4284 (23)	4438 (24)	
18,000–30,999 EUR	19,052 (25)	4637 (24)	4825 (25)	4897 (26)	4693 (25)	
30,999–51,999 EUR	20,012 (27)	5254 (28)	5118 (27)	4954 (26)	4686 (25)	
>52,000 EUR	20,287 (27)	5701 (30)	5104 (27)	4823 (25)	4659 (25)	
Drinking status						< 0.001
Never	3699 (4.2)	1029 (4.7)	968 (4.4)	912 (4.2)	790 (3.6)	
Previous	2999 (3.4)	701 (3.2)	832 (3.8)	750 (3.4)	716 (3.3)	
Current	81,079 (92)	20,216 (92)	20,146 (92)	20,283 (92)	20,434 (93)	
Smoking status						< 0.001
Never	48,434 (55)	13,149 (60)	12,267 (56)	11,614 (53)	11,404 (52)	
Previous	30,021 (34)	6914 (32)	7433 (34)	7828 (36)	7846 (36)	
Current	9098 (10)	1835 (8.4)	2195 (10)	2446 (11)	2622 (12)	
Systolic blood pressure, mmHg	137 (18)	134 (18)	137 (18)	138 (18)	139 (19)	< 0.001
Diastolic blood pressure, mmHg	82 (10)	81 (10)	82 (10)	83 (10)	83 (10)	< 0.001
BMI, kg/m^2	27.7 (4.7)	27.0 (4.5)	28.0 (4.7)	28.3 (4.9)	27.3 (4.8)	< 0.001
Underweight	371 (0.4)	95 (0.4)	90 (0.4)	64 (0.3)	122 (0.6)	
Normal	27,984 (32)	8214 (38)	6291 (29)	5762 (26)	7717 (35)	
Overweight	38,053 (43)	9265 (42)	9950 (45)	9732 (44)	9106 (42)	
Obese	21,260 (24)	4358 (20)	5576 (26)	6368 (29)	4958 (23)	
Total energy intake, kcal/day	2124.1 (658.7)	2119.8 (657.4)	2132.7 (657.2)	2135.3 (661.0)	2018.8 (658.6)	< 0.001
Total carbohydrate intake, g/day	256.8 (92.9)	257.8 (92.9)	258.3 (92.7)	257.6 (93.3)	253.4 (92.7)	< 0.001
Total protein intake, g/day	82.9 (26.3)	82.6 (26.2)	83.2 (26.3)	83.5 (26.5)	82.3 (26.4)	< 0.001
Total fat intake, g/day	78.3 (30.9)	77.9 (30.8)	78.6 (30.9)	78.9 (31.2)	77.9 (30.9)	0.007
Physical activity, MET-min/week	1794 (805, 3699)	1810 (836, 3705)	1752 (777, 3588)	1764 (758, 3610)	1886 (838, 3855)	< 0.001
Low	13,317 (19)	3239 (18)	3443 (19)	3552 (20)	3083 (18)	
Moderate	36,146 (51)	9280 (51)	9054 (51)	8983 (50)	8829 (50)	
High	22,019 (31)	5543 (31)	5332 (30)	5401 (30)	5743 (33)	
Comorbidity						
Hypertension	26,048 (30)	5309 (24)	6596 (30)	7446 (34)	6697 (31)	< 0.001
Diabetes	4198 (4.8)	528 (2.4)	935 (4.3)	1610 (7.3)	1125 (5.1)	< 0.001
Cardiovascular Disease	5713 (6.5)	1105 (5.0)	1518 (6.9)	1737 (7.9)	1353 (6.2)	< 0.001
Medication use						
Antihypertensive agents	18,618 (21)	3549 (16)	4699 (21)	5570 (25)	4800 (22)	
RAAS blocker	11,192 (13)	2017 (9.2)	2781 (13)	3467 (16)	2927 (13)	< 0.001
Beta-blockers	5610 (6.4)	1198 (5.5)	1572 (7.2)	1741 (7.9)	1099 (5.0)	< 0.001
CCB	4910 (5.6)	830 (3.8)	1201 (5.5)	1472 (6.7)	1407 (6.4)	< 0.001
Diuretics	6083 (6.9)	1038 (4.7)	1507 (6.9)	1878 (8.5)	1660 (7.6)	< 0.001
Statin	13,493 (15)	2400 (11)	3465 (16)	4272 (19)	3356 (15)	< 0.001
Laboratory parameters						
eGFR, mL/min/1.73 m^2	95.5 (11.9)	96.5 (11.8)	95.4 (12.0)	94.8 (12.0)	95.0 (11.8)	< 0.001
Serum albumin, g/L	45 (3.0)	45 (3.0)	45 (3.0)	45 (3.0)	46 (3.0)	< 0.001

(*continued on next page*)

Table 1 (*continued*)

	Overall (n = 87,899) (10.5–370.3 μmol/L)	Quartile of log-transformed plasma total KB level				P for trend
		Q1 (n = 21,975) (< 46.7 μmol/L)	Q2 (n = 21,975) (46.7–62.2 μmol/L)	Q3 (n = 21,975) (62.2–91.7 μmol/L)	Q4 (n = 21,974) (> 91.7 μmol/L)	
Total cholesterol, mmol/L	5.7 (1.1)	5.6 (1.1)	5.7 (1.1)	5.7 (1.2)	5.8 (1.2)	< 0.001
LDL-C, mmol/L	3.6 (0.9)	3.5 (0.8)	3.6 (0.9)	3.6 (0.9)	3.6 (0.9)	< 0.001
HDL-C, mmol/L	1.4 (0.4)	1.5 (0.4)	1.4 (0.4)	1.4 (0.4)	1.5 (0.4)	< 0.001
Triglyceride, mmol/L	1.7 (1.0)	1.6 (0.8)	1.8 (1.0)	2.0 (1.2)	1.6 (1.0)	< 0.001
hs-CRP, nmol/L	12.5 (6.2, 25.3)	10.4 (5.2, 20.6)	12.8 (6.4, 25.7)	13.9 (7.0, 28.3)	13.0 (6.3, 27.0)	< 0.001
Urine albumin-to-creatinine ratio, mg/mmol	1.2 (0.7)	1.2 (0.7)	1.2 (0.7)	1.2 (0.7)	1.3 (0.7)	< 0.001

Note: All continuous variables are expressed as mean and standard deviation. All categorical variables are expressed as number and percentage. The median values of plasma total KB, β-hydroxybutyrate, acetoacetate, acetone, Townsend deprivation index, and hs-CRP are shown with interquartile ranges in parentheses. As the summary statistics are for the complete dataset, numbers for categorical variables may not add up.

Abbreviations: KB, ketone body; BMI, body mass index; MET, metabolic equivalent; RAAS, renin-angiotensin-aldosterone system; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein.

SI conversion factors: Conversion factor for converting g/dL to g/L for serum albumin is 10; mmol/L to mg/dL for total cholesterol, LDL-C, and HDL-C is 0.0259; mmol/L to mg/dL for triglyceride is 0.0113; nmol/L to mg/L for hs-CRP is 9.5238; mg/mmol to mg/g for urine albumin-to-creatinine ratio is 0.01505.

eGFR < 60 mL/min/1.73m^2 over a period of at least 90 days, whichever came first.

Finally, we tested the effect modification of the association between plasma total KB level quartiles and the composite outcome in pre-specified subgroups according to age (< 60 vs. ≥ 60 years), sex (male vs. female), BMI (< 25 vs. ≥ 25 kg/m^2), alcohol (no vs. yes), smoking status (no vs. yes), presence of CVD (no vs. yes), presence of diabetes (no vs. yes), and hs-CRP (< 1.0 vs. ≥ 1.0 mg/L). Statistical significance was defined as $P < 0.05$. Data were analyzed using STATA (version 15; STATA Corp), and R language (version 4.1.0; R Foundation for Statistical Computing).

Results

Baseline characteristics

After excluding participants according to the pre-specified exclusion criteria, a total of 87,899 participants were enrolled in this study (Figure S1; see supplementary materials associated with this article on line). Baseline characteristics of the final study population, categorized according to plasma total KB level quartiles, are presented in Table 1. The mean age was 56 years, and 45,902 (52 %) participants were male. The median plasma total KB level was 62 μmol/L, and the mean baseline eGFR was 96 mL/min/1.73 m^2. Participants with higher plasma total KB level were more likely to be current smokers, have higher systolic and diastolic blood pressures, and have higher total and low-density lipoprotein (LDL)-cholesterol levels (all P for trend < 0.001). These participants tended to have slightly but significantly lower intake of total energy and carbohydrate than those with lower plasma total KB levels.

Outcome event rates according to plasma total KB level quartiles

During 1013,644 person-years of follow-up (median follow-up of 11.9 years), a total of 8145 participants developed the composite outcome (8.0/1000 person-years). When participants were grouped according to plasma total KB level quartiles, the composite outcome occurred in 1511 (5.9/1000 person-years), 1972 (7.8/1000 person-years), 2325 (9.3/1000 person-years), and 2337 (9.3/1000 person-years) participants in Q1, Q2, Q3, and Q4, respectively (Table 2). In the Kaplan-Meier plot, the cumulative incidence of the composite outcome significantly differed among all four quartile groups (Fig. 1).

The overall incidence rates of individual outcomes of incident CKD and all-cause mortality were 3.8, and 4.6 per 1000 person-years, respectively (Table 2). The highest incidence rate for incident CKD was observed in the Q3 group (4.5/1000 person-years). For all-cause mortality, there was an incremental increase in incidence rates across the quartiles, with the highest incidence rate observed in Q4 group (5.5/1000 person-years). The cumulative incidence curves for each study outcome showed similar results (Fig. 1).

Association between plasma total KB level and study outcomes

In Cox proportional hazards models, there were statistically significant associations between total KB and the composite outcome, in both the continuous and categorical forms of log-transformed total KB (Table 3). For a 1-SD log increase in log-transformed total KB, the adjusted HR (aHR) (95 % CI) for the composite outcome was 1.07 (1.05–1.10). When treating the total KB as a categorical variable, there was a graded relationship between total KB and the primary outcome. Compared with Q1, the aHRs (95 % CIs) for Q2, Q3, and Q4 were 1.06 (0.99–1.13), 1.10 (1.02–1.17), and 1.18 (1.11–1.27), respectively.

Secondary outcome analyses yielded similar results (Table 3). In continuous models after full adjustment, the aHRs (95 % CIs) per a 1-SD log increase in log-transformed total KB were 1.04 (1.01–1.07) and 1.10 (1.07–1.13) for incident CKD, and all-cause mortality, respectively. In categorical models, there was a graded relationship between total KB

Table 2
Outcome event rates by plasma total KB level quartiles.

	Overall (n = 87,899) (10.5–370.3 μmol/L)	Q1 (n = 21,975) (<46.7 μmol/L)	Q2 (n = 21,975) (46.7–62.2 μmol/L)	Q3 (n = 21,975) (62.2–91.7 μmol/L)	Q4 (n = 21,974) (>91.7 μmol/L)	P [c]
Composite outcome [a]						
Person-years	1013,644	257,383	254,017	251,155	251,089	
Events, n	8145	1511	1972	2325	2337	
Incidence rate, per 1000 person-years	8.0 (7.9–8.2)	5.9 (5.6–6.2)	7.8 (7.4–8.1)	9.3 (8.9–9.6)	9.3 (8.9–9.7)	< 0.001
Incident CKD [b]						
Person-years	1033,145	260,993	258,657	256,507	256,958	
Events, n	3888	747	934	1146	1061	
Incidence rate, per 1000 person-years	3.8 (3.6–3.9)	2.9 (2.7–3.1)	3.6 (3.4–3.9)	4.5 (4.2–4.7)	4.1 (3.9–4.4)	< 0.001
All-cause mortality						
Person-years	1036,967	262,094	259,701	257,797	257,376	
Events, n	4723	821	1157	1322	1423	
Incidence rate, per 1000 person-years	4.6 (4.4–4.7)	3.1 (2.9–3.4)	4.5 (4.2–4.7)	5.1 (4.9–5.4)	5.5 (5.2–5.8)	< 0.001

[a] Defined as the composite of incident CKD and all-cause mortality.
[b] Defined as eGFR < 60 mL/min/1.73 m^2.
[c] P value estimated by log-rank test.
Abbreviations: KB, ketone bodies; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

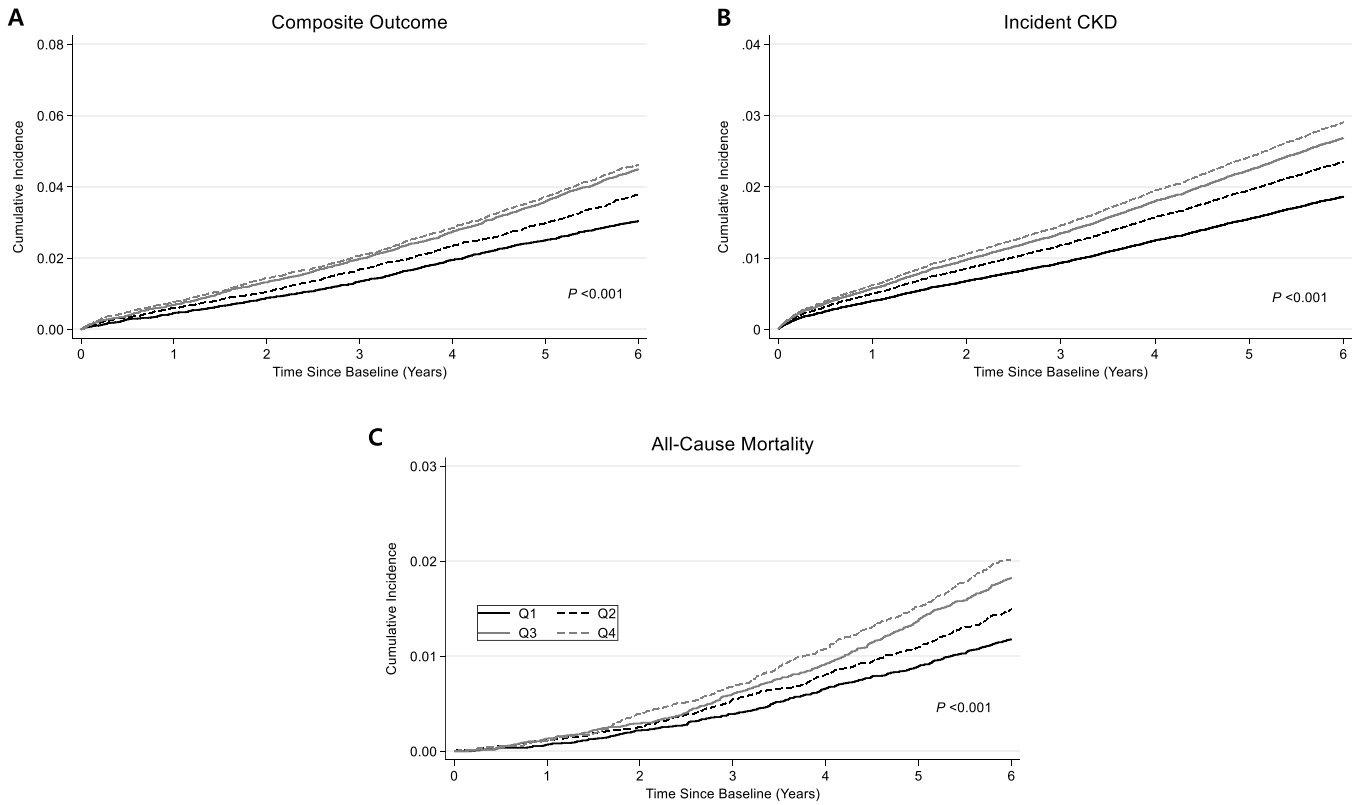

Fig. 1. Cumulative incidences of (A) composite outcome, (B) incident CKD, and (C) all-cause mortality according to log-transformed plasma total KB level quartiles.
Note: P-values were derived using the log-rank test for composite outcome and all-cause mortality, and Gray's test for incident CKD.
Abbreviations: CKD, chronic kidney disease; KB, ketone body.

and both incident CKD and all-cause mortality.

Association between β-hydroxybutyrate, acetoacetate and the study outcomes

We separately analyzed the association of individual components of KBs with risk of the study outcome. In Cox proportional hazards models after adjusting for demographics, comorbidities, laboratory parameters, and medications, a 1-SD log increase in log-transformed β-hydroxybutyrate was associated with an 8 % (aHR, 1.08; 95 % CI, 1.05–1.11) higher risk of the composite outcome (Table S3; see supplementary materials associated with this article on line). Compared with Q1, the aHRs (95 % CIs) for Q2, Q3, and Q4 were 1.01 (0.94–1.08), 1.11 (1.04–1.18), and 1.17 (1.10–1.25), respectively. In secondary outcome analyses, statistical significance was also observed for both incident CKD and all-cause mortality. A 1-SD log increase in log-transformed β-hydroxybutyrate was associated with a 5 % (aHR, 1.05; 95 % CI, 1.02–1.09) higher risk of incident CKD, and a 11 % (aHR, 1.11; 95 % CI, 1.07–1.14) higher risk of all-cause mortality. Compared to Q1, the aHRs (95 % CI) for Q4 were 1.10 (1.00–1.21) and 1.23 (1.13–1.34) for incident CKD and all-cause mortality, respectively.

In the analysis with acetoacetate, the aHR (95 % CI) per a 1-SD increase in log-transformed acetoacetate for the composite outcome was 1.05 (1.02–1.07; Table S4; see supplementary materials associated with

Table 3
Hazard ratios for study outcomes according to plasma total KB levels, and plasma total KB level quartiles.

	Model 1		Model 2		Model 3	
	HR (95 % CI)	*P*	HR (95 % CI)	*P*	HR (95 % CI)	*P*
Composite outcome [a]						
Per 1 SD log (per 0.6)	1.16 (1.13–1.18)	< 0.001	1.07 (1.05–1.10)	< 0.001	1.07 (1.05–1.10)	< 0.001
Q1 (<46.7 μmol/L)	Reference		Reference		Reference	
Q2 (46.7–62.2 μmol/L)	1.33 (1.24–1.42)	< 0.001	1.07 (1.00–1.15)	0.044	1.06 (0.99–1.13)	0.120
Q3 (62.2–91.7 μmol/L)	1.59 (1.49–1.70)	< 0.001	1.12 (1.05–1.20)	< 0.001	1.10 (1.02–1.17)	0.010
Q4 (>91.7 μmol/L)	1.60 (1.50–1.70)	< 0.001	1.20 (1.12–1.28)	< 0.001	1.18 (1.11–1.27)	< 0.001
Incident CKD [b]						
Per 1 SD log (per 0.6)	1.11 (1.08–1.14)	< 0.001	1.04 (1.01–1.07)	0.019	1.04 (1.01–1.07)	0.023
Q1 (<46.7 μmol/L)	Reference		Reference		Reference	
Q2 (46.7–62.2 μmol/L)	1.26 (1.15–1.39)	< 0.001	1.06 (0.96–1.17)	0.239	1.03 (0.93–1.13)	0.599
Q3 (62.2–91.7 μmol/L)	1.56 (1.42–1.71)	< 0.001	1.16 (1.06–1.28)	0.002	1.11 (1.00–1.22)	0.032
Q4 (>91.7 μmol/L)	1.44 (1.31–1.58)	< 0.001	1.14 (1.04–1.26)	0.005	1.12 (1.02–1.24)	0.016
All-cause mortality						
Per 1 SD log (per 0.6)	1.21 (1.17–1.24)	< 0.001	1.10 (1.07–1.13)	< 0.001	1.10 (1.07–1.13)	< 0.001
Q1 (<46.7 μmol/L)	Reference		Reference		Reference	
Q2 (46.7–62.2 μmol/L)	1.43 (1.31–1.57)	< 0.001	1.11 (1.02–1.22)	0.021	1.11 (1.01–1.21)	0.027
Q3 (62.2–91.7 μmol/L)	1.66 (1.52–1.81)	< 0.001	1.10 (1.01–1.20)	0.034	1.09 (1.00–1.20)	0.048
Q4 (>91.7 μmol/L)	1.79 (1.64–1.95)	< 0.001	1.27 (1.16–1.38)	< 0.001	1.26 (1.15–1.37)	< 0.001

Model 1: unadjusted model.
Model 2: adjusted for age, sex, race, Townsend deprivation index, income level, physical activity, alcohol intake, smoking status, BMI, comorbidities of hypertension, diabetes, and cardiovascular disease.
Model 3: model 2, with additional adjustments for baseline eGFR, UACR, LDL-cholesterol, triglycerides, hs-CRP, use of ACEis, ARBs, statins, total daily dietary carbohydrate, protein, fat intake, and total daily energy intake.
[a] Defined as the composite of incident CKD and all-cause mortality.
[b] Defined as eGFR < 60 mL/min/1.73 m^2.
Abbreviations: KB, ketone bodies; SD, standard deviation; CKD, chronic kidney disease; HR, hazard ratio; CI, confidence interval; BMI, body mass index; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; ACEi, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker.

this article on line). Additionally, compared with Q1, the aHRs (95 % CI) for Q2, Q3, and Q4 were 1.04 (0.97–1.11), 1.03 (0.96–1.10), and 1.09 (1.02–1.16), respectively. In secondary outcome analyses, statistical significance was particularly observed for mortality outcome. A 1-SD log increase in log-transformed acetoacetate was associated with a 6 % (aHR, 1.06; 95 % CI, 1.02–1.09) higher risk of all-cause mortality. In the categorical model, compared with Q1, the aHRs (95 % CIs) for Q2, Q3, and Q4 were 1.02 (0.94–1.11), 1.06 (0.97–1.15), and 1.12 (1.03–1.22), respectively. For the CKD outcome, a 1-SD log increase in log-transformed acetoacetate was associated with a higher risk of incident CKD (aHR, 1.04; 95 % CI, 1.00–1.07), but this association was weaker in the categorical model.

Sensitivity analysis

In the additional analyses where a stricter definition of CKD was applied, the results were largely similar (Table S5 and S6; see supplementary materials associated with this article on line). During 196,316 person-years of follow-up in 33,011 participants, a total of 4735 participants developed the composite outcome (24.1/1000 person-years; Table S5; see supplementary materials associated with this article on line). There were statistically significant associations between total KB and the composite outcome, in both the continuous and categorical models (Table S6; see supplementary materials associated with this article on line). The aHR per a 1-SD log increase in log-transformed total KB was 1.09 (1.06–1.12). In the categorical model, compared with Q1, the aHRs (95 % CIs) for Q2, Q3, and Q4 were 1.09 (1.00–1.19), 1.15 (1.06–1.25), and 1.26 (1.15–1.37), respectively. Secondary outcome analyses yielded similar results (Table S6; see supplementary materials associated with this article on line). In continuous models, the aHRs (95 % CIs) per a 1-SD log increase in log-transformed total KB were 1.07 (1.03–1.11) and 1.12 (1.07–1.18) for incident CKD and all-cause mortality, respectively. In categorical models, there was a graded relationship between total KB and both incident CKD and all-cause mortality. The highest risk was observed in Q4, with the corresponding aHRs (95 % CI) 1.21 (1.10–1.34) and 1.31 (1.13–1.51) for incident CKD and all-cause mortality, respectively.

Subgroup analysis

When we tested the interactions among the pre-specified subgroups to evaluate the effect modification of various subgroups on the primary composite outcome, no significant interactions were found, suggesting that the signification association between plasma total KB levels and the primary composite outcome existed across the aforementioned subgroups (Fig. 2).

Discussion

In this large population-based cohort study of individuals without established CKD, higher plasma KB levels, as indicated by both higher quartiles of log-transformed KB levels and log-transformed KB levels in its continuous form, were independently associated with higher risk of incident CKD and death. A 1-SD log increase in total KB was associated with a consistently higher risk of the primary composite outcome and secondary outcomes. Compared to Q1, higher quartiles of log-transformed KB were significantly associated with a higher risk of these clinical outcomes. Applying a stricter definition of CKD yielded similar findings. Given the lack of epidemiologic data that has looked into the clinical implications of KBs, the findings of the present study may provide insight into how KBs may affect the risk of developing CKD and death.

Our findings have several important clinical implications. Findings from cellular and animal models have suggested a beneficial role of ketones in distinct pathological conditions such as renal ischemia and reperfusion injury, renal aging, and diabetic nephropathy [3,19,20]. For instance, in an aging mouse model, continuous infusion of β-hydroxybutyrate attenuated renal ischemia and reperfusion injury through anti-pyroptotic effects [19], and daily subcutaneous administration of β-hydroxybutyrate for 30 days decreased levels of pro-inflammatory

Subgroup		Number	Events	HR (95% CI)	*P* for interaction
Age, years					0.060
<60	Q1	14,819	576	Reference	
	Q2	13,299	708	1.14 (1.01-1.30)	
	Q3	12,157	747	1.19 (1.05-1.35)	
	Q4	11,917	728	1.29 (1.14-1.46)	
≥ 60	Q1	7,156	935	Reference	
	Q2	8,676	1,264	1.02 (0.93-1.13)	
	Q3	9,818	1,578	1.02 (0.93-1.12)	
	Q4	10,057	1,609	1.13 (1.03-1.24)	
Sex					0.242
Male	Q1	13,251	806	Reference	
	Q2	10,429	810	1.10 (0.98-1.23)	
	Q3	9,842	862	1.07 (0.95-1.20)	
	Q4	12,380	1,030	1.13 (1.01-1.27)	
Female	Q1	8,724	705	Reference	
	Q2	11,546	1,162	1.06 (0.95-1.18)	
	Q3	12,133	1,463	1.10 (0.99-1.22)	
	Q4	9,594	1,307	1.25 (1.13-1.39)	
BMI, kg/m^2					0.546
< 25	Q1	8,304	422	Reference	
	Q2	6,375	442	1.11 (0.95-1.29)	
	Q3	5,824	444	1.08 (0.93-1.26)	
	Q4	7,832	625	1.24 (1.08-1.43)	
≥ 25	Q1	13,671	1,089	Reference	
	Q2	15,600	1,530	1.05 (0.96-1.15)	
	Q3	16,151	1,881	1.07 (0.98-1.17)	
	Q4	14,142	1,712	1.16 (1.06-1.27)	
Alcohol					0.300
No	Q1	1,730	176	Reference	
	Q2	1,800	210	0.99 (0.78-1.26)	
	Q3	1,662	262	1.16 (0.92-1.47)	
	Q4	1,506	212	1.08 (0.84-1.38)	
Yes	Q1	20,216	1,333	Reference	
	Q2	20,146	1,760	1.08 (1.00-1.17)	
	Q3	20,283	2,059	1.07 (0.99-1.16)	
	Q4	20,434	2,119	1.20 (1.11-1.30)	
Smoking					0.175
No	Q1	20,063	1,308	Reference	
	Q2	19,700	1,683	1.09 (1.00-1.18)	
	Q3	19,442	1,943	1.08 (0.99-1.17)	
	Q4	19,250	1,872	1.17 (1.08-1.27)	
Yes	Q1	1,835	195	Reference	
	Q2	2,195	275	0.96 (0.77-1.20)	
	Q3	2,446	366	1.05 (0.85-1.30)	
	Q4	2,622	448	1.22 (0.99-1.49)	
CVD					0.751
No	Q1	20,870	1,323	Reference	
	Q2	20,457	1,649	1.05 (0.97-1.14)	
	Q3	20,238	1,912	1.07 (0.99-1.16)	
	Q4	20,621	2,020	1.18 (1.09-1.28)	
Yes	Q1	1,105	188	Reference	
	Q2	1,518	323	1.20 (0.97-1.49)	
	Q3	1,737	413	1.18 (0.96-1.45)	
	Q4	1,353	317	1.24 (1.00-1.55)	
Diabetes					0.653
No	Q1	21,447	1,443	Reference	
	Q2	21,040	1,770	1.05 (0.97-1.14)	
	Q3	20,365	1,992	1.07 (0.99-1.16)	
	Q4	20,849	2,070	1.17 (1.08-1.27)	
Yes	Q1	528	68	Reference	
	Q2	935	202	1.39 (1.01-1.89)	
	Q3	1,610	333	1.26 (0.93-1.69)	
	Q4	1,125	267	1.46 (1.08-2.00)	
hs-CRP, mg/L					0.940
< 1.0	Q1	10,173	543	Reference	
	Q2	8,426	559	1.04 (0.91-1.19)	
	Q3	7,753	613	1.07 (0.94-1.22)	
	Q4	8,529	634	1.16 (1.01-1.32)	
≥ 1.0	Q1	11,802	968	Reference	
	Q2	13,549	1,413	1.08 (0.98-1.19)	
	Q3	14,222	1,712	1.08 (0.98-1.19)	
	Q4	13,445	1,703	1.20 (1.09-1.32)	

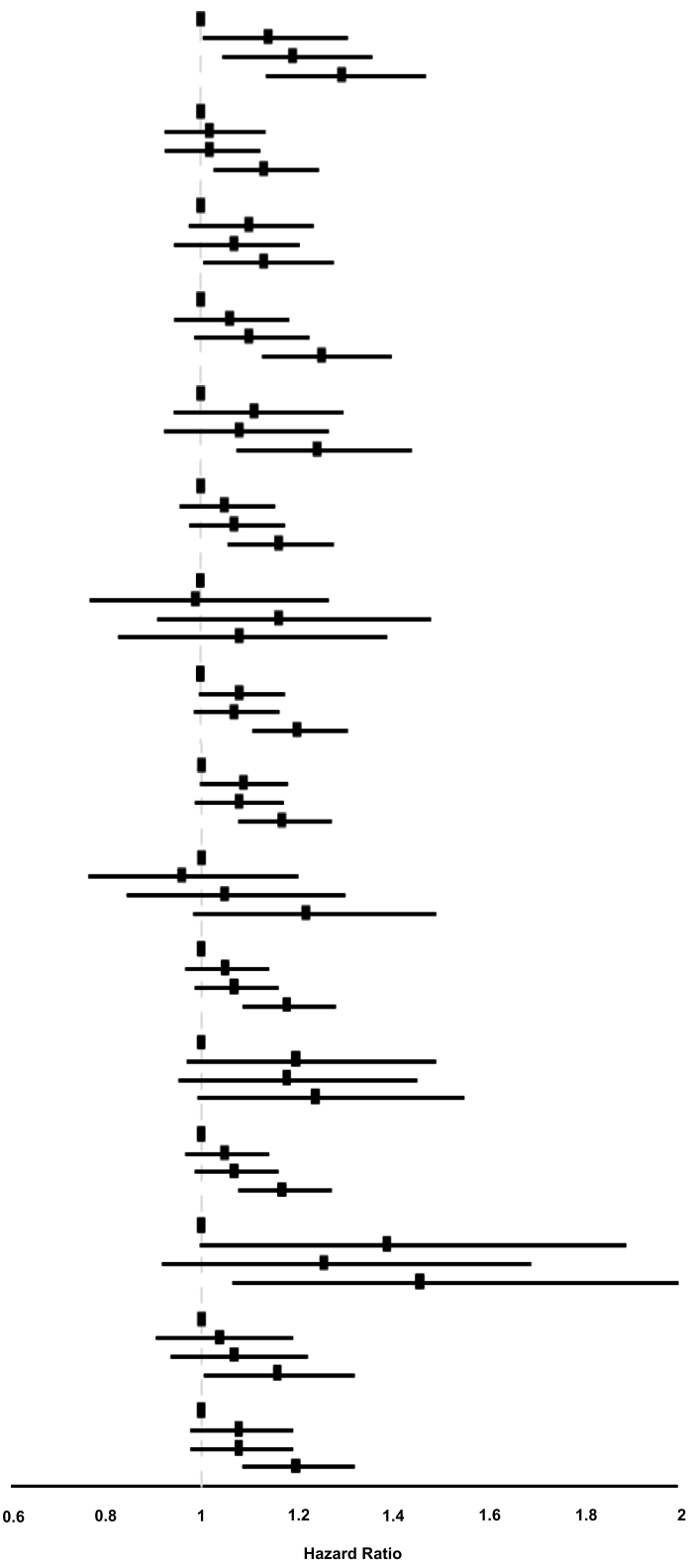

Fig. 2. Forest plot showing the effect of plasma total KB levels on the risk of the primary composite outcome.
Note: HRs were adjusted for age, sex, race, Townsend deprivation index, income level, physical activity, alcohol intake, smoking status, BMI, comorbidities of hypertension, diabetes, CVD, baseline eGFR, UACR, LDL-cholesterol, triglycerides, hs-CRP, and use of ACEis, ARBs, and statins.
Abbreviations: KB, ketone body; HR, hazard ratio; CI, confidence interval; BMI, body mass index; CVD, cardiovascular disease; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; LDL, low-density lipoprotein; ACEi, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker.

proteins, as well as reactive oxygen and nitrogen species levels in the kidney [3]. Moreover, feeding a 1,3-butanediol-rich diet in diabetic mice diminished macrophage infiltration, interstitial fibrosis, apoptosis, and albuminuria [20]. However, findings from clinical studies have been scarce. To the best of our knowledge, no population-based studies to date have reported on the clinical implications of KBs focusing on the development of CKD. The findings from the present study are the first to suggest that, in contrast to the findings of cellular and animal models, elevated KBs may be associated with adverse kidney outcomes.

A possible explanation for this discrepancy between the findings from animal models and the findings of this study may lie in the duration of exposure to KBs. In most experimental models, KBs were either exogenously administered KBs [3,19] or through high-fat, low-carbohydrate ketogenic diet for research purposes [20]. Thus, the fasting state induced by experimental design may have only resulted in acute changes in KB levels, whereas it is highly unlikely that participants from a population-based cohort would have intentionally been in a fasting state or ketogenic diet at the time of study enrollment. Presumably, participants who had higher KB levels at baseline are more likely to be attributed to chronic and long-term exposure to high KB levels. For example, as shown in the subgroup analysis, the association between KB levels and the primary outcome appeared to be more evident among alcohol drinkers, although the *P*-for-interaction was > 0.05. It is possible that regular alcohol consumers were chronically exposed to mild ketosis, potentially resulting in higher KB levels. Another explanation could be that the underlying disease process leading to an increase in KB, rather than KB levels per se, may have prognostic significance. Therapeutic modulation of KB levels by ketogenic diet or administration of SGLT2is may be beneficial [21] while chronically elevated KB levels in an otherwise healthy population may be more reflective of underlying pathological stimulus and thus, associated with poorer clinical outcomes.

The profound kidney protective effects of the SGLT2i class are hypothesized to be due, in part, to a metabolic switch in favor of KBs and free fatty acids [21,22]. By promoting glucosuria through inhibition of renal glucose reabsorption, SGLT2is create an energy-deficient metabolic state that stimulates endogenous production of KBs as a compensatory energy source. Animal models have suggested that physiological levels of KBs exert anti-inflammatory and antioxidative effects [2,3,20]. As mentioned earlier, a 30-day consecutive subcutaneous injection of β-hydroxybutyrate substantially reduced inflammatory mediators and oxidative stress in the kidneys in aging mouse models [3]. In diabetic mice, ketogenic diet reversed unfavorable features of diabetic nephropathy, and both endogenous ketone production and mTOR inhibition mediated the benefits of SGLT2i in mice [20]. In clinical studies, among users of empagliflozin, mean plasma ketone levels were slightly but significantly elevated [21–23] suggesting a potential protective role of ketones. However, the results of the present study contradict previous findings. KBs, when assessed in both plasma total KB and in separate components of β-hydroxybutyrate and acetoacetate, were all associated with higher risk of incident CKD and all-cause mortality. However, since the present study did not have information related to the use of SGLT2is, the underlying potential relationship between SGLT2is, KBs, and favorable clinical outcomes remains unclear.

The findings of our study are in agreement with previous studies that have investigated the association between KB levels and all-cause mortality. In a recent study of 6796 participants without a history of CVD from the Multi-Ethnic Study of Atherosclerosis, elevated endogenous KB was associated with an 81 % higher risk of all-cause death when adjusted for various potential confounders that included baseline kidney function, physical activity, and total calorie intake [6]. Similarly, in a retrospective cohort study of 405 stable hemodialysis patients, increased serum β-hydroxybutyrate levels were associated with a significantly higher risk of all-cause death. When compared to Q1, the highest β-hydroxybutyrate quintile was associated with an approximately five-fold higher risk of all-cause mortality [7]. In line with these findings, we confirmed that plasma total KB levels were associated with a higher risk of all-cause death in a large population-based cohort. Therefore, future studies should focus on addressing the knowledge gap between findings from epidemiologic research studies and that of preclinical studies.

This study has several limitations. First, due to the observational nature of the study, concerns of uncertain causality and the possibility of residual confounding may persist, therefore the results should be interpreted with caution. Second, as KB levels were only measured once at the time of enrollment, the association between varying levels of total KB and study outcomes could not be assessed. Additionally, KB levels can vary from person to person and be influenced by many factors such as dietary intake. However, we obtained robust results even after adjustments for dietary factors that included total daily dietary carbohydrate, protein, fat intake, and total daily energy intake. Third, as this was a population-based cohort that enrolled participants before the approval of SGLT2i, a drug that can modulate endogenous KB levels, we were unable to account for the use of SGLT2i that may affect KB levels. However, the proportion of patients with diabetes at baseline was only 4.1 % and SGLT2is are typically prescribed to patients at high risk of cardiovascular and kidney diseases. In addition, there was no significant interaction between KB levels and the presence of diabetes for the primary outcome. Therefore, it is less likely that the association of KB levels with outcomes was influenced by SGLT2is. Finally, although this was a population-based cohort that consisted of participants with diverse backgrounds, the majority of participants were enrolled in the UK, and therefore, regional differences in environmental exposures, dietary patterns, and social factors may have been unaccounted for in the present study.

In conclusion, in this large population-based cohort, higher KB levels were associated with higher risk of incident CKD and all-cause death. Given the conflicting data regarding the clinical significance of KBs, further validation studies are warranted to test the clinical utility of KBs in various clinical settings.

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CRediT authorship contribution statement

Chan-Young Jung: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Hee Byung Koh:** Data curation. **Ga Young Heo:** Data curation. **Byounghwi Ko:** Formal analysis, Data curation. **Hyung Woo Kim:** Supervision. **Jung Tak Park:** Supervision. **Tae-Hyun Yoo:** Supervision. **Shin-Wook Kang:** Supervision. **Seung Hyeok Han:** Writing – review & editing, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

Dr. Han reports serving as a subeditor of *Nephrology* and servicing as a scientific advisor and member of the Korean Society of Nephrology. All remaining authors have nothing to disclose.

Data availability

Publicly available data from the UK Biobank study was analyzed in this study. The datasets are available to researchers through an open application via https://www.ukbiobank.ac.uk/register-apply/.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diabet.2024.101527.

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