ORIGINAL RESEARCH

Association Between Circulating Ketone Bodies and Worse Outcomes in Hemodialysis Patients

Masaru Obokata, MD, PhD; Kazuaki Negishi, MD, PhD, FACC, FAHA, FASE, FESC; Hiroaki Sunaga, PhD; Hideki Ishida, RN; Kyoko Ito, MD, PhD; Tetsuya Ogawa, MD, PhD; Tatsuya Iso, MD, PhD; Yoshitaka Ando, MD; Masahiko Kurabayashi, MD, PhD

***Background*—**Cardiovascular disease is the leading cause of morbidity and mortality in patients receiving hemodialysis. Systemic metabolic perturbation is one of the hallmark abnormalities in patients at high cardiovascular risk. We sought to determine the relationship between circulating ketone body and clinical outcomes in patients with prevalent hemodialysis.

***Methods and Results*—**We retrospectively assessed the relationship between serum β-hydroxybutyrate (βOHB), the most abundant ketone body in the circulation, and prognosis in 405 stable hemodialysis patients. During a mean follow-up of 3.2±0.9 years, there were 54 major adverse cardiovascular events (defined as cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, and hospitalization attributed to heart failure) and 67 all-cause deaths. Major adverse cardiovascular events rates increased from 11.1 per 1000 person-years in the lowest βOHB quintile (<89 μmol/L) to 80.1 per 1000 person-years in the highest quintile (>409 μmol/L). After adjusting for demographic characteristics, coronary artery disease, and atrial fibrillation, the highest βOHB quintile was associated with increased risk of major adverse cardiovascular events compared with the lowest quintile (hazard ratio, 10.2; 95% confidence interval [3.35–44.0]; $P<0.001$). Increased quintiles of βOHB were independently and incrementally associated with major adverse cardiovascular events over the model based on an established risk score (the second Analyzing Data, Recognizing Excellence and Optimizing Outcomes cohort score) and N-terminal pro-B-type natriuretic peptide (chi square 39.9 versus 21.7; $P<0.001$; c-statistics, 0.713). Sensitivity analyses also confirmed the robustness of association between βOHB and all-cause death.

***Conclusions*—**Increased serum βOHB levels were independently associated with cardiovascular events and all-cause death in patients receiving hemodialysis. These results highlight the need for future studies to understand the mechanisms underlying these observations. **(*J Am Heart Assoc*. 2017;6:e006885. DOI: 10.1161/JAHA.117.006885.)**

Key Words: hemodialysis • ketone body • metabolism • prognostic factor • β-hydroxybutyrate

Cardiovascular disease is common among patients with end-stage renal disease (ESRD), especially in those who require maintenance hemodialysis, and substantially contributes to mortality and morbidity in this population.[1–4]

There are multiple mechanisms that link to the worse clinical outcome, including sympathetic nervous system (SNS) activation, inflammation, insulin resistance, hypertension, and dyslipidemia, in ESRD patients. Growing evidence indicates that increased fatty acids flux and perturbed tissue metabolism of fatty acids are consistent features in the patients at cardiovascular risk.[5,6] However, the prognostic potential of abnormal lipid metabolism in ESRD patients remains unclear.

Ketone bodies are a crucial alternative metabolic fuel source for extrahepatic organs in some physiological states, such as fasting, exercise, and pregnancy.[7] Ketone bodies are predominantly produced in the liver from fatty acids and are transported to extrahepatic tissues. It has been increasingly recognized that ketone body metabolism is increased in a myriad of pathological conditions, including insulin resistance, inflammation, heart failure (HF), and ischemic heart disease,[8,9] that are prevalent in hemodialysis populations. This led us to hypothesize that elevated levels of circulating ketone body (β-hydroxybutyrate; βOHB) would be related to cardiovascular adverse outcomes in dialysis patients. To explore this

From the Department of Cardiovascular Medicine, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan (M.O., K.N., H.S., T.I., M.K.); Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia (K.N.); Hidaka Hospital, Takasaki, Gunma, Japan (H.I., K.I., T.O., Y.A.); Department of Nephrology, Heisei-Hidaka Clinic, Takasaki, Gunma, Japan (K.I.); Department of Medicine, Tokyo Women's Medical University Medical Center East, Tokyo, Japan (T.O.).

Correspondence to: Kazuaki Negishi, MD, PhD, FACC, FAHA, FASE, FESC, Department of Cardiovascular Medicine, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan. E-mail: kazz.negishi@nifty.com

Received June 8, 2017; accepted August 3, 2017.

Clinical Perspective

What Is New?

- Hemodialysis patients had elevated β-hydroxybutyrate levels, the most abundant ketone body in the circulation.
- Increased levels of β-hydroxybutyrate were independently associated with both adverse cardiovascular events and all-cause death in patients receiving maintenance hemodialysis.
- Addition of β-hydroxybutyrate significantly improved prognostic performance over the model based on an established mortality risk prediction tool and N-terminal pro-B-type natriuretic peptide.

What Are the Clinical Implications?

- These data suggest that serum β-hydroxybutyrate levels serve as a novel biomarker predicting both survival and incidents of cardiovascular events in hemodialysis patients, which could be utilized further risk stratification in this population.

hypothesis, we performed a retrospective cohort study in patients receiving hemodialysis.

Methods

Study Population

The sample for this ancillary study was drawn from a previously published prospective study designed to determine the prognostic cardiac biomarkers in hemodialysis patients.[10] Briefly, we enrolled 437 hemodialysis patients who consented to the prospective study and had been on maintenance dialysis treatment at Hidaka Hospital (Takasaki, Japan) for at least 3 months between April 2013 and June 2013. Participants were hemodynamically stable and hemodialysis was performed 3 times weekly (3–5 h/day). Exclusion criteria included active malignancy at enrollment (n=5). Serum βOHB levels were not able to be measured in 27 subjects because of insufficient blood sample volume, and thus 405 dialysis patients were analyzed. The authors had full access to the data and take responsibility for its integrity. The study protocol was approved by the Institutional Medical Ethics Committee of Hidaka Hospital (Takasaki, Japan) with waiver of additional consent for this analysis.

Data Collection

We collected information about clinical and demographic characteristics and clinical variables related to the delivery of hemodialysis. A total of 12 predialysis blood pressures (BPs) during the 4 weeks preceding enrollment were averaged. Blood sampling was performed before a dialysis session after an overnight fast. Samples were centrifuged and stored at −80°C before assay. Serum βOHB levels were measured by a commercially available calorimetric assay (BioAssay Systems, Hayward, CA). Serum blood urea nitrogen, creatinine, hemoglobin, calcium, phosphate, ferritin, albumin, and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were also measured by routine automated laboratory procedures. Malnutrition was assessed by the Geriatric Nutritional Risk Index.[11] An established 2-year mortality risk score in a hemodialysis cohort (the the second Analyzing Data, Recognizing Excellence and Optimizing Outcomes cohort [AROii] risk score) was used for assessment of overall mortality risk.[12] This score is based on 13 clinical and laboratory parameters (age, smoking status, body mass index, cardiovascular disease history, cancer history, chronic kidney disease etiology, vascular assess, and blood flow rates, and levels of hemoglobin, ferritin, C-reactive protein, serum albumin, creatinine, and calcium).

We collected echocardiographic data from our database for subanalyses. The following echocardiographic parameters were used for analysis among patients who underwent echocardiographic examinations within 6 months of the study: left ventricular (LV) mass index (n=201); LV ejection fraction (n=281); and the ratio of peak early diastolic mitral inflow velocity to peak early diastolic mitral annular velocity (n=187).

Follow-up

All patients were followed to record outcomes. The primary end point was major adverse cardiovascular events (MACE), including cardiac deaths, nonfatal myocardial infarction, nonfatal stroke, and hospitalization because of HF. The secondary end point was all-cause death. Critical events were classified based on strict definitions and were manually collected from the medical records by a qualified cardiologist (M.O.). Diagnosis of acute myocardial infarction was defined as a cardiac event accompanied by ST-segment changes and elevation of cardiac biomarkers of myocardial necrosis (cardiac troponin). HF was defined as dyspnea and pulmonary edema on chest X-ray requiring extra hemodialysis. Stroke was diagnosed by either neurologists or neurosurgeons and was confirmed by either magnetic resonance imaging or computed tomography. Patients with transient ischemic attacks or negative imaging findings were excluded. Sudden cardiac death was defined as death attributed to cardiac causes which occurred within the previous 1 hour. Unexpected deaths which happened during asleep were also categorized as “sudden cardiac death” if deceased patients were found by family members living with the patients. Other unwitnessed deaths were recorded as “unknown.” Death was classified as noncardiac if cardiovascular events were

Table 1. Baseline Characteristics According to Quintile of Serum Levels of βOHB

	Quintile of Serum Level of βOHB (μmol/L)					*P* Value
	Q1 (<89)	Q2 (89–162)	Q3 (163–250)	Q4 (251–409)	Q5 (>409)	
Subjects, n	82	80	82	80	81	
Median βOHB, μmol/L	61 (44, 76)	123 (109, 136)	208 (181, 231)	301 (272, 385)	564 (484, 808)	<0.001
Age, y	69±11	68±12	66±13	65±10	65±12	0.08
Male, n (%)	57 (70)	52 (65)	58 (71)	60 (75)	48 (59)	0.25
Body mass index, kg/m^2	23.4±4.1	22.3±3.9	22.2±3.1	23.0±3.4	22.6±4.8	0.22
Hemodialysis data						
Dialysis duration, y	4.4 (1.8, 8.7)	6.3 (2.7, 13.2)	6.7 (2.8, 12.4)	4.9 (2.1, 14.0)	6.8 (2.2, 14.4)	0.11
Causes of end-stage renal disease, %						
Diabetes mellitus	39 (48)	36 (45)	34 (41)	41 (51)	39 (48)	0.81
Glomerulonephritis	26 (32)	25 (31)	29 (35)	23 (29)	33 (41)	
Hypertension	6 (7)	5 (6)	4 (5)	3 (4)	3 (4)	
Cystic kidney disease	2 (2)	5 (6)	4 (5)	2 (3)	2 (2)	
Other	9 (11)	9 (11)	11 (13)	11 (14)	4 (5)	
Dry weight, kg	58.0±13.5	55.6±12.8	55.8±10.3	57.3±10.2	56.0±15.6	0.68
Predialysis weight, kg	61.1±13.9	58.3±13.4	58.6±10.8	60.3±10.8	59.1±16.3	0.64
Predialysis SBP, mm Hg	151±20	150±18	150±17	150±19	147±19	0.68
Predialysis DBP, mm Hg	73±13	75±14	76±10	77±10	73±12	0.28
GNRI	97±10	94±8	95±8	95±9	94±11	0.26
Comorbidities, n (%)						
Diabetes mellitus	38 (46)	39 (49)	38 (46)	40 (50)	38 (47)	0.99
Hypertension	70 (85)	70 (88)	71 (87)	72 (90)	63 (78)	0.24
Current or ex-smoker	38 (46)	38 (48)	43 (52)	50 (63)	36 (44)	0.15
Coronary artery disease	19 (23)	11 (14)	6 (7)	14 (18)	20 (25)	0.02
Atrial fibrillation	8 (10)	9 (11)	8 (10)	9 (11)	20 (25)	0.02
HF hospitalization	6 (7)	2 (3)	2 (2)	4 (5)	8 (10)	0.17
Medications, n (%)						
Antiplatelets	35 (43)	30 (38)	25 (30)	33 (41)	41 (51)	0.12
ACEI or ARB	50 (61)	52 (65)	46 (56)	49 (61)	46 (57)	0.78
Beta-blocker	30 (37)	21 (26)	18 (22)	23 (29)	31 (38)	0.12
Calcium-channel blocker	48 (59)	42 (53)	40 (49)	45 (56)	41 (51)	0.72
Loop diuretic	23 (28)	8 (10)	17 (21)	22 (28)	15 (19)	0.03
25-hydroxy vitamin D	24 (29)	34 (43)	36 (44)	33 (41)	27 (33)	0.23
Phosphate binders	55 (67)	62 (78)	62 (76)	61 (76)	60 (74)	0.58
Laboratories						
Serum creatinine, mg/dL	9.6±3.2	10.2±2.8	10.3±3.1	10.3±2.6	9.9±3.1	0.57
BUN, mg/dL	60±15	61±14	59±14	60±14	62±17	0.71
Calcium, mg/dL	8.6±0.7	8.4±0.7	8.6±0.7	8.5±0.8	8.6±0.9	0.74
Phosphate, mg/dL	5.0±1.4	5.1±1.2	5.2±1.2	5.1±1.2	5.1±1.2	0.93
Albumin, g/dL	3.7±0.3	3.6±0.3	3.7±0.3	3.6±0.4	3.6±0.3	0.31
LDL-cholesterol, mg/dL	83±25	82±24	80±26	78±25	84±25	0.65
HDL-cholesterol, mg/dL	39±11	43±13	42±12	42±12	40±13	0.23

Continued

Table 1. Continued

	Quintile of Serum Level of βOHB (μmol/L)					
	Q1 (<89)	Q2 (89–162)	Q3 (163–250)	Q4 (251–409)	Q5 (>409)	*P* Value
Triglyceride, mg/dL	95 (66, 143)	80 (60, 105)	89 (61, 124)	97 (57, 141)	99 (69, 146)	0.06
Glucose, mg/dL	129±40	141±49	132±44	144±46	143±54	0.16
Hemoglobin, g/dL	11.0±1.1	10.5±0.8	10.8±1.0	11.0±1.0	11.0±0.9	0.02
Ferritin, ng/mL	40 (17, 63)	42 (22, 81)	37 (16, 66)	39 (22, 84)	39 (22, 77)	0.50
C-reactive protein, mg/dL	0.07 (0, 0.26)	0.14 (0.02, 0.3)	0.11 (0, 0.21)	0.10 (0, 0.38)	0.13 (0.06, 0.35)	0.12
NT-proBNP, pg/mL	3090 (1445, 8463)	3780 (2425, 7400)	3660 (1988, 7488)	4095 (1718, 8368)	4540 (2210, 13 550)	0.27
AROii risk score	4.0 (2.8, 7.0)	5.0 (3.0, 8.0)	3.5 (1.0, 6.0)	4.0 (2.0, 7.0)	4.0 (2.0, 7.0)	0.20
Echocardiography						
LV mass index, g/m^2	118±37	106±30	103±27	115±35	109±33	0.22
LV ejection fraction, %	65±9	62±13	64±7	62±12	61±15	0.20
E/e′ ratio	13 (10, 16)	13 (10, 17)	13 (9, 17)	11 (10, 16)	13 (9, 19)	0.96

Data are mean±SD, median (interquartile range), or n (%). Final column reflects overall group differences. ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; AROii risk score, the second Analyzing Data, Recognizing Excellence and Optimizing Outcomes cohort score; BUN, blood urea nitrogen; DBP, diastolic blood pressure; E/e′ ratio, the ratio of peak early diastolic mitral inflow velocity to peak early diastolic mitral annular velocity; GNRI, Geriatric Nutritional Risk Index; HDL, high-density lipoprotein; HF, heart failure; LDL, low-density lipoprotein; LV, left ventricular; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic blood pressure; βOHB, β-hydroxybutyrate.

excluded as causes of death. Patients were censored on the day that they had the last dialysis visit if they left the dialysis unit because of relocation.

Statistical Analysis

All continuous variables are presented as mean±SD or median (interquartile range). Comparisons between groups were performed using a chi-square, ANOVA, or Kruskal–Wallis tests, as appropriate. Correlations were assessed by Pearson's correlation, where non-normally distributed variables were log-transformed. Kaplan–Meier curves were created based on quintiles of βOHB. Univariable and multivariable Cox proportional hazards models were used to assess the prognostic value of βOHB, where βOHB was used as a categorical variable with patients in the lowest quintile as the reference category. Variables put into multivariable models were selected based on the associations in the univariable models and previous studies.[12] The proportional hazards assumption, assessed by the goodness-of-fit approach for each independent variable,[13] was not violated. The incremental value for the prediction of outcomes was evaluated by sequential Cox proportional hazard analysis using nested models. The first step consisted of AROii score and then NT-proBNP, and βOHB quintiles were included in the next steps. The change in overall −2 log likelihood ratios of the models was used to assess the increase in predictive power after adding a subsequent parameter. Sensitivity analyses, using our secondary end point (all-cause death), were performed to confirm the robustness of the findings from our primary end point, as well as to elucidate whether these associations are specific to cardiovascular outcomes alone. Two-sided $P<0.05$ was accepted as indicating statistical significance. All data were statistically analyzed using JMP software (version 10.0.0; SAS Institute Inc, Cary, NC).

Results

Clinical Characteristics

Overall, the mean age was 66±12 years and the median dialysis duration was 5.9 (interquartile range, 2.4, 12.2) years. Participants were predominantly male (68%) and 47% had diabetic nephropathy. The hemodialysis patients had elevated βOHB levels (median, 207 [108–351] μmol/L) compared with normal controls in a previous study.[14] Clinical characteristics of the study population according to quintile of βOHB levels are shown in Table 1. Age, sex, body mass index, dialysis duration, cause of ESRD, dry weight, predialysis BPs, and Geriatric Nutritional Risk Index were similar among the groups. Atrial fibrillation was more common in the highest βOHB quintile whereas coronary artery disease was less frequent in quintile 3. Patients in quintile 2 were less likely to receive diuretics therapy. Laboratory data were similar across all groups, including albumin and NT-proBNP levels. The AROii risk score and echocardiographic parameters did not differ among all groups. Interestingly, βOHB was not associated with nutrition status parameters, including dry weight ($r=-0.03$; $P=0.52$), body mass index ($r=-0.05$; $P=0.27$), albumin ($r=-0.04$; $P=0.40$), Geriatric Nutritional Risk Index ($r=-0.07$; $P=0.17$), hemoglobin ($r=0.05$; $P=0.27$), glucose ($r=0.08$; $P=0.12$), triglyceride ($r=-0.01$; $P=0.88$), or total-

Table 2. Incidence Rates and Hazard Ratios for MACE According to Quintile of Serum Levels of βOHB

	Quintile of Serum Level of βOHB (μmol/L)				
	Q1 (<89)	Q2 (89–162)	Q3 (163–250)	Q4 (251–409)	Q5 (>409)
Subjects, n	82	80	82	80	81
Events, n (%)	3 (3.7)	8 (10.0)	11 (13.4)	13 (16.3)	19 (23.5)
Incidence rate (per 1000 person-years)	11.1	30.4	42.0	49.7	80.1
Models					
1: Unadjusted	1 [Ref]	2.77 (0.80–12.7)	3.79 (1.18–16.7)*	4.67 (1.51–20.4)*	7.42 (2.53–31.6)*
2: Age+sex	1 [Ref]	2.77 (0.80–12.7)	4.25 (1.32–18.8)*	5.62 (1.80–24.6)*	9.80 (3.28–42.1)*
3: Age+sex+predialysis SBP	1 [Ref]	2.80 (0.81–12.8)	4.32 (1.34–19.1)*	5.79 (1.85–25.4)*	10.5 (3.52–45.5)*
4: Age+sex+predialysis SBP+albumin+NT-proBNP	1 [Ref]	2.57 (0.74–11.7)	4.34 (1.35–19.2)*	4.58 (1.43–20.3)*	9.64 (3.19–41.7)*
5: Age+sex+predialysis SBP+CAD+AF	1 [Ref]	3.37 (0.96–15.5)	5.61 (1.71–25.2)*	6.49 (2.06–28.6)*	10.2 (3.35–44.0)*

Values are hazard ratios (95% confidence interval). AF indicates atrial fibrillation; CAD, coronary artery disease; MACE, major adverse cardiovascular events; Ref, reference group; βOHB, β-hydroxybutyrate.
*P<0.05 vs Q1 (ref).

cholesterol (r=−0.04; P=0.39). There were no correlations or very weak correlations between βOHB and each of the other prognostic hemodialysis-related and echocardiographic parameters: AROii risk score (r=−0.05; P=0.32); C-reactive protein (r=0.11; P=0.07); ratio of peak early diastolic mitral inflow velocity to peak early diastolic mitral annular velocity (r=0.01; P=0.87); LV mass index (r=−0.01; P=0.90); systolic BP (r=−0.05; P=0.31); dialysis duration (r=0.11; P=0.03); NT-proBNP (r=0.12; P=0.02); and LV ejection fraction (r=−0.14; P=0.02). When dividing the patients into 2 groups with and without cardiovascular diseases at enrollment (history of HF hospitalization, ischemic heart disease, and atrial fibrillation),[12] circulating βOHB levels were similar between the groups (n=82, 250 [82, 467] μmol/L versus n=323, 200 [115, 317] μmol/L; P=0.39).

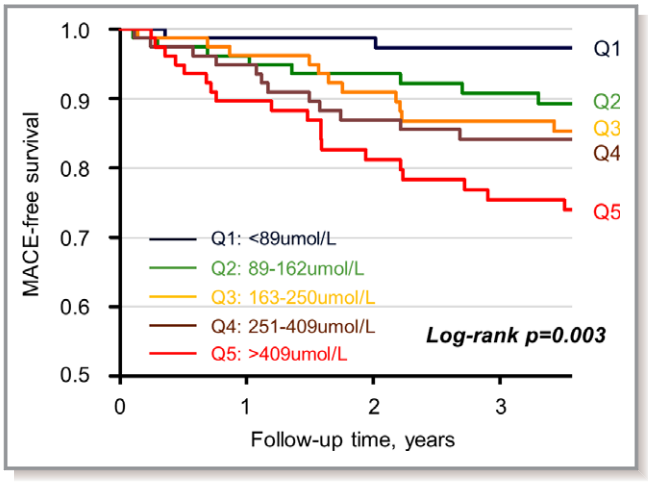
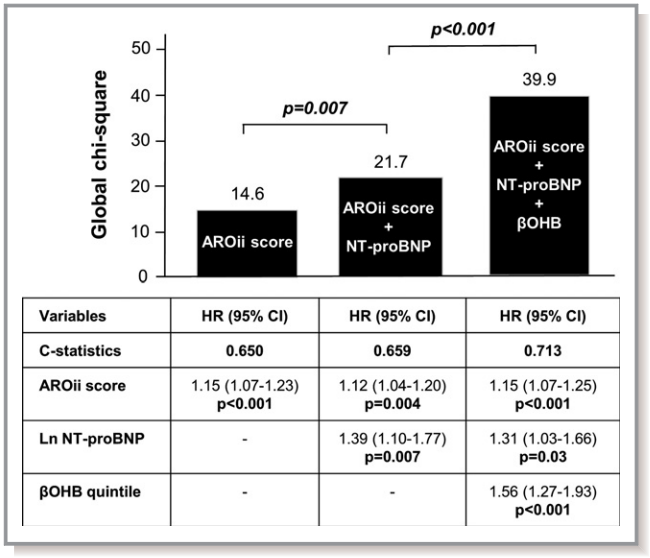


Figure 1. Kaplan–Meier survival curves for major cardiovascular adverse events stratified by quintiles of β-hydroxybutyrate. MACE indicates major cardiovascular adverse events.

Association Between βOHB and MACE

During a mean follow-up of 3.2±0.9 years, there were 54 MACE, including 21 cardiovascular deaths, 3 nonfatal myocardial infarctions, 13 HF hospitalizations, and 17 strokes. MACE rates monotonically increased from 11.1 per 1000 person-years in the lowest βOHB quintile to 80.1 per 1000 person-years in the highest quintile (Table 2). Kaplan–Meier analysis showed a dose-dependent worsening of MACE-free survival among βOHB quintiles (Figure 1). In an unadjusted Cox model, patients in the highest βOHB quintile (Q5) had 7-fold increased risk of adverse outcomes compared with those in the lowest quintile (Q1; hazard ratio [HR], 7.42; 95% confidence interval [CI; 2.53–31.6]; P<0.001; Table 2). After adjusting for age, sex, and predialysis systolic BP, risk estimates for MACE associated with βOHB quintiles remained significant and were substantially increased (Q5 versus Q1; HR, 10.5; 95% CI [3.52–45.5]; P<0.001). After further adjustment for albumin and NT-proBNP levels, the association remained significant. Even after adjusting for coronary artery disease and atrial fibrillation, point estimates found little attenuation (Q5 versus Q1; HR, 10.2; 95% CI [3.35–44.0]; P<0.001). In a subgroup of patients with echocardiography, βOHB quintile remained significant after adjustment for age, sex, and any of LV mass index, LV ejection fraction, or ratio of peak early diastolic mitral inflow velocity to peak early diastolic mitral annular velocity (adjusted P values for βOHB quintile were 0.004, 0.002, or 0.03, respectively).

A single-unit increase in the established risk score (AROii score) was associated with increased risk of MACE (HR, 1.15; 95% CI [1.07–1.23]; P<0.001), but its prognostic significance was modest (c-statistics, 0.650; Figure 2). Addition of NT-proBNP levels significantly improved the model based on the AROii score (chi square, 21.7 versus 14.6; P=0.007; c-



Variables	HR (95% CI)	HR (95% CI)	HR (95% CI)
C-statistics	0.650	0.659	0.713
AROii score	1.15 (1.07-1.23) p<0.001	1.12 (1.04-1.20) p=0.004	1.15 (1.07-1.25) p<0.001
Ln NT-proBNP	-	1.39 (1.10-1.77) p=0.007	1.31 (1.03-1.66) p=0.03
βOHB quintile	-	-	1.56 (1.27-1.93) p<0.001

Figure 2. Incremental value of β-hydroxybutyrate for prediction of major cardiovascular adverse events. AROii score indicates the second Analyzing Data, Recognizing Excellence and Optimizing Outcomes cohort score; CI, confidence interval; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; βOHB, β-hydroxybutyrate. For details, see the text.

statistics, 0.659). Further incremental prognostic value was observed by adding βOHB quintile to the model based on the AROii score and NT-proBNP (chi square, 39.9 versus 21.7; $P<0.001$; c-statistics, 0.713).

Sensitivity Analysis (All-Cause Death)

We next evaluated the association between βOHB levels and the secondary end point (all-cause death). There were 67 all-cause deaths. Mortality rates were at the lowest (22.3 per 1000 person-years) in the lowest βOHB quintile (Q1) and at the highest (96.9 per 1000 person-years) in the highest quintile (Q5; Table 3). Kaplan–Meier analysis revealed early separation of the survival curves within the first few months between the highest βOHB quintile and the others (Figure 3). In contrast to the result in the primary end point, the association was not dose dependent across the range of βOHB quintiles. In an unadjusted model, subjects with the highest βOHB quintile had 4-fold increased risk of death compared with those in the lowest quintile (Q1; HR, 4.32; 95% CI, [1.88–11.7]; $P<0.001$; Table 3). After adjusting for age, sex, and diabetic nephropathy, the association between βOHB and mortality remained significant. Even after adjustment for either predialysis systolic BP, albumin, and NT-proBNP (model 4), or predialysis systolic BP, presence of coronary artery disease, and atrial fibrillation (model 5), the inferences remained unchanged. In a subgroup of patients with echocardiographic data, βOHB quintile remained significant after adjustment for age, sex, predialysis systolic BP, and any of LV mass index, LV ejection fraction, or ratio of peak early diastolic mitral inflow velocity to peak early diastolic mitral annular velocity (adjusted P values for βOHB quintile were 0.004, 0.002, or 0.002, respectively).

Consistent with results in the primary outcome, the AROii score was associated with mortality (HR, 1.25; 95% CI [1.17–1.33]; $P<0.001$; c-statistics, 0.730; Figure 4). Adding NT-proBNP levels significantly increased the chi-square value based on the AROii score (chi square, 57.1 versus 48.7; $P=0.002$; c-statistics, 0.747). Addition of βOHB quintile further improved the model based on the AROii score and NT-proBNP (chi square, 68.8 versus 57.1; $P<0.001$; c-statistics, 0.740).

Table 3. Incidence Rates and Hazard Ratios for All-Cause Deaths According to Quintile of Serum Levels of βOHB

	Quintile of Serum Level of βOHB (μmol/L)				
	Q1 (<89)	Q2 (89–162)	Q3 (163–250)	Q4 (251–409)	Q5 (>409)
Subjects, n	82	80	82	80	81
Events, n (%)	6 (7.3)	14 (17.5)	14 (17.1)	10 (12.5)	23 (28.4)
Incidence rate (per 1000 person-years)	22.3	53.1	53.4	38.2	96.9
Models					
1: Unadjusted	1 [Ref]	2.38 (0.95–6.72)	2.38 (0.95–6.72)	1.71 (0.64–5.03)	4.32 (1.88–11.7)*
2: Age+sex	1 [Ref]	2.33 (0.94–6.59)	2.75 (1.10–7.78)*	2.14 (0.79–6.32)	5.58 (2.40–15.2)*
3: Age+sex+predialysis SBP	1 [Ref]	2.35 (0.94–6.63)	2.78 (1.11–7.87)*	2.11 (0.78–6.24)	5.39 (2.31–14.7)*
4: Age+sex+predialysis SBP+albumin+NT-proBNP	1 [Ref]	2.16 (0.87–6.11)	2.83 (1.13–8.04)*	1.39 (0.48–4.33)	5.19 (2.20–14.3)*
5: Age+sex+predialysis SBP+CAD+AF	1 [Ref]	2.41 (0.96–6.80)	2.92 (1.16–8.34)*	2.10 (0.77–6.21)	4.92 (2.08–13.5)*

Values are hazard ratios (95% confidence interval). AF indicates atrial fibrillation; CAD, coronary artery disease; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic blood pressure; βOHB, β-hydroxybutyrate.
*$P<0.05$ vs Q1 (ref).

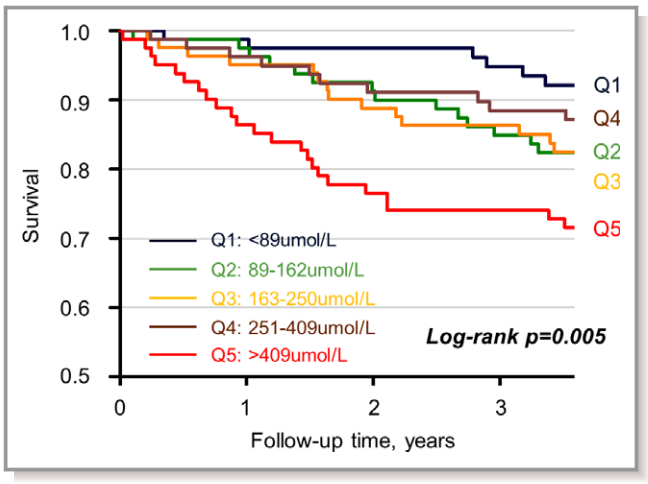
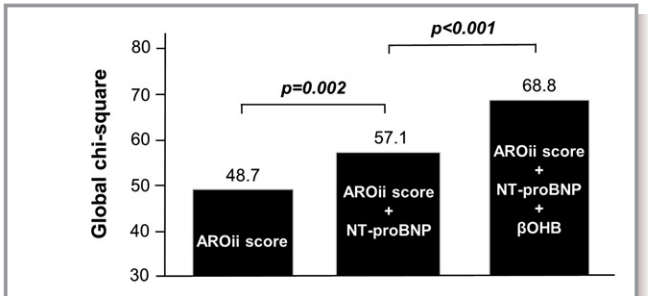


Figure 3. Kaplan–Meier survival curves for all-cause death stratified by quintiles of β-hydroxybutyrate.

Discussion

In this study, we demonstrated a significant and independent association of circulating levels of βOHB with adverse cardiovascular events in the prevalent hemodialysis patients. To our knowledge, this is the first study that evaluates circulating βOHB in a cohort of routine hemodialysis patients. A robust association was observed between βOHB and cardiovascular events after adjustment for many covariates, including age, sex, predialysis systolic BP, albumin, and NT-proBNP levels. Of importance, βOHB significantly improved prognostic performance beyond an established mortality risk prediction tool, AORii, and further improved prognostic power over AORii plus NT-proBNP–based score (C-statistics, 0.740; $P<0.001$). Moreover, our sensitivity analyses using all-cause death underpin the relationship between ketone body level and prognosis. These results suggest that serum βOHB levels could serve as a novel biomarker predicting both survival and incidents of cardiovascular events in patients with hemodialysis.



Variables	HR (95% CI)	HR (95% CI)	HR (95% CI)
C-statistics	0.730	0.747	0.740
AROii score	1.25 (1.17-1.33) p<0.001	1.22 (1.14-1.30) p<0.004	1.25 (1.16-1.34) p<0.001
Ln NT-proBNP	-	1.39 (1.13-1.71) p=0.002	1.32 (1.07-1.62) p=0.008
βOHB quintile	-	-	1.38 (1.15-1.64) p<0.001

Figure 4. Incremental value of β-hydroxybutyrate for prediction of all-cause death. AROii score indicates the second Analyzing Data, Recognizing Excellence and Optimizing Outcomes cohort score; CI, confidence interval; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; βOHB, β-hydroxybutyrate. For details, see the text.

Possible Mechanisms Underlying the Association of Increased Serum βOHB and MACE

The specific role of βOHB in the development of cardiovascular outcome in ESRD remains unknown at present. One of the potential mechanistic links between βOHB and cardiovascular events may be relevant to the mechanisms that increase βOHB production, rather than to the direct effects of βOHB on the cardiovascular system. Ketogenesis occurs primarily in the liver using free fatty acids as precursors that arise from lipolysis of triacylglycerol in adipose tissue.[15] The fatty acid oxidation product, acetyl-CoA, is the major substrate for ketogenesis, and the rate of ketogenesis is proportional to total fatty acid oxidation rate.[7] In addition, there is a consistent evidence of increased serum levels of ketone bodies and free fatty acids in correlation to the severity of HF in humans.[8,16] Given the systemic increase in sympathetic tone in the patients with HF and the evidence for an apparent increase in SNS activation as well as lipolysis in chronic kidney disease,[17,18] we propose that an increase in SNS tone may play a central role as the mechanism responsible for increased βOHB levels in hemodialysis patients with future cardiovascular events. However, it should be noted that ≈20% of patients in the present study had apparent cardiovascular diseases at enrollment (history of HF hospitalization, ischemic heart disease, and atrial fibrillation), and no clear relationship was observed between βOHB and history of HF. Accordingly, SNS overdrive attributed to HF at baseline may not fully account for the increased βOHB levels in the patients with hemodialysis who ultimately suffer cardiovascular events and all-cause mortality. Further studies should be warranted to evaluate the ability of βOHB to assess the magnitude of generalized SNS activation.

Inflammation may also induce an additional non-neuronal source of catecholamines,[19] although there was no significant association between βOHB and C-reactive protein in our study. Malnutrition may be another possible mechanism to explain the association between increased ketone bodies and adverse outcomes. Although our statistical analysis could not identify a significant association among βOHB levels and nutritional parameters, such as albumin and Geriatric

Nutritional Risk Index, the possibility of malnutrition as a causative role in increased βOHB levels cannot be excluded. Furthermore, insulin resistance, which commonly exists in chronic kidney disease patients, in conjunction with an increase in glucagon, may also contribute to increased ketogenesis. However, the absence of the association between elevation of ketone bodies and diabetes mellitus in our study suggests that insulin resistance may not significantly contribute to the significant link between high βOHB levels and MACE.

Ketone Body: A Friend or Foe in the Cardiovascular System

The strong prognostic potential of βOHB may reflect its role in the pathophysiology of cardiovascular disease. Ketone bodies have long been known as a better fuel than glucose or fatty acids because of their capability of providing more cellular energy per unit of oxygen consumed.[20,21] Recent studies in human and animal models provide clear evidence indicating that myocardial ketone body utilization is increased by an induction of the gene expression responsible for this pathway whereas the fatty acid oxidation pathway is disrupted in HF.[8,9] The data from animal models in which ketone oxidation is defective suggest that the ability of the heart to increase ketone body utilization is protective against HF. Schugar et al described that cardiomyocyte-specific deletion of the rate-limiting enzyme, succinyl-CoA:3-oxoacidic-CoA transferase, encoded by the nuclear *OXCT1* gene, that catalyzes the ketone body flux is detrimental when the heart is subjected to pressure overload in succinyl-CoA:3-oxoacidic-CoA transferase–deficient mice.[22] In that study, reactive oxygen species production was increased in succinyl-CoA:3-oxoacidic-CoA transferase knockout mice.

Besides the role as an alternative fuel for the extrahepatic organs, emerging evidence indicates that ketone bodies, particularly βOHB, possess a variety of capabilities, including inhibition of the activity of histone deacetylases, activation of particular G-protein-coupled receptors, anti-inflammatory activity, and antioxidative stress activity.[7] Cumulatively, these data suggest the importance of appropriate ketonemia and a shift from fatty acid oxidization to ketone oxidation in the heart as a metabolic adaptation. Further studies to prove this assumption should be warranted.

Study Limitations

Our study has several limitations. This study was an exploratory analysis of a single-center, retrospective, observational study and, as such, has inherent flaws relating to selection bias, spurious observations, and unmeasured covariates. Blood samples were obtained after overnight fasting, so circulating levels of ketone bodies would be slightly increased during this time period. Myocardial ketone levels were not assessed. Not all patients underwent echocardiographic examination. Nevertheless, this is the first study to determine the association between circulating βOHB levels and adverse cardiovascular outcomes in hemodialysis patients.

Conclusions

In the present study, we demonstrate a strong association between higher circulating βOHB levels and cardiovascular events after adjustment for a variety of traditional prognostic factors, including laboratory and echocardiographic parameters, in the patients with prevalent hemodialysis. This study highlights the prognostic importance of the metabolic perturbation that induces ketogenesis in patients with hemodialysis. Future studies to determine the mechanisms underlying the association between βOHB and increased cardiovascular morbidity and mortality will contribute to the development of efficient preventive strategies in these patients.

Acknowledgment

The authors thank Tomiko Shimoda, RN, for her study management.

Disclosures

Dr Obokata received research funding from Kureha Corporation. Dr Negishi is supported by an award from the Select Foundation, which had no role in the preparation of this article. Dr Kurabayashi and Dr Iso are supported by grants from Japan Society for the Promotion of Science, the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and the Gunma University Initiative for Advanced Research.

References

1. United States Renal Data System (USRDS). Annual Data Report 2015: International Comparisons. 2015. https://www.usrds.org/2015/view/
2. National Kidney and Urologic Diseases Information Clearinghouse (NKUDIC). Kidney Disease Statistics for the United States 2012. https://www.niddk.nih.gov/health-information/kidney-disease
3. The Japanese Society of Dialysis Therapy. 2012 statistics of maintainance dyalysis therapy in japan. 2013. http://docs.jsdt.or.jp/overview/index2013.html
4. Foley RN, Collins AJ. End-stage renal disease in the United States: an update from the United States Renal Data System. *J Am Soc Nephrol*. 2007;18:2644–2648.
5. Quehenberger O, Dennis EA. The human plasma lipidome. *N Engl J Med*. 2011;365:1812–1823.
6. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M, Kangas AJ, Kettunen J, Kaikkonen J, Mikkila V, Jula A, Kahonen M, Lehtimaki T, Lawlor DA, Gaunt TR, Hughes AD, Sattar N, Illig T, Adamski J, Wang TJ, Perola M, Ripatti S, Vasan RS, Raitakari OT, Gerszten RE, Casas JP, Chaturvedi N, Ala-Korpela M, Salomaa V.

Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation*. 2015;131:774–785.

7. Puchalska P, Crawford PA. Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics. *Cell Metab*. 2017;25:262–284.
8. Bedi KC Jr, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, Rame JE. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation*. 2016;133:706–716.
9. Aubert G, Martin OJ, Horton JL, Lai L, Vega RB, Leone TC, Koves T, Gardell SJ, Kruger M, Hoppel CL, Lewandowski ED, Crawford PA, Muoio DM, Kelly DP. The failing heart relies on ketone bodies as a fuel. *Circulation*. 2016;133:698–705.
10. Obokata M, Sunaga H, Ishida H, Ito K, Ogawa T, Ando Y, Kurabayashi M, Negishi K. Independent and incremental prognostic value of novel cardiac biomarkers in chronic hemodialysis patients. *Am Heart J*. 2016;179:29–41.
11. Bouillanne O, Morineau G, Dupont C, Coulombel I, Vincent JP, Nicolis I, Benazeth S, Cynober L, Aussel C. Geriatric Nutritional Risk Index: a new index for evaluating at-risk elderly medical patients. *Am J Clin Nutr*. 2005;82:777–783.
12. Floege J, Gillespie IA, Kronenberg F, Anker SD, Gioni I, Richards S, Pisoni RL, Robinson BM, Marcelli D, Froissart M, Eckardt KU. Development and validation of a predictive mortality risk score from a European hemodialysis cohort. *Kidney Int*. 2015;87:996–1008.
13. Lee KL, Pryor DB, Harrell FE, Califf RM, Behar VS, Floyd WL, Morris JJ, Waugh RA, Whalen RE, Rosati RA. Predicting outcome in coronary disease. Statistical models versus expert clinicians. *Am J Med*. 1986;80:553–560.
14. Hansen JL, Freier EF. Direct assays of lactate, pyruvate, beta-hydroxybutyrate, and acetoacetate with a centrifugal analyzer. *Clin Chem*. 1978;24:475–479.
15. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70:243–251.
16. Lommi J, Kupari M, Koskinen P, Naveri H, Leinonen H, Pulkki K, Harkonen M. Blood ketone bodies in congestive heart failure. *J Am Coll Cardiol*. 1996;28:665–672.
17. Floras JS. Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. *J Am Coll Cardiol*. 2009;54:375–385.
18. Schlaich MP, Socratous F, Hennebry S, Eikelis N, Lambert EA, Straznicky N, Esler MD, Lambert GW. Sympathetic activation in chronic renal failure. *J Am Soc Nephrol*. 2009;20:933–939.
19. Flierl MA, Rittirsch D, Nadeau BA, Chen AJ, Sarma JV, Zetoune FS, McGuire SR, List RP, Day DE, Hoesel LM, Gao H, Van Rooijen N, Huber-Lang MS, Neubig RR, Ward PA. Phagocyte-derived catecholamines enhance acute inflammatory injury. *Nature*. 2007;449:721–725.
20. Kashiwaya Y, Sato K, Tsuchiya N, Thomas S, Fell DA, Veech RL, Passonneau JV. Control of glucose utilization in working perfused rat heart. *J Biol Chem*. 1994;269:25502–25514.
21. Veech RL, Chance B, Kashiwaya Y, Lardy HA, Cahill GF Jr. Ketone bodies, potential therapeutic uses. *IUBMB Life*. 2001;51:241–247.
22. Schugar RC, Moll AR, Andre d'Avignon D, Weinheimer CJ, Kovacs A, Crawford PA. Cardiomyocyte-specific deficiency of ketone body metabolism promotes accelerated pathological remodeling. *Mol Metab*. 2014;3:754–769.