Association of Higher Arterial Ketone Body Ratio (acetoacetate/β-hydroxybutyrate) with Relevant Nutritional Marker in Hemodialysis Patients

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Abstract

Background: An association of higher levels of β-hydroxybutyrate (β-HB) in serum with greater mortality in hemodialysis (HD) patients has been reported. This study examined the significance of arterial ketone body ratio (AcAc/β-HB), a relevant marker of energy state, in HD patients.

Methods: The levels of arterial AcAc and β-HB, and AcAc/β-HB ratio were determined in 49 HD patients just before undergoing an HD session. Additionally, their changes during the session were examined to investigate their associations with clinical nutritional markers.

Results: Arterial β-HB, but not AcAc, was significantly higher at the baseline in 25 patients with diabetes mellitus (DM) as compared to 24 non-DM patients, with a significant reduction in arterial AcAc/β-HB ratio seen in those with DM. Although the arterial AcAc/β-HB ratio before the HD session was significantly higher in the non-DM group, it did not differ significantly after the session between the groups, indicating a faster rate of β-HB disappearance from circulation in non-DM HD patients during the interdialytic period. Multiple regression analysis, which included age, gender, presence/absence of DM, log HD duration, log β-HB, and log AcAc/β-HB ratio as independent variables, revealed an independent and significant association of log AcAc/β-HB ratio, but not log β-HB, with serum albumin and uric acid.

Conclusion: We found that a decreased AcAc/β-HB ratio resulting from increased β-HB, but not increased β-HB itself, was significant factor independently associated with decreased serum albumin and uric acid, which are both known as risks for higher mortality in HD patients. Furthermore, it is possible that higher mortality in DM HD patients might be explained by their lower arterial AcAc/β-HB ratio.

Introduction

Patients undergoing hemodialysis (HD) are known to exhibit a significant increase in serum ketone bodies [acetoacetate (AcAc), β-hydroxybutyrate (β-HB)] during a single HD session (1). Ketone bodies have long been understood as a better fuel source than glucose or fatty acids (2, 3). However, it was recently reported that higher serum β-HB was independently associated with increased cardiovascular disease (CVD) events and cases of all-cause death in HD patients in Japan (4). It has been shown that a reduction in arterial ketone body ratio, defined by determining arterial AcAc/β-HB ratio, a non-invasive mean of evaluating hepatic energy charge (5), is a novel independent CVD risk factor (6), thus it is important to examine whether higher β-HB by itself or a reduction in arterial AcAc/β-HB ratio resulting from higher β-HB as well contributes to worse outcome of HD patients. Furthermore, it is interesting to investigate the significance of changes in arterial AcAc, β-HB, and AcAc/β-HB ratio occurring during an HD session. Also, because of the effect of insulin to suppress production of arterial ketone bodies (7), it is possible that the stimulation of ketone body production resulting from inhibition of insulin secretion treated with dialysate containing 125 mg/dL glucose might differ between DM and non-DM HD patients.

These background factors prompted us to examine (i) whether arterial AcAc/β-HB ratio or β-HB might be better to predict nutritional state, and thus clinical outcome in HD patients, and (ii) the mechanism of changes in serum β-HB and AcAc together with arterial AcAc/β-HB ratio during an HD session by enrolling both DM and non-DM HD patients.
Patients And Methods

Patients

For the present study, 49 HD patients (25 DM, 24 non-DM) receiving four-hour treatments three times per week at the outpatient clinic of Minami-Osaka Hospital Kidney Center, Japan, were enrolled. Written informed consent was obtained from each prior to participation. The protocol was approved by the Ethics Review Committee of Minami-Osaka Hospital (Approval #2015-10) and conducted in accordance with the principles of the Declaration of Helsinki. Patients who had been undergoing HD therapy for less than one year or more than 21 years were excluded, as previously described (8, 9). The underlying nephropathy type was diabetic nephropathy (n = 25), chronic glomerulonephritis (n = 10), nephrosclerosis (n = 9), polycystic kidney disease (n = 1), other disease (n = 3), and unknown disease (n = 1). All patients were free from significant acute illness or malignancy considered to have influence on metabolic status, as noted in our previous report (10).

Sample collection

Arterial blood samples were drawn twice consecutively from the arteriovenous fistula just before a Monday or Tuesday morning HD session, three days after the previous HD session and following an overnight fast (11). Furthermore, to assess changes in arterial blood AcAc, β-HB, and ketone body ratio, defined by arterial AcAc/β-HB ratio, during a single four-hour HD session, blood samples were also obtained just after the session had finished. A portion of the sampled arterial blood was used for measurements of acid-base parameters, while the remaining specimen was kept at 4 °C for 1 hour, then centrifuged at 1000 x g for 10 minutes and stored in aliquots at −80 °C until being assayed. Prior to the assay, the frozen sample was thawed and measurements were performed immediately thereafter.

Laboratory measurements

Just before and after the HD session, in addition to standard parameters, ketone bodies (AcAc, β-HB) were measured in arterial serum samples obtained from the arteriovenous fistula using commercially available kits. Arterial blood samples obtained simultaneously were measured for acid-base parameters (pH, bicarbonate) using a blood gas analyzer. Arterial ketone body (AcAc/β-HB) ratio was estimated as redox state in liver mitochondria capable of producing ATP (12). Glycoalbumin (GA), a clinically relevant parameter for glycemic control in HD patients uninfluenced by either the presence of anemia or usage of an erythropoiesis-stimulating agent (9), in contrast to HbA1c, was measured as previously described (13).

Statistical analysis

Values are shown as the mean ± standard deviation (SD) or median with interquartile range (IQR), depending on the presence or absence of a normal distribution. Comparisons of mean and median values between the DM and non-DM patients were performed using Student’s t test and Mann-Whitney’s U test, respectively. Correlations were assessed by Pearson’s correlation test or a nonparametric Spearman’s rank correlation test. Multiple regression analysis was performed to determine the independent associations of AcAc, β-HB, and
arterial AcAc/b-HB ratio with various clinical parameters. Because of the skewed distribution of the values for these ketone bodies, they were entered into multiple regression analysis after logarithmic transformation. $P$ values less than 0.05 were considered to indicate statistical significance. All calculations were performed using a Windows personal computer with the StatView V statistics software package (SAS Institute Inc., Cary, NC, USA).

**Results**

**Clinical characteristics of HD patients just prior to starting HD session**

The baseline clinical characteristics of the 49 enrolled HD patients (24 non-DM, 25 DM), measured just prior to a Monday or Tuesday morning HD session, three days after the previous session, are shown in Table 1. There were no significant differences for age, gender, HD duration, or inter-dialytic weight gain between the non-DM and DM groups, while BMI and albumin were significantly higher in the DM HD patients. Serum creatinine did not differ significantly between the groups. Casual plasma glucose and GA, parameters for glycemic control, were significantly higher in the DM group.
|                          | All patients (n = 49) | Non-DM (n = 24) | DM (n = 25) | P       |
|--------------------------|-----------------------|----------------|-------------|---------|
| Age, years               | 66.7 ± 11.5           | 68.6 ± 11.3    | 64.8 ± 11.6 | 0.2220  |
| Gender, male/female      | 32/17                 | 13/11          | 19/6        | 0.1085  |
| BMI, kg/m²               | 22.7 ± 4.5            | 21.3 ± 4.3     | 24.0 ± 4.3  | 0.0494* |
| HD duration, year        | 4.5 (2.5–6.3)         | 4.7 (3.4–7.1)  | 4.3 (2.2–5.5) | 0.1497  |
| Interdialytic BW gain, % | 5.3 (4.3–5.9)         | 5.4 (4.5–6.9)  | 5.2 (4.1–5.6) | 0.2041  |
| Serum urea nitrogen, mg/dL | 60.5 ± 16.1          | 64.4 ± 18.3    | 56.8 ± 12.5 | 0.1096  |
| Cre, mg/dL               | 10.0 ± 2.8            | 10.0 ± 2.7     | 10.1 ± 2.8  | 0.9045  |
| Alb, g/dL                | 3.6 (3.4–3.8)         | 3.5 (3.2–3.7)  | 3.7(3.5–3.9) | 0.0141  |
| Casual plasma glucose, mg/dL | 122.0 (101.8–152.3)  | 115.5 (93.0–134.0) | 145.0 (117.8–163.8) | 0.0117* |
| Glycoalbumin, %          | 16.8 ± 3.0            | 15.2 ± 2.2     | 18.2 ± 3.0  | 0.0003* |
| CRP, mg/dL               | 0.11 (0.04–0.34)      | 0.11 (0.03–0.33) | 0.11 (0.04–0.35) | 0.6237  |
| LDL-C, mg/dL             | 78.9 (56.3–102.0)     | 83.0 (58.5–107.0) | 75.0 (51.8–95.8) | 0.3127  |
| Uric acid, mg/dL         | 5.9 (5.3-7.0)         | 6.2 (5.3–7.1)  | 5.8 (5.0-6.8) | 0.3023  |
| cCa, mg/dL               | 8.4 ± 0.6             | 8.5 ± 0.6      | 8.4 ± 0.6   | 0.6378  |
| Pi, mg/dL                | 5.5 (4.9–6.1)         | 5.6 (4.9–6.1)  | 5.5 (4.8–6.2) | 0.9920  |
| Arterial blood           |                       |                |             |         |
| pH                       | 7.34 (7.32–7.36)      | 7.34 (7.31–7.36) | 7.34 (7.33–7.36) | 0.5754  |
| HCO₃, mEq/L              | 19.9 ± 2.2            | 19.4 ± 2.6     | 20.4 ± 1.7  | 0.2844  |
| AcAc, µmol/L             | 26.0 (21.8–43.3)      | 26.0 (20.0-36.5) | 28.0 (22.0-49.3) | 0.3839  |
| β-HB, µmol/L             | 20.0 (14.8–42.0)      | 17.0 (11.5–36.0) | 29.0 (17.0-59.5) | 0.0134* |
| AcAc/β-HB ratio, µmol/µmol | 1.18 (0.76–1.51)    | 1.35 (1.06–2.17) | 0.91 (0.73–1.24) | 0.0155* |

Values in parentheses show range. *indicates significant difference between non-DM and DM HD patients with p value < 0.05.

As shown in Fig. 1, arterial β-HB was significantly higher in the DM HD [29.0 (range 17.0-59.5) µmol/L] as compared to the non-DM [17.0 (11.5–36.0) µmol/L] patients, whereas arterial AcAc was not significantly different between the groups [28.0 (22.0-49.3) vs. 26.0 (20.0-36.5) µmol/L], resulting in a significantly lower arterial AcAc/β-HB ratio in the DM HD [0.91 (0.73–1.24) µmol/L] than in the non-DM HD [1.35 (1.06–2.17) µmol/L].
µmol/L] patients. An arterial AcAc/β-HB ratio < 1.0, known to indicate significant risk for organ dysfunction (liver, heart) and increased mortality (12), was noted in 13 (56%) of the DM HD patients, which was significantly higher as compared to the non-DM group (n = 5, 20.8%) (p < 0.05, χ² test). Neither arterial blood pH nor HCO₃⁻ was significantly different between the groups.

**Regression analysis of correlation of arterial serum AcAc, β-HB, and arterial AcAc/β-HB ratio with various clinical parameters**

Arterial AcAc, β-HB, and AcAc/β-HB ratio were examined for their correlation with various clinical parameters in all of the present cohort, as well as separately in the non-DM and DM groups (Table 2). Arterial AcAc was correlated with serum Pi in all as well as in the non-DM HD patients, and arterial β-HB was correlated with the rate of interdialytic weight gain, and urea nitrogen and uric acid in serum in all patients, and with casual plasma glucose and LDL-C in the non-DM HD patients. Additionally, arterial AcAc/β-HB ratio was correlated with rate of interdialytic weight gain as well as urea nitrogen, creatinine, and uric acid in serum in all HD patients, and also with albumin, casual plasma glucose, and LDL-C in the non-DM HD patients. Notably, casual plasma glucose before the HD session was significantly correlated in a negative manner with β-HB and in a positive manner with AcAc/β-HB ratio in the non-DM, but not the DM group. The only significant correlations noted in the DM group was between arterial β-HB and rate of interdialytic weight gain, and between arterial AcAc/β-HB ratio and serum urea nitrogen and Pi.
### Table 2
Correlations of arterial AcAc, β-HB, and AcAc/β-HB ratio in non-DM and DM HD patients

|                         | All patients (n = 49) | Non-DM (n = 24) | DM (n = 25) |
|-------------------------|-----------------------|-----------------|-------------|
| Age                     | 0.092                 | 0.035           | -0.039      |
| BMI                     | -0.023                | -0.068          | 0.160       |
| HD duration             | 0.043                 | -0.162          | 0.190       |
| Interdialytic BW gain   | -0.243                | -0.472          | 0.360*      |
| Serum urea nitrogen     | -0.095                | -0.350          | 0.434*      |
| Cre                     | -0.031                | -0.267          | 0.343*      |
| Alb                     | 0.140                 | 0.021           | 0.112       |
| Casual plasma glucose   | 0.196                 | -0.013          | 0.207       |
| Glycoalbumin            | 0.088                 | 0.166           | -0.163      |
| LDL-C                   | 0.054                 | 0.193           | -0.231      |
| Uric acid               | 0.057                 | -0.296          | 0.474*      |
| Pi                      | -0.338*               | -0.242          | 0.204       |
| AcAc                    |                       |                 |             |
| β-HB                    |                       |                 |             |
| AcAc/β-HB               |                       |                 |             |

Correlation (r) was examined using Spearman’s Rank Correlation test.

*p < 0.05 (*) was considered to indicate statistically significant.

**Increased arterial ketone body levels during a single HD session**

During a single session, all of the present HD patients exhibited significant increases in arterial levels of AcAc from 26.0 (range: 20.0-36.5) µmol/L to 82.0 (range: 39.8-157.8) µmol/L (p < 0.0001) (Fig. 1A) as well as β-HB from 20.0 (14.8-42.0) µmol/L to 104.0 (32.0-297.3) µmol/L (p < 0.0001) (Fig. 1B). Those increases resulted in a significant reduction in arterial AcAc/β-HB ratio from 1.18 (0.76-1.51) (µmol/µmol) to 0.70 (0.44-1.13) (µmol/µmol) (p < 0.0001) (Fig. 1C). When all HD patients were divided into the DM and non-DM groups, non-DM HD patients retained significant increases in arterial AcAc from 26.0 (20.0-36.5) µmol/L to 57.0 (37.5-162.5) µmol/L (p < 0.0001) (Fig. 1D) and β-HB from 17.0 (11.5-36.0) to 80.0 (28.5-291.5) µmol/L (p < 0.0001) (Fig. 1E). In DM HD patients, arterial AcAc and β-HB were significantly increased from 28.0 (22.0-49.3) µmol/L to 96.0 (54.0-158.0) µmol/L (p < 0.0001) (Fig. 1G) and from 29.0 (17.0-59.5) µmol/L to 105.0 (63.5-309.0) µmol/L (p < 0.0001), respectively. Of interest, the reduction in arterial AcAc/β-HB ratio in the non-DM HD patients (Fig. 1F) during an HD session [from 1.35 (1.06-2.17) µmol/µmol to 0.70 (0.44-1.41) µmol/µmol]
became statistically significant (p < 0.0001), while that change in DM HD patients (Fig. 1I) [from 0.91 (0.73–
1.24) µmol/µmol to 0.68 (0.44–1.06) µmol/µmol] did not (p = 0.1078). As a result, though the arterial AcAc/β-
HB ratio was significantly higher in the non-DM HD as compared to the DM HD patients before (p = 0.0134), it
did not differ significantly between those groups after the HD session. Furthermore, arterial β-HB was found to
be significantly lower in the non-DM HD patients only before but not after the session. Together, these results
suggest that the rate of increase in AcAc/β-HB ratio during the inter-dialytic period was higher in the non-DM
HD patients due possibly to a greater rate of β-HB reduction.

Changes in arterial blood pH and HCO₃⁻ were not significantly correlated with those of arterial AcAc, β-HB, or
AcAc/β-HB ratio in the full cohort, as well as after dividing into the DM and non-DM groups (data not shown).
Notably, the baseline levels of both casual plasma glucose and glycoalummin before the HD session were
significantly correlated in a negative manner with change in AcAc/β-HB ratio during the HD session in the non-
DM HD patients (Fig. 2), but not in the DM HD patients (data not shown).

**Multiple regression analysis of β-HB and arterial AcAc/β-HB ratio with serum log albumin, Pi, and UA**

Multiple regression analysis was performed to examine whether arterial β-HB or AcAc/β-HB ratio had a
significant association with serum levels of albumin and uric acid in all of the present HD patients. When log
β-HB was included as an independent variable, in addition to age, gender, presence/absence of DM, and log
HD duration (Model 1), it emerged as a significant and independent factor showing an association with uric
acid, but not albumin. When log β-HB was replaced with log AcAc/β-HB ratio (Model 2), that ratio showed a
significant positive relationship with both albumin and uric acid. When log β-HB and log AcAc/β-HB ratio were
simultaneously included as independent variables in Model 3, log AcAc/β-HB ratio, but not log β-HB, retained
its independent and significant association in a positive manner with albumin and uric acid, clearly indicating
that arterial AcAc/β-HB ratio is superior to arterial β-HB as a clinically relevant marker for nutritional status in
HD patients.

**Discussion**

Results of the present study demonstrated an independent association of lower arterial AcAc/β-HB ratio, but
not higher arterial β-HB, with reduced levels of albumin and uric acid in serum (Table 3), suggesting that lower
arterial AcAc/β-HB ratio rather than higher arterial β-HB is a clinically relevant measurement for determining
nutritional status in HD patients. Since both lower serum albumin (14) and uric acid (15) have been
established as having an association with higher mortality in HD patients, the present findings suggest
that a lower arterial AcAc/β-HB ratio prior to starting an HD session may be an indicator of risk for malnutrition
in HD patients and thus possibly increased mortality.
Table 3
Multivariate analysis to elucidate factors associated with arterial β-HB and AcAc/β-HB ratio in all HD patients (n = 49)

|                      | Log albumin |                         | Uric acid |                         |
|----------------------|-------------|--------------------------|-----------|--------------------------|
|                      | Model 1     | Model 2                 | Model 3   | Model 1                 | Model 2 | Model 3 |
| Age                  | -0.172      | -0.164                   | -0.165    | 0.058                   | 0.067   | 0.067   |
| Gender (M/F, 1/2)    | -0.0004     | 0.059                    | 0.042     | 0.163                   | 0.221   | 0.217   |
| DM (-/+, 1/2)        | 0.313       | 0.386*                   | 0.368*    | -0.0004                 | 0.073   | 0.068   |
| Log (HD duration)    | -0.256      | -0.300*                  | -0.319*   | 0.310*                  | 0.237   | 0.232   |
| Log β-HB             | -0.092      | –                        | 0.244     | -0.333*                 | –       | 0.061   |
| Log AcAc/ β-HB ratio | –           | 0.300*                   | 0.463*    | –                       | 0.533*  | 0.578*  |
| p(R²)                | 0.0582      | (0.214)                  | 0.0128    | (0.278)                 | 0.0159  | (0.299) |
|                      |             |                          | 0.0159    | (0.299)                 | 0.0404  | (0.230) |
|                      |             |                          |           |                         | 0.0013  | (0.362) |
|                      |             |                          |           |                         | 0.0029  | (0.364) |

p < 0.05 (*) was considered to indicate statistically significant.

An independent association of higher serum β-HB with greater number of cardiovascular events and all-cause mortality was recently shown in Japanese HD patients (4). It is known that β-HB, an energy-rich short-chain (4-carbon) organic acid that can be freely diffused across the cell membrane, is capable of transporting energy to the heart and brain (16). Furthermore, since β-HB is utilized as an energy source in the human heart in individuals either with or without DM (2), it has been suggested that a higher level of arterial β-HB should improve cardiac function by serving as a greater source of energy for the heart (17). The present study clearly showed that a reduction in arterial AcAc/β-HB ratio resulting from higher arterial β-HB, but not increased β-HB by itself, provides a better indicator for poor nutritional status in HD patients. β-HB is formed through the reduction of AcAc in liver mitochondria by 3-hydroxybutyrate dehydrogenase, which requires oxidation of NADH to NAD⁺, indicating an arterial AcAc/β-HB ratio lowered to < 1.0 as a result of highly reduced state of hepatic mitochondria (i.e., the NADH/NAD⁺ ratio) and reflects the reduced capacity of ATP synthesis within hepatic mitochondria (18). It has also been shown that a normal level of arterial AcAc/β-HB ratio is usually > 1.0 (5), while a decrease to < 1.0 has been reported in various diseases associated with malnutrition, such as diabetic ketoacidosis, severe hypoxia, end-stage liver disease, hepatic ischemia, various metabolic disorders, and multiple organ failure (19), and it is also recognized as the metabolic basis for hepatocyte dysfunction (20) as well as lethal outcome in pediatric patients following heart surgery (12). Furthermore, recovery of arterial AcAc/β-HB ratio to > 1.0 was found to accompany normalization of graft metabolic function after liver transplantation (21).

In the present study, the arterial AcAc/β-HB ratio was significantly lower in our DM HD as compared to non-DM patients due to a significantly higher level of arterial β-HB (Table 1). Furthermore, because of a significant reduction of arterial AcAc/β-HB ratio during an HD session in non-DM, but not in DM HD patients, we consider
that the arterial AcAc/β-HB ratio is increased to a lesser degree in DM HD patients during the interdialytic period, mainly due to the smaller decrease in arterial β-HB in those patients. Diabetic ketoacidosis was previously reported to be associated with a decrease in arterial AcAc/β-HB ratio with a relatively high level of generation of β-HB and that insulin treatment decreases serum β-HB long before serum AcAc in diabetic ketoacidosis, resulting in an increased AcAc/β-HB ratio (5). Therefore, it is likely that the insulin deficiency seen in DM HD patients is responsible for higher β-HB and lower AcAc/β-HB ratio, and possibly unremarkable change in arterial AcAc/β-HB ratio during the interdialytic period in contrast with non-DM HD patients. Indeed, the proportion of DM HD patients with an arterial AcAc/β-HB ratio ≤1.0 was significantly greater than that of non-DM HD patients in our study. Notably, the baseline level of casual plasma glucose as well as glycoalbumin was significantly correlated in a negative manner with change in AcAc/β-HB ratio during the HD session only in the non-DM group (Fig. 2).

Increases in arterial AcAc and β-HB can be mainly explained by three mechanisms; (i) induction of alkalosis by dialysis with bicarbonate-containing dialysate, (ii) inhibition of insulin secretion by a reduced level of 125 mg/dL glucose-containing dialysate, and (iii) stimulation of AcAc production by dialysis with acetate-containing dialysate. Therefore, the reduction in plasma glucose during the HD session was greater in the in non-DM patients with worse glycemic control. Since non-DM HD patients retained insulin secretory capacity, those with poorer glycemic control, who are exposed to a higher level of suppressive effect of insulin secretion during an HD session, might cause a greater AcAc/β-HB ratio due to a greater increase in β-HB. In contrast, it is possible that, in DM HD patients with severely impaired insulin secretor capacity, poorer glycemic control did not have an effect on the degree with which insulin secretion was inhibited during the HD session.

It has been shown that alkalosis stimulates lipolysis (22) to produce free fatty acid with a resultant increase in ketone production (23). However, the absence of a correlation of arterial blood pH or bicarbonate either at the baseline or change during the HD session with arterial ketone bodies might clearly negate the involvement of alkalotic change in increased arterial ketone bodies during a session. Although it has been reported that a high concentration of acetate in dialysate increases serum AcAc and β-HB after an HD session (24,25), we found that changes in AcAc and β-HB levels in the present HD patients were not significantly different between those treated with acetate-free Carbostar dialysate and 8.0 mM of acetate-containing Kindaly 4E dialysate, clearly demonstrating that 8.0 mM of acetate in the dialysate did not contribute to an increase in arterial ketone bodies during the HD session.

As demonstrated in multivariate analysis (Table 3), even after the inclusion of presence/absence of DM as an independent variable, arterial β-HB, arterial AcAc/β-HB ratio retained its significant association with serum albumin and uric acid, both markers for nutrition and mortality in HD patients (14, 15). Those results support the notion that lower arterial AcAc/β-HB ratio is a clinically relevant marker for poor nutritional status, also in HD patients.

This study has some limitations. First, the sample size was small and all subjects had Japanese ethnicity. On the other hand, strong points include performance by a single institution and dialysis performed with one dialysate under the same situation managed by the same staff.

**Conclusions**
The present results clearly indicate that alteration of arterial AcAc/β-HB ratio is mainly due to an increase in arterial β-HB. Therefore, together with the independent significance of arterial AcAc/β-HB ratio shown in multivariate analysis, the previously reported association of higher serum β-HB with greater cardiovascular mortality in HD patients might be explained by a reduction in arterial AcAc/β-HB ratio due to higher β-HB, but not by higher serum β-HB itself.

**Abbreviations**

AcAc: acetoacetate; Alb: albumin; ATP: adenosine triphosphate; b-HB: b-hydroxybutyrate; BMI: body mass index; Bw: body weight; Ca: calcium; Cre: creatinine; CRP: c-reactive protein; CVD: cardiovascular disease; DM: diabetes mellitus; GA: glycoalbumin; HD: hemodialysis; IQR: interquartile range; LDL-C: low density lipoprotein cholesterol; NAD: nicotinamide adenine dinucleotide; PG: plasma glucose; Pi: phosphate; SD: standard deviation

**Declarations**

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**Authors’ Contributions**

Research idea and study design: MI, YK. Data acquisition: CH, KN, EY. Data analysis/interpretation: MI, YK, SY, NT. Supervision or mentorship: YF, ME. YO. Wrote the paper: MI.

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**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Conflicts of interest**

The authors declare that they have no relevant financial interests.

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Figures
Changes in arterial AcAc, β-HB, and AcAc/β-HB ratio during 4-hour hemodialysis session in all patients, as well as the non-DM, and DM groups. All HD patients, as well as after dividing into with or without DM, exhibited significant increases in arterial levels of AcAc and β-HB during a single 4-hour HD session. Arterial AcAc/β-HB ratio was significantly reduced in all (C) and non-DM (F), but not in DM HD (I) patients. As a result, though the arterial AcAc/β-HB ratio was significantly higher in non-DM than DM HD patients before the HD session (p=0.0134), it was not significantly different between those groups after the HD session (p=0.1078).
Correlation of change in AcAc/β-HB ratio during HD session with casual PG and glycoalbumin in non-DM HD patients. The change in AcAc/β-HB ratio during the HD session was significantly and negatively correlated with casual PG (A) ($\rho=-0.537$, $p=0.0097$) and glycoalbumin (B) ($\rho=-0.625$, $p=0.0027$) in the non-DM group.