Correction of Acidosis in Hemodialysis Decreases Whole-Body Protein Degradation

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Abstract. Correction of acidosis in hemodialysis (HD) decreases protein degradation. The effect of the correction of chronic metabolic acidosis in chronic renal failure patients treated with HD was determined from the kinetics of infused L-[1-^{13}C]leucine. Six HD patients were studied before (acid) and after (bicarbonate) correction of acidosis (pH: acid 7.36 ± 0.01, bicarbonate 7.40 ± 0.01, $P < 0.005$). Leucine appearance from body protein (PD) and leucine disappearance into body protein (PS) decreased significantly with correction of acidosis (PD: acid 180.6 ± 7.3, bicarbonate 130.9 ± 7.2 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, $P < 0.005$; PS: acid 172.3 ± 6.8, bicarbonate 122.0 ± 6.8 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, $P < 0.005$). There was no significant change in leucine oxidation or plasma amino acid concentrations. These results demonstrate that optimal correction of acidosis in HD is beneficial in terms of protein turnover and may improve long-term nutritional status in HD. (J Am Soc Nephrol 8: 632–637, 1997)

Metabolic acidosis in chronic renal failure (CRF) results from impaired excretion of hydrogen ions derived principally from the metabolism of sulphur-containing amino acids (1). One of the aims of treatment with hemodialysis (HD) is to provide sufficient buffer in the dialysate to correct the acidosis; bicarbonate and, to a lesser extent, acetate are the primary buffers used. Acidosis in CRF increases protein degradation and amino acid oxidation (2,3), impairs glucose tolerance (4), and has adverse affects on bone metabolism (5,6). Most patients are prescribed a bicarbonate buffer in a concentration of 35 mmol/L, but a significant number remain acidotic as evidenced by predialysis bicarbonate values (7,8). Therefore, optimal correction of acidosis in HD may reduce protein degradation (9), and such an effect might be responsible for the increase in lean body mass seen after the optimal correction of acidosis using bicarbonate dialysis (10).

In this study, the hypothesis that optimal correction of acidosis in HD reduces protein degradation and increases lean body mass has been tested by measuring whole-body protein degradation, using primed continuous infusions of the stable isotope L-[1-^{13}C]leucine, and by assessing body composition using anthropometry and measurements of whole-body potassium. These studies have been performed before and after the optimal correction of acidosis by using high-bicarbonate dialysis (HBD).

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Materials and Methods

Subjects

Six stable HD patients were investigated (Table 1). All had evidence of metabolic acidosis at the time they were recruited, with mean total CO_2 (tCO_2) levels of <22 mmol/L. No patient had diabetes mellitus or was taking corticosteroids. Written informed consent was obtained from each subject, and the protocol was approved by the Joint Ethics Committee of the Newcastle Health Authority and the University of Newcastle upon Tyne.

Isotopes

L-[1-^{13}C]leucine (99 mole% ^{13}C) and $NaH^{13}CO_3$(99 mole% ^{13}C) were purchased from Promochem (St. Albans, Herts, UK). They were diluted with normal saline under sterile conditions in the Pharmacy Manufacturing Department of the Royal Victoria Infirmary, Newcastle upon Tyne, UK, and were tested for sterility and pyrogenicity.

Experimental Design

The study consisted of two 4-wk periods separated by a 2-wk washout period. During one period, subjects were prescribed a final dialysate concentration of 35 mmol/L, the standard buffer concentration used in our unit. During the other period, 40 mmol/L bicarbonate was prescribed. No other changes in the dialysis prescription were made. During the washout period, 35 mmol/L bicarbonate was prescribed. Subjects were randomly allocated to the order of treatment. If, after 2 wk on HBD, the target predialysis tCO_2 level of >23 mmol/L was not achieved, oral sodium bicarbonate starting at 2 g per day was prescribed. This was necessary for patients 2 and 5. For the 3 days before the isotope infusion, subjects were asked to keep a food diary; these diaries were subsequently analyzed by a renal dietitian.

At the time of admission for the infusion study, each subject (in stockinged feet) was weighed and his/her height was measured. Skinfold thickness was measured at the biceps, triceps, suprailiac, and subscapular sites. As close as possible to the day of infusion, total body potassium (TBK) was estimated using a shadow shield device (11).

Table 1. Patient details at time of entry into study[a]

Subject	Sex	Age	tCO_2 (mmol/L)	Renal Disease
1	M	72	18.0	Unknown
2	F	42	17.4	GN
3	M	30	21.5	CPN
4	F	22	16.8	CPN
5	M	70	19.0	Unknown
6	M	40	18.5	GN

[a] GN, glomerulonephritis; CPN, chronic pyelonephritis.

L-[1–13C]Leucine Infusion

After an overnight fast, subjects were admitted to a metabolic research laboratory on a midweek nondialysis day, *i.e.*, the day after a standard dialysis session. At 8.00 a.m., a cannula (18G Vasculon; Viggo-Spectrometer, Sweden) was placed retrogradely in a dorsal hand vein for blood sampling. The patient's hand was then placed in a heated chamber, which was maintained at 60°C, to allow sampling of arterialized blood (12,13). A second cannula (18G Venflon; Viggo-Spectrometer) was placed in a forearm vein in the contralateral arm for the primed constant infusion of L-[1-^{13}C]leucine (Model 940 infusion pump; Harvard Apparatus, Millis, MA). Both cannulas were inserted under local anesthetic (1% lignocaine).

Samples of blood and expired air were collected 30, 15, and 1 min before the infusion of L-[1-^{13}C]leucine, to determine the basal ^{13}C enrichment of plasma α-ketoisocaproate (KIC) and CO_2. Samples were also taken for measurement of electrolyte, plasma amino acid, glucose, and intermediary metabolite levels.

Priming doses of L-[1-^{13}C]leucine (0.5 mg/kg) and $NaH^{13}CO_3$ (0.087 mg/kg) were then given, and a 4-h constant infusion of L-[1-^{13}C]leucine (0.5 $mg \cdot kg^{-1} \cdot h^{-1}$) commenced.

During the last hour of the infusion, five heparinized blood samples were obtained at 15-min intervals and centrifuged at 7°C, and plasma aliquots were stored at −70°C until subsequent analysis for ^{13}C enrichment for plasma KIC. At the same time points during the last hour of the infusion, five samples of expired air were collected at 15-min intervals into evacuated glass Vacutainer tubes (Becton Dickinson, Rutherford, NJ) and stored at room temperature until analyzed for ^{13}C enrichment of CO_2.

Immediately before the last hour of the constant infusion, carbon dioxide production (VCO_2) was measured continuously for 30 min by open-circuit indirect calorimetry, using a ventilated hood system (Deltatrac MBM-100; Datex Instrumentation, Helsinki, Finland).

Three separate samples of arterialized blood were collected at 30-min intervals during the last hour of the infusion for the measurement of PCO_2, PO_2, and pH values.

Analytic Methods

The trimethylsilylquinoxalinol derivative was created as previously described (14). Electron ionization–gas chromatography–mass spectrometry (Finnigan 1020B quadrupole instrument; MAT, Hemel Hempstead, UK) was used to measure the ions (mass per unit charge = 232 and 233) (15). The ^{13}C enrichment of CO_2 in expired air was determined by isotope ratio mass spectrometry (Finnigan Delta D instrument) (16).

Plasma amino acids were analyzed using a Beckman 6300 amino acid analyzer (Beckman, Palo Alto, CA), using ion-exchange chromatography and post-column detection after reaction with ninhydrin. Blood samples for glycerol, pyruvate, acetoacetate, and β-hydroxybutyrate were taken in 5 mL of chilled $HClO_4$ (500 mM) and assayed by an automated fluorometric enzymatic method (17). Electrolyte levels were measured on a Technicon SMAC system, and glucose was measured with a glucose oxidase electrode.

PCO_2, PO_2, and pH values in arterialized blood were measured with a blood gas analyzer (Model 178; Dow Corning, Corning, NY).

Calculations

The model and calculations of leucine metabolism have been described previously (18).

Leucine carbon flux was calculated from the enrichment of KIC by ^{13}C because this has been reported to give a more accurate estimate of the intracellular enrichment of leucine (19) in muscle.

Anthropometric Assessment

Skinfold measurements were taken at four sites—the biceps, triceps, suprailiac, and subscapular areas.

Each measurement was taken three times, and the mean of the results was recorded. Body density was calculated from the regression equations of Durnin and Womersley (20). Percentage of body fat was calculated from the equation developed by Siri (21). Fat-free body mass was estimated from the percentage of body fat and body weight.

Statistical Analysis

Rates are expressed as micromoles per kilogram per hour. Values are reported as means ± SE and analyzed using paired *t* tests. $P < 0.05$ was considered statistically significant.

Results

Plasma tCO_2 and pH

The pH values of arterialized blood and venous tCO_2 increased after correction of acidosis (pH: acidotic 7.36 ± 0.01 *versus* bicarbonate 7.40 ± 0.01, $P < 0.005$; tCO_2: acidotic 18.5 ± 0.7 *versus* bicarbonate 24.8 ± 0.7 mmol/L, $P < 0.001$) (Table 2).

Glucose and Intermediate Metabolites

There were no significant differences in the postabsorptive values of glucose and intermediary metabolites in the acidotic and corrected states (Table 3).

Table 2. tCO_2 and pH values before (acid) and after correction of acidosis (HCO_3), taken at time of leucine infusion on a midweek nondialysis day[a]

Subject	tCO_2 (mmol/L)		pH	
	Acid	HCO_3	Acid	HCO_3
1	18.0	27.2	7.38	7.43
2	17.4	23.4	7.36	7.38
3	21.5	25.6	7.38	7.40
4	16.8	23.6	7.40	7.46
5	19.0	26.3	7.31	7.35
6	18.5	23.0	7.35	7.38
Mean ± SE	18.5 ± 0.7	24.8 ± 0.7	7.36 ± 0.01	7.40 ± 0.01

[a] Statistical significance: tCO_2, acid *versus* HCO_3, $P < 0.001$; pH, acid *versus* HCO_3, $P < 0.005$.

Table 3. Glucose and intermediate metabolite values[a]

	Acid	Bicarbonate
Glucose (mM)	4.8 ± 0.6	5.2 ± 0.7
Pyruvate (μM)	73.2 ± 12	62.0 ± 8.8
Lactate (μM)	812 ± 240	720 ± 160
3-Hydroxybutyrate (μM)	50.4 ± 24.2	61.5 ± 33.6
Glycerol (μM)	54.0 ± 8.8	51.9 ± 7.9

[a] Values are means ± SE.

Leucine Kinetics

Leucine appearance from protein breakdown and leucine disappearance into body proteins were significantly decreased after correction of acidosis (Table 4). There was no significant change in leucine oxidation after correction of acidosis.

Plasma Amino Acids and Plasma Urea

There were no significant changes in the postabsorptive amino acid levels after correction of acidosis (Table 5). The plasma urea level was significantly lower after the correction of acidosis (acid: 26.5 ± 3.5 *versus* bicarbonate 22.6 ± 2.8 mmol/L, $P < 0.01$).

Dietary Intake

There was no significant change in protein or carbohydrate intake after correction of acidosis (carbohydrate: acid 1380 ± 156 *versus* bicarbonate 1430 ± 168 kcal/24 h; protein: acid 71.0 ± 2.9 *versus* bicarbonate 76.4 ± 6.9 g/24 h).

Total Body Potassium

There was no significant change in lean body mass as assessed by TBK measurements before and after the correction of acidosis (Table 6).

Anthropometric Assessment and Body Mass Index

There was no significant change in body density, fat-free mass, or body mass index after the correction of acidosis (Table 6).

Table 5. Postabsorptive amino acid concentration (μmol/L)[a]

Amino Acid	Acid	HCO_3
Alanine	273 ± 4	249 ± 8
Arginine	88 ± 1	88 ± 1
Asparagine	24 ± 6	18 ± 7
Aspartate	16 ± 8	25 ± 4
Citrulline	187 ± 12	197 ± 16
Glutamate	59 ± 10	88 ± 20
Glutamine	563 ± 17	596 ± 60
Glycine	515 ± 30	596 ± 52
Histidine	43 ± 7	56 ± 14
Hydroxyproline	51 ± 6	40 ± 5
Isoleucine	43 ± 5	37 ± 12
Leucine	68 ± 14	98 ± 20
Lysine	130 ± 10	131 ± 20
Methionine	23 ± 8	22 ± 4
3-Methylhistidine	25 ± 11	30 ± 10
Ornithine	114 ± 25	89 ± 18
Phenylalanine	78 ± 16	90 ± 16
Proline	320 ± 50	360 ± 46
Serine	123 ± 8	112 ± 6
Taurine	29 ± 8	42 ± 10
Threonine	145 ± 36	103 ± 14
Tyrosine	46 ± 22	53 ± 14
Valine	150 ± 22	157 ± 12

[a] Values are means ± SE.

Discussion

This study examined the effect on leucine kinetics and body composition of correcting chronic metabolic acidosis in HD. The kinetics of a primed constant infusion of L-[1-^{13}C]leucine have been used as a model of whole-body protein metabolism. For estimating whole-body protein turnover, L-[1-^{13}C] leucine is the optimal amino acid to use. It is a stable, nonradioactive tracer and an essential amino acid, and therefore cannot be synthesized *de novo*; it is principally metabolized in skeletal muscle, the body's major protein store; and it has a regulatory

Table 4. Leucine kinetics before and after the correction of acidosis[a]

Subject	PD		PS		O	
	Acid	HCO_3	Acid	HCO_3	Acid	HCO_3
1	189.3	135.6	184.8	127.6	4.9	8.0
2	190.6	132.4	181.2	124.8	9.4	7.6
3	185.7	110.2	178.3	105.5	7.4	4.7
4	165.6	151.6	158.4	141.2	7.2	10.4
5	200.8	144.8	190.1	134.6	10.7	10.2
6	152.7	109.2	141.0	98.5	11.7	10.7
Mean ± SE	180.8 ± 7.3	130.6 ± 7.2	172.3 ± 7.6	122.0 ± 6.8	8.5 ± 1.0	8.6 ± 1.0

[a] Values shown are for leucine derived from protein degradation (PD), leucine incorporated into body protein via synthesis (PS), and leucine oxidation (O) given in $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. Statistical significance: PD, acid *versus* bicarbonate, $P < 0.005$; PS, acid *versus* bicarbonate, $P < 0.005$; O, acid *versus* bicarbonate, P = not statistically significant.

Table 6. Anthropometric data, body mass index, and whole-body potassium

Subject	BMI (kg/m^2)		%Fat		FFM (kg)		TBK (g)	
	Acid	HCO_3	Acid	HCO_3	Acid	HCO_3	Acid	HCO_3
1	20.7	20.6	1.9	2.7	62.3	61.3	97.8	95.7
2	31.5	31.4	30.9	31.0	54.4	54.2	92.4	93.0
3	25.2	25.5	18.6	15.8	55.3	58.0	88.6	88.0
4	20.9	20.5	22.8	22.7	37.7	37.0	80.4	80.0
5	21.4	21.4	20.3	20.4	49.4	49.3	93.2	94.0
6	25.3	25.5	26.6	26.7	57.5	60.0	141.6	140.2
Mean ± SE	24.2 ± 1.7	24.2 ± 17	20.2 ± 4.1	19.9 ± 4.0	52.7 ± 3.4	53.3 ± 3.7	99.0 ± 8.8	98.5 ± 8.6

[a] BMI, body mass index; %Fat, percentage of body fat; FFM, fat-free mass; TBK, total body potassium. Statistical significance: BMI, acid *versus* bicarbonate, not statistically significant; %Fat, acid *versus* bicarbonate, not statistically significant; FFM, acid *versus* bicarbonate, not statistically significant; TBK, acid *versus* bicarbonate, not statistically significant.

effect on other branched-chain amino acids. This model, shown in Figure 1 and originally proposed by Golden and Waterlow (22), shows that the flux of an amino acid Q in a steady-state condition is equivalent to dietary intake (I) plus the input from protein degradation (B) and the input from synthesis (N). Flux is also equal to the incorporation of amino acid into body protein (S) plus oxidation (C) and other forms of metabolism (M). By using labeled leucine infusion, the model is simplified because leucine is an essential amino acid (therefore, $N = 0$) and is irreversibly metabolized to α-ketoisocaproic acid (15) (therefore, $M = 0$). If the study is performed under fasting condition, $I = 0$, and therefore leucine flux $(Q) = B = S + C$.

In this study, to correct acidosis, the concentration of bicarbonate in the dialysate was increased from 35 to 40 mmol/L, and oral sodium bicarbonate (2 g per day) was added if tCO_2 was persistently < 23 mmol/L. The results demonstrate that correction of chronic acidosis in HD is associated with a reduction in PD and PS but not O (oxidation). There was no change in percentage of body fat or in lean body mass after the correction of acidosis.

Other studies have suggested that protein degradation decreases with the correction of acidosis. Nitrogen balance improves when the acidosis associated with CRF (23) and prolonged fasting (24) is corrected, and L-[1-^{13}C]leucine infusion studies have confirmed that correcting acidosis in CRF patients not yet requiring dialysis reduces PD, PS, and O (2). Although it is known that the HD process itself is a net catabolic event (25), this is the first study to demonstrate that correction of acidosis in HD decreases protein degradation. We speculate that the correction of acidosis, and its beneficial effect on protein metabolism, may contribute to the increase in body mass seen in other studies (10). We were unable to confirm that HBD is associated with an increase in fat-free mass or TBK in this study, probably because of the short duration of HBD (4 wk). This small a period of time is unlikely to show major changes in body composition but was chosen in this study because it minimizes the possible effects of other factors on leucine kinetics (such as a change in dietary protein intake, or an intercurrent illness).

Correction of acidosis in these subjects did not alter the concentration of plasma amino acids; this is consistent with the study by Reaich *et al.* (2). In keeping with other studies, there is a fall in plasma urea after the correction of acidosis (9,23), reflecting the reduction in protein degradation.

In this study, correction of acidosis was not accompanied by a decrease in amino acid oxidation. As a result, the balance between whole-body protein synthesis and breakdown was unchanged. This is at variance with previous findings in pre-

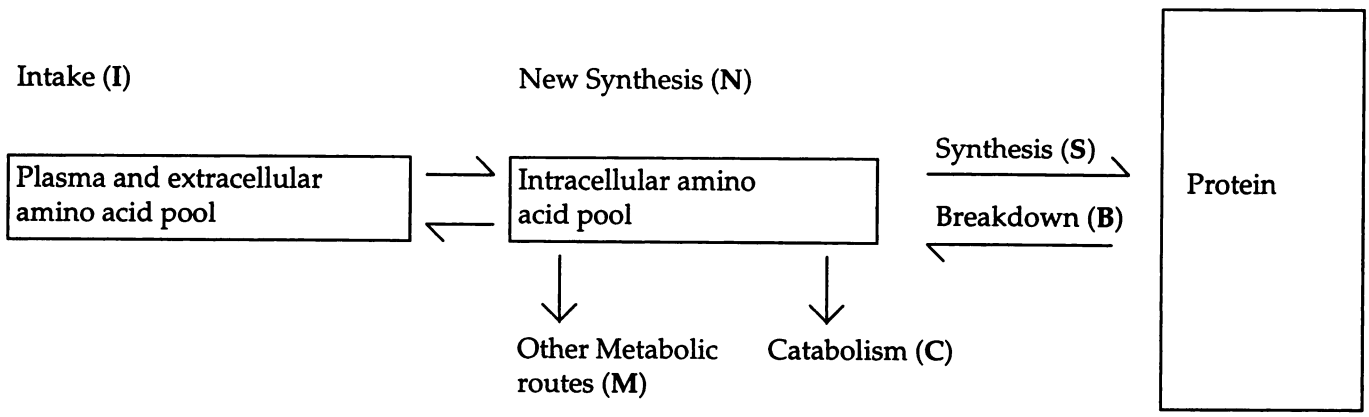

Figure 1. Model of whole-body protein turnover. Flux $Q = I + B + N = S + M + C$.

dialysis patients (2,4). Because our study was undertaken while patients were fasting, it is impossible to extrapolate to the full 24-h period, and it is the 24-h balance between whole-body protein breakdown and synthesis that results in changes in body protein and urea clearance. Whether correction of acidosis changes the response of protein metabolism during feeding has not yet been addressed. It is interesting to note that in a study of branched-chain amino acid metabolism conducted in CRF and HD subjects (26), leucine oxidation—although diminished in CRF—was no different in HD compared with healthy control subjects (O: CRF 7.58 ± 2.05, HD 13.16 ± 2.3; healthy control subjects 12.42 ± 3.29 $\mu mol^{-1} \cdot kg^{-1} \cdot h^{-1}$). Although the acid-base status of the two groups was not stated in the article, it is possible that HD has a direct effect on leucine oxidation and that this effect is unrelated to acid-base perturbations.

What is the mechanism for this acidosis-induced catabolism seen in HD? Animal studies suggest that acidosis activates the ATP-dependent ubiquitin proteolytic pathway (27). Whether a similar mechanism operates in HD patients remains to be determined.

We have demonstrated that correction of acidosis in HD is associated with decreased protein degradation. This finding is likely to be of long-term benefit to HD patients because poor nutritional status is associated with increased morbidity and mortality (28). What, therefore, is the optimal acidosis correction procedure for HD patients? Acidosis is better corrected in patients on long-term bicarbonate dialysis than in those patients undergoing acetate dialysis (29). The predialysis tCO_2 level is a better indicator of acid-base balance in HD patients because at the onset of renal replacement therapy with HD, the buffer deficit accumulated during a period of positive hydrogen balance must be replenished (30). During this time, the predialysis tCO_2 level will increase only slightly, if at all, even though an optimal postdialysis tCO_2 is eventually reached (31). The postdialysis tCO_2 level must also be optimized to prevent lethargy, mental confusion, and anxiety (32), which are associated with alkalosis. For these reasons, the bicarbonate dialysate concentration should be individualized to increase predialysis tCO_2 levels to >23 mmol/L. This may require an increase in dialysate bicarbonate concentration and administration of oral bicarbonate supplements.

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