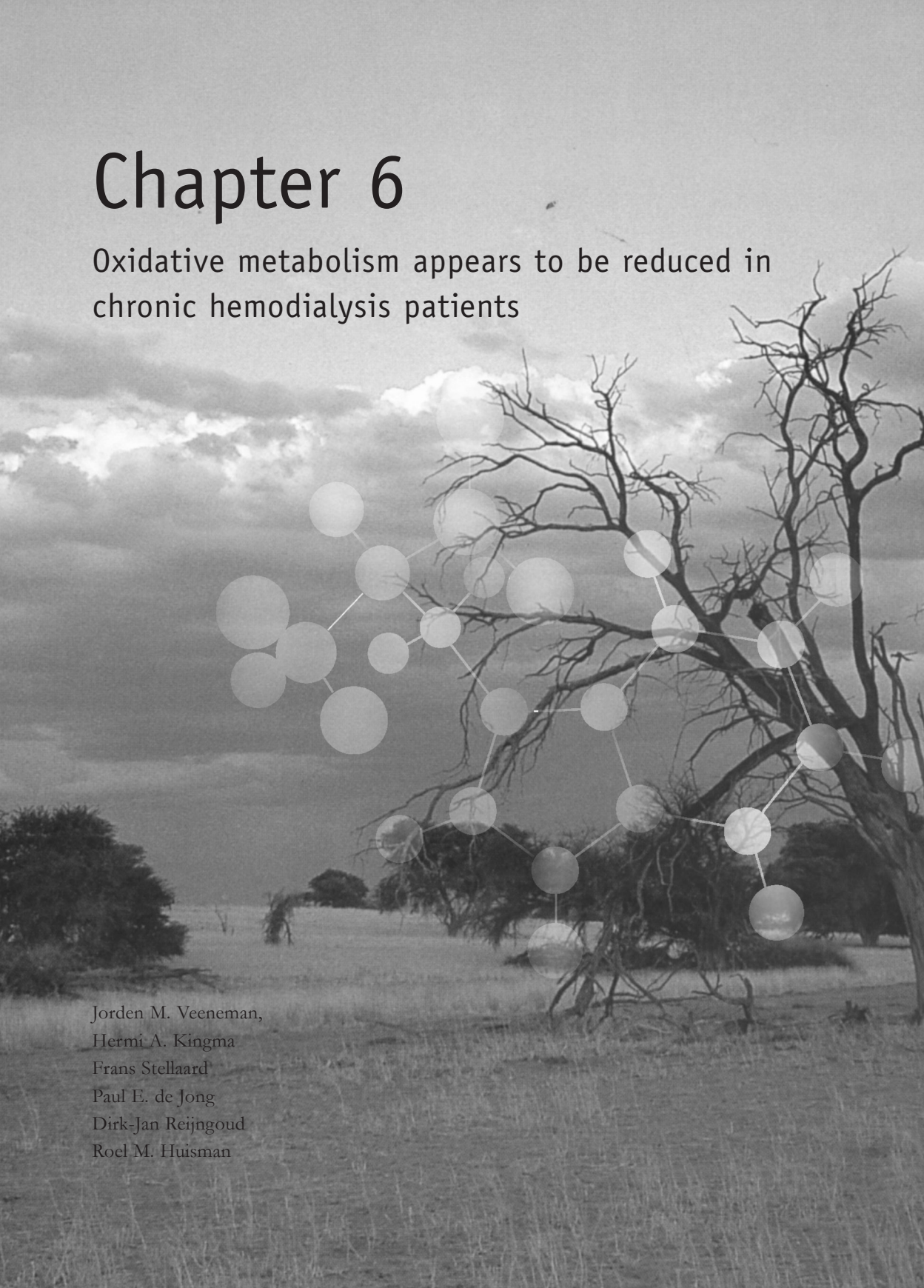
Chapter 6

Oxidative metabolism appears to be reduced in chronic hemodialysis patients



Jorden M. Veeneman,
Hermi A. Kingma
Frans Stellaard
Paul E. de Jong
Dirk-Jan Reijngoud
Roel M. Huisman

Abstract

Background. As part of a study of whole body protein metabolism in hemodialysis patients, we obtained values of whole body bicarbonate production in control subjects and hemodialysis patients before and during dialysis using stable isotopically labelled bicarbonate. Indirect calorimetry measurements have shown a normal or increased energy expenditure in hemodialysis patients, which has been used to explain the malnutrition present in many of these patients. This method however, becomes inaccurate when the dynamics of whole body bicarbonate production change during measurement, as is the case in hemodialysis patients during dialysis.

Methods. Six control subjects (C), 9 patients, on a non-dialysis day (HD-) and 8 during a hemodialysis session (HD+) respectively were measured using biocompatible membranes. The clinical condition of the patients had been stable for 6 months prior to the study protocol. Whole body bicarbonate production was measured by primed-constant infusion of $NaH^{13}CO_3$ of 1 h and ^{13}C-abundance of expired CO_2 was measured by isotope ratio mass spectrometry.

Results. CO_2 production was 141 ± 12, 123 ± 11*, and 148 ± 19 μmol kg^{-1} min^{-1} for C, HD- and HD+ respectively (* = $p<0.05$ compared to control subjects). Values of energy expenditure were derived and were 29.1 ± 2.4, 24.9 ± 2.1*, and 32.6 ± 2.0 kcal kg^{-1} day^{-1} respectively (* = $p<0.05$ compared to control subjects).

Conclusion. Whole body oxidation in hemodialysis patients is reduced compared to control subjects. During dialysis, bicarbonate turnover is increased and expiration of CO_2 increased due to the influx of bicarbonate from the dialyzer. Increased energy expenditure is no likely cause of energy malnutrition in chronic hemodialysis patients.

Keywords: calorimetry, energy expenditure, and stable isotope.

Introduction

Malnutrition is a strong predictor of mortality and morbidity in chronic hemodialysis patients (1). Nutritional status of these patients is affected by low food intake, more frequent episodes of illnesses, and dialysis related factors such as amino acid losses and consequent changes in whole body metabolism. On the other hand, it is known that hemodialysis patients have the ability to maintain a stable body weight over long periods of time (2). Besides a reduced energy intake, increased energy expenditure might also contribute to the observed malnutrition in these patients. There are several studies indicating that energy expenditure in hemodialysis patients is not different from control subjects (3-5). In one study (6), energy expenditure was increased in hemodialysis patients during dialysis. The authors concluded that this phenomenon plays a role in protein-energy malnutrition seen in these patients. In these 4 studies indirect calorimetry was used to assess energy expenditure. This method measures whole body O_2 consumption and CO_2 production, which are then converted into energy expenditure values (7). Although this method can easily be applied, interpretation of results becomes complex when changes take place in dynamics of whole body bicarbonate metabolism during the measurement. Measurement of energy expenditure by ^{13}C-bicarbonate tracer methodology offers an alternative approach. Different from indirect calorimetry, the ^{13}C-bicarbonate tracer method depends on the dynamics of individual bicarbonate fluxes constituting whole body CO_2 production. This aspect might offer a possibility to monitor changes in bicarbonate fluxes in HD patients during dialysis with bicarbonate containing solutions.

As part of earlier studies, we used an independent infusion of $NaH^{13}CO_3$ to estimate whole body bicarbonate production prior to infusion of [1-^{13}C]-valine, which was used in the calculation of whole body protein metabolism (8). This experimental set-up provided us also with a data set on whole body bicarbonate production in HD patients when dialysing and on a non-dialysis day, and offered us the possibility to derive estimates of energy expenditure. These preliminary data allowed us to address the following questions: (i) how does dialysis change the dynamics of whole body bicarbonate production in HD patients. (ii) Is energy expenditure increased in HD patients when compared to healthy control subjects? (iii) Does dialysis invoke an increase in whole body oxidation and energy expenditure in HD patients?

Subjects and Methods

As part of a study on protein metabolism, we separately infused ^{13}C-labeled bicarbonate during a 1-hour period in control subjects and stable hemodialysis patients in the absence and presence of dialysis (8). In this report we will use the data from the ^{13}C-labeled bicarbonate infusion period only to calculate the bicarbonate fluxes constituting whole body bicarbonate metabolism and derive preliminary estimates of the energy expenditure of HD patients under these conditions.

Study subjects

The recruitment and clinical and demographic characteristics of the healthy control subjects and hemodialysis patients have been described in detail in previous publications (8). Six control subjects, 9 patients during the HD- protocol, and 8 patients during the HD+ protocol were studied. The medical ethical committee from the University of Groningen approved all studies and written consent was obtained from all participants. No patients received hormone or immunosuppressive agents for 6 month before the study protocol. The patients were dialysed with low flux biocompatible dialyzers for 4 hours three times weekly. Blood flow ranged from 250 to 350 ml min^{-1} (300 ± 35 ml min^{-1}) and dialysate flow was 500 ml min^{-1}. Standard dialysate with 140 mEq Na^+, and 34 mEq bicarbonate was used for all patients. Glucose content in dialysate was 5.6 mM in 3 patients and 11,2 mM in 5 patients for the patients studied during the HD+ protocol. Dialysate temperature was 37 ºC. Residual renal function was 3 ml min^{-1} or less.

Materials

$NaH^{13}CO_3$, >99% atom percent enrichment was purchased from Cambridge Isotope Laboratories, Inc (Andover, MA, USA). Chemical purity was confirmed before use. Pyrogen and bacteria free solutions of $NaH^{13}CO_3$ were prepared in sterile saline by the hospital dispensary the afternoon before the study day.

Experimental design

Three weeks prior to the study, all subjects visited the Dialysis Centre Groningen for a dietary interview and instructions on dietary recording. All subjects consumed a constant protein diet (1.0 g kg^{-1} day^{-1}) for three weeks and were requested to refrain from any physical heavy exercise (this was especially important for the control subjects). During the study day, overnight fasted subjects remained seated in supine position from 15 minutes before the experiment until the end of the study period.

HD- protocol

Whole body bicarbonate production was measured during a mid-week day for control subjects and a mid-week non-dialysis day for HD patients. Subjects were fasted overnight for 10 to 12 hours. Subjects were admitted to the hospital research unit at approximately 7.30 AM. A catheter was inserted into the dorsal vein of the hand of the shunt arm to collect two baseline blood samples. Two single breath samples (9) were taken simultaneously with the blood sample to measure initial background breath ^{13}C-abundance. The $NaH^{13}CO_3$ infusion was started at 8.00 AM (5 μmol kg^{-1} bolus followed by a continuous infusion of 0.083 μmol kg^{-1} min^{-1}). Four breath samples were taken between 30 and 60 minutes after the start of the $NaH^{13}CO_3$ infusion at 10-minute interval. From these data, bicarbonate turnover was estimated while the study day continued for the measurement of protein metabolism, which is described elsewhere (8).

HD+ protocol

Effects of dialyzer on ^{13}C abundance. With regard to the effects of dialysis on ^{13}C-abundance of blood bicarbonate 2 observations were made, pertinent to the modelling of whole body bicarbonate metabolism and its measurement by label dilution. First, dialysis by itself was found to increase the ^{13}C abundance in expired air gradually because of entrance of bicarbonate with a high natural ^{13}C-abundance from the dialysate (δ= –4.0 ± 0.3 ‰ versus Pee Dee Belemnite (PDB) standard). Therefore, background ^{13}C-abundance in expired breath was measured independently in 5 patients during a dialysis session without tracer infusion prior to the infusion studies. Starting at δ=–25.4±1.2‰, background ^{13}C abundance increased rapidly till δ= –21.9±0.9‰ at 30 min after which it remained constant (δ= –21.4±0.7‰ at 60 min). From these measurements a curve was constructed depicting the fractional change in background ^{13}C abundance relative to the initial background ^{13}C-abundance as a function of time. Between 30 and 60 min after the start of the dialysis the background ^{13}C-abundance reached steady state at 0.84 ± 0.01 of the initial background ^{13}C abundance.

Second, it was found that the efficiency of the dialyzer to equilibrate blood and dialysate bicarbonate was limited. We observed a limited increase in background ^{13}C-abundance of blood bicarbonate from –25.4±1.2‰ proximal to the dialyzer to –11.1±0.3‰ distally, instead of –4.0±0.3‰, which was the background ^{13}C-abundance of dialysate bicarbonate. This indicates that effectively, only 67±3% of blood bicarbonate was replaced by dialysate bicarbonate. The consequence for the clearance of infused $NaH^{13}CO_3$ will be that also 67% of the infused-labelled bicarbonate will be replaced by dialysate bicarbonate.

In other words, venous blood still holds an amount of labelled bicarbonate equivalent to 0.33 times the infusion rate of $NaH^{13}CO_3$, which is returned to the body.

^{13}C bicarbonate infusion experiment. Prior to dialysis, patients were fasted for 12 to 14 hours and all patients were dialysed 2 days before the experimental hemodialysis session. The experimental dialysis session started at approximately 12.00 PM. A catheter was inserted in the shunt and baseline blood samples were drawn. Two single breath samples were taken simultaneously with the blood sample to measure initial background ^{13}C abundance in expired air. The continuous infusion of $NaH^{13}CO_3$ was administered for one hour in the venous side of the artificial kidney (5 μmol kg^{-1} bolus followed by 0.083 μmol kg^{-1} min^{-1}). Four breath samples were taken between 30 and 60 minutes after the start of the $NaH^{13}CO_3$ infusion at 10 minute interval. An arterial blood sample was drawn at 0 minutes and arterial and venous blood samples were drawn at 30 and 60 minutes to measure bicarbonate concentration and enrichment in whole blood. True blood flow was measured using ultrasound and dilution (transonic-flow QC, Ithaca, NY, USA).

Analytical procedures

Four ml of blood was drawn for each sample in liquid heparinized vacuum tubes and was immediately analysed for the concentration and enrichment of bicarbonate. Concentration of $NaHCO_3$ was measured using an automated blood gas analyser (Radiometer ABL 700, Copenhagen, Denmark) while enrichment was measured by injecting 1 ml of whole blood into a gas testing tube (Exetainer 10 ml, Labco Limited, High Wycombe, UK), which was stored at room temperature until analysis. Breath sample collection was performed using a gas testing tube as described earlier (9). Subjects exhaled normally through a straw into the glass container. After exhalation was complete, tubes were closed immediately and stored at room temperature until analysis.

Measurement of ^{13}C-abundance in expired breath

Measurement of $^{13}CO_2$ tracer ratio was performed directly in breath with a Finnigan TracerMat (Finnigan MAT, Bremen Germany) continuous flow isotope ratio mass spectrometer as described by Vonk *et al.* (9). Values of ^{13}C enrichments in expired CO_2 were obtained as δ-values relative to PDB limestone and were subsequently converted in atom percent enrichment (APE), expressed as %.

Evaluation of primary data

Total rate of appearance of unlabeled bicarbonate into plasma, R(a), was calculated according to:

$$R(a) = \{APE_{HCO3}(infusate) \text{ X } i_{HCO3}\} / \{APco_2(plateau) - APco_2(background)_{ini}\} \quad eq(1)$$

In which, APE_{HCO3}(infusate) is the isotopic ^{13}C-enrichment of bicarbonate in the infusate in atom percent enrichment (99 %), i_{HCO3} is the $NaH^{13}CO_3$ infusion rate (0.083 µmol ^{13}C kg^{-1} min^{-1}), $APco_2$(plateau) is the isotopic abundance of $^{13}CO_2$ in expired air in atom percent during last 30 minutes of the HCO_3 infusion, while $APco_2(background)_{ini}$ is the initial background ^{13}C abundance of CO_2 in expired air in atom percent before the start of the infusion.

During dialysis, R(a) was calculated according to:

$$R(a) = \{APE_{HCO3}(infusate) \text{ x } d_{HCO3}\} / \{(APco_2(background*) - APco_2(plateau)\} \quad eq(2)$$

In which d_{HCO3} is the rate of delivery of $NaH^{13}CO_3$ corrected for the finite exchange of blood for dialysate bicarbonate and $APco_2$(background*) is the background ^{13}C abundance at steady state during dialysis corrected for the change induced by dialysis in atom percent.

The rate of delivery of bicarbonate was calculated according to:

$$d_{HCO3} = 1.33 \text{ X } i_{HCO3} \quad eq(3)$$

In which 1.33 is the factor that accounts for limited ability of the dialyzer to exchange blood and dialysate bicarbonate. The value of $APco_2$(background*) was calculated according to:

$$APco_2(background*) = 0.84 \text{ X } APco_2(background)_{ini} \quad eq(4)$$

In which 0.84 is the fractional correction factor derived from the measurement of the change in background ^{13}C abundance during dialysis in the absence of infusion of labelled bicarbonate (see Subjects and Methods).

Calculation of bicarbonate fluxes

In figure 1, a schematic representation of the steady-state isotope dilution model of whole body bicarbonate homeostasis is shown including the situation of HD patients during a dialysis session. In this model 3 pools were defined i.e. a plasma bicarbonate pool, a dialyzer pool, and a large pool comprising bicarbonate fixed in metabolically active

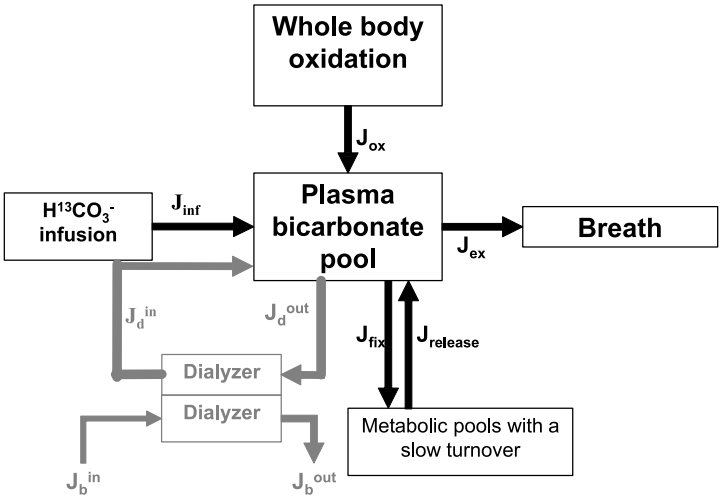


Figure 1. *Model of the flux of bicarbonate in the human body. Whole body oxidation produces all the bicarbonate in the plasma pool. In control subjects and hemodialysis patients on a non-dialysis day, bicarbonate fluxes are depicted in black while during dialysis, there is a second influx of bicarbonate from the dialysis machine which is represented in grey.*

pools which release bicarbonate slowly. The following fluxes are assumed to exist, J_{ox} (substrate oxidation), J_{exp} (expiration of CO_2), J_{fix} (fixation of HCO_3- and CO_2 in pools with a relatively slow turnover), $J_{release}$ (the release of bicarbonate from these pools), Jd^{out} (the flux of bicarbonate out of the plasma bicarbonate pool into the dialyzer), Jd^{in} (the flux of bicarbonate from the dialyzer into the plasma bicarbonate pool), Jb^{out} (the flux of bicarbonate in dialysate out of the dialyzer), and Jb^{in} (the flux of bicarbonate from the dialyzer into dialysate). J_{inf} is the infusion of $NaH^{13}CO_3$, which is less than 2 ‰ compared to the other bicarbonate fluxes, thus it is neglected in the actual calculations. Steady state was assumed to exist between 30 and 60 minutes of the measurement. This implies that for each of the compartments the following relation holds (10):

$$\sum_i J_i = 0 \qquad \text{eq(5)}$$

in which J_i is any flux into or out of the compartment under consideration. For the plasma bicarbonate pool:

$$J_{inf} + J_{release} + Jd^{in} + J_{ox} - J_{fix} - Jd^{out} - Jex = 0 \qquad \text{eq(6)}$$

For the dialyzer:

$$Jd^{out} + Jb^{in} - Jd^{in} - Jb^{out} = 0 \qquad \text{eq(7)}$$

For the large pool of bicarbonate fixated in pools with a slow turnover,

$$J_{fix} - J_{release} = 0 \qquad \text{eq(8)}$$

In this model R(a) is related to the influxes of unlabeled bicarbonate into plasma as follows:

$$R(a) = J_{inf} + J_{release} + Jd^{in} + J_{ox} \qquad \text{eq(9)}$$

A separate problem concerns recovery of infused ^{13}C-bicarbonate in expired air and how to accommodate this observation in the bicarbonate isotope dilution model presented in Fig 1. In measurements in fasted control subjects, 77 % of the infused ^{13}C-bicarbonate label could be recovered as $^{13}CO_2$ in expired air during a short-term ^{13}C-bicarbonate infusion (11). The remainder of 23 % is presumably removed from the plasma bicarbonate pool into pools with a slow turnover from which the label does not reappear into the circulation. This flux of irreversibly removed ^{13}C-bicarbonate is assumed to be associated with J_{fix}. In order to maintain steady state in bicarbonate pools with a slow turnover of bicarbonate this flux needs to be compensated. This compensatory flux of unlabeled bicarbonate from these large body pools of bicarbonate into plasma is represented by $J_{release}$. As a consequence, an empirical relationship between R(a) and J_{fix} can be derived to account for the irreversible loss of infused ^{13}C-bicarbonate:

$$J_{fix} = J_{release} = 0.23 \text{ X } R(a) \qquad \text{eq(10)}$$

During HD- protocol

When measurements are made in both control subjects and patients in the absence of dialysis, Jd^{in} and Jd^{out} do not exist. The relation between R(a), J_{ox} and J_{exp} can be derived starting from eq (5), substituting eq (9) and ignoring J_{inf}:

$$J_{ox} = J_{exp} = 0.77 \text{ X } R(a) \qquad \text{eq(11)}$$

During HD+ protocol

By combining eq (9) and eq (10) and rearranging a relation can be derived between J_{fix}

and J_{ox}:

$$J_{fix} = J_{release} = 0.30 \text{ X } J_{ox} \quad \text{eq(12)}$$

in which 0.30 is the constant 0.23 divided by 0.77. We will assume that this relation holds under the HD(+) protocol, as it does for the measurements under the HD(-) protocol. The relation between R(a), J_{ox} and J_{exp} can now be derived by substituting eq (11) in eq (5) and rearranging:

$$J_{ox} = \{R(a) - J_d^{in}\} \text{ X } 0.77 \quad \text{eq(13)}$$

$$J_{exp} = \{R(a) - J_d^{out}\} - 0.30 \text{ X } J_{ox} \quad \text{eq(14)}$$

with

$$J_d^{out} = [HCO_3]art \text{ X } flow_{art} \quad \text{eq(15)}$$

In which $[HCO_3]$art is the concentration of HCO_3 in arterial blood in mM and $flow_{art}$ is the blood flow from the patient to the dialyzer in ml min^{-1} and

$$J_d^{in} = [HCO_3]_{ven} \text{ X } flow_{ven} \quad \text{eq(16)}$$

In which $[HCO_3$-]ven is the concentration of HCO_3 in venous blood in mM and $flow_{ven}$ is the bloodflow from the dialyzer into the patient defined as $flow_{art}$ minus the ultrafiltration in ml min^{-1}. J_d^{in} and J_d^{out} were expressed as μmol kg^{-1} min^{-1}.

Energy Expenditure

With the calculated values of J_{ox} and J_{exp}, one can estimate the influence of the dialysis procedure on energy expenditure. Energy expenditure was calculated using the abbreviated Weir Equation adopted from Wolfe (12):

$$EE = [3.9 \text{ X } (VO_2) + 1.1 \text{ X } (VCO_2)] \text{ X } 1.44 \quad \text{eq(17)}$$

In which VO_2 equals the oxygen uptake and VCO_2 equals the carbon dioxide output both in ml kg^{-1} min^{-1}. VCO_2 was calculated according to:

$$VCO_2 = (25.6/1000) \text{ X } J_{exp} \quad \text{eq(18)}$$

In which 25.6 is the gas constant in l mol^{-1} at 38°C. VO_2 is calculated according to:

$$VO_2 = VCO_2/RQ \qquad \text{eq(19)}$$

In which RQ is the estimated respiratory quotient. We used the following published values for RQ: 0.84 for control subjects, 0.86 for HD patients during the HD- protocol and 0.90 for HD patients during the HD+ protocol (6).

Statistics

All values are given as means ± SD. Statistical analysis was done using SPSS 10.0 (SPSS Inc, Chicago, Illinois). Differences between control subjects and dialysis patients during the control situation and during dialysis were tested using ANOVA and Tukey's post-hoc test was performed to study differences between specific groups. Differences in energy expenditure between control subjects and dialysis patients were also studied using analysis of covariance (ANCOVA) to control for confounders (age, body weight, dialysate glucose and caloric intake). Statistical significance was assumed when $p<0.05$.

Results

Demographic data and dialysis parameters

Table 1 shows the demographic and clinical data for control subjects and patients. Patients were in a good nutritional status and were dialysed adequately as is clear from the albumin concentrations and Kt/V values. There were no significant differences in age and body mass index between control subjects and patients. During the 3 weeks preceding the day of the study, habitual energy intake (EI) was 29.2 ± 8.2 in control subjects and 26.3 ± 6.1 kcal kg^{-1} day^{-1} in patients (average of the two study protocols, NS). In HD patients, CRP levels were not elevated (<2 mg l^{-1}). Plasma bicarbonate concentrations were 22.3 ± 2.1 mM during the HD- protocol at the start of the next dialysis session after the experimental study day. At the start of the experimental dialysis session, the arterial bicarbonate concentration was 22.7 ± 2.4 mM and reached a new steady state after 30 min of dialysis at 26.5 ± 2.1 mM. Bicarbonate concentrations in venous blood remained constant during the experiment at 30.5 ± 1.8 mM. Blood flow was 305 ± 35 ml/min at the arterial side of the dialyzer and 295 ± 35 ml/min venous of the dialyzer.

Bicarbonate enrichment

In Fig 2, ^{13}C-abundances of CO_2 in expired air before and at 30, 40, 50, and 60 min

Table 1: Demographic, nutritional, and dialysis status of the studied HD patients and control subjects[1].

Subjects	Control	HD -	HD +
age (years)	45 ± 12	54 ± 10	60 ± 4
sex (male/female)	5/1	6/3	5/3
Weight (kg)	80 ± 16	75 ± 12	76 ± 12
FFM (kg)	65 ± 14	60 ± 12	60 ± 11
BMI (wt/m^2)	23.6 ± 3.3	23.9 ± 3.4	25.6 ± 3.3
Albumin (g/l)	43.8 ± 1.2	40.6 ± 2.52	39.3 ± 2.1[2]
Kt/V		1.2 ± 0.2	1.3 ± 0.1
P.I. (g/kg/d)	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2
E.I. (kcal/kg/d)	29.2 ± 8.2	26.5 ± 6.8	26.1 ± 5.5

Mean ± SD. BMI: Body Mass Index; Kt/V: Measure of dialysis adequacy; P.I.: Protein intake measured by dietary recording; E.I.: Energy intake measured by dietary recording. [2]significantly different from control subjects, $p<0.05$.

after the start of the primed constant rate infusion of ^{13}C-bicarbonate are shown for controls, HD patients during the HD- and HD patients during the HD+ protocol. Changes in background ^{13}C-abundance during the first hour of dialysis reached steady state after 30 min after the start of the experiment. In both experimental protocols with a primed-continuous infusion of $NaH^{13}CO_3$ the plasma bicarbonate pool was overprimed (data not shown) and isotopic steady state in expired air was reached between 30 and 60 min after the start of the infusion. Initial background ^{13}C-abundance was not significantly different between the study groups with –26.5±0.6 ‰ for control subjects, –25.6±0.7 ‰ for hemodialysis patients in the control situation, and –27.4±2.2 ‰ just before the hemodialysis session. During the $NaH^{13}CO_3$ infusion, ^{13}C-abundance of expired CO_2 at isotopic steady state was +13.7 ± 3.1‰ for control subjects (corresponding to 0.045 ± 0.003 APE), +20.4 ± 3.7 ‰ for hemodialysis patients on a non-dialysis day (HD-, corresponding to 0.052 ± 0.004 APE), and +8.3±1.6 ‰ during dialysis (HD+, corresponding to 0.035 ± 0.004 APE, $p<0.05$ for comparison between all groups).

Bicarbonate production

All mean values of the fluxes comprising whole body bicarbonate metabolism are given in table 2. R(a) in HD patients during the HD- protocol was significantly lower compared to control subjects. As a consequence, the bicarbonate fluxes associated with oxidation (J_{ox}), expiration (J_{exp}), and with bicarbonate fixated in pools with a slow turnover (J_{fix},

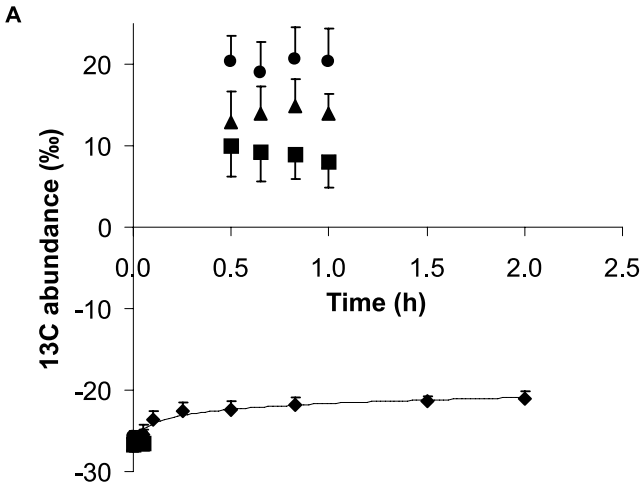


Figure 2. *Time course of the ^{13}C-abundance of expired CO_2 during the first hour of hemodialysis and the change in background enrichment during the first 2 hours of hemodialysis. Circles: HD- patients with $NaH^{13}CO_3$ infusion, triangles: control subjects with $NaH^{13}CO_3$ infusion, squares: HD+ patients with $NaH^{13}CO_3$ infusion, diamonds: dialysis without $NaH^{13}CO_3$ infusion.*

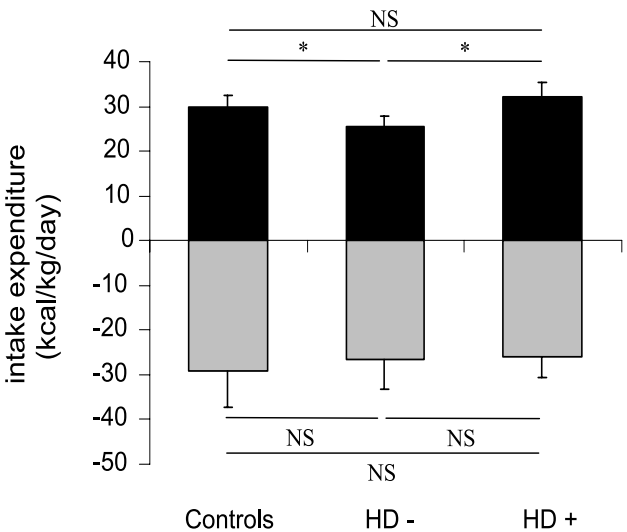


Figure 3*. Calculated energy expenditure in black bars for control subjects (controls), HD patients during a non-dialysis day (HD -) and HD patients during dialysis (HD +) on the positive axis. Energy intake for the 3 groups is shown on the negative axis in grey bars. *: p<0.05. NS: not significant.*

Table 2. Calculation of bicarbonate fluxes using the isotope steady state model for whole body bicarbonate homeostasis presented in Fig 1. For the derivation of the equations, see the section Subjects and Methods.

subjects	R(a)	Jd^{in}	Jd^{out}	$J_{fix}/J_{release}$	J_{ox}	J_{exp}
Control	184 ± 15			42 ± 3	141 ± 12	141 ± 12
HD-	160 ± 14*			37 ± 3*	123 ± 11*	123 ± 11*
HD+	313 ± 32#*	124 ± 16	105 ± 15	44 ± 6#	145 ± 19#	165 ± 15#*

All values mean ± SD and expressed as µmol/kg/min * P < 0.05 compared to control group # P < 0.05 compared to HD- group

$J_{release}$) were also significantly lower. Patients during the HD+ protocol had a significantly higher R(a) compared to both control subjects and patients during the HD- protocol. Furthermore, whereas the rate of oxidation (J_{ox}) remained almost equal to that observed in control subjects, the rate of expiration (J_{exp}) increased significantly (Control and HD– *vs.* HD+, $p<0.05$).

Resting energy expenditure

In figure 3, calculated energy expenditure (EE) is given for the control subjects and the hemodialysis patients both during the HD- and the HD+ protocol. Hemodialysis patients on a non-dialysis day have a significantly lower EE (24.9 ± 2.1 kcal kg^{-1} min^{-1}) than control subjects (29.1 ± 2.4 kcal kg^{-1} day^{-1}, $p < 0.05$). However, EE of HD patients during hemodialysis (32.6 ± 2.0 kcal kg^{-1} day^{-1}, $p < 0.05$) was significantly higher than in HD patients during the HD- protocol but not significantly different from control subjects. Also, energy intake is shown for all three groups. A multivariate analysis was performed for the differences in energy expenditure between the groups. Age, caloric intake, glucose concentration in dialysate or body weight, as covariates, did not change the significant differences between the groups. Finally, the differences in EE remained significant when they were calculated on the basis of FFM or when expressed as absolute values/min (data not shown).

Discussion

The results of this study demonstrate that during the period in between dialyses, patients have lower energy expenditure compared to control subjects. The difference is significant during the non-dialysis period. Dialysis induced a large increase in bicarbonate turnover,

mainly due to a net influx of bicarbonate from the dialysate. Total body oxidation, as compared to the non-dialysis period increased during dialysis but it did not increase beyond the level of control subjects.

Since we are the first to present data, albeit preliminary, on whole body bicarbonate production in CHD patients during dialysis, we would like to start with a discussion of some of the assumption underlying the model. In our study, we used a primed-continuous infusion where the prime was approximately 70 times larger than the continuous infusion, as is usually done in most other studies using a primed continuous infusion of ^{13}C-bicarbonate (cf. (13)). Therefore, in all experiments the bicarbonate pool was over-primed and reached rapid equilibration due to the high turnover of bicarbonate in the body even during dialysis. Two observations had to be incorporated in our model: (1) the observation in literature that recovery of infused bicarbonate in expired air is limited and (2) the observation by us that the dialyzer exchanged arterial bicarbonate only to a limited extent. Firstly, we reinterpreted the observed recovery factor of 0.77 of infused-labelled bicarbonate in expired air in stable fasting resting subjects (11). The remaining unrecovered bicarbonate (0.23) was defined in our model as J_{fix}. We assumed that there existed a fixed relation between J_{fix} and J_{ox}, since we did not measure how much of the infused label left the patient in expired air and in spent dialysate. On the other hand, this assumption seems reasonable in view of the observations that recovery of CO_2 increases during exercise (14) or food intake (15). Therefore, the control subjects in our study were asked to refrain from severe activity before the experiments but it is likely that the general activity level of the control subjects in our study was still higher than that of the HD patients. Secondly, the dialyzer was unable to fully replace blood bicarbonate for dialysate bicarbonate. This became apparent when we measured background ^{13}C-abundance of bicarbonate in venous blood and compared that to background ^{13}C-abundance of dialysate bicarbonate. In the present study, an empirical approach was chosen to account for this limited exchange of bicarbonate across the dialysing membrane. The efficacy of exchange was calculated on the basis of our observations on the limited increment of background ^{13}C-abundance of bicarbonate in venous blood. Then, we added the remainder to the rate of infusion of labelled bicarbonate. We showed that during dialysis, the dynamics underlying whole body bicarbonate homeostasis changed considerably. The turnover of bicarbonate in blood greatly increased. This increase was due to the unidirectional influx of bicarbonate from the dialyzer. Bicarbonate homeostasis was maintained by a large unidirectional return flux of bicarbonate to the dialyzer. The net A-V difference in bicarbonate fluxes across the dialyzer was compensated by an increased expiration of CO_2. As a consequence, the appearance of $^{13}CO_2$ in expired air, J_{exp}, was higher than what would have been expected

from the oxidation (J_{ox}). As shown in table 2, the A-V difference in bicarbonate fluxes ($\Delta J_d = J_d^{in} - J_d^{out}$) across the dialyzer is negative and is equal to 13 μmol $kg^{-1}min^{-1}$, represented approximately 8 % of total expired CO_2. This observation indicates that measurements of VCO_2 by indirect calorimetry during dialysis will always overestimate the VCO_2 due to oxidation by an amount equivalent to this A-V difference. Accordingly, VCO_2 values have to be corrected for that amount of CO_2 expired equivalent to this A-V difference to obtain the RQ value due to oxidation. This would imply that indirect calorimetry can still be used under the condition of dialysis if appropriate corrections are made.

Short 1-h $NaH^{13}CO_3$ infusion experiments allowed us to accurately estimate energy expenditure. In our control subjects the calculated values of energy expenditure were almost identical to energy intake estimated from the dietary record. Furthermore, energy expenditure in our control group was also comparable to that in a 24-h study using indirect calorimetry and stable isotopes (11). Energy expenditure was, however, lower compared to another study (16). In that study, control subjects were studied with an average age of 45 and were allowed to perform some exercise. These differences in study protocol might offer an explanation for the observed discrepancies. In HD patients in the absence of dialysis energy expenditure was significant lower compared to control subjects. Furthermore, energy intake was also lower, albeit not significant. This is in agreement with earlier reports (17).

A more difficult problem arises when discussing the EE values in dialysing HD patients. We observed a higher EE in CHD patients on a dialysis day in comparison with a non-dialysis day. As we discussed, measured RQ values can not be used without correction for the A-V difference in net influx of bicarbonate across the dialyzer into the patient. When CO_2 expiration is an estimated 8 % larger than bicarbonate production due to oxidation, RQ values will also increase by 8 % if O_2 consumption does not change. Literature data on O_2 consumption are contradictory. Lim *et al.* (18) showed that O_2 consumption did not increase in CHD patients upon dialysis, in contrast to Symreng *et al.* (19) who observed an increase in O_2 consumption during dialysis in CHD patients. In the present study we used values published by Ikizler *et al.* (6) in our calculations. In the study by Ikizler, a lower VCO_2 and a higher RQ would decrease their energy expenditure values. An increase in VCO_2 of CHD patients upon dialysis and subsequent increase in RQ, was also observed by Symreng *et al.* These authors interpreted this as a shift in utilisation of oxidative substrate to glucose. If our model is correct, it is more likely that the increase in RQ resulted from a continuous net flux of bicarbonate from the dialyzer to the patient, without a shift in oxidative substrate utilisation.

This does not exclude the possibility that increased energy expenditure can occur in HD patients during periods of infection or with other medical complications. Also, it has been suggested that HD patients require a higher than normal energy intake due to the loss of high quality nutrients during dialysis (8;20). Amino acid losses account for 6 g/dialysis session, which is 24 kcal/dialysis session. For 3 times weekly dialysis this is approximately 10 kcal/day. This is a minimal estimate since the replacement of high quality nutrients probably requires larger amounts of energy (21). This could result in apparent lower energy expenditure since these dialysate losses are not accounted for in energy expenditure measurements but the magnitude of this pathway seems limited.

In summary, our results show that energy expenditure is decreased in stable hemodialysis patients compared to healthy control subjects. The notion that an increase in energy expenditure is responsible for the protein-energy malnutrition is not supported by this work. Low food intake during medical complications appears a more likely cause of malnutrition in chronic hemodialysis patients.

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