Effects of feeding on albumin synthesis in hypoalbuminemic hemodialysis patients

JONATHAN D. LOUDEN, KIM BARTLETT, DAVID REAICH, RACHEL EDSON, CLARE ALEXANDER, and TIMOTHY H.J. GOODSHIP

School of Clinical Medical Sciences and Biomedical Mass Spectrometry Unit, University of Newcastle upon Tyne, Newcastle-upon-Tyne, and Renal Unit and Department of Nutrition and Dietetics, James Cook University Hospital, Middlesbrough, England, United Kingdom

Effects of feeding on albumin synthesis in hypoalbuminemic hemodialysis patients.

Background. Hypoalbuminemia is a powerful predictor of morbidity and mortality in hemodialysis (HD) patients and results from a reduction in albumin synthesis. It is not known if this is associated with any impairment of the normal response to feeding.

Methods. Protein turnover and albumin synthesis were measured in the fasting and fed state using a primed constant infusion of L-[1-13C]leucine in seven hypoalbuminemic (albumin ≤ 36 g/L) HD patients (HHD), seven normoalbuminemic (albumin ≥ 40 g/L) HD patients (NHD) and nine age-matched normal controls.

Results. The increase in albumin synthesis on feeding was impaired in HHD patients (fasting 15.0 ± 1.5 vs. fed 17.7 ± 2.9%, P = NS) compared to NHD (fasting 13.7 ± 0.9 vs. fed 17.4 ± 1.0%, P < 0.05) and controls (fasting 12.9 ± 0.6 vs. fed 15.2 ± 0.6%, P < 0.05). In addition, body mass index and percent body fat were significantly (P < 0.05) lower in HHD (20.8 ± 1.3 kg/m², 23.4 ± 2.0%) than NHD (26.7 ± 1.3 kg/m², 33.1 ± 3.2%) or controls (26.2 ± 1.1 kg/m², 32.6 ± 1.8%). There was no difference in dietary protein or energy intake in the three groups.

Conclusions. There are differences of body composition and protein metabolism in HHD patients that may be related to an impaired metabolic response to feeding.

That a low serum albumin is associated with increased morbidity and mortality in hemodialysis patients is well established [1]. A low serum albumin may be a consequence of an increase in the size of the plasma pool, increased catabolism, increased distribution into the interstitial space, abnormal losses (urinary, gastrointestinal or in the dialysate) or a decrease in albumin synthesis. It has been shown that albumin synthesis is the predominant factor controlling the plasma concentration of albumin. Factors that increase albumin synthesis include insulin [2], amino acids [3] and a low plasma oncotic pressure [4], while cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) [5, 6] have been shown to decrease synthesis. To date there have been no studies in which albumin synthesis has been directly measured in hypoalbuminemic hemodialysis patients. Kaysen et al measured albumin catabolism with 125I-labeled albumin and, taking into account external losses, estimated albumin synthesis [7]. Their estimate assumed that this system was in a steady state and, therefore, albumin synthesis equaled the albumin catabolism plus external losses. In Kaysen’s study, both fractional and absolute albumin synthesis rates were decreased in hypoalbuminemic patients. Because the technique used is derived from blood samples taken over several days, the measurement reflects a composite of both basal and feeding-induced albumin synthesis. It has been shown that the albumin fractional rate of synthesis (FSR) is increased approximately twofold during a mixed glucose-amino acid meal [8], and this may contribute a significant proportion of the area under the curve for a measurement of albumin synthesis derived from the decay of 125I albumin. In hemodialysis patients with a normal serum albumin, fasting albumin synthesis has been measured directly from the incorporation of 5,5,5-D3-L-leucine derived from a primed constant infusion. While FSR was not different from controls, the absolute synthesis rate (ASR) was significantly greater because of an increased plasma volume in the dialysis patients [9]. The aim of this study was to examine whether the response of albumin synthesis to feeding in hypoalbuminemic hemodialysis patients is impaired. Albumin fractional synthesis rates were measured in both the fasting and fed state in hypoalbuminemic and normoalbuminemic hemodialysis patients and in normal controls. The results show an impairment of meal-induced albumin synthesis in hypoalbuminemic hemodialysis patients.

Key words: protein turnover, body composition, diet and HD, metabolism, serum albumin in HD, fasting and feeding, dialysis.

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<table>
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<th>Table 1. Patient details</th>
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<td><strong>Subject</strong></td>
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<tr>
<td>NHD6</td>
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<tr>
<td>NHD7</td>
</tr>
</tbody>
</table>

Abbreviations are: HHD, hypoalbuminemic; NHD, normoalbuminemic.

**METHODS**

**Subjects**

Seven hypoalbuminemic (HHD) and seven normoalbuminemic hemodialysis (NHD) patients, and nine control subjects with normal renal function were studied (Table 1). Subjects were matched for age and sex (HHD, 5 males/2 females, mean age 56 ± 4 years; NHD, 5 males/2 females, mean age 52 ± 4 years; controls, 5 males/4 females, mean age 52 ± 3 years). The patients all had been treated by HD for at least 90 days and all were undergoing conventional low flux dialysis with Fresenius F8 dialyzers (Fresenius Medical Care, Bad Homburg, Germany). The urea reduction ratio (URR) in all patients was greater than 60% on entering the study and was maintained constant. Hypoalbuminemia was defined as a mean serum albumin less than or equal to 36 g/L and normoalbuminemia as a mean serum albumin of greater than or equal to 40 g/L during the previous three months. None of the patients had significant proteinuria.

Subjects with chronic inflammatory conditions and hepatic or neoplastic disease were excluded from the study. Dietary prescription for all HD patients included appropriate phosphate and potassium restrictions and routine guidance on protein and calorie requirements (1.2 g/kg/day protein, 35 kcal/kg/day).

Written informed consent was obtained from each subject. The experimental protocol was approved by the South Tees Research Ethics Committee.

**Isotopes**

L-[1-13C] leucine (99 mol% 13C) was purchased from Promochem (Welwyn Garden City, Herts, UK), diluted with normal saline under sterile conditions in the Pharmacy Manufacturing Department of the Royal Victoria Infirmary (Newcastle-upon-Tyne, UK), and tested for sterility and pyrogenicity.

**Experimental design**

Each subject underwent a primed constant infusion of L-[1-13C] leucine on two occasions. The first infusion was performed in the postabsorptive state and the second two weeks later during hourly oral meal feeding as described below.

Subjects kept a food diary for the three days before each infusion and were asked to follow their usual diet during this time. During the fed study, subjects were given six hourly oral meals, the first being given at the start of the infusion. These collectively provided 50% of daily requirements (1.2 g/kg/day protein, 35 kcal/kg/day). In calculating dietary requirements an adjustment was made for non-ideal body weight so that subjects were fed according to an adjusted body weight (mean of actual body weight and ideal body weight at body mass index of 23 kg/m²).

**Anthropometry**

Weight and height were measured in light indoor clothing without shoes. Skinfold thicknesses were measured at the triceps, biceps, subscapular and supra-iliac sites. Body density was calculated using the equations of Durnin and Womersley [10], and the percentage body fat was calculated from the equation developed by Siri [11]. Fat free mass was calculated from the body weight and percentage fat. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. A normalized index of fat free mass, the fat free mass index (FFMI) was calculated as the fat free mass in kilograms divided by the height in meters squared [12].

**Isotope infusions**

On the day of each isotope infusion, subjects were admitted to an outpatient investigation unit after an overnight fast. At 8:00 am 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, maintained at 60°C, to allow sampling of arterialized blood. A second cannula (18G Venflon; Viggo-Spectrometer) was placed antegradely into a vein on the contralateral arm for isotope infusion. Both cannulae were placed under local anesthetic (1% Lidocaine). Arterialized blood samples were collected at −15, −10 and −5 minutes before the infusion of L-[1-13C]leucine, to determine the basal 13C enrichment of plasma α-ketoisocaproic acid (KIC) and leucine derived from albumin hydrolysates. Samples were also taken for the measurement of bicarbonate, glucose, albumin and C-reactive protein (CRP) before the start of each isotope infusion. During both the fasting and fed study samples were taken for the measurement of insulin before (basal) and during (120, 180, 240 and 300 min) feeding (fed).

A priming dose of L-[1-13C]leucine (0.9 mg/kg) was administered followed by a six-hour constant rate infusion (0.9 mg/kg/h; model 940 infusion pump; Harvard
Apparatus, MA, USA). Heparinized blood samples were obtained at hourly intervals from two hours onwards for four hours. These were centrifuged immediately and plasma aliquots stored at −80°C until subsequent analysis.

Measurement of 13C enrichment of KIC

The trimethylsilylquinaxalinol derivative of plasma KIC was created as described previously [13]. Gas chromatography-mass spectrometry (GCMS) (Finnegan Voyager, Hemel Hempstead, Herts, UK) was used to measure the ions m/z 232 and 233. The criteria used to determine that isotopic equilibrium had been achieved were that (a) the CV of the points used were less than 5% and (b) that the slope of a linear regression line through the points was not significantly different from zero.

Albumin isolation and purification

Albumin was isolated from plasma samples by alcohol 10% trichloroacetic acid (TCA) extraction. Free leucine contaminating albumin was removed through a series of washing procedures using TCA. The purity of isolated albumin was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); a single band at molecular weight 65,000 Daltons on silver staining confirmed pure albumin [14]. Albumin was hydrolyzed by incubation in 6 mol/L HCl for 18 hours at 110°C and dried under nitrogen. N-acetyl propyl (NAP) derivatives of the constituent amino acids were created and injected onto a gas chromatograph (Hewlett Packard 960; Hewlett Packard, Palo Alto, CA, USA). The separated amino acids were carried directly to a combustion furnace maintained at 800°C where combustion to CO2 and water occurred. The CO2 derived from leucine was directed to an isotope ratio mass spectrometer to determine the 13C enrichment of CO2.

Analytical methods

Serum CRP concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Serum albumin concentrations were measured by an automated version of the Bromocresol Green method [15]. Serum insulin was assayed by double antibody radioimmunoassay [16, 17], blood glucose and bicarbonate with an automated analyzer (Technicon DAX-72; Technicon, Tarrytown, NY, USA).

Calculation of whole body leucine metabolism

The model and calculations of leucine metabolism have been described previously [18]. Leucine flux is equal to the sum of leucine disappearance into body proteins (PS) plus leucine oxidation (O), and to the sum of leucine appearance from protein degradation (PD) plus dietary intake of leucine (I). Therefore, leucine flux equals:

\[ Q = PS + O = PD + I \]  

Leucine appearance from protein degradation (PD) is equal to Q in the fasting state; in the fed state PD is calculated as Q minus the dietary intake of leucine (I).

Calculation of albumin fractional synthesis rate

The [13C]leucine incorporation rate into albumin was calculated between the 120 and 360 minutes by least square regression analysis. The FSR was obtained by dividing the slope of the incorporation (corrected for 24 hours) by the plasma [13C]KIC enrichment at isotopic equilibrium [19].

Statistical analysis

Values are reported as means ± SE and analyzed using analysis of variance (ANOVA). The values for albumin, CRP and bicarbonate are the means of the results from both the fasting and the fed study. The fed insulin values are the means of the values at 120, 180, 240 and 300 minutes. Differences were considered significant if P < 0.05.

RESULTS

Albumin, CRP, insulin, glucose and bicarbonate

Serum albumin measured on the day of study was significantly (P < 0.05) lower in HHD patients (33 ± 2 g/L) than in both NHD patients (43 ± 1 g/L) and controls (43 ± 1 g/L). C-reactive protein was significantly (P < 0.05) higher in both groups of HD patients (HHD 5.6 ± 2.1 mg/l, NHD 13.0 ± 7.1 mg/l) than in controls (1.0 ± 0.5 mg/l). There was, however, no significant difference between the two groups of HD patients. Basal blood glucose concentrations on the day of the fed study were not significantly different in the three groups (HHD 4.1 ± 0.7, NHD 3.6 ± 0.2, control 4.0 ± 0.2 mmol/L).

Insulin concentrations were not significantly different in the three groups either basal or during feeding (HHD, basal 4.7 ± 0.3 and fed 27.4 ± 12.0; NHD, basal 10.4 ± 2.9 and fed 52.2 ± 12.0; control, basal 5.7 ± 0.8 and fed 61.2 ± 24.0 mU/L). Basal insulin-to-glucose ratios were not significantly different in the three groups (HHD 1.1 ± 0.3, NHD 0.5 ± 0.1, control 0.8 ± 0.1 mU/mmole glucose). There was no difference in the bicarbonate concentration of the three groups (HHD 22.3 ± 1.1, NHD 21.0 ± 1.4, control 22.5 ± 0.6 mmol/L).

Anthropometry

Hypoalbuminemic hemodialysis patients weighed significantly less (60.3 ± 5.8 kg) than the other two groups (Table 2), but there was no difference in weight between NHD patients (78.5 ± 5.6 kg) and controls (78.4 ± 5.1 kg). Body mass index and percent body fat were significantly lower in HHD patients (20.8 ± 1.3 kg/m², 23.4 ± 2.0%) than in the other groups, with no difference be-
Table 2. Anthropometry

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Weight kg</th>
<th>Body mass index kg/m²</th>
<th>Body fat content % body weight</th>
<th>Fat free mass index kg/m²</th>
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<tbody>
<tr>
<td>Hypoalbuminemic HD</td>
<td>60.3 ± 5.8</td>
<td>20.8 ± 1.3</td>
<td>23.4 ± 2.0</td>
<td>15.9 ± 0.9</td>
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<tr>
<td>Normalbuminemic HD</td>
<td>78.5 ± 5.6</td>
<td>26.7 ± 1.3</td>
<td>33.1 ± 3.2</td>
<td>17.7 ± 0.9</td>
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<tr>
<td>Controls</td>
<td>78.4 ± 5.1</td>
<td>26.2 ± 1.1</td>
<td>32.6 ± 1.8</td>
<td>18.4 ± 0.8</td>
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</tbody>
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Values are mean ± SE.

*P < 0.05 vs. normalbuminemic and P < 0.001 vs. control

Table 3. Daily dietary intake

<table>
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<th>Subject group</th>
<th>Protein</th>
<th>Energy</th>
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<tr>
<td></td>
<td>g/kg ABW</td>
<td>g/kg FFM</td>
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<tr>
<td>Hypoalbuminemic HD</td>
<td>1.23 ± 0.15</td>
<td>1.58 ± 0.16</td>
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<tr>
<td>Normalbuminemic HD</td>
<td>0.87 ± 0.08</td>
<td>1.30 ± 0.11</td>
</tr>
<tr>
<td>Controls</td>
<td>1.02 ± 0.09</td>
<td>1.49 ± 0.13</td>
</tr>
</tbody>
</table>

Values are mean ± SE and are corrected for actual body weight (ABW), fat free mass (FFM) and ideal body weight (IBW).

between NHD patients (26.7 ± 1.3 kg/m², 33.1 ± 3.2%) and controls (26.2 ± 1.1 kg/m², 32.6 ± 1.8%).

Fat free mass and FFMI were not significantly different between groups, but there was a trend for them to be lower in HD patients, lowest in the hypoalbuminemic group.

Dietary intake

There were no significant differences between the groups in either protein or energy intake (Table 3). This applied whether the values were corrected for actual body weight, fat free mass or ideal body weight.

Fractional albumin synthesis rates

Feeding was associated with a significant increase in albumin FSR in NHD patients (fasting 13.7 ± 0.9 vs. fed 17.4 ± 1.0%, P < 0.05) and controls (fasting 12.9 ± 0.6 vs. fed 15.2 ± 0.6%, P < 0.05; Fig. 1). There was no significant change in albumin FSR on feeding in the HHD patients (fasting 15.0 ± 1.5 vs. fed 17.7 ± 2.9%, P = NS). There were no differences between the three groups in mean albumin FSR in either the postabsorptive or the fed state.

Leucine flux

The rate of appearance of leucine from protein degradation (PD) was significantly greater in hypoalbuminemic patients in the fasting state when corrected for actual body weight (Table 4). This difference was not present when corrected for fat free mass.

DISCUSSION

The results document that the normal increase in albumin synthesis seen with feeding is impaired in HD patients with hypoalbuminemia. However, there was no significant difference between the three groups in albumin FSR in either the fasting or fed state. CRP levels were significantly higher in both groups of patients compared to controls, but there was no difference between the hypoalbuminemic and normalbuminemic patients. Anthropometric measurements showed that BMI and percent body fat was significantly lower in the hypoalbuminemic patients compared to the normalbuminemic patients and normal controls. There were no significant differences in either dietary protein or energy intake between the three groups. Whole body leucine flux was not significantly different from controls in the normalbuminemic patients. In the hypoalbuminemic patients it was significantly greater when corrected for actual body weight, but this difference was not present when cor-
rected for fat free mass. This study did not take into account removal of leucine from the splanchnic bed in our calculation of protein degradation, because we were interested in the response between groups rather than the absolute response. Of course this assumes that there was no difference in the removal of leucine by the splanchnic bed between the three groups of subjects.

A major strength of this study is that we have been able to measure not only albumin synthesis, but also undertake a comprehensive nutritional assessment in three well-matched groups of subjects. To our knowledge, this is also the first study to directly measure albumin synthesis in hypoalbuminemic patients. A weakness of the study is that plasma volume was not directly measured, which would have enabled the calculation of absolute albumin synthesis rates. The results of our study are also compromised by the variability in the response to feeding in the hypoalbuminemic patients. The percent increase in the fractional synthesis rate induced by feeding in the three groups was 21% in normoalbuminemic patients, 15% in controls, and 15% in hypoalbuminemic patients. These percentages alone might suggest that there was no impairment of feeding induced albumin synthesis in the hypoalbuminemic patients compared to controls, but the response to feeding in this group showed a substantially greater variability than in the two other groups. The mean difference between FSR in the fasting and fed state in the hypoalbuminemic groups was 2.7% per day. To detect such a difference at a two-sided significance level of 0.05 with a power of 80% at least 98 subjects would have to be studied.

Only one previous study has been published in which albumin synthesis was measured in hypoalbuminemic hemodialysis patients [7]. In that study albumin synthesis rates were determined from the kinetics of a single intravenous dose of $^{[125]I}$ human albumin. The plasma radioactivity disappearance curve was constructed from measurements taken until plasma $^{[125]I}$ levels decreased to 10% of original counts; this ranged from 25 to 38 days. The total albumin turnover derived from the integration of this curve therefore reflects a composite of albumin metabolism. Assuming that neither the total or plasma albumin mass is changing during the period of the measurement albumin synthesis can be calculated as the difference between albumin turnover and extracorporeal losses of albumin (urine and dialysate). However, albumin synthesis rates are in a dynamic state with increases particularly associated with feeding. In humans, increases in albumin FSR of 18% [14] and 48% [8] have been reported, while in the pig increases of approximately 30% have been demonstrated [20]. Differences in albumin synthesis rates measured with $^{[125]I}$ human albumin therefore may be due to changes in either basal or feeding induced albumin synthesis. The aim of our study was to measure both components of composite albumin synthesis. Albumin synthesis in the basal state has previously been measured directly in hemodialysis patients [9]. FSR was not different from control subjects but ASR was significantly greater, primarily because of an increase in the plasma albumin pool. This was secondary not to a difference in serum albumin, but because the plasma volume, measured with Evans blue, was 24% higher in the hemodialysis patients. In our study it is the response to feeding that is impaired in hypoalbuminemic patients, and this may be an important determinant of composite albumin synthesis. This is in contrast to cancer cachexia where the response to feeding is maintained [21]. The absolute values for albumin FSR reported in our study are higher than those reported in previous studies in both HD patients [9] and normal subjects [8]. We presume that this represents different study populations and analytical techniques, but it does emphasize the importance of using appropriate control groups in these types of studies.

The anthropometric data from our study clearly show a difference in body composition of the hypoalbuminemic patients characterized by a BMI at the lower end of the normal range and a lower body fat. There was also a trend for the lean body mass, represented by the fat free mass index, to be lower. It is unlikely that these changes in body composition were secondary to dietary intake because there was no difference in either protein or energy intake corrected for actual body weight, ideal body weight and fat free mass. These results are in keeping with the recent suggestion that there are two types of malnutrition seen in patients with chronic renal failure [22]. Type 1 is characterized by loss of lean body mass, a low dietary intake and a normal serum albumin

<table>
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<tr>
<th>Table 4. Leucine flux, fasting and fed, corrected for both actual body weight and fat free mass</th>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Hypoalbuminemic HD</td>
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<tr>
<td>Normoalbuminemic HD</td>
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</tbody>
</table>

Values are mean ± SE.

*P < 0.05 vs. normoalbuminemic, P = 0.072 vs. control
whereas type 2 is associated with a low serum albumin and normal dietary intake. Our data suggest that type 2 also is associated with altered body composition in the presence of a normal dietary intake.

Whole body leucine flux was not significantly different from normal in the normoalbuminemic patients whereas previous reports have documented an increased flux [9]. Correction for fat free mass rather than actual body weight showed there to be no difference in flux in any of the three groups, and it is probably more appropriate to correct leucine flux in this way and thus remove the compounding effect of the fat mass.

What are the possible mechanisms underlying the changes that we have documented? Chronic inflammation associated with increased levels of pro-inflammatory cytokines is now thought to be an important cause of type 2 malnutrition. It is also well established that there is an association between pro-inflammatory cytokines such as IL-6 and insulin resistance [23]. Insulin resistance is a well documented feature of chronic renal failure [24], while insulin is an important regulator of muscle protein synthesis and breakdown [25], and albumin synthesis [2, 26]. Although insulin levels, both basal and during feeding, were not significantly different between the hypoalbuminemic and normoalbuminemic groups, this does not exclude the presence of insulin resistance. It is tempting to speculate that cytokine-induced insulin resistance may play a pivotal role in the pathogenesis of type 2 malnutrition.

In summary, this study suggests that there are differences of body composition and albumin metabolism in hypoalbuminemic hemodialysis patients that may be related to an impaired metabolic response to dietary intake.

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Reprint requests to Dr. T.H.J. Goodship, Royal Victoria Infirmary, Newcastle-upon-Tyne, NE1 4LP England, United Kingdom. E-mail: t.h.j.goodship@ncl.ac.uk

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