Dialysate Improves Protein Anabolism in Renal Failure Patients on Automated Peritoneal Dialysis

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Protein-energy malnutrition as a result of anorexia frequently occurs in dialysis patients. In patients who are on peritoneal dialysis (PD), dialysate that contains amino acids (AA) improves protein anabolism when combined with a sufficient oral intake of calories. It was investigated whether protein anabolism can be obtained with a mixture of AA plus glucose (G) as a source of proteins and calories during nocturnal automated PD (APD). A random-order cross-over study was performed in eight APD patients to compare in two periods of 7 d each AA plus G dialysate obtained by cycler-assisted mixing of one bag of 2.5 L of AA (Nutrineal 1.1%, 27 g of AA) and four bags of 2.5 L of G (Physioneal 1.36 to 3.86%) versus G as control dialysate. Whole-body protein turnover was determined using a primed continuous infusion of l-[1-13C]leucine, and 24-h nitrogen balance studies were performed. During AA plus G dialysis, when compared with control, rates of protein synthesis were 1.20 ± 0.4 and 1.10 ± 0.2 μmol/kg per min leucine (mean ± SD), respectively (NS), and protein breakdown rates were 1.60 ± 0.5 and 1.72 ± 0.3 μmol/kg per min (NS). Net protein balance (protein synthesis minus protein breakdown) increased on AA plus G in all patients (mean 0.21 ± 0.12 μmol leucine/kg per min; P < 0.001). The 24-h nitrogen balance changed by 0.96 ± 1.21 g/d, from −0.60 ± 2.38 to 0.35 ± 3.25 g/d (P = 0.061, NS), improving in six patients. In conclusion, APD with AA plus G dialysate improves protein kinetics. This dialysis procedure may improve the nutritional status in malnourished PD patients.

Received May 19, 2004. Accepted February 22, 2005.
Published online ahead of print. Publication date available at www.jasn.org.
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Materials and Methods
Patients
Eight APD patients (Table 1) were recruited from the Peritoneal Dialysis Unit of the Erasmus MC. Inclusion criteria called for stable patients who were on PD >3 mo and had weekly Kt/V >1.7. Exclusion criteria were peritonitis, other infectious or inflammatory diseases in the previous 6 wk, malignancy, and life expectancy <6 mo. The study was approved by the Medical Ethics Committee, and written informed consent was obtained from all patients.
Protein turnover study (PTO) was carried out on day 3. Nitrogen balance took place during days 5 to 7 and 12 to 14. During 5 d (days 3 to 7 and 10 to 14). Collection of materials for amino acids plus glucose (AAG) or with glucose (G) was performed. A controlled hospital-supplied diet was prescribed that consisted of two consecutive study periods of 7 d each. In two consecutive periods of 7 d each, a dialysis scheme using dialysate-containing AAG (Nutrineal 1.1% plus Physioneal 1.36 to 3.86%; Baxter BV, Utrecht, The Netherlands) was compared with a control scheme that contained G (Physioneal 1.36 to 3.86%). Before the study, all patients used G-based dialysis fluid (Dianeal or Physioneal; Baxter BV).

The study was performed on an outpatient basis, except for the first dialysis scheme by drawing one of eight sealed envelopes.

Primary end points of the study were whole-body protein turnover (WBPT) and 24-h nitrogen balance (NB). Secondary end points were changes in acid-base homeostasis and blood chemistry.

Before the study and at the end of the first and second weeks (day 7 and day 14), venous blood samples were taken for chemistry and an acid-base profile. On the third day of each period, patients were admitted to the metabolic ward, where WBPT was determined during an overnight stay. The NB study was carried out on an outpatient basis.

Study Design

The study was a single-center, open-label, randomized, crossover study of 14 d duration (Figure 1). In two consecutive periods of 7 d each, a dialysis scheme using dialysate-containing AAG (Nutrineal 1.1% plus Physioneal 1.36 to 3.86%; Baxter BV, Utrecht, The Netherlands) was compared with a control scheme that contained G (Physioneal 1.36 to 3.86%). Before the study, all patients used G-based dialysis fluid (Dianeal or Physioneal; Baxter BV).

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Dialysis Procedures

Six nighttime exchanges were performed automatically using a cycler (HomeChoice; Baxter BV). In the daytime, there were one or two exchanges with G (Dianeal or Physioneal) and/or polyglucose-containing (Extraneal, Baxter BV) dialysate.

During the study, the APD schedule for each patient was similar to that used before the study to meet adequacy and ultrafiltration targets. The cyclo- regulated mixing of AA and G. The AAG dialysate was obtained after mixing one bag of 2.5 L of Nutrineal 1.1%, which contained 27 g of AA, and four bags of 2.5 L of Physioneal, 1.36 to 3.86% G, depending on ultrafiltration targets. In one patient (patient 8), only bags with 2.0 L were used. The AA and G solutions need to be mixed such that at each cycle, AA are given together with a sufficient amount of energy. To obtain an AAG mixture from the first cycle onward, we applied an “empty bag procedure,” while all bags were hung with the undersides on the same level. For the first cycle, a weighed amount of AA solution was mixed in the so-called heater bag (the bag where the solutions are mixed) with the G solutions to a final ratio of 1:4. For the NB studies, when the patients were dialyzed at home, mixing during the other cycles was regulated automatically by the cycler. This mixing procedure was tested in an in vitro experiment by labeling the AA solution with methylene blue. A proper mixing for each cycle was found (interbag coefficient of variation for methylene blue concentrations, 7%). During the WBPT studies, in each cycle, the heater bag first was filled by the research nurse with the AA solution in an exactly weighed amount, whereupon the cycler filled the bag with the required amount of G solution so that exactly the same amount of AA was obtained after mixing one bag of 2.5 L of Nutrineal 1.1%, which contained 27 g of AA, and four bags of 2.5 L of Physioneal, 1.36 to 3.86% G, depending on ultrafiltration targets. In one patient (patient 8), only bags with 2.0 L were used. The AA and G solutions need to be mixed such that at each cycle, AA are given together with a sufficient amount of energy. To obtain an AAG mixture from the first cycle onward, we applied an “empty bag procedure,” while all bags were hung with the undersides on the same level. For the first cycle, a weighed amount of AA solution was mixed in the so-called heater bag (the bag where the solutions are mixed) with the G solutions to a final ratio of 1:4. For the NB studies, when the patients were dialyzed at home, mixing during the other cycles was regulated automatically by the cycler. This mixing procedure was tested in an in vitro experiment by labeling the AA solution with methylene blue. A proper mixing for each cycle was found (interbag coefficient of variation for methylene blue concentrations, 7%). During the WBPT studies, in each cycle, the heater bag first was filled by the research nurse with the AA solution in an exactly weighed amount, whereupon the cycler filled the bag with the required amount of G solution so that exactly the same amount of AA was obtained after mixing one bag of 2.5 L of Nutrineal 1.1%, which contained 27 g of AA, and four bags of 2.5 L of Physioneal, 1.36 to 3.86% G, depending on ultrafiltration targets. In one patient (patient 8), only bags with 2.0 L were used. The AA and G solutions need to be mixed such that at each cycle, AA are given together with a sufficient amount of energy. To obtain an AAG mixture from the first cycle onward, we applied an “empty bag procedure,” while all bags were hung with the undersides on the same level. For the first cycle, a weighed amount of AA solution was mixed in the so-called heater bag (the bag where the solutions are mixed) with the G solutions to a final ratio of 1:4. For the NB studies, when the patients were dialyzed at home, mixing during the other cycles was regulated automatically by the cycler. This mixing procedure was tested in an in vitro experiment by labeling the AA solution with methylene blue. A proper mixing for each cycle was found (interbag coefficient of variation for methylene blue concentrations, 7%).

The composition of the AA 1.1% dialysis solution (g/L) was 0.714 histidine, 0.850 isoleucine, 1.020 leucine, 0.955 lysine-HCl, 0.850 methionine, 0.570 phenylalanine, 0.646 threonine, 0.270 tryptophane, 1.393 valine, 1.071 arginine, 0.951 alanine, 0.595 proline, 0.510 glycine, 0.510 serine, and 0.300 tyrosine. The electrolyte and buffer composition (mmol/L) were 132 Na, 105 Cl, 1.25 Ca, 0.25 Mg, and 40 lactate. The electrolyte and buffer concentrations (mmol/L) were 132 Na, 105 Cl, 1.25 Ca, 0.25 Mg, 25 bicarbonate, 15 and lactate.

Figure 1. The study design was a randomized, crossover study that consisted of two consecutive study periods of 7 d each. During these periods (days 1 to 7 and 8 to 14), dialysis with amino acids plus glucose (AAG) or with glucose (G) was performed. A controlled hospital-supplied diet was prescribed during 5 d (days 3 to 7 and 10 to 14). Collection of materials for nitrogen balance took place during days 5 to 7 and 12 to 14. Protein turnover study (PTO) was carried out on day 3.

Table 1. Characteristics of the patients

<table>
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<tr>
<th>Patient</th>
<th>Primary Diagnosis of Renal Disease</th>
<th>Time on PD (mo)</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (wt/ht2)</th>
<th>Kt/V</th>
<th>PET</th>
<th>nPNA (g/kg per d)</th>
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<td>78</td>
<td>185</td>
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*BMI, body mass index; Kt/V, value per week; PET, peritoneal equilibrium test; nPNA, normalized protein equivalent of nitrogen appearance (PD adequate 2.0, software Baxter); HA, high average; LA, low average.*

Figure 1. The study design was a randomized, crossover study that consisted of two consecutive study periods of 7 d each. During these periods (days 1 to 7 and 8 to 14), dialysis with amino acids plus glucose (AAG) or with glucose (G) was performed. A controlled hospital-supplied diet was prescribed during 5 d (days 3 to 7 and 10 to 14). Collection of materials for nitrogen balance took place during days 5 to 7 and 12 to 14. Protein turnover study (PTO) was carried out on day 3.
**WBPT Studies**

In the two study periods, rates of WBPT during nocturnal dialysis were determined with a primed continuous intravenous infusion of \[^{13}\text{C}\text{-leucine}} (25). WBPT was studied on day 3, at the end of the dialysis between 2:30 and 5:00 a.m. (Figure 2). To create baseline conditions, patients were instructed to drain all dialysate 12 h before starting the APD, leaving the abdomen empty. Thus, only during day 3, when WBPT was performed, the patients had a dry day. At 5:00 p.m., two catheters were inserted into superficial veins on both arms, one for continuous infusion of the tracer solution and the other for repeated blood sampling. Dialysis started at 8:30 p.m. (T0). Baseline blood samples and expiratory breath samples were collected in duplicate at 150 min, after priming and starting the tracer infusion. Indirect calorimetry (Deltatrac metabolic monitor; Datex, Helsinki, Finland) was performed simultaneously in duplicate at T480, T495, and T510 min, after priming and starting the tracer infusion. Indirect calorimetry was designed, isonitrogenous and isocaloric to the prestudy habitual diet of the patient’s habitual dietary intake was determined. A balanced diet food diary. On these food records and a subsequent dietary interview, the research nurse delivered the hospital-prepared diet, leaving the abdomen empty. Thus, only during day 3, when WBPT was performed, the patients had a dry day. At 5:00 p.m., two catheters were inserted into superficial veins on both arms, one for continuous infusion of the tracer solution and the other for repeated blood sampling. Dialysis started at 8:30 p.m. (T0). Baseline blood samples and expiratory breath samples were collected in duplicate at 2:30 a.m. (6 h from the start of the dialysis, T360), and priming doses of \[^{-[1-13\text{C}\text{-leucine}} (3.8 \text{ mol/kg}) and \[^{13}\text{C}\text{NaHCO}_3 (1.7 \text{ mol/kg}) were given to label the leucine and \[^{12}\text{C}\text{CO}_2 pools. Then, a continuous infusion of \[^{-[1-13\text{C}\text{-leucine}} (infusion rate 0.063 \text{ mol/kg per min}) was started and continued for 150 min until the end of the nocturnal dialysis at T510. For measuring plateau plasma keto-isocaproic acid (KIC) and \[^{13}\text{C}\text{CO}_2 enrichment, blood and expired air samples were collected simultaneously in duplicate at T480, T495, and T510 min, i.e., at 120, 135, and 150 min, after priming and starting the tracer infusion. Indirect calorimetry (Deltatrac metabolic monitor; Datex, Helsinki, Finland) was performed to measure \[^{12}\text{C}\text{CO}_2 production. Patients were not allowed to eat during the isotope studies, but noncaloric beverages were permitted.

**Diet**

A renal dietitian instructed the patients on how to complete a 4-d food diary. On these food records and a subsequent dietary interview, the patient’s habitual diet was determined. A balanced diet was designed, isonitrogenous and isocaloric to the prestudy habitual diet. Meals were prepared and deep-frozen in the Erasmus MC according to the prescription of the dietitian. The patients took nothing but this food during days 3 through 7 of each period. The patients recorded all food intake in the diaries.

**NB Studies**

On day 3 of each week, patients started the individually tailored diets and continued them until the end of day 7. During days 5, 6, and 7, all dialysate and all urine produced per 24-h period were collected. On the daily patient visits, the research nurse delivered the hospital-prepared food, supervised the study procedures, checked for changes in body weight, and returned to the hospital all collected materials (urine, spent dialysate) and the remaining food of the previous day. The dietitian weighed the remaining food to calculate its protein and energy content. An aliquot of every collection was stored at –20°C until later analysis.

**Analytical Determination**

Dialysate and urine nitrogen content were determined by a continuous flow elemental analyzer (Carlo Erba NC-1500; Interscience BV, Breda, The Netherlands). In brief, triplicate samples are weighed in tin containers, freeze-dried, and combusted at 1020°C; the resulting nitrogen gas is measured. This is an automation of the Dumas combustion method (26).

Leucine carbon flux was calculated from the \[^{13}\text{C}\text{-enrichment of KIC} (27). In brief, the sample was deproteinized with sulfo-salicylic acid and the supernatant was put on a cation exchange column to isolate the AA. The effluent that contained the KIC was reacted with phenyl-diamine to form quinoxalinols. These derivatives were extracted with a mixture of dichloromethane/hexane, dried, and silylated with N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide. The \[^{13}\text{C}\text{-enrichment was determined by gas chromatography–mass spectrometry by measuring the fragments 259 and 260 of natural and \[^{13}\text{C}\text{KIC, respectively. Gas chromatography–mass spectrometry analyses were carried out on a Carlo Erba GC8000 gas chromatograph coupled to a Fisons MD800 mass spectrometer (Interscience BV) by injecting 1 \mu l of test material with a split ratio of 50:1 on a 25-m \times 0.22-mm fused silica capillary column, coated with 0.11 \mu m of HT5 (SGE, Victoria, Australia). Oxidation of \[^{-[1-13\text{C}\text{-leucine}} was determined by measuring breath \[^{12}\text{CO}_2 \[^{13}\text{C}\text{-enrichment (Automatic Breath Carbon Analyser; Europa Scientific, Crewe, Great Britain).}

**Blood Chemistries**

Fasting blood samples were taken before the morning exchange, before the study, at the end of each study period, and at the start of WBPT studies. Serum urea, creatinine, phosphate, albumin, G, bicarbonate (standardized at 40 mmHg), insulin, glucagon, and 24-h dialysate contents of urea and protein were measured by routine laboratory procedures. Insulin was measured by a chemiluminescent immunometric assay (Immulum 2000 Insulin, DPC, Los Angeles, CA). Glucagon was measured by means of a radioimmunochemical method (Eurodiagnostics, Apeldoorn, The Netherlands).

**Calculations of NB**

The classical NB was calculated, with the equation \(N_{\text{bal}} = N_{\text{in}} - N_{\text{out}}\). Then, the mean of the 3 study days was calculated. The supply of nitrogen \(N_{\text{in}}\) consisted of the sum of the calculated daily dietary nitrogen intake and the dialysate nitrogen content \((i.e., \text{nitrogen in the infused dialysate})\). The loss of nitrogen \(N_{\text{out}}\) included the measured nitrogen content of all peritoneal drainage fluid and the urinary nitrogen losses. For fecal and integumental nitrogen losses, fixed values of 1.5 and 0.5 g/d, respectively, were assumed. The differences between the study periods were evaluated by subtracting the NB on G from that on AAG. No correction was made of the NB for potential changes in the body urea-N pool. To convert the results of the NB \((g\text{ of N/24 h})\) to its protein equivalent \((g\text{ protein/24 h})\), it was assumed that 1 g of N corresponds to 6.25 g of protein.

**Calculations of Whole-Body Turnover**

Leucine carbon flux was calculated as described previously (25). Leucine carbon flux \((Q)\) is equal to the sum of endogenous leucine appearance from protein breakdown \((B)\) plus exogenous leucine appearance via oral intake and via dialysate \((I)\). At metabolic equilibrium \((\text{steady state}), Q\) is also equal to the sum of leucine disappearance into body proteins \((S)\) plus leucine oxidation \((O)\). Therefore, \(Q = S + O = B + I\). Leucine flux in \text{mol/kg per hr} is calculated as \(Q = \text{Inf} (E_{\text{plasma KIC}} - 1)\), where \(\text{Inf}\) is the leucine infusion rate \((\text{mol/kg per hr})\), \(E_{\text{plasma KIC}}\) is the \[^{13}\text{C}\text{-enrichment of plasma KIC as measured at isotopic equilibrium. Isotopic steady state (plateau plasma \[^{13}\text{KIC enrichment) was assumed between T_{480} and T_{510} min. Leucine oxidation (O, in \text{mol/kg per hr}) is}

\[
\begin{align*}
\text{Dialysis AAG or G} & \quad \begin{cases}
0 & 360 \\
510 \text{ min} & \\
\end{cases} \\
\text{Primed constant infusion of} & \quad \begin{cases}
\text{L-}\left[^{1-13\text{C}}\text{-L-Leucine} \right. & \\
\end{cases}
\end{align*}
\]

**Figure 2.** Schematic diagram of the PTO study protocol. Arrowheads denote time points of blood and breath sampling during automated peritoneal dialysis (0 to 510 min).
calculated as $O = F^{13} \text{CO}_2 \times (1/E_{\text{KIC}} - 1/E) \times 100$, where $F^{13} \text{CO}_2$ (in $\mu$mol $^{13}$C/kg per hr) is the rate of expired $^{13}$CO$_2$ calculated from CO$_2$$^{13}$C enrichment in expired air and from CO$_2$ production. Leucine absorption from dialysate was calculated by subtracting the amount of leucine in spent dialysate from that in fresh dialysate.

**Statistical Analyses**

Data were analyzed using the statistical program SPSS, version 10.0, for Windows (SPSS Inc., Chicago, IL). Data are expressed as mean ± SD. The paired t test was used to compare differences between the two treatment regimens (AAG versus G dialysis) after verifying that there were no significant carryover or period effects. All tests of significance were two sided, and differences were considered statistically significant at $P < 0.05$.

**Results**

Table 1 shows the baseline characteristics of the eight patients, three of whom were anuric. Apart from the use of medications that are taken regularly by PD patients, patients 4, 5, and 7 used prednisone in a dose of 5, 7.5, and 2.5 mg/d, respectively. The treatment protocol was performed easily and well tolerated by all patients. There were no complaints of loss of appetite or nausea, and there were no other adverse reactions reported during the use of AA-containing dialysis fluid. None of the patients dropped out of the study.

**WBPT**

During dialysis with AAG, protein synthesis increased (1.20 ± 0.4 versus 1.10 ± 0.2 $\mu$mol leucine/kg per min; mean difference 0.10 ± 0.31 $\mu$mol leucine/kg per min; NS) and protein breakdown decreased (1.60 ± 0.5 versus 1.72 ± 0.3 $\mu$mol leucine/kg per min; mean difference 0.11 ± 0.30 $\mu$mol leucine/kg per min; NS) compared with the use of G. Net protein balance (S minus B) was negative in all patients (fasting state conditions). With the use of the AAG mixture, net protein balance was invariably less negative by a mean of 0.21 ± 0.12 $\mu$mol leucine/kg per min ($P = 0.001$) compared with G dialysis.

**Energy and Protein Intake**

The prestudy (i.e., habitual) dietary protein intake was 0.9 ± 0.2 g/kg per d; only one of the eight patients had an intake of 1.2 g protein/kg per d. Also, dietary energy intake was low (21.1 ± 6.2 kcal/kg per d). The prescribed diet contained on average 0.9 ± 0.2 g protein/kg per d and 22.1 ± 5.5 kcal/kg per d. During the NB energy intake, including G absorbed from dialysate was 25.4 ± 7.0 kcal/kg per d with the AAG dialysate versus 27.0 ± 6.7 kcal/kg per d with the G dialysate ($P = 0.10$, NS). Protein intake calculated as the sum of protein from diet and AA absorbed from dialysate (on average 47%) was 1.0 ± 0.2 g/kg per d on AAG and 0.85 ± 0.2 g/kg per d on G dialysis ($P = 0.002$).

**NB**

Mean values of NB were ± 0.35 ± 3.25 and ± 0.60 ± 2.38 g of N/24 h (mean ± SD) for AAG and G, respectively (Table 3). The strongly negative values in both series in one patient (patient 6) are primarily responsible for the large SD. In six patients, NB improved with the AAG compared with G solu-

<table>
<thead>
<tr>
<th>Patient</th>
<th>AGG Flux</th>
<th>Oxidation</th>
<th>Intake</th>
<th>Synthesis</th>
<th>Breakdown</th>
<th>Net Protein Balance</th>
<th>G Flux</th>
<th>Oxidation</th>
<th>Intake</th>
<th>Synthesis</th>
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<td>1.05</td>
<td>1.50</td>
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<td>1.43</td>
<td>0.54</td>
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<td>1.38</td>
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<tr>
<td>Mean</td>
<td>1.84</td>
<td>0.64</td>
<td>0.24</td>
<td>1.20</td>
<td>1.60</td>
<td>-0.41$^b$</td>
<td>1.72</td>
<td>0.62</td>
<td>0</td>
<td>1.10</td>
<td>1.72</td>
<td>-0.62</td>
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| SD      | 0.50    | 0.18      | 0.03   | 0.40      | 0.50      | 0.16                | 0.29   | 0.20      | 0      | 0.16      | 0.29      | 0.20                

$^a$Data are expressed in $\mu$mol leucine/kg per min as mean ± SD; AAG, combined amino acids plus glucose dialysis; G, glucose dialysis.

$^b$Net protein balance is synthesis minus breakdown. $P = 0.001$ for net protein synthesis on AAG versus G dialysis.
There was no significant treatment period interaction for NB were no appreciable changes in body weight in any patient.

Discussion

Our results show that combined intraperitoneal administration of AA and G improves protein anabolism in APD patients. Recently, the importance of supplying calories simultaneously with intraperitoneal AA to stimulate protein metabolism was demonstrated in CAPD patients (22). In that daytime study, calories were taken orally. However, poor appetite may restrain patients from ingesting enough food including calories. Giving AAG as dialysate during regular APD would be a practical approach.

We found that protein synthesis increased and breakdown decreased during AAG dialysis. Although neither component attained statistical significance, net protein balance (i.e., synthesis minus breakdown) during AAG dialysis improved in all APD patients.

This is the first study to measure WBPT during AAG dialysis. A previous daytime study that involved CAPD patients and used an automated cycler showed an increase in muscle protein turnover (24), whereas a similar study performed during one night in children showed an increase in AA levels without concomitant rise in blood urea nitrogen levels (23).

Anorexia is an important factor in the development of malnutrition (28). In our patients, we noticed a low habitual dietary energy intake and a mean daily protein intake below the Kidney Disease Outcomes Quality Initiative–advised 1.2 g of N/kg per d. In only one patient were Kidney Disease Outcomes Quality Initiative standards actually met. We did not notice any interference of the AA dialysis with appetite or daily food intake. The clinical relevance of the increase in net protein balance (0.21 μmol/kg per min) can be appreciated when one calculates that during 8.5 h of dialysis with AAG mixture, a 70-kg person would gain an average of 13 g of body protein. We supplied 27 g of AA during the night, approximately 47% of which was absorbed. This suggests that virtually all of the absorbed AA were utilized for protein synthesis. This gain in protein exceeds the usual 24-h protein and AA losses via dialysate (10). A stimulatory effect of intraperitoneal AA on protein synthesis was also found previously (22). The slow rate of AA supply in our study (27 g during 8.5 h) might explain the small increase in protein synthesis rate. In Delarue’s study, the additional supply of oral calories simultaneously with AA dialysate induced a decrease in protein breakdown, probably mediated through insulin secretion (16,22,23,29).

Our study suggests that combining AA with the G solution inhibits protein breakdown and stimulates protein synthesis. Human feeding experiments have shown that AA augment the insulin-mediated inhibition of protein degradation in addition to stimulating protein synthesis. Such an inhibitory effect of AA levels on endogenous AA appearance minimizes oxidation and maximizes protein utilization (30,31). Our study does not take into account retention of peritoneally absorbed leucine in the splanchnic bed during AA dialysis. Ignoring splanchnic retention may have resulted in overestimation of the entry rate of absorbed leucine in the plasma pool (i.e., exogenous leucine appearance) and thereby in underestimation of protein breakdown (i.e., endogenous leucine appearance) as the latter is calculated as flux (Q) minus exogenous appearance. A reliable assessment of splanchnic sequestration of AA (leucine) is difficult, and values of 10 to 40% have been reported (22,30).

However, even if a value as high as 40% for splanchnic retention had been present with AA dialysis, the net protein balance observed in our study still would have improved in all patients.
The improvements in protein anabolism during nocturnal APD are acute effects in the fasting state. The results of the 24-h NB studies suggest an improvement in nitrogen retention with the AAG mixture; however, this change was not statistically significant (P = 0.061). We performed the classical NB (N_{bal} = N_{in} - N_{out}), which describes changes over time in body nitrogen content. Whether a positive balance indicates a gain in body protein or an increase in another nitrogenous compound, such as urea, cannot be judged from this method, as any change in urea N pool are not taken into account. However, that body weight did not change appreciably and plasma urea at the end of each study week was not different from the values at the start tends to suggest that there was no increase in the body urea pool. Nevertheless, our results do not provide conclusive evidence of a gain in body protein over 24 h. Improvement in NB was reported previously in malnourished CAPD patients who were treated with an AA-based dialysis fluid (11).

In our study, the proportion of energy and protein given via dialysate varied between 160 and 340 kcal/g N overnight. This suggests that some patients received a considerable surplus of energy in proportion to protein than is present in the normal West-European diet (approximately 150 to 200 kcal/g N). AA were given in a fixed amount of 27 g. The variation in calorie supply resulted from the G concentrations in the dialysate, which were chosen to meet ultrafiltration targets. The best energy-to-protein ratio for optimal protein accretion is unknown and remains to be determined.

Various studies have reported increased urea levels with AA dialysis in CAPD patients (11–14). In contrast, our study showed similar plasma urea levels and urea excretion into dialysate in both study periods. This is in line with an effective utilization of the intraperitoneally administered AA. We also found a decrease in serum phosphate levels, suggesting a shift of phosphate toward the intracellular space, which is another indication that AAG dialysis induced an anabolic response (11,17). With the use of the AAG, serum bicarbonate levels were
slightly lower than on G dialysis but remained within the normal range. In six of eight patients, the levels of serum bicarbonate were even higher than the prestudy levels; this may be attributable to the use of dialysis fluids that contained lower buffer concentrations (35 instead of 40 mmol/L) in some patients in the prestudy period. Furthermore, in the prestudy period, some patients performed fewer than six cycles during nightly dialysis. This suggests that when AA are given in a dose of 27 g in which sufficient amounts of buffer are also present, acidosis can be prevented.

In summary, APD with dialysate composed of a mixture of AAG improves protein anabolism. This finding promises an improvement of nutritional status of PD patients with inadequate protein intake. Studies in larger groups, especially in those with malnourishment, inflammation, and anorexia, are needed to evaluate the long-term clinical relevance of this concept.

Acknowledgments
This study was supported by grants from Baxter Europe and Baxter Benelux.

We thank the patients for participation and the PD nurses for cooperation in the study. We thank Wim Hop, statistician, for statistical advice.

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