Dialysate as Food

The anabolic effects of combined amino acid and glucose solutions in peritoneal dialysis

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Dialysaat als voeding

De anabole effecten van aminozuren en glucose bevattende mengsels bij peritoneale dialyse

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In memory to my beloved parents
To ‘my’ patients, residents and medical students

In der Beschränkung zeigt sich der Meister

Johann Wolfgang von Goethe (1749-1832)

Contents

Chapter 1

Introduction and aim of the thesis

The central theme of this thesis is the concept that it is possible to improve the nutritional state of patients with chronic renal failure undergoing peritoneal dialysis by adding nutrients to the dialysis fluid.

Peritoneal dialysis

In 1923 Ganter performed the first peritoneal dialysis (PD) in a woman with renal failure.[1] It was only in the 1960s that PD became an accepted renal replacement treatment in end-stage renal failure.[2] Following the introduction of continuous ambulatory PD (CAPD) in 1975 by Popovich and Monrief, and the introduction of automated PD (APD) in 1980,[3-5] the number of PD patients increased rapidly worldwide. In the Netherlands, 25.7% of dialysis patients were on PD in 2005.[6] In CAPD 2 liters of fresh dialysis fluid are usually infused into the abdomen via a peritoneal catheter. After a dwell time of 4 to 8 h, during which retained body fluid and metabolites diffuse from the blood into the peritoneal cavity and vice versa, the dialysis fluid is drained. After drainage, fresh dialysis fluid is infused. A common prescription for CAPD is three to four exchanges during daytime and one long (usually 6 to 12 h) overnight exchange. In contrast, in APD an automated cycler regulates the infusions of the dialysis solutions (usually 2 liters) into the abdominal cavity. The cycler is programmed to perform three to four (or more) exchanges during the night. In the morning, 2 to 2.5 liters of fluid is left in the abdomen for 12 to 16 h. There is usually no additional exchange during the daytime, but in some patients one or two additional exchanges are performed during the day to improve clearance or ultrafiltration.

Ideally, a peritoneal dialysis solution should have a maximal and sustained ultrafiltration capacity and a predictable solute clearance, with minimal absorption of the osmotic agents, and deliver a minimal caloric load; it should have no systemic toxicity, it should not accumulate in the body or in body compartments, it should be non-toxic to the peritoneum, non-immunogenic, and it should be easy and inexpensive to manufacture.[7] The first solute in PD fluid to be used commercially on a large scale was glucose. However, it did not fulfill all requirements. After the first commercial dialysis solution became available in 1959, several substances including glycerol, amino acids, and various mixtures of low molecular weight osmotic agents as well as the high molecular weight icodextrin have been studied as alternatives to glucose.[7,8] Despite the long-term disadvantages of glucose such as

obesity, hyperglycemia, hypertriglyceridemia and bioincompatibility, it is still the most widely used osmotic agent for PD. The standard peritoneal dialysis solutions contain 1.36%, 2.27% or 3.86% of anhydrous dextrose/glucose as an osmotic agent in an aqueous solution of electrolytes similar to blood, which also contains a buffer, usually either lactate or a combination of lactate and bicarbonate. In spite of improvements in the composition of PD fluids and in the care of dialysis patients, however, the morbidity and mortality rates remain high.[9] Atherosclerotic cardiovascular disease is a major cause of morbidity and mortality in patients with chronic renal failure, including those treated with dialysis.[10,11] Among the many factors that affect outcome in the PD population, malnutrition plays an important role.[12-14] Since insufficient intake of protein and calories due to a poor appetite and protein loss into the dialysate are major risk factors for the development of malnutrition in PD patients, intraperitoneal provision of amino acids (AA) and glucose (calories) is a logical approach to improve the nutritional state of these patients. This will be outlined in the next sections.

Malnutrition in peritoneal dialysis patients

Protein and energy malnutrition (PEM) is common in patients with chronic renal failure, including those undergoing peritoneal dialysis.[15] The reported prevalence of PEM in PD patients varies from 18% to 56%, depending on the characteristics of the included patients, the time on dialysis treatment, and the nutritional assessment tools and nutritional markers used.[16-18] There is abundant evidence in patients with chronic renal failure for a strong association between malnutrition, inflammation, and atherosclerosis (known as MIA syndrome), but how these factors interact to cause malnutrition is not clear.[19] Although PEM is rarely the direct cause of death, it is an important risk factor for morbidity and mortality in PD patients.[20,21] Prevention and treatment of malnutrition have been advocated to reduce malnutrition-related complications and to improve the quality of life and life expectancy. Although it seems self-evident, the beneficial effect of improving nutritional status on the prognosis of PD patients still needs to be confirmed. Effective strategies to prevent and treat malnutrition in PD patients should be based on an understanding of the pathogenesis. In the past decade two types of PEM in dialysis patients have been identified. Type-1 malnutrition is mainly caused by inadequate nutrient

intake. This type of malnutrition should benefit from nutritional supplements. In contrast, type-2 malnutrition is thought to be induced by a catabolic state due to inflammation and coexisting illnesses, rather than only a low nutrient intake.[22] The terms wasting or malnutrition-inflammation complex syndrome or malnutrition-inflammation-cachexia syndrome (MICS) have been proposed for this latter condition.[23] In type-2 malnutrition, in addition to sufficient nutrient intake, treatment of the underlying illnesses and inflammation is essential. Most malnourished dialysis patients probably suffer from a combination of the two types of malnutrition. Factors thought to be involved in the pathogenesis of PEM in PD patients[24] will be summarized in the next paragraphs.

Low dietary nutrient intake

A large proportion of chronic renal failure patients, including patients on PD, suffer from anorexia.[25,26] Factors involved in the suppression of appetite in PD patients include uremic toxicity, metabolic acidosis, inflammation, fluid overload, dialysis procedures, hormonal and gastrointestinal disturbances, and complicating illnesses. The mechanisms of appetite suppression are poorly understood.[27] A relationship between the progression of renal failure and spontaneous dietary protein intake, especially below a creatinine clearance of 25 ml/min, has been documented.[28,29] In the Modification of Diet in Renal disease (MDRD) study group, patients with a GFR of 24 ml/min/1.73 m^2 or lower tended to lose body mass.[29] It has been shown that the protein requirements for CAPD patients are considerably higher than those for normal individuals.[30,31] After about one year after commencing dialysis therapy, the protein intake of a substantial proportion of PD patients remains far below an intake of 1.2 to 1.3 g/kg body weight per day that is recommended for clinically stable chronic PD patients by the National Kidney Foundation Dialysis Outcome Quality Initiative (NKF/DOQI) guidelines.[32,33] In spite of the additional energy supplied by glucose absorbed from the dialysis fluid, the actual energy intake is lower than the recommended total (diet and dialysate) energy intake of 35 kcal/kg body weight per day.[34] In the few studies reporting resting energy expenditure (REE) in PD patients, no difference in REE was found compared with healthy subjects.[35,36]

Dialysis procedure

The number of dialysate exchanges per day, the glucose concentration of each exchange and plasma glucose concentration, the dwell time, and patient's membrane

characteristics determine the amount of glucose absorbed from the dialysate. Generally, CAPD patients absorb on average 100 to 200 g glucose per day from dialysate, accounting for 12% to 34% of the total daily energy intake.[37] Continuous glucose absorption from the dialysate may lead to hyperinsulinemia, hyperglycemia and hypertriglyceridemia, and may also suppress appetite.[37,38] When oral intake is not reduced, in some patients glucose absorption may lead to obesity.[39,40]

A total daily loss of 3.4 ± 1.2 (SD) g of amino acids and 5 to 15 g of protein through peritoneal clearance has been reported in various studies.[41-43] Factors which are involved in the quantity of the protein lost into the dialysate include the composition of the infused dialysis fluid, the peritoneal membrane transport characteristics, the serum protein concentration, and the patient's clinical state. An early washout phenomenon due to residual peritoneal fluid may explain why in intermittent peritoneal dialysis the protein concentration is usually highest in the first exchange.[44] The protein and amino acid losses can be compensated by an adequate protein intake.[41] A poor appetite, however, prevents a considerable proportion of PD patients from consuming sufficient protein, resulting in protein deficiency and a decrease in lean body mass. Various factors may play a role in the development of PEM including loss of residual renal function, fluid overload, underdialysis with accumulation of poorly defined uremic toxins, such as middle molecular weight waste products of proteins.[45-48] A relationship between peritoneal membrane permeability characteristics and nutritional state has also been suggested. A high permeability state may be a risk factor for PEM in PD patients.[49,50]

Metabolic acidosis

Metabolic acidosis is often overlooked in patients on PD, but may reduce appetite. Another major consequence of chronic metabolic acidosis is activation of the ATP-dependent ubiquitin-proteasome pathway and increased branched-chain keto-acid dehydrogenase activity, which results in breakdown of muscle protein and inhibition of protein synthesis.[51,52] Several studies have shown that correction of metabolic acidosis in maintenance dialysis prevents the up-regulation of the ubiquitin-proteasome pathway and reduces protein degradation, and is associated with an improvement in nutritional status.[53,54] The optimal level of serum bicarbonate for correcting acidemia has not been established. The NKF/DOQI guidelines for maintenance dialysis recommend a serum bicarbonate level of 22 meq/L or higher.

Inflammation and hypoalbuminemia

Inflammation is a common feature of end-stage renal failure as a cause of PEM.[55,56] Infectious peritonitis and low-grade inflammation of the peritoneum induced by the presence of glucose in the dialysis fluid are likely to contribute to the increased levels of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukins 1β (IL-1β), and 6 (IL-6) in dialysate of PD patients.[57-59] Pro-inflammatory cytokines may suppress appetite, cause muscle wasting, hypoalbuminemia, and increased C-reactive protein (CRP) concentrations.[60,61] As mentioned above, a pathophysiologic link between malnutrition, inflammation, and atherosclerosis has been proposed in this patient population.[19,62]

Inflammation, alone or in combination with a low protein intake or transperitoneal losses of albumin, plays a significant role in causing hypoalbuminemia in PD patients.[63] A strong association between hypoalbuminemia and a high mortality rate has also been reported in PD patients.[64,65] Hypoalbuminemia, however, reflects the presence of systemic disease rather than malnutrition, and serum albumin is a poor nutritional parameter in PD patients.[66]

Gastrointestinal disturbances

An increase in abdominal pressure due to the presence of dialysis fluid, sometimes combined with impaired gastric emptying, has inconsistently been reported as a factor contributing to anorexia and malnutrition in PD patients.[67,68] There is evidence for impaired small intestinal protein assimilation in end-stage renal disease, which seems to correlate with the severity of the MIA syndrome.[69] Finally, an association between infection with Helicobacter pylori and anorexia and malnutrition has been recently reported.[70]

Hormonal disturbances

Chronic renal failure of any cause is associated with insulin resistance of peripheral tissues with hyperinsulinemia and glucose intolerance.[71] Resistance to the anabolic effects of other hormones such as insulin-like growth factor (IGF-1) may also contribute to PEM in maintenance dialysis patients.[72,73] A reduced serum IGF-1 has been found in malnourished uremic patients, and it has been suggested to be an early indicator of malnutrition in end-stage renal disease.[74,75] Another hormone that may play a role in the development of PEM is leptin. However, whether leptin contributes to malnutrition in uremic patients

has not yet been clarified. An association between an elevated serum leptin with inflammation and a decrease in lean body mass during PD has been recently reported.[76]

Comorbidity

Pre-existing illnesses such as diabetes mellitus are highly prevalent in the PD population and may be the most important determinant of morbidity and mortality.[77] There is a strong association between co-morbidity and poor nutrition with a lower dietary protein and calorie intake.[78]

Methods to assess nutritional state in peritoneal dialysis patients

Nutritional assessment is essential for the early recognition and treatment of protein and calorie malnutrition (PEM) in PD patients. Unfortunately there is no single perfect tool for evaluating the nutritional status of PD patients, and in clinical practice we have to make do with a combination of objective and subjective methods. These methods should preferably being simple and inexpensive. The original subjective global assessment (SGA) is such a simple and inexpensive method, initially validated as a screening tool for surgical patients.[79] The SGA has been repeatedly modified and tailored to the needs of the PD patients, as recently reviewed.[80,81] However, the best version of the SGA as a screening tool of the nutritional state of PD patients has still to be defined. In our studies we used the original SGA. Other approaches such as the body mass index (BMI), four-site skinfold anthropometry (FSA), bioelectrical impedance analysis (BIA), and total body dual energy x-ray absorptiometry (DEXA) have been used to measure body composition in PD patients. These methods will be discussed in chapter 2.

Protein homeostasis

Protein homeostasis is a continuous process in which proteins are synthesized from amino acids and degraded back to them, a process of protein turnover. A 'nitrogen or amino acids pool' is considered to exist in the body that is in dynamic

equilibrium with tissue proteins (Figure 1). Amino acids (AA) are continually taken from the pool for protein synthesis and replaced by dietary and tissue AA through proteolysis. In a healthy adult, the total amount of protein in the body is rather constant, so that the long-term rate of protein synthesis is equal to the long-term rate of protein breakdown. In an average 70 kg person, about 300 g of protein is synthesized each day and 300 g is degraded.[82] About 80% of the amino acids from degraded protein are reutilized for protein synthesis. Individual proteins turn over at different rates. For example, some liver and plasma proteins have a half-life of 180 days or more, while enzymes and hormones may be recycled in a matter of minutes or hours. All cellular and tissue protein has a biological function. Since surplus non-functional protein cannot be stored, any amino acids that are not immediately used are oxidized and their nitrogen converted to urea, which is excreted from the body. There is a diurnal pattern of protein and energy metabolism in the human body due to the cyclic pattern of food intake.[83] The discontinuous pattern of nutrition consists of a post-absorptive phase (fasting state) of negative net protein balance (*i.e.* protein synthesis minus breakdown), while in a post-prandial phase (fed state) repletion of the post-absorptive losses occurs, resulting in a positive balance after ingestion of food. Various studies of the effects of administration of insulin and amino acids (AA) on protein metabolism have been published.[84- 89] In healthy human subjects, insulin and AA have different effects on protein dynamics in the splanchnic bed and skeletal muscle.[90,91] After an overnight fast (post-absorptive state), muscle is in a catabolic state and provides AA for synthesis of essential proteins in other parts of the body, *e.g.* the splanchnic bed. Insulin has been shown to have an inhibitory effect on protein breakdown (PB) in skeletal muscle and supplying AA did not enhance this inhibitory effect. In contrast, in the splanchnic bed insulin had no inhibitory effect on PB, while AA had a profound inhibitory effect on PB. In muscle, insulin and AA had an additive effect on protein synthesis (PS), whereas no such additive effect was found in the splanchnic bed. After a mixed meal a net positive AA balance is achieved mainly by the insulin-induced inhibition on muscle PB and the AA-induced stimulation of muscle PS. The net positive AA balance in splanchnic bed was a consequence of an AA-induced inhibition of PB and an AA-induced stimulation of PS. This effect of AA is independent of insulin, as studies of subjects with type 1 diabetes have shown that in the insulin deficiency state, high circulating AA are associated with increased AA uptake in the splanchnic bed and an increase in PS rate.[92] Taken together, these findings

indicate that AA are predominant stimulants of splanchnic PS.[91] It is likely that the increase in splanchnic PS results from an increase in gut PS, as infusion of AA together with insulin did not stimulate PS in the liver.[93] Thus, protein anabolism in muscle is mostly due to the insulin effect on muscle protein breakdown; insulin alone plays little or no role in stimulating muscle protein synthesis. By contrast, protein anabolism in the splanchnic bed is largely determined by amino acids through stimulating protein synthesis and inhibiting protein breakdown. A differential effect of insulin on the synthesis rates of various liver proteins has also been reported.[93] The results, however, are conflicting.[94,95] In human studies, insulin infusion decreased splanchnic protein synthesis in non-diabetic and type 1 diabetic subjects.[90,92] Although insulin may stimulate the synthesis of some liver proteins[95] like albumin, the synthesis of other liver proteins, such as fibrinogen, is suppressed.[96] To maintain the synthesis rate of albumin, insulin together with a supply of amino acid is necessary.[93, 95,97]

Insulin resistance is common in patients with CRF, including those on PD.[71] In CRF patients, the insulin-mediated suppression of muscle protein breakdown is normal,[98] but the ability of amino acids and insulin to augment protein synthesis during AA-infusion seems to be impaired.[99] Conversely, in CAPD patients the post-absorptive reduction of protein synthesis is reversed by supplying amino acids, which suggests a normal anabolic response to protein feeding.[100]

Methods of measuring whole-body protein metabolism

Overall protein metabolism in an organism can be studied by techniques that enable quantification of biological pathways (of amino acids) involved in protein synthesis and breakdown.

Nitrogen balance

The classical nitrogen balance (N-balance) is the oldest method and is the traditional reference technique for assessing nutritional efficacy in protein metabolism, despite its limitations.[101] It measures the net change in total body protein, *i.e.* the difference between the amount of nitrogen entering the body and the amount leaving the body. It is calculated using measured nitrogen intake, measured fecal and urinary nitrogen losses, while fixed values of 1.5 and 0.5 g/d for nitrogen losses via feces and via the skin respectively are usually assumed.

When applied to patients with renal failure, nitrogen balance must be adjusted for changes in the body nitrogen pool, because of a large urea N pool.[101,102] Such changes are detected by daily measurement of serum urea nitrogen.

Whole-body protein turnover

Since stable isotopes became available in 1939, they have been extensively used in diagnostic tests and in clinical research.[103] In 1949, Sprinson and Rittenberg were the first to publish the end-product method using a single oral dose of [^{15}N]glycine and measuring ^{15}N enrichment of urinary urea as end product to estimate whole-body protein turnover (WBPT).[104] This method has subsequently been modified several times. Waterlow introduced the single oral [^{15}N]glycine method with ammonia as end product.[105,106] Fern et al made a correction by measuring the body urea pool during WBPT assessment.[107] The various modifications of the end-product methods of measuring WBPT have been reviewed recently by Duggleby and Waterlow.[108] A technique that developed after the single-dose method makes use of continuous infusion of stable isotopic tracers of amino acids and is known as the precursor method. The precursor method with continuous infusion of L-[1-^{14}C]leucine has previously been compared with the end-product method using [^{15}N]glycine. It was concluded that the results of both methods were comparable.[109] The precursor method,[110] however, provided more appropriate results than those obtained with the end-product method. In this thesis, the precursor method with L-[1-^{13}C]leucine, administered as a primed continuous intravenous infusion, is used as the reference tracer method for measuring WBPT. To our knowledge there is no study comparing the two methods in PD patients. In this thesis, the end-product method with [^{15}N]glycine given as a single oral dose is compared with the precursor method using a primed continuously intravenous infusion of L-[1-^{13}C]leucine for measuring WBPT to have a methological comparison in PD patients. The precursor method will be described in chapters 3 and 4, and the comparison between both methods will be discussed in chapter 6.

Principles of the whole-body protein turnover-methods

A model consisting of simple homogeneous pools as shown in Figure 1, restricted by some assumptions, represents the human whole body.

In this model there is one amino acid pool and one protein pool. It is considered that each of these pools is mixed homogeneously. The following

assumption is that there is equilibrium between these pools during the time of sampling for measurements. Amino acids are continuously entering the pool via diet and/or dialysate (I) and as a consequence of tissue protein breakdown (B). At the same time, amino acids are leaving the pool for new protein synthesis (S) and are susceptible to oxidation (O). The total flow through this system is called the flux (Q). The ideal situation to be reached during measurements for protein turnover is that there is a steady state for the system during which the pools do not change in size. In this steady state, the total rate of AA appearance in the pool is equal to the total rate of disappearance. In this situation the total flux Q can be represented as Q = S + O. But at the same time, the flux can be represented with the formula: Q = I + B. Because there is only one flux per definition, the formula can be written as Q = S + O = I + B. When known variables are substituted, the unknown processes as synthesis and breakdown can be calculated by this method. This simple model can be used in two different ways to find synthesis and breakdown of protein: the precursor method using continuous infusion of L-[1-^{13}C]leucine or the end-product method using a single oral dose of [^{15}N]glycine. Both methods are shortly explained hereafter.

In the precursor method L-[1-^{13}C]leucine is infused after a priming dose, which leads to an equilibrium (plateau concentration) of the infused material. Instead of L-[1-^{13}C]leucine itself the measurements are based on [^{13}C]ketoisocaproic acid (KICA) which is a metabolite representing closely the L-[1-^{13}C]leucine value inside the tissue cells where protein synthesis actually takes place.[111] The rate of oxidation of amino acid is calculated from the $^{13}CO_2$ in expired air. The flux, protein synthesis, and breakdown rates are calculated from these results. Since part of the absorbed amino acid is taken up by the splanchnic tissues (*i.e.* first pass uptake), the splanchnic retention has to be taken into account as reported,[112,113] and as will be discussed in chapters 3 and 4.

The end-product method uses [^{15}N]glycine as a tracer, which is orally ingested as one single dose. In the period following, this amino acid is diluted in the pool and is subject to metabolism along with the other amino acids. After this 'pulse label' is metabolized (usually 9 h), the end products urea and ammonia can be analyzed for [^{15}N] and lead to a prediction of the amounts of N used for synthesis (S) or breakdown (B) of protein. Both methods are based on different assumptions and calculation procedures, which are reviewed in detail elsewhere.[108]

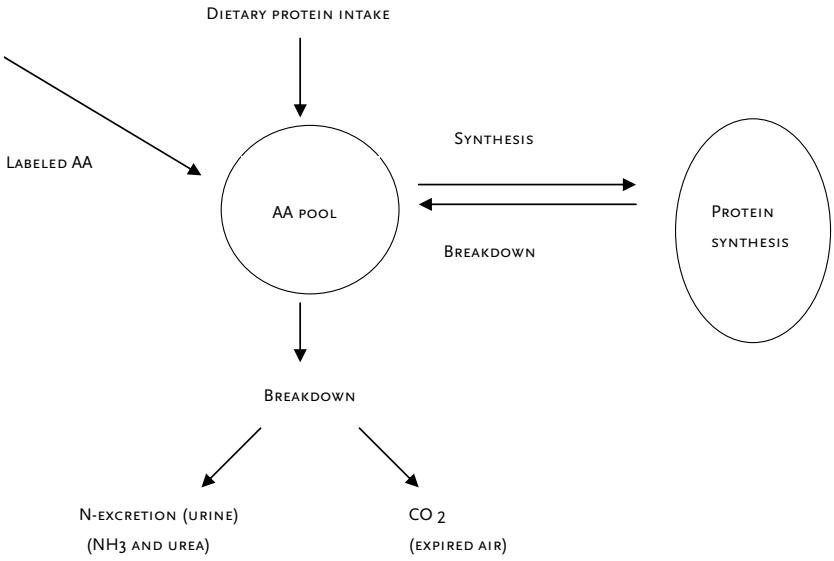

Figure 1. A general model of protein metabolism used in the whole-body turnover method[82]

Dialytic approach to nutritional intervention

Intraperitoneal nutrition has been proposed as an alternative approach to overcome protein deficiency in PD patients, and dialysis solutions, which contain amino acids, have become commercially available.

Amino acid-based dialysate

Gjessing (1968) was the first to evaluate the effects of adding amino acids (AA) to the dialysis fluid in patients under regular PD regimens for chronic renal failure. He noticed considerable absorption of AA by the peritoneum and a diminished reduction of serum protein.[114] Oreopoulos et al (1979) was the first to use AA-based dialysate in CAPD patients.[115] The use of AA-based peritoneal dialysis solutions has been extensively reviewed.[116-118] To summarize, peritoneal solute transport and ultrafiltration during dialysis with solutions containing 1% AA and containing 1.36% glucose are similar. The average molecular weight of AA included in the AA-containing PD solutions is about 140 daltons. In short-term studies it has been found that AA-based PD solutions are capable of providing sufficient ultrafiltration, although the period of effective ultrafiltration is rather

short due to the more rapid absorption of AA than that of glucose. The amount of absorbed AA depends on the AA concentration in the PD solutions, dwell time and dwell volume, and a patient's membrane transport characteristics. In CAPD an absorption ranging from 60 to 90% is reported during a 4 to 6 h dwell. Plasma AA concentrations rise significantly during the use of AA-containing solutions. After peritoneal delivery, peak plasma levels of most AA were comparable with the levels that are normally found after ingestion of a large protein meal or after mixed meals with a protein ingestion similar to that observed after a protein-only meal. An increased peritoneal protein loss with AA solutions has been reported in studies with 1% and 2.7% AA-based solutions respectively.[119,120] The composition of the AA-PD solutions and the buffer component have been changed over time and adjusted to the needs of CRF patients. In the earlier studies an AA-PD solution (Travasol, Baxter Healthcare, Deerfield, Il, USA) was used. The pH of the solution was 6.0 to 6.5 and the buffer was lactate at a concentration of 33 mmol/liter either alone or combined with acetate at 7 mmol/liter. Studies with a small number of patients showed a wide discrepancy in nutritional effects, in part due to a great diversity in the nutritional parameters examined, while there was a little overall nutritional benefit. Uremic symptoms and metabolic acidosis were reported. In subsequent studies performed with RenAmin (Baxter Healthcare), the AA composition was changed, with an increase in the essential AA. The total AA concentration remained at 1%, the lactate concentration was increased to 35 mmol/liter and the pH maintained at 6.0 to 6.5. These studies in a small number of patients demonstrated an overall positive nutritional benefit, but an increase in BUN and mild metabolic acidosis persisted. The solution was well tolerated. The current 1.1% AA-PD solution (Nutrineal, Baxter Healthcare) approaches the assumed optimal balance between essential and non-essential AA required for a CRF patient. It contains all nine essential AA, six non-essential AA, and the buffer component is lactate at a level of 40 mmol/liter (Table 1). Studies conducted with the 1.1% AA-based PD solutions encompass a larger number of patients. The gain of amino acids exceeds the losses of amino acids, *i.e.* there is a net gain.[121] An increase in serum urea and, with the use of more than one bag of 1.1% AA dialysis solutions per day, mild metabolic acidosis has been found. Generally one bag of 1.1% AA dialysis solutions is well tolerated.

The beneficial nutritional effects of the AA-based solutions have not been consistently found, which may due in part to differences in study design, patient characteristics such as the presence or absence of malnutrition and the

nutritional parameters being used.[122-130] An important factor underlying the lack of conclusive positive results, however, might be that the use of the AA-containing solution was not accompanied by an adequate supply of calories. Moreover, it has been reported recently that 1.1% AA-containing dialysate produced an increased glucose transport from blood to dialysate, which may contribute to loss of glucose from the body.[131] It has been shown that simultaneous administration of sufficient oral calories is essential to achieve an optimal utilization of intraperitoneally administered AA.[132] Since a poor appetite prevents PD patients from taking sufficient proteins and calories, PD solutions containing a mixture of AA plus glucose as a source of food would be a practical approach to circumvent the insufficient nutrient intake in these patients.

Table 1. The composition of 1.1% amino acid-peritoneal dialysis solution (Nutrineal)

	Amino acids				
Essential amino acids (g/L)	Histidine	0.714	Non-essential amino acids (g/L)	Arginine	1.071
	Isoleucine	0.850		Alanine	0.951
	Leucine	1.020		Proline	0.595
	Lysine-HCL	0.955		Glycine	0.510
	Methionine	0.850		Serine	0.510
	Phenylalanine	0.570		Tyrosine	0.300
	Threonine	0.646			
	Tryptophane	0.270			
	Valine	1.393			
Electrolytes and buffer (mmol/L)	Sodium	132			
	Chloride	105			
	Calcium	1.25			
	Magnesium	0.25			
	Lactate	40			
pH		6.7			
Osmolality	365 mOsmol				

Aim of the thesis

Patients treated with peritoneal dialysis (PD) often develop protein and calorie malnutrition (PEM), which is an important risk factor for morbidity and mortality. This thesis examines the potential of a specific approach to the prevention and treatment of PEM, the administration of protein and calories via dialysis fluid. The underlying idea is to circumvent a number of factors, such as the loss of appetite and the gastrointestinal problems that reduce food intake. The amounts of food administered via the dialysis fluid are simple to quantify and, in contrast to parenteral nutrition, direct access to the bloodstream is not needed. Dialysis solutions containing amino acids (AA) plus glucose are expected to compensate for the low dietary protein and calorie intake in these patients.

In **chapter 2** we assessed the nutritional state of patients with chronic renal failure who were being treated in the Erasmus MC using the original subjective global assessment (SGA).

In **chapter 3** we investigate the metabolic effects of dialysis solutions containing AA plus glucose in patients treated with automated PD (APD) in the fasting state overnight.

A substantial part of PD patients, however, are still treated by continuous ambulatory PD (CAPD) during the day while they consume their meals. These patients might also benefit from the use of AA-containing solutions in addition to their dietary food intake. In **chapter 4** we investigate whether dialysis solution containing AA plus glucose may also contribute to an improvement in protein metabolism in CAPD patients.

In **chapter 5** we examine whether dialysis solution containing AA plus glucose combined with food intake is capable of increasing the fractional synthesis rate of albumin (FSR-albumin) in parallel to an improvement of the whole-body protein synthesis in PD patients in the fasting (APD) and the fed state (CAPD).

There is a need for a validated method to measure whole-body protein metabolism, which can be applied repeatedly in different patient populations and in an outpatient setting. In **chapter 6** we examine whether the end-product method with a single oral dose of [^{15}N]glycine is such a method and whether it can replace the precursor method with primed continuous infusion of [^{13}C] leucine.

Little is known about the protein and AA losses into dialysis effluent in APD patients, and as AA-based dialysis solutions could conceivably induce inflammation of the peritoneum, resulting in increased peritoneal protein

losses, in **chapter 7** we describe the measurements of peritoneal protein losses and cytokines in plasma and dialysate with the use of combined AA plus glucose dialysis solutions, and additionally the amino acid losses into the dialysis effluents with standard (glucose) dialysis solution in APD patients.

In the final chapter the results are discussed and summarized.

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Chapter 2

Subjective global assessment of nutritional state of peritoneal dialysis patients

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Submitted

Abstract

Early detection of malnutrition in uremic patients is essential as malnutrition is associated with high morbidity and mortality. We compared the results of subjective global assessment (SGA) of nutrition state of peritoneal dialysis (PD) patients with other nutritional parameters.

An observational cross-sectional study was performed in 30 adults (20 males) on PD. Nutritional state was assessed by SGA, and related to body mass index (BMI), four skin-fold anthropometry (FSA), bioelectrical impedance analysis (BIA), and dual energy x-ray absorptiometry (DEXA).

Using SGA 37% of all patients were malnourished (CI: 20 % to 56 %), with 27% in SGA-B (moderate) and 10% in SGA-C (severe). High correlations were found between the graded SGA on one hand and BMI ($r = -0.75$, $P < 0.001$), fat mass (FM) index using FSA ($r = -0.75$, $P < 0.001$), BIA ($r = -0.71$, $P < 0.001$), DEXA ($r = -0.76$, $P < 0.001$) on the other hand. In conclusion, a high prevalence of malnutrition was found in PD patients, using SGA as a screening tool. SGA correlated well with body composition measurements.

Introduction

Malnutrition is a risk factor for morbidity and mortality in PD patients.[1,2] Early detection of malnutrition in PD patients is crucial if effective nutritional strategies are to be initiated. Reliable methods to assess the nutritional status such as underwater weighing to estimate body composition, prompt neutron activation analysis to measure total body nitrogen, and total body potassium counting to measure fat free mass (FFM) are only available in a few centers and require costly equipment.[3-5] Alternative methods for the assessment of the body composition have been proposed, including anthropometry, bioelectrical impedance analysis (BIA), and dual energy x-ray absorptiometry (DEXA).[3,6-9] The subjective global assessment (SGA) has a fundamentally different approach. It is a simple method, which is easy to apply in routine clinical practice. It is a reproducible and inexpensive tool, which has been shown to be a valid method for nutritional assessment in different populations.[10-13] SGA takes clinical findings into account and reflects changes in the nutritional state in the preceding months (*i.e.* change in body weight), whether it is compromised at the present time (edema), or whether it affects function (muscular weakness). The other methods give information about the actual nutritional state by measuring body composition. Initially, SGA was used to assess nutritional state in preoperative surgical patients to predict risk of postoperative complications.[14] The original SGA was subsequently modified in an attempt to increase its predictive value and reproducibility for dialysis patients.[15-18] Up to now, however, none of these modifications for PD patients have been validated in a large study.[19,20]

The primary purpose of this study was to assess the nutritional state of PD patient by using the original SGA. Secondly we analyzed relationships between the SGA and measurements of body composition.

Subjects and Methods

Patients

Patients were recruited from the Peritoneal Dialysis Unit of the Erasmus MC, Rotterdam, the Netherlands. The enrollment period was from July to September

2005. Thirty out of thirty-three consecutive PD patients were included in this study. Three patients did not participate in the evaluation because of non-medical reasons (refusal and non-compliance). The study was performed according to the guidelines of the local medical ethical committee. All participating patients gave informed consent.

Study design

We performed an observational cross-sectional study to assess nutritional state. In each patient all measurements were carried out on a single day. On the study day all patients came to the hospital at least two hours after their last meal and before the next exchange of dialysate. One observer performed the SGA, the anthropometric measurements, the BIA, and the DEXA in all patients. Weighing, BIA and DEXA measurements were carried out with an empty peritoneal cavity. The daily intake of protein and calories was based on food records and a dietary interview by a renal dietician.

Measurements

Subjective global assessment (SGA): the SGA method was used as described by Detsky et al.[21] Using data from the history (anorexia, nausea, vomiting, diarrhea and weight loss in the preceding 6 months) and physical examination (loss of subcutaneous fat over the triceps and mid-axillary line on lateral chest wall, muscle wasting in the deltoids and quadriceps, and the presence of ankle edema), the patients were classified into three categories: category A = good nutritional state, category B = moderate malnutrition, category C = severe malnutrition. The SGA was performed in all patients by one single observer.

Anthropometric measurements: body height was measured to the nearest 0.5 cm with the patient standing against a fixed stadiometer. Body weight was measured to the nearest 0.1 kg, in light clothing without shoes using a weight scale (Seca Corp. Scale, USA). Body mass index (BMI) was calculated as weight (kg) divided by height square (m^2).

Four-site skinfold anthropometry (FSA): skinfold measurements were performed to estimate total body fat mass using a Harpenden calliper (British Indicators Ltd, West Sussex, UK). Skinfold thickness was measured at four sites (biceps, triceps, subscapular and suprailiac) on the non-dominant arm to the nearest 0.1 mm. Three measurements were performed at each site and the averaged value is presented. Body fat mass (FM) and fat free mass (FFM)

were estimated from the sum of the four skinfold thickness and body density according to the method of Durnin and Wormersley.[22]

Bioelectrical impedance analysis (BIA): measurements were performed using a bioelectrical impedance analyser (model BIA-101 RJL/Akern Systems, Detroit, MI, USA). After five minutes in supine position, a low-amplitude, single-frequency, imperceptible current of 800 mA at 50 kHz was introduced at the distal electrodes. Resistance and reactance were measured as parameters of impedance. Software supplied by LeanBody RJL Systems (Detroit, MI, USA) was used to provide data of the FM and FFM derived from the impedance parameters.

Total body dual energy x-ray absorptiometry (DEXA): the DEXA was performed using a Lunar Prodigy system (General Electric Corporation, Madison, WI, USA). The data were calculated using software version 7.53. For clarity the LBM measured with DEXA is presented as FFM.

Residual renal function (RRF): the RRF was defined as the mean of renal urea and creatinine clearances (ml/min) and derived from PD adequest 2.0, software Baxter.

Calculations

The following formulas were used to calculate the fat free mass index (FFMI) and the fat mass index (FMI): FFM divided by the square of the height (kg/m^2) and FM divided by the square of the height (kg/m^2), respectively.

Statistical analysis

Data are expressed as mean ± SD or range and median. Correlations between the SGA classifications and the RRF and the body composition measurements were evaluated using Spearman's rank correlation. The two-independent sample *t* test was used to compare differences regarding the RRF as well as body composition measurements between both nutritional states. Differences were considered statistically significant when the two-sided P value was < 0.05. Receiver operating characteristic (ROC) curve of BMI, with SGA as reference, was constructed.

To assess the agreement between different methods of measuring body compositions intraclass correlation coefficients (ICC) were calculated.

Data were analyzed using the statistical program SPSS version 11.0, for Windows (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the baseline characteristics of the patients. Hypertensive nephropathy was the most prevalent cause of renal failure. There was only one patient with diabetic nephropathy. Six patients (4 males, 2 females) were anuric, four patients had a urine production between 50 and 320 ml per day. In 5 patients RRF was not determined as they were less than 3 months on PD.

We found that nineteen patients (11 men, 8 women) were well-nourished, classified as SGA-A (63%), eight patients (6 men, 2 women) were moderately malnourished, classified as SGA-B (27%) and three patients (all men) were severely malnourished, classified as SGA-C (10%). For the whole group significant correlations were found between the SGA classifications (A, B, C) and the following parameters: BMI ($r = 0.75$, $P < 0.001$), FMI measured using FSA ($r = -0.75$, $P < 0.001$), using BIA ($r = -0.71$, $P < 0.001$), and using DEXA ($r = -0.76$, $P < 0.001$). Regarding the FFMI, only BIA showed a significant correlation between the SGA and the FFMI-values ($r = -0.47$, $P = 0.009$). In men: FFMI measured with all three methods correlated significantly with SGA (FSA: $r = -0.51$, $P = 0.027$; BIA: $r = -0.79$, $P < 0.001$; DEXA: $r = -0.62$, $P = 0.004$). Table 2 presented the results

Table 1. Baseline characteristics of the study patients (N = 30)[a]

Gender (M/F)	20 /10
Age (yr ± SD)	54 ± 16.2
Cause of ESRD	10 hypertension
	5 unknown
	5 glomerulopathy
	3 obstructive nephropathy
	2 chronic pyelonephritis
	2 reflux nephropathy
	1 diabetic nephropathy
	1 hyperoxaluria
	1 APKD
Time on PD (mo median with range)	17.5, 1-91
PET	8 H, 19 HA, 2 LA
Protein intake (g/kg/d)	1.0 ± 0.3
Energy intake (kcal/kg/d)	23 ± 7.0

[a]M, male; F, female; ESRD, end-stage renal disease; PD, peritoneal dialysis; mo, month; APKD, adult polycystic disease; PET, peritoneal equilibrium test, H, high; HA, high average; LA, low average.

Table 2. Nutritional variables according to SGA-classification[a]

	Males			Females		
	Well-nourished n =11	Malnourished n = 9	95% CI	Well-nourished n = 8	Malnourished n = 2	95% CI
BMI (kg/m²)	27.8 ± 3.7	21.3 ± 2.3	3.5 to 9.5	27.7 ± 2.9	19.5 ± 1.0	3.2 to 13.1
FSA-FMI (kg/m²)	8.6 ± 2.1	4.4 ± 2.1	2.1 to 6.3	11.5 ± 1.3	5.3 ± 0.03	4.0 to 8.3
BIA-FMI (kg/m²)	8.7 ± 2.5	5.1 ± 1.4	1.6 to 5.5	11.8 ± 1.9	5.7 ± 1.4	2.7 to 9.5
DEXA-FMI (kg/m²)	8.1 ± 2.2	4.1 ± 2.0	2.1 to 6.1	11 ± 1.6	3.8 ± 1.0	4.4 to 10.1
FSA-FFMI (kg/m²)	19.2 ± 2.4	17.3 ± 1.0	0.03 to 3.8	16.2 ± 1.9	14.2 ± 1.0	-1.4 to 5.3
BIA-FFMI (kg/m²)	19.1 ± 1.5	16.2 ± 1.1	1.6 to 4.1	15.8 ± 1.2	13.8 ± 0.5	-0.5 to 4.2
DEXA-FFMI (kg/m²)	18.7 ± 2.1	16.4 ± 1.2	0.6 to 3.9	15 ± 2.9	15 ± 0.2	-4.8 to 5.0

[a]Data are expressed as mean (± SD); 95% CI, 95% confidence interval for difference; SGA, subjective global assessment (A, well nourished; B + C, malnourished); FM, fat mass; FFM, fat free mass; BMI, body mass index; FSA, four skinfold anthropometry; BIA, bioelectrical impedance analysis; DEXA, dual energy x-ray absorptiometry.

of the comparison between nutritional state and the objective measurements for both gender. FMI measured with all three body composition methods was significantly higher in female patients than in male patients: using FSA 10.2 ± 2.8 kg/m^2 *versus* 6.8 ± 3.0 kg/m^2, P = 0.006, using BIA 10.6 ± 3.1 kg/m^2 *versus* 7.1 ± 2.7 kg/m^2, P = 0.004, using DEXA 9.6 ± 3.4 kg/m^2 *versus* 6.3 ± 2.9 kg/m^2, P = 0.011. Malnourished patients showed approximately 50% lower FMI and 10% lower FFMI. The percentage of cases with SGA equal to B or C did not significantly differ between both genders, 45% (9/20) for men *versus* 20% (2/10) for women (Fisher exact test, P = 0.25). None of the differences between SGA-A (well-nourished) and SGA-B+C (malnourished) with regard to the nutritional variables (BMI, FSA-FMI or FFMI, BIA-FMI or FFMI, DEXA-FMI or FFMI) depended on gender (effect modification: all P > 0.09 using Anova).

Using the ROC curve and the SGA as reference standard, we found a good discriminating value between SGA-A *versus* SGA-B+C for BMI (AUC > 0.90). A cut-off level of BMI of < 23.7 kg/m^2 was able to predict malnourishment with a sensitivity of 90% and a specificity of 91% for the whole group. There was a very good agreement between FSA and BIA (ICC = 0.90), FSA and DEXA (ICC = 0.93) and between BIA and DEXA (ICC = 0.91).

Furthermore we found that the SGA-classification A, B, C was significantly correlated with the RRF (r = - 0.51, P = 0.009). Mean RRF varied between 0 and 8.53 ml/min/1.73m^2 (median 3.7 ml/min/1.73m^2) in the well-nourished patients (n = 17) and between 0 and 5.10 ml/min/1.73m^2 (median 0 ml/min/1.73m^2) in the malnourished patients (n = 8).

Discussion

We assessed the nutritional state of patients with chronic renal failure who were being treated with PD in our center using the three-point SGA scale as originally described to identify malnourished PD patients. A generally accepted standard clinical method to assess nutritional status of PD patients is not available at present. The SGA has been used in many populations including dialysis patients.[10-13] The SGA is reproducible and easy to perform in daily practice. In addition, evidence that the SGA has the potential to guide nutritional intervention resulting in a better clinical outcome has recently been reported.[15]

Using the SGA, malnutrition was observed in 37% of our study population while 10% were even severely malnourished. This is in accordance with the literature, which reports a prevalence of malnutrition of up to 40% in PD patients.[11]

We compared the SGA with the results of the body composition measurements using anthropometry, BIA, and DEXA. There is no consensus in the literature on which method is the best to assess body composition in PD patients. We found a high ICC between the different methods in our study (0.90-0.94), in line with what has been reported previously by others.[23]

FSA is another simple and inexpensive method to assess nutritional state. It has been reported that with trained observers the estimation of FM and FFM using FSA correlated reasonably well with results from DEXA.[23,24] Unfortunately, FSA does not work as well in routine clinical practice as in research, since this method is subject to a high intra-observer and inter-observer variability and is not easy to perform in obese subjects.[3] BIA is considered a valid method to assess the nutritional state of stable dialysis patients.[7] It has been reported that BIA measurements are influenced by the presence of peritoneal dialysis fluid.[25] We have also found a statistically significant, albeit very small difference, between the BIA measurements before and after drainage of dialysis fluid (data not shown). These findings suggest that BIA measurements in PD patients should be taken under well-defined and standardized conditions. The same goes for the DEXA measurements. DEXA is an expensive method and not widely available. BIA and DEXA give information only about the actual body composition. SGA differs from the other methods discussed above in that it takes clinical and functional findings into account and moreover, it reflects the nutritional state as it developed in the preceding months, since it includes changes in body weight.

In our study we found that in male patients the SGA was strongly correlated with all measured objective parameters and discriminated the malnourished from the well-nourished patients as defined by body composition techniques. In female patients there were no significant correlations between SGA and FFM index. The reason for this discrepancy is not well understood. In line with the literature we found a significantly higher FM in women than in men.[24] Furthermore we observed an approximately 50% lower FM and 10% lower FFM in malnourished patients than in well-nourished patients. This suggests that in malnourished PD patients lean body mass is preferentially conserved relative to body fat similar to patients undergoing bariatric surgery.[26]

For malnutrition we showed a cut-off value for BMI of < 23 kg/m^2. This finding indicates that a BMI that falls within the normal range does not exclude malnutrition. Measurement of BMI fails to take into account the separate contributions of fat mass and fat free mass to body weight. A high BMI can be due to either an excess of adipose tissue or an increase in FFM or a combination of both. Similarly, a low BMI may be due to a decrease in FFM or adipose tissue or both.[27] Generally a BMI < 18.5 kg/m^2 is considered to reflect malnutrition in the normal population (World Health Organization, 1995). However, the use of currently available normal anthropometric values in PD patients are of questionable value, since age-, sex-, and race- or ethnicity-specific reference data are not available for the PD population.[28] Recently Deurenberg et al reported that the relationship between the body fat percentage and BMI is different between Asians and Caucasians, and that body fat in relation to BMI is higher in Asians than in Caucasians.[29] PD patients have higher visceral fat for a given BMI when compared with the normal population.[30]

Interestingly, we found a strong correlation between the SGA and the RRF, in particular in male patients. This may indicate that a decline in residual renal clearance may play a role in the development of malnutrition in PD patients.[11,31,32]

In conclusion, based on the SGA we observed a high prevalence of malnutrition in PD patients. This cross-sectional study shows that conventional SGA is a practical screening tool to assess malnutrition in PD patients, and that BMI, FSA, BIA, or DEXA have a high correlation with the SGA. BIA and DEXA do, however, provide additional information on body composition and may be used to follow up changes in lean body or fat mass. Although several modifications of the SGA have been proposed, none has convincingly been shown to be superior to the original three-point SGA scale. In the absence of further studies to define the best SGA for PD patients, clinicians should probably continue to use the version of SGA with which they are familiar.

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Chapter 3

Dialysate as Food: combined amino acid and glucose dialysate improves protein anabolism in renal failure patients on automated peritoneal dialysis

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Abstract

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 and glucose (G) as a source of proteins and calories during nocturnal automated PD (APD). A random-order crossover study was performed in eight APD patients to compare in two periods of 7 d each AA and 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-^{13}C]leucine, and 24 h nitrogen balance studies were performed. During AA and 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 and 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 and G dialysate improves protein kinetics. This dialysis procedure may improve the nutritional status in malnourished PD patients.

Introduction

Protein-energy malnutrition (PEM) is frequently found in patients with chronic renal failure, including those who are on dialysis.[1-3] There is increasing evidence of a strong association among malnutrition, inflammatory processes, and cardiovascular mortality.[4-7] Many factors are involved in the development of PEM, in particular inadequate intake of proteins and calories through anorexia. Although many strategies have been proposed to improve dietary nutrient intake in peritoneal dialysis (PD) patients, actual protein intake is frequently below the recommended amount of 1.2 g/kg body wt.[8,9] In continuous ambulatory PD (CAPD) patients, amino acids (AA)-containing dialysate has been used to compensate for a low dietary protein intake and loss of AA and proteins through peritoneal clearance.[10] Until now, however, no convincing clinical benefits have been demonstrated.[11-19] AA dialysate may lead to significant increase in serum urea levels[11-14] and metabolic acidosis,[11,12,15] a protein catabolic stimulus.[20,21] To date, AA dialysates are not routinely used in PD. Recently, it was shown convincingly that simultaneous ingestion of calories is essential to obtain an optimal anabolic effect of intraperitoneal AA.[22-24] However, anorexia may impede patients from taking enough calories with intraperitoneally supplied AA. Giving AA intraperitoneally during nightly dialysis without calories had not shown beneficial nutritional effects.[14]

Because the utilization of intraperitoneally supplied AA can be optimized by giving them simultaneously with glucose (G), we put forward the hypothesis that in patients who are on nightly automated peritoneal dialysis (APD), a dialysis solution that contains a mixture of AA and G (AAG), as part of a regular dialysis schedule, could improve protein metabolism. We conducted a randomized crossover study in APD patients to compare AAG dialysis with G dialysis as control. To assess the effects on protein metabolism, we measured whole-body protein turnover and nitrogen balance as endpoints.

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 weeks, malignancy, and life expectancy <6 mo. The study was approved by the Medical Ethics Committee, and written informed consent was obtained from all patients.

Study Design

The study is 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% and 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 whole-body turnover study. Patients were randomized to start with AAG or G as 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 week (day 7 and day 14), venous blood samples were taken for chemistry and an acid-base profile. On

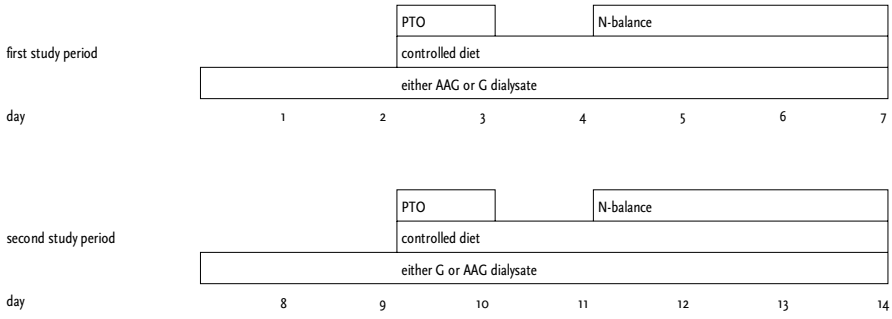

Figure 1. The study design was a randomized, crossover study that consisted of two consecutive study periods of 7 d each. During these periods (days 1 to 7 and 8 to 14), dialysis with amino acids plus glucose (AAG) or with glucose (G) was performed. A controlled hospital-supplied diet was prescribed 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.

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.

Dialysis Procedures

Six nighttimes 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 cycler 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 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 supplied and the steady-state conditions could be met in each cycle. During the G period, five bags of 2.5 L of Physioneal 1.36% to 3.86% were infused, individualized per patient depending on ultrafiltration targets.

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 concentrations (mmol/L) were 132 Na, 105 Cl, 1.25 Ca, 0.25 Mg, and 40 lactate. The electrolyte and buffer composition (mmol/L) of the 1.36%, 2.27%, or 3.86% G was 132 Na, 95 Cl, 1.25 Ca, 0.25 Mg, 25 bicarbonate, and 15 lactate.

WBPT Studies

In the two study periods, rates of WBPT during nocturnal dialysis were determined with a primed continuous intravenous infusion of ^{13}C-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. (T_0). Baseline blood samples and expiratory breath samples were collected in duplicate at 2.30 a.m.(6 h from the start of the dialysis, T_{360}), and priming doses of L-[1-^{13}C]leucine (3.8 μmol/kg) and of $NaH^{13}CO_3$ (1.7 μmol/kg) were given to label the leucine and CO_2 pools. Then, a continuous infusion of L-[1-^{13}C]leucine (infusion rate 0.063 μmol/kg per min) was started and continued for 150 minutes until the end of the nocturnal dialysis at T_{510}. For measuring plateau plasma keto-isocaproic acid (KIC) and CO_2 ^{13}C enrichment, blood and expired air samples were collected simultaneously in duplicate at T_{480}, T_{495} and T_{510} 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 CO_2 production. Patients were not allowed to eat during the isotope studies, but noncaloric beverages were permitted.

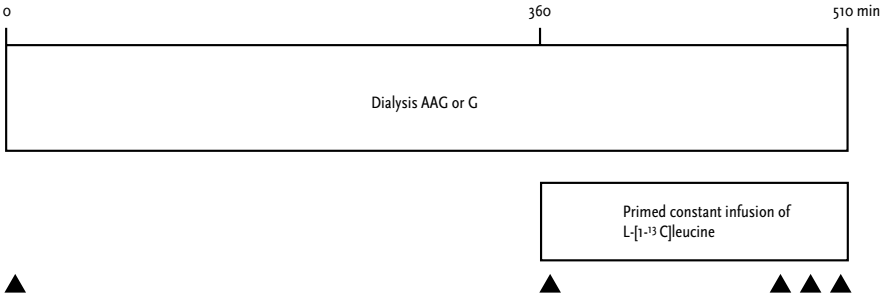

Figure 2. Schematic diagram of the PTO study protocol. Arrowheads denote time points of blood and breath sampling during automated peritoneal dialysis (0 to 510 min)

Diet

A renal dietician 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 dietary intake 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 day 5, 6, and 7, all dialysate and all urine produced per 24 h period were collected. On the daily patients 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}C enrichment of KIC.[27] In brief, the sample was deproteinized with sulfosalicylic acid and the supernatant was put on a cation exchange column to isolate the AA. The effluent that contained the KIC was reacted with phenylene-diamine to form quinoxalinols. These derivatives were extracted with a mixture of dichloromethane/hexane, dried, and silylated with N-methyl-N-(tert-butyldimethylsilyl)-trifluoracetamide. The ^{13}C enrichment was determined by gas chromatography-mass spectrometry by measuring the fragments 259 and 260 of natural and ^{13}C 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 µl of test material with a split ratio of 50:1 on a 25-m x 0.22-mm fused silica capillary

column, coated with 0.11μm of HT5 (SGE, Victoria, Australia). Oxidation of L-[1-^{13}C]leucine was determined by measuring breath CO_2 ^{13}C- 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 mm Hg), insulin, glucagon, as well as 24 h dialysate contents of urea and protein were measured by routine laboratory procedures. Insulin was measured by a chemiluminescent immunometric assay (Immulite 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 $N_{bal} = N_{in} - N_{out}$. Then, the mean of the 3 study days was calculated. The supply of nitrogen (N_{in}) consisted of the sum of the calculated daily dietary nitrogen intake and the dialysate nitrogen content (*i.e.*, nitrogen in the infused dialysate). The loss of nitrogen (N_{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 of N/24 h) to its protein equivalent (g protein /24 h), it was assumed that 1 g of N corresponds to 6.25 g of protein.

Calculations of Whole-Body Protein 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 (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 μmol/ kg per hr is calculated as $Q = Inf (E_i/E_{plasma\ KIC} - 1)$, where Inf is the leucine infusion rate (μmol/ kg per hr), E_i is the ^{13}C enrichment of the L-[1-^{13}C]leucine infused, and E_{KIC} is the ^{13}C enrichment of plasma KIC as measured

at isotopic equilibrium. Isotopic steady state (plateau plasma ^{13}KIC enrichment) was assumed between T_{480} and $T_{510\ min}$. Leucine oxidation (O, in μmol/kg per hr) is calculated as $O = F^{13}CO_2\ (1/E_{KIC}\text{-}1/E_i) \times 100$, where $F^{13}CO_2$ (in μmol ^{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 analysis

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, patient 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 μmol leucine/ kg per min; mean difference 0.10 ± 0.31 μmol leucine/kg per min; NS) and protein breakdown decreased (1.60 ± 0.5 *versus* 1.72 ± 0.3 μmol leucine/ kg per min; mean difference 0.11 ± 0.30 μmol 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 μmol leucine/kg per min ($P = 0.001$) compared with G dialysis in all patients. The oxidation of leucine remained unchanged also during the supply of AA (Table 2). Net peritoneal absorption of

Table 1. Characteristics of the patients[a]

Patient	Primary Diagnose of Renal Disease	Time on PD (mo)	Gender	Age (yr)	Weight (kg)	Height (cm)	BMI (wt/ht²)	Kt/V	PET	nPNA (g/kg/d)
1	Nephrosclerosis	14	M	57	69	170	23.9	1.95	HA	0.80
2	Reflux nephropathy	8	M	43	85	184	25.1	1.85	HA	0.90
3	Alport disease	63	M	35	84	180	25.9	1.82	High	0.72
4	Rapidly progressive glomerulonephritis	45	M	56	78	185	22.8	2.04	High	0.86
5	Periarteritis nodosa	5	M	47	67	174	22.1	1.76	HA	0.92
6	Unknown kidney disease	9	M	66	94	176	30.4	2.49	High	0.91
7	Morbus Wegener	12	F	45	76	166	27.6	2.24	LA	0.69
8	Polycystic disease	36	F	45	80	163	30.1	1.98	HA	0.62
Mean		24		49.3	79.1	174	26.0	2.0		0.8
SD		21		9.8	8.8	8.1	3.1	0.2		0.1

[a]BMI, body mass index; Kt/V, value per week; PET, peritoneal equilibrium test; nPNA, normalized protein equivalent of nitrogen appearance (PD adequest 2.0, software Baxter); HA, high average; LA, low average.

Table 2. Whole-body protein turnover[a]

	AAG						G					
Patient	Flux	Oxidation	Intake	Synthesis	Breakdown	Net Protein Balance	Flux	Oxidation	Intake	Synthesis	Breakdown	Net Protein Balance
1	2.19	0.95	0.27	1.25	1.92	– 0.68	2.04	0.82	0	1.22	2.04	– 0.82
2	1.91	0.62	0.25	1.29	1.67	– 0.38	1.93	0.60	0	1.33	1.93	– 0.60
3	2.89	0.78	0.23	2.11	2.66	– 0.55	2.14	0.87	0	1.27	2.14	– 0.87
4	1.79	0.71	0.24	1.08	1.55	– 0.47	1.54	0.57	0	0.97	1.54	– 0.57
5	1.51	0.68	0.29	0.83	1.23	– 0.40	1.71	0.85	0	0.86	1.71	– 0.85
6	1.59	0.49	0.20	1.10	1.39	– 0.29	1.50	0.45	0	1.05	1.50	– 0.45
7	1.43	0.54	0.25	0.89	1.18	– 0.29	1.38	0.41	0	0.97	1.38	– 0.41
8	1.42	0.38	0.19	1.04	1.23	– 0.19	1.49	0.37	0	1.11	1.49	– 0.37
Mean	1.84	0.64	0.24	1.20	1.60	– 0.41[b]	1.72	0.62		1.10	1.72	– 0.62
SD	0.50	0.18	0.03	0.40	0.50	0.16	0.29	0.20		0.16	0.29	0.20

[a]Data are expressed in μmol 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.

AA was approximately 47% of infused AA. The amount of [^{13}C]leucine lost into the dialysate was not significantly different between AAG and G (<1 % of the dose).

As shown in Figure 3, isotopic steady state was reached in the time frame in which sampling was done (T_{480} - T_{510}). The gain through change in net protein balance (0.21 μmol leucine / kg per min) during the 8.5 h of dialysis can be expressed as grams of protein by taking a molecular weight of 131 for leucine, and assuming a leucine content of muscle protein of 7.8%. There was no significant treatment period interaction for the WBPT studies (P = 0.71)

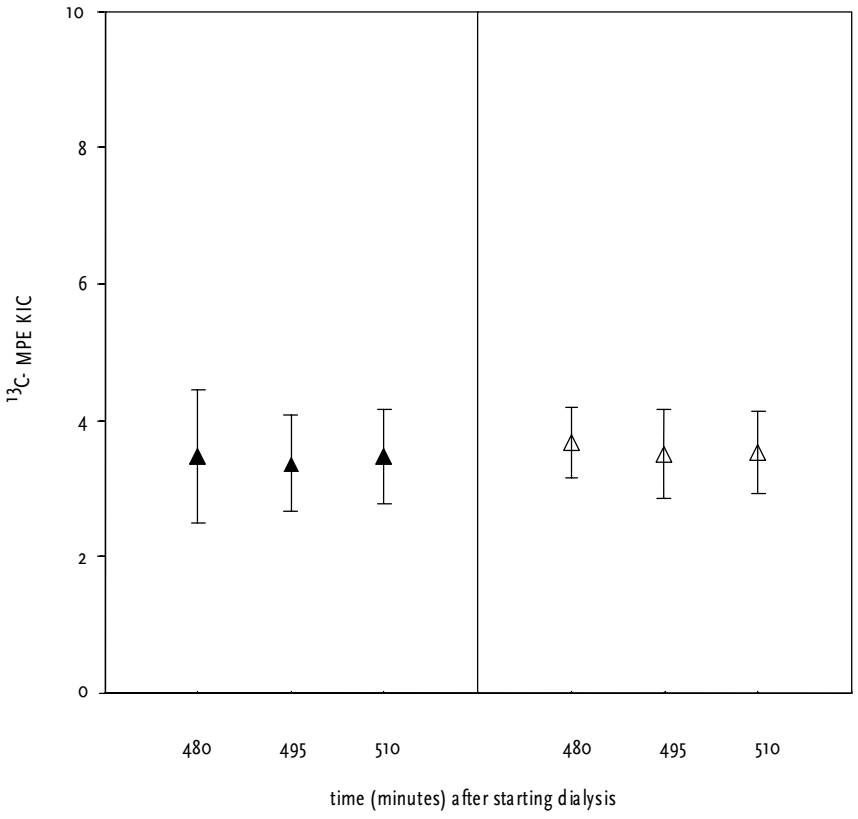

Figure 3. Plateau in plasma ^{13}C-keto-isocaproic acid (KIC) enrichment during PTO study. ^{13}C-KIC values (mean ± SD) in eight patients in moles percent excess(MPE). ▲, AAG; △, G dialysis.

Energy and Protein Intake

The pre-study (*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 pre-study calorie supply via peritoneal dialysate was estimated to be approximately 5.6 ± 3.0 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 nitrogen balance were + 0.35 ± 3.25 and - 0.60 ± 2.38 gN/24h (mean ± SD) for AAG and G, respectively (Table 3). The strongly negative values in both series in one patient (nr 6) are primarily responsible for the large SD. In six patients, NB improved with the AAG compared with G solution, whereas in two patients NB showed a slight decline with AA-based dialysis. Overall, the difference in NB with AAG compared to G dialysis was 0.96 ± 1.2 g of N/24 h (P = 0.061, NS), corresponding to 6.0 ± 7.6 g of protein/d. There were no appreciable changes in body weight in any patient. There was no significant treatment period interaction for NB measurements (P = 0.36).

Biochemical Parameters

As shown in Table 4, mean serum concentrations of creatinine and urea did not show a statistically significant difference at the end of either study period. Phosphate levels were significantly lower after treatment with AAG compared with G dialysate. Mean serum venous bicarbonate concentrations before to the study were in the (low) normal range. After treatment with AAG dialysate, serum bicarbonate concentrations showed a slight and statistically significant decreased compared with G dialysate. Mean serum levels of albumin and glucose remained unchanged. Mean fasting insulin and glucagon were not significant different at the end of both study periods. The total excretion of urea in dialysate and urine during AAG dialysis did not show a significant difference compared with G dialysis. Losses of protein via dialysate were not different.

Table 3. Nitrogen balance[a]

Patient	AAG Dialysis						G Dialysis						Difference (g N/d)
	Energy Intake		Nitrogen Intake		Excretion (g N/d)	Balance (g N/d)	Energy Intake		Nitrogen Intake		Excretion (g N/d)	Balance (g N/d)	
	Diet (kcal/kg per d)	Dialysate (kcal/kg per d)	Diet (g/d)	Dialysate (g/d)			Diet (kcal/kg per d)	Dialysate (kcal/kg per d)	Diet (g/d)	Dialysate (g/d)			
1	20	12.3	10.19	4.25	8.99	3.44	19	14.3	10.03	0	7.77	0.26	3.18
2	19	3.4	9.17	4.25	11.40	0.02	24	4.5	11.47	0	10.11	-0.65	0.67
3	24	6.4	12.53	4.25	11.51	3.28	25	9.3	12.96	0	9.22	1.74	1.54
4	26	5.3	11.63	4.25	12.58	1.29	24	5.7	10.88	0	7.88	1.00	0.29
5	27	5.5	12.37	4.25	12.59	2.04	26	5.7	12.37	0	10.15	0.22	1.82
6	13	2.7	9.65	4.25	18.53	-6.63	14	2.9	9.87	0	13.89	-6.02	-0.61
7	18	3.3	10.51	4.25	11.93	0.83	20	3.5	10.51	0	8.59	-0.09	0.92
8	13	4.1	6.29	3.40	9.14	-1.44	14	4.1	6.77	0	6.06	-1.28	-0.16
Mean	20.0	5.4	10.29	4.14	12.08	0.35	20.8	6.3	10.61		9.21	-0.60	0.96[b]
SD	5.4	3.1	2.03	0.30	2.95	3.25	4.8	3.8	1.89		2.32	2.38	1.21

[a]Data are expressed as mean value of 3 d in g of N/d. Intake is diet plus input via dialysate; excretion is output via dialysate plus urine plus 2.0 g of N (fecal, integumental losses). Difference is balance on AAG minus balance on G dialysis.

[b]P = 0.061, NS.

Table 4. Serum biochemistry before study and at the end of each study period[a]

	Prestudy	End AAG Dialysis Period	End G Dialysis Period
Bicarbonate (mmol/L)	22.8 ± 2.2[b]	24.5 ± 1.7[c]	26.3 ± 1.0
Urea (mmol/L)	22.7 ± 7.1	21.1 ± 4.1	21.1 ± 5.1
Creatinine (μmol/L)	915 ± 251	878 ± 242	770 ± 237
Phosphate (mmol/L)	2.0 ± 0.4	1.9 ± 0.6[d]	2.1 ± 0.4
Albumin (g/L)	38 ± 3.0	38 ± 3.0	39 ± 3.0
Glucose (mmol/L)	4.4 ± 0.6	4.2 ± 0.5	4.6 ± 1.0
Insulin (mU/L)	32.1 ± 9.9	37.0 ± 10.9	37.8 ± 11.6
Glucagon (ng/L)	67.9 ± 21.7	71.4 ± 46.5	72.3 ± 31.4
Dialysate urea (mmol/24h)	ND	264 ± 85	247 ± 71
Dialysate protein (g/24h)	ND	7.5 ± 3.2	6.8 ± 2.6

[a]Data are mean ± SD. ND, not determined.
[b]P = 0.022 versus G
[c]P = 0.005 versus G.
[d]P = 0.04 versus G.

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 would still 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 gram. 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 out of eight patients, the levels of serum bicarbonate were even higher than the pre-study 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.

Acknowledgements

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Chapter 4

Peritoneal dialysis with solutions containing amino acids plus glucose promotes protein synthesis during oral feeding

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Abstract

Inadequate food intake plays an important role in the development of malnutrition. Recently, an increased rate of protein anabolism was shown in fasting state in patients who were on automated peritoneal dialysis with combined amino acids (AA) and glucose (G) dialysate serving as a source of both proteins and calories. This study investigated the effects of such a dialysis procedure in the daytime in fed state in patients who were on continuous ambulatory peritoneal dialysis (CAPD). A crossover study was performed in 12 CAPD patients to compare, at 7 d intervals, a mixture of AA (Nutrineal 1.1%) plus G (Physioneal l.36 to 3.86 %) *versus* G only as control dialysate. Whole-body protein turnover was studied by primed constant intravenous infusion of ^{13}C-leucine during the 9 h dialysis. For meeting steady-state conditions during whole-body protein turnover, frequent exchanges with a mixture of AA plus G were done using an automated cycler. Fed-state conditions were created by identical liquid hourly meals. Using AA plus G dialysate, as compared with the control, rates of protein synthesis increased significantly (2.02 ± 0.08 *versus* 1.94 ± 0.07 μmol leucine/kg per min (mean ± SEM); P = 0.039). Rates of protein breakdown and net protein balance did not differ significantly between AA plus G and G. In conclusion, dialysate that contains AA plus G also improves protein synthesis in fed CAPD patients. The use of such a mixture may contribute to long-term improvement of the nutritional status in malnourished CAPD patients with deficient food intake.

Introduction

Many patients who are on peritoneal dialysis (PD) develop protein-energy malnutrition.[1,2] Inflammation, acidosis, insulin resistance, insufficient intake of proteins and calories as a result of anorexia, and peritoneal losses of proteins and amino acids (AA) contribute to protein-energy malnutrition.[3-5] A strong association between malnutrition, inflammatory parameters and cardiovascular mortality has been reported.[6-10] However, it is not understood completely how these factors are related causally.

Dialysate that contains AA was introduced to compensate for low protein intake and protein losses.[11-13] The use of AA-containing dialysate was shown to be most effective when intraperitoneal AA were supplied simultaneously with sufficient oral calories.[14-18] However, anorexia in continuous ambulatory PD (CAPD) patients prevents adequate oral intake of protein and calories. Recently, we showed improved protein anabolism in fasting patients who were on PD with dialysate that contained AA plus glucose (G).[18]

The daily cycle of fasting and feeding plays a key role in whole-body protein homeostasis. In this study, we examined the metabolic effects of AA plus G (AAG) dialysis in PD patients to determine whether the AAG mixture could contribute to protein anabolism in the fed state. This could be of clinical relevance especially when daily protein and calorie intake of CAPD patients is insufficient. Effects of AAG dialysate on protein metabolism were studied using whole-body protein turnover (WBPT). Because metabolic steady-state conditions are not achieved with a standard CAPD scheme, frequent dialysate exchanges were done using an automated cycler. The dialysis took place during the day while the patients consumed liquid food hourly in identical portions. This was a random-order crossover study in 12 patients to compare AAG dialysate with G dialysate as a control at 1 wk intervals.

Materials and Methods

Patients

Twelve CAPD patients were recruited from the PD Unit of the Erasmus Medical Center. Inclusion criteria called for stable patients who were on PD for >3 mo

and had a 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.

Study Design

The study was a single-center, open-label, randomized, crossover study of 2 d with 1 wk interval to compare a dialysis scheme using either dialysate that contained AAG (Nutrineal 1.1% plus Physioneal 1.36% to 3.86%, Baxter BV, Utrecht, the Netherlands) or a control scheme that contained G only (Physioneal 1.36% to 3.86%). Dialysis was performed during a 9 h period (Figure 1). The end point of the study was WBPT. Before the study, all patients had a dialysis scheme of four exchanges of G-based dialysis fluid (Physioneal 1.36% to 3.86%) in the daytime and a nighttime dwell of Physioneal1.36% to 3.86% or Extraneal (Baxter BV). The night before the study days, all patients underwent dialysis with Extraneal. Patients were randomly assigned by drawing one of 12 sealed envelopes to determine whether patients started with either AAG or G on the first study day. Six patients each were allocated to start with AAG dialysis and G dialysis. On the 2 study days, the patients stayed at the Department of Nephrology of the Erasmus MC. They came at 8.00 a.m. and they left at the end of the study after inflow of their usual nightly dialysate. At the end of each study day, they continued their usual dialysis

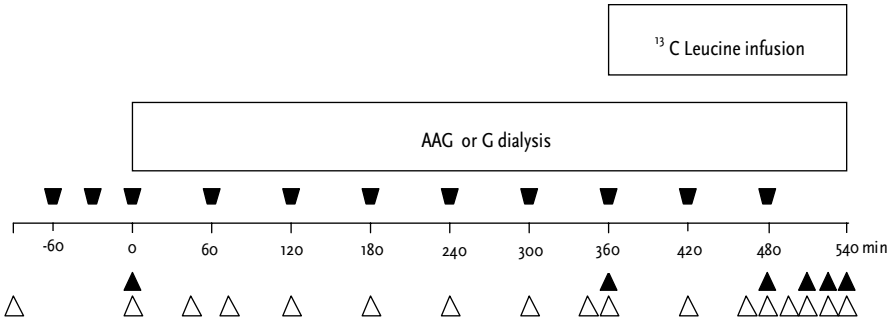

Figure 1. Schematic diagram of the study-day protocol. ▼, portions of the liquid food, the first two portions given half-hourly and thereafter hourly; ▲, time points of blood sampling; △, time points of breath sampling.

scheme. During the 2 study days, the patients consumed a liquid complete diet comparable in nitrogen and energy content to their habitual diet. In each patient, dietary counseling had been performed at least once per 6 mo as a standard practice in our department.

Subjective Global Assessment

Subjective global assessment (SGA) as described by Detsky et al.[19] is based on clinical parameters. Patients are classified according to their nutritional status into three groups: A, good nutritional status; B, moderate malnutrition; or C, severe malnutrition.

Dialysis Procedures

Six daytime exchanges were performed automatically using a cycler (HomeChoice; Baxter BV). The AAG dialysate was obtained by mixing one bag of 2.5 L of Nutrineal 1.1% (containing 27.5 g of AA), and four bags of 2.5 L of Physioneal 1.36 % to 3.86 % G. For G dialysis, five bags of 2.5 L of Physioneal 1.36% to 3.86% were infused. G concentrations were individualized depending on ultrafiltration targets. The composition of the AA 1.1% dialysis solution and the precise mixing procedures that were required to obtain metabolic steady-state conditions for WBPT studies were performed as described previously.[18]

WBPT Studies

Rates of WBPT were determined with a primed continuous intravenous infusion of ^{13}C-leucine, which was carried out at the end of the 9 h dialysis period between 3:30 p.m. and 6:30 p.m. At approximately 8:20 a.m., two catheters were inserted into superficial veins on both arms, one for continuous infusion of the tracer solution, the other for repeated blood sampling. Baseline blood samples and expiratory breath samples were collected before starting the oral feeding at 8:20 a.m. The dialysis started at 9:30 a.m. (T_0). At 3:30 p.m., priming doses of L-[1-^{13}C]leucine (3.8 μmol/kg) and of $NaH^{13}CO_3$ (1.7 μmol/kg) were given to label the leucine and CO_2 pools, followed by a continuous infusion of L-[1-^{13}C]leucine (infusion rate 0.063 μmol/kg per min) and continued for 180 min until the end of the dialysis period at 6:30 p.m. (T_{540}). For measurement of plateau plasma keto-isocaproic acid (KIC) and CO_2 ^{13}C enrichment, blood and expired air samples were collected simultaneously at $T_{480,}$ T_{510}, T_{525} and T_{540} min. (*i.e.*, at 120, 150, 165 and 180

min) after priming and starting the tracer infusion. Indirect calorimetry (Deltatrac metabolic monitor; Datex, Helsinki, Finland) was performed to measure CO_2 production. Patients were not allowed to eat except their liquid diet during the whole study day; however, noncaloric beverages were permitted.

Liquid Diet

A renal dietician instructed the patients to keep a 4 d food diary. On the basis of these food records and a subsequent dietary interview, the appropriate calorie and protein content of the study diet was calculated. The prescribed study diet consisted of a combination of two commercially available complete liquid nutritional products (Nutridrink and Fortimel; Nutricia, Zoetermeer, the Netherlands). The total food volume was divided into 11 equal portions. The first two meal portions were given at half-hourly intervals, and the remaining nine portions were given at hourly interval thereafter (Figure 1).

Analytical Determination

Leucine carbon flux was calculated from the ^{13}C enrichment of KIC as described previously.[18,20] In brief, the ^{13}C enrichment was determined by gas chromatography-mass spectrometry by measuring the fragments 259 and 260 of natural- and ^{13}C-KIC, respectively. Oxidation of L-[1-^{13}C]leucine was determined by measurement of breath CO_2 ^{13}C enrichment.

Blood Chemistries

Blood samples were taken before the first dialysate exchange (fasting state) and at the end of the study days. Insulin was measured by a chemiluminescent immunometric assay (Immulite 2000 Insulin; DPC, Los Angeles, CA). The other determinations were performed by routine laboratory procedures.

Calculations of WBPT

Metabolic leucine carbon flux was calculated as described previously.[21] On the basis of the one-pool model at equilibrium, leucine carbon flux (Q) is equal to the sum of endogenous leucine appearance from protein breakdown (B) plus exogenous leucine appearance through oral intake (I_o) and *via* dialysate (I_d). At metabolic equilibrium, Q also is equal to the sum of leucine disappearance into body proteins (S) plus leucine oxidation (O). Thus, $Q = S + O = B + I_o +$

I_d. Plasma KIC enrichment provides a better estimate of intracellular leucine enrichment than does plasma leucine enrichment because KIC is derived from intracellular leucine metabolism.[20] Leucine flux in μmol/kg per hr is calculated as Q = Inf (E_i / $E_{plasma\ KIC}$ -1), where Inf is the leucine infusion rate (μmol/kg per hr), E_i is the ^{13}C enrichment of the L-[1-^{13}C]leucine infused, and E_{KIC} is the ^{13}C enrichment of plasma KIC as measured at isotopic equilibrium. Isotopic steady state (plateau plasma ^{13}KIC enrichment) was found between T_{480} and $T_{540\ min}$. Leucine oxidation (O; μmol/kg per hr) is calculated as O = F^{13}CO2 (1/E_{KIC}-1/E_i) x 100, where F$^{13}CO_2$ (in μmol ^{13}C/kg per hr) is the rate at which ^{13}CO2 is expired as calculated from CO_2 ^{13}C enrichment in expired air and from CO_2 production. Leucine absorption from dialysate (I_d) was calculated by subtracting the amount of leucine in spent dialysate from that in fresh dialysate. Loss of leucine (L_d) in G dialysate was included in the total rate of disappearance of leucine. Thus, during G dialysis, Q = B + I_o = S + O + L_d. During AAG dialysis, Q = B + I_o + I_d = S + O. A fraction of 0.25 of absorbed leucine was assumed for the retention in the splanchnicus bed.[22] This correction factor was applied for leucine both from diet and dialysate. A fraction of 0.32 was assumed for the oxidation of leucine taken up by the splanchnic region.[22] Therefore, leucine fluxes and rates of whole-body protein synthesis and breakdown corrected for splanchnic uptake were calculated as follows: Q_c = measured flux + (I_o + I_d) x 0.25, where Q_c is corrected flux. The corrected oxidation (O_c) = measured oxidation + [(I_o + I_d) x 0.25] x 0.32. The corrected protein synthesis = Q_c - O_c. The corrected breakdown B_c = Q_c - (I_o + I_d).

Statistical Analyses

Data were analyzed using the statistical program SPSS, version 11.0, for Windows (SPSS Inc., Chicago, IL). Data are expressed as mean ± SEM or indicated otherwise. The paired *t* test was used to compare differences between the two treatment regimens (AAG *versus* G dialysis). This comparison also was done in the subgroup of malnourished patients. The unpaired *t* test was used to compare differences regarding protein synthesis, protein breakdown and net protein balance between the present fed state study and the previous published fasting state study. Differences were considered statistically significant when the two-sided P value was < 0.05.

Power-calculations, based on our previous study.[18] had led to 12 patients for this crossover study.

Results

The baseline characteristics of the 12 patients are shown on Table 1. Three patients (1, 3, and 5) were anuric, whereas most of the other patients had a substantial residual renal function. According to the SGA classification in this study, four out of the 12 patients were moderately malnourished. None of the patients used prednisone. The study protocol was well tolerated by all patients. No complaints of nausea or other adverse reactions were reported during the AAG dialysis. None of the patients dropped out of the study.

WBPT

Essential conditions for measuring WBPT were met as shown in Figures 2 and 3, showing that isotopic steady state in plasma ^{13}C-KIC and exhaled breath $^{13}CO_2$ was reached within the time frame of sampling (T_{480} to T_{540}). There was a slight increase in breath $^{13}CO_2$ enrichment on feeding with liquid food. However, a new steady state was reached before sampling for analytical purposes was started (Figure 3). In this study, we measured that net peritoneal absorption of leucine was 39.3 ± SD 7.1% of the supplied amount.

Protein turnover results in Table 2 show that the leucine flux with AAG mixture was increased compared with G dialysis (2.90 ± 0.13 *versus* 2.67 ± 0.11 μmol leucine/kg per min; mean difference 0.23 ± 0.05 μmol leucine/kg per min; P = 0.001). Leucine oxidation increased significantly with AAG dialysate compared with G dialysate (0.88 ± 0.06 *versus* 0.74 ± 0.06 μmol leucine/kg per min, mean difference 0.14 ± 0.02 μmol leucine/kg per min; P < 0.001). Protein synthesis increased significantly with the mixture (2.02 ± 0.08 *versus* 1.94 ± 0.07 μmol leucine/kg per min; mean difference 0.08 ± 0.04 μmol leucine/kg per min; P = 0.039). Overall, net protein balance was not significantly increased using AAG dialysate compared with G dialysate (mean difference 0.03 ± 0.03 μmol leucine/kg per min, P = 0.347). There also were no significant differences regarding protein breakdown (P = 0.346).

Within the subgroup of malnourished patients (Table 1, SGA classification B, n = 4), the net protein balance with AAG was significantly higher than with G dialysis (mean difference 0.13 ± 0.02 μmol leucine/kg per min; P = 0.0063). This difference was significantly higher (unpaired *t* test P = 0.0058) than the corresponding value in the group of eight well-nourished patients (difference -0.02 ± 0.03 μmol leucine/kg per min).

Table 1. Baseline characteristics of the patients[a]

Patient	Primary Diagnosis of Renal Disease	Time on PD (mo)	Gender	Age (yr)	BMI (wt/ht²)	SGA	PET	Kt/V	nPNA (g/kg per d)	Protein (g/kg per d)	Energy (kcal/kg per d)
1	Unknown kidney disease	28	M	69	32.8	B	H	1.91	0.89	0.59	11.1
2	Chronic pyelonephritis	20	F	43	22.0	B	HA	2.73	0.97	0.75	16.7
3	IgA nephropathy	15	M	33	21.4	B	H	1.86	0.69	0.90	32.1
4	Unknown kidney disease	9	M	59	24.5	A	HA	2.64	1.37	1.14	19.4
5	Hypertensive nephropathy	72	F	62	28.6	A	HA	2.02	0.76	0.68	16.0
6	Membranous glomeruloathy	5	M	56	25.8	A	LA	3.10	0.89	0.73	17.8
7	Hypertensive nephropathy	4	M	70	23.7	B	HA	2.00	0.79	0.93	23.4
8	Chronic pyelonephritis	15	F	52	23.7	A	HA	2.68	1.27	1.38	26.4
9	Nephrolithiasis	9	M	35	32.6	A	H	2.46	1.15	1.01	26.6
10	Hypertensive nephropathy	6	M	63	25.5	A	HA	2.65	1.01	0.98	26.5
11	Hypertensive nephropathy	4	F	53	28.6	A	HA	2.61	0.73	1.27	25.8
12	Diabetic nephropathy	4	M	62	27.7	A	HA	2.21	0.56	0.90	19.8
	Mean	15.9		54.8	26.4			2.41	0.92	0.94	22.8
	SD	19.2		12.2	3.8			0.40	0.24	0.24	6.0

[a]A, good nutritional status; B, moderate malnutrition; BMI, body mass index; H, high; HA, high average; LA, low average; nPNA, normalized protein equivalent of nitrogen appearance (PD Adequest 2.0 software; Baxter); PET, peritoneal equilibrium test; SGA, subjective global assessment.

Table 2. Whole-body protein turnover[a]

	AAG						G					
Patient	Flux[b]	Oxidation[c]	Intake	Synthesis[d]	Breakdown[e]	Net Protein Balance[f]	Flux	Oxidation	Intake	Synthesis	Breakdown	Net Protein Balance
1	2.55	0.66	0.87	1.88	1.68	0.20	2.56	0.62	0.71	1.94	1.85	0.09
2	2.60	0.62	1.14	1.98	1.46	0.52	2.40	0.54	0.89	1.86	1.51	0.35
3	3.54	0.88	1.61	2.65	1.93	0.72	3.33	0.82	1.39	2.51	1.95	0.56
4	2.98	1.07	1.50	1.91	1.48	0.42	2.95	1.00	1.33	1.95	1.61	0.34
5	2.42	0.73	1.09	1.69	1.34	0.36	2.20	0.54	0.86	1.66	1.34	0.32
6	2.24	0.62	1.02	1.61	1.22	0.39	2.21	0.55	0.88	1.66	1.33	0.33
7	2.77	0.85	1.31	1.92	1.46	0.45	2.63	0.76	1.13	1.87	1.50	0.36
8	3.58	1.34	1.81	2.23	1.76	0.47	3.30	1.18	1.63	2.12	1.67	0.46
9	3.14	0.98	1.34	2.16	1.80	0.36	2.90	0.73	1.22	2.16	1.68	0.49
10	3.08	0.94	1.38	2.14	1.70	0.44	2.59	0.75	1.21	1.84	1.37	0.47
11	3.30	1.06	1.72	2.23	1.57	0.66	2.72	0.79	1.53	1.93	1.19	0.75
12	2.66	0.80	1.21	1.86	1.45	0.41	2.31	0.54	1.07	1.77	1.24	0.53
Mean	2.90	0.88	1.33	2.02	1.57	0.45	2.67	0.74	1.16	1.94	1.52	0.42
SD	0.43	0.21	0.29	0.28	0.21	0.14	0.38	0.20	0.29	0.24	0.24	0.16

[a]Individualized data are expressed in μmol leucine/kg per min. AAG, combined amino acids plus glucose dialysis; G, glucose dialysis.
[b]$P = 0.001$ for flux on AAG versus G dialysis.
[c]$P < 0.001$ for oxidation on AAG versus G dialysis.
[d]$P = 0.039$ for synthesis on AAG versus G dialysis.
[e]$P = 0.346$ for breakdown on AAG versus G dialysis.
[f]$P = 0.347$ for net protein balance (is synthesis minus breakdown) on AAG versus G dialysis.

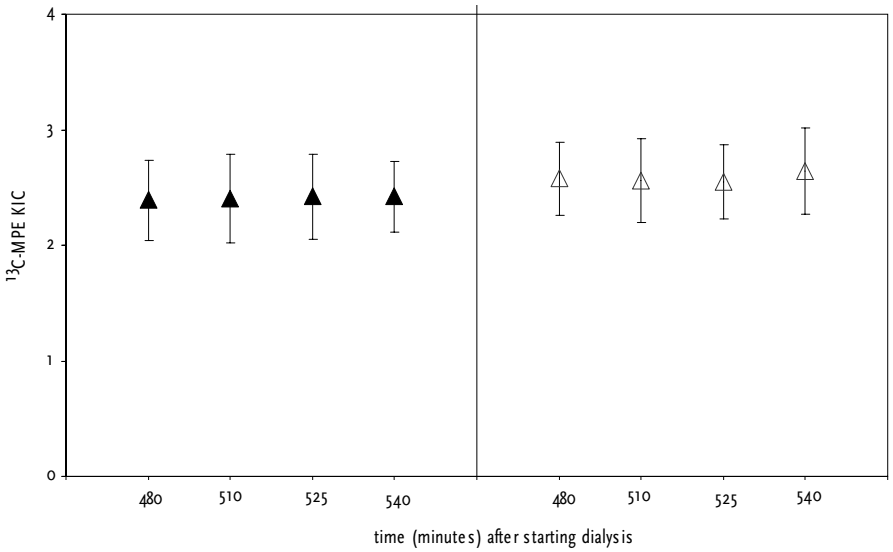

Figure 2. Plateau ^{13}C-ketoisocaproic acid (KIC) enrichment during whole-body protein turnover (WBPT) study. ^{13}C-KIC values (mean ± SD) in 12 patients in moles percent excess (MPE). ▲, amino acids plus glucose (AAG) dialysis; △, glucose dialysis.

Protein and Energy Intake

Table 1 shows the average prestudy (*i.e.*, habitual) dietary protein and calorie intake estimates based on a dietary interview and 4 d food records. The proportion of dietary energy and protein varied between 107 and 157 kcal /gN (mean 141 kcal/gN). The liquid food contained on average 0.96 ± 0.2 g protein/kg per d and 22 ± 5.0 kcal/kg per d, which was consumed completely and was well tolerated in all patients. The proportion of dialysate energy and protein ranged from 124 to 243 kcal/gN (mean 161 kcal/gN).

Biochemical Parameters

As shown in Table 3, serum bicarbonate, creatinine, urea, G, and insulin concentrations did not show a statistically significant difference between AAG and G dialysis. Insulin levels significantly increased during both AAG and G dialysis as compared with fasting baseline conditions. During dialysis, serum creatinine decreased. Mean plasma leucine concentrations were significantly increased with AAG compared with G dialysate ($P = 0.003$).

The loss of leucine into dialysate during G dialysis was 0.65 ± 0.11 μmol/L. Protein loss and excretion of urea in dialysate did not differ with either type of dialysate.

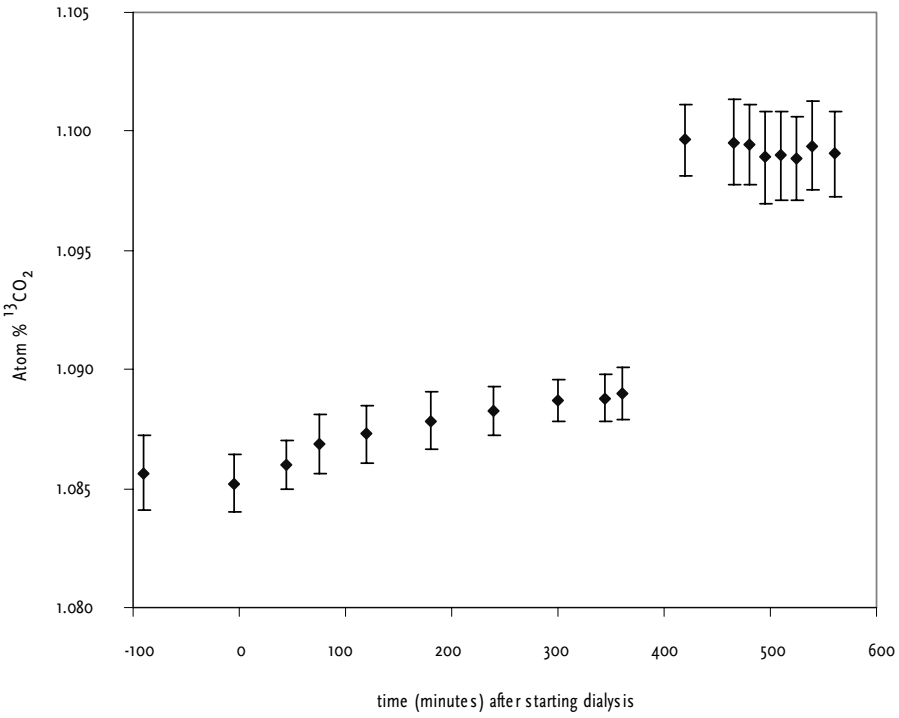

Figure 3. The course of the breath $^{13}CO_2$ levels during consumption of the liquid meals and during the WBPT study with AAG dialysate. Values (mean ± SD) in 12 patients are expressed in Atom % $^{13}CO_2$

Discussion

Recently, we reported that intraperitoneal administration of calories combined with AA to fasting automated peritoneal dialysis patients as part of their regular nightly dialysis scheme improved protein anabolism.[18] In this study, we investigated whether AAG dialysate could serve as a useful extra supply of nutrients to CAPD patients in a fed state, considering that many of these patients have deficient

Table 3. Serum biochemistry at baseline and at the end of dialysis[a]

	Baseline AAG Dialysis	End AAG Dialysis	Baseline G Dialysis	End G Dialysis
Bicarbonate (mmolL)	23.7 ± 3.3	25.6 ± 3.3[b]	24.1 ± 3.6	25.8 ± 2.8[c]
Urea (mmol/L)	22.0 ± 7.6	21.8 ± 6.5	20.5 ± 7.6	20.0 ± 6.9
Creatinine (µmol/L)	841 ± 225	762 ± 213[b]	835 ± 240	773 ± 208[b]
Glucose (mmol/L)	4.8 ± 1.0	6.2 ± 2.6[c]	4.2 ± 1.1	5.7 ± 2.1[c]
Insulin (pmol/L)	134 ± 76	495 ± 339[b]	133 ± 86	458 ± 243[b]
CRP (mg/L)	12.2 ± 6.9	12.2 ± 7.8	9.1 ± 10.6	10.8 ± 13.3
Plasma leucine (µmol/L)	108 ± 17.4	198 ± 40.8[b]	102 ± 10.3	172 ± 37[b,d]
Dialysate leucine (µmol/L)	ND	ND	ND	0.65 ± 0.11
Dialysate urea (mmol/9h)	ND	174 ± 59.1	ND	163 ± 61.1
Dialysate protein (g/9h)	ND	3.9 ± 2.9	ND	4.0 ± 2.8

[a]Data are mean ± SD. CRP, C-reactive protein; ND, not determined.
[b]$P < 0.01$ and [c]$P < 0.05$ versus baseline.
[d]$P < 0.01$ versus end AAG dialysis.

intake of proteins and calories as a result of anorexia. WBPT studies were carried out in fed-state CAPD patients in the daytime using a primed constant infusion of a stable isotope. Instead of manual changes of dialysis fluids, AAG solutions frequently were exchanged using an automated cycler to achieve the steady-state conditions that were required for the WBPT studies.

Administration of AAG dialysate increased both leucine flux and leucine oxidation significantly compared with G dialysate. Although the amount of AA absorbed from the peritoneal cavity was small in comparison with dietary AA intake, there were significant metabolic effects. These metabolic responses are in line with other studies that reported that an increased protein supply stimulates both flux and oxidation. In contrast, in the fasting state,[18] AAG dialysate did not stimulate oxidation, which agrees with studies that showed that low protein intake does not increase oxidation.[23,24] Despite the increased oxidation, extra intraperitoneal AA stimulated protein synthesis significantly. Protein breakdown and net protein balance (*i.e.*, synthesis minus breakdown) did not change significantly with AA dialysate.

Retention of absorbed leucine in the splanchnic bed may have influenced the results of the net protein balance. Splanchnic extraction cannot be measured directly, and a reliable assessment is difficult. Retention of 10% to 32% of gut-absorbed dietary leucine has been reported.[22,25-28] Little is known about

splanchnic retention of peritoneally absorbed AA.[15] We assumed a value of 25% for both orally and peritoneally absorbed AA.[22] Assuming increasing values of splanchnic retention brings about that the effects of protein intake shift from inhibition of protein breakdown to stimulation of protein synthesis.

The peritoneal absorption of leucine, calculated as the difference between the amounts in fresh and spent dialysate, ranged from 29 to 51% with a dwell time of approximately 1 h. These values are similar to those reported elsewhere.[15,17] In the standard CAPD procedure with a dwell time of 4 to 6 h, AA absorption is considerably higher.[15,29,30] Plasma leucine levels increased significantly with the AA dialysis fluid. The higher plasma leucine levels as a result of feeding may have caused a slightly lower absorption rate of intraperitoneal AA as a result of a decreased gradient compared with the fasting state.

Calories have an inhibitory effect on protein breakdown via stimulation of insulin secretion, whereas AA may exert a separate and additive effect.[31,32] In this study, the intake of calories was virtually the same during AAG and G dialysis. Intraperitoneal AA did not lead uniformly to lower protein degradation; neither was it associated with a different insulin response.

Comparing the results of this and previous published study[18] in similar patients, we found that protein synthesis rates were significantly higher in the fed state as compared with the fasting state with both AAG and G dialysis. The synthesis rates (mean values in μmol leucine/kg per min) using AAG dialysate were 2.02 (fed state; $n = 12$) as compared with 1.20 (fasting state; $n = 8$; $P < 0.001$) and using G only dialysate were 1.94 (fed state; $n = 12$) *versus* 1.10 (fasting state; $n = 8$; $P < 0.001$). Protein breakdown did not significantly differ in response to feeding between the two studies with both AAG and G dialysis. This suggests that ingested protein was used mainly for stimulation of protein synthesis. That G-containing dialysate has been found to inhibit protein breakdown in fasting patients through moderate hyperinsulinemia may explain why feeding did not inhibit proteolysis further.[16] Net protein balance was negative during fasting but became positive in all patients who were taking oral food, as also has been demonstrated in healthy people and hemodialysis patients. [23,33]

Giving dialysis fluid that contains a mixture of AA and G guarantees simultaneous supply of both protein and calories when oral intake of proteins and calories is deficient in anorectic CAPD patients. In this study, most patients were not malnourished according to the SGA classification.

In a subgroup analysis, we compared the results of WBPT in the four malnourished patients (SGA-B) with the eight well-nourished ones (SGA-A). In malnourished patients, the AAG dialysate showed a statistically significant increase in net protein balance compared with the G dialysate. This suggests that the nutritional state may play a role in the metabolic effects of AAG dialysis.

This study is the first to examine the metabolic effects of AA absorbed from AAG-containing dialysate on WBPT in fed CAPD patients. The results represent acute effects that were obtained in a relatively well-nourished and stable CAPD population. Further work is needed to evaluate the metabolic effects of AAG dialysate in the presence of inflammation or in malnourished patients.

In conclusion, even in a fed state, dialysis solutions that contain AAG improve protein synthesis in CAPD patients. These solutions could function as a nutritional supplement and may help to improve the nutritional state in CAPD patients with deficient intake of both proteins and calories. For assessment of the long-term effects on nutritional status, morbidity, and mortality in different subgroups, studies in a large number of patients are needed.

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Chapter 5

Albumin and whole body protein synthesis respond differently to intraperitoneal and oral amino acids

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Abstract

Patients with peritoneal dialysis are at risk for malnutrition and hypoalbuminemia, which are indicators of poor outcome. Recently, it was shown that dialysis solutions containing amino acids (AA) and glucose improve protein anabolism in peritoneal dialysis patients. We determined if the same solutions could increase the fractional synthetic rate of albumin along with whole-body protein synthesis. Changes in the fractional albumin synthesis rate reflect acute changes in hepatic albumin synthesis. A random-order cross-over study compared the effects of Nutrineal (AA source) plus Physioneal (glucose) dialysate with Physioneal alone dialysate. Eight patients in the overnight fasting state were compared to 12 patients in the daytime-fed state. Fractional albumin synthetic rate and whole-body protein synthesis were determined simultaneously using a primed-continuous infusion of L-[1-^{13}C]leucine. Fractional albumin synthesis on AA plus glucose dialysis did not differ significantly from that on glucose alone in the fasting state or the fed state. Protein intake by itself (fed *versus* fasting) failed to induce a significant increase in the fractional synthetic rate of albumin. Conversely, the oral protein brought about a significant stimulation of whole-body protein synthesis. Our findings show that the supply of AA has different effects on whole-body protein synthesis and the fractional synthetic rate of albumin.

Introduction

Hypoalbuminemia has been recognized as a strong predictor of increased risk of morbidity and mortality in peritoneal dialysis patients.[1-3] Because in protein-energy malnutrition albumin synthesis is reduced, hypoalbuminemia traditionally has been attributed to poor nutritional intake.[4] In terms of kinetics, plasma albumin level is determined by the synthetic rate, the catabolic rate plus external losses, and volume of distribution of albumin. Various clinical conditions apart from protein-energy malnutrition affect its concentrations including inflammatory states, volume expansion, and increased losses of albumin.[5-9] In PD patients, considerable amounts of albumin are lost via dialysate. Plasma albumin concentration has been found to be a poor marker of protein-energy malnutrition.[10] Most of the studies evaluating the acute effects of nutritional intervention in patients treated with PD, could not demonstrate appreciable alterations in the serum albumin concentration.[11]

Recently, we found a stimulation of protein synthesis in PD patients in the fasting and fed state with dialysate containing a mixture of amino acids (AA) plus glucose (G).[12,13] Improvements in whole-body protein synthesis by supply of AA may be, partly, due to an increase in the fractional synthesis rate of albumin (FSR-albumin). Alterations in the FSR-albumin represent acute changes in hepatic albumin synthesis.

In this study, we analysed in PD patients whether combined AA plus G dialysate could increase FSR-albumin in PD patients both during the night in the fasting state as well as during the day in the fed state. Moreover, by comparing the fasting and fed state, we aimed to study the effects of food *per se*. Using stable isotope infusion technique, the FSR-albumin was measured simultaneously with the whole-body protein synthesis (WBPS).

Material and Methods

Patients

Eight patients with end-stage renal failure undergoing Automated Peritoneal Dialysis and 12 patients on Continuous Ambulatory Peritoneal Dialysis were

recruited from the Peritoneal Dialysis Unit of the Erasmus Medical Center. Inclusion criteria were for stable patients, undergoing PD for more than 3 months and who had a weekly Kt/V ≥ 1.7. Exclusion criteria included peritonitis, other infectious or inflammatory diseases in the previous 6 weeks, malignant disease, and life expectancy of less than 6 months. The local Medical Ethics Committee approved the study protocol, and written informed consent was obtained from all study patients. The baseline characteristics of the patients included in both studies are summarized in Table 1.

Table 1. Baseline characteristics of the patients in both studies[a]

	Fasting-state study	Fed-state study
Number of patients	8	12
Gender (M/F)	6/2	8/4
Age (year)	49.3 ± 9.8	54.8 ± 12.2
Time on PD (month)	24 ± 21	15.9 ± 19.2
BMI (kg/m^2)	26 ± 3.1	26.4 ± 3.8
Kt/V	2.0 ± 0.2	2.41 ± 0.40
nPNA (g/kg/day)	0.8 ± 0.1	0.92 ± 0.24

BMI, body mass index; F, female; Kt/V, value per week; M, male; nPNA, normalized protein equivalent of nitrogen appearance (PD adequest 2.0, software Baxter); PD, peritoneal dialysis.
[a]Data are mean ± SD

Study Design

The plasma FSR-albumin study protocol was designed as an extension of our previous studies on the effects of peritoneal dialysis fluids containing AA and glucose on whole-body protein metabolism.[12,13] This was an open-label, randomized, cross-over single-centre study, comparing FSR-albumin using a dialysis scheme with dialysate containing AA plus G (Nutrineal©1.1% plus Physioneal© 1.36% to 3.86%; Baxter BV, Utrecht, the Netherlands) and a control solution containing only G (Physioneal© 1.36% to 3.86%). The study was conducted both in the fasting and fed state (Figure1) and was performed using an automated cycler (HomeChoice©, Baxter BV) to obtain steady-state condition. Each patient was studied on two separate days, with a 1 week interval. The patients were randomly assigned to start with AA plus G or G dialysis. The results of WBPS were all derived from our previously published studies.[12,13]

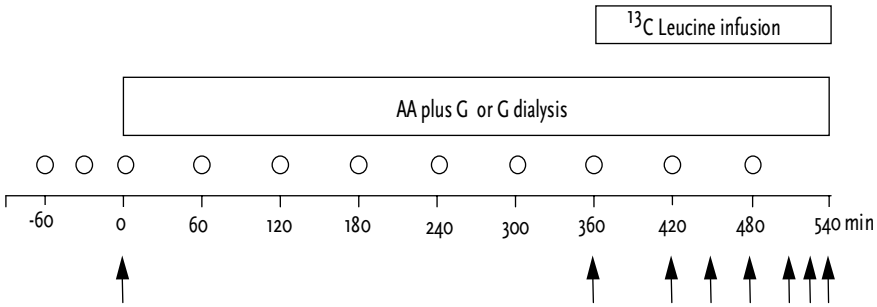

Figure 1. Schematic diagram of the study-day protocol of the FSR-albumin in the fed state. ○ denotes portions of the liquid food, ▲ denotes time points of blood sampling.

Dialysis Procedure

In the fasting-state study, the dialysis procedure was performed in eight patients on Automated Peritoneal Dialysis during a period of 8.5 h at night. Six exchanges either with AA plus G or G dialysate were carried out automatically using a cycler. In the daytime, there were one or two exchanges with G (Dianeal 1.36% to 3.86% or Physioneal; Baxter BV) and/or polyglucose-containing dialysate (Extraneal, Baxter BV). Before the study, all Automated Peritoneal Dialysis patients used glucose-based dialysis fluid (Dianeal© or Physioneal©).

In the fed-state study, the dialysis procedure was carried out during a period of 9 h at daytime. Six exchanges either with AA plus G or G dialysate were performed automatically using a cycler to obtain steady-state condition during the WBPT study, liquid food was given in equal hourly aliquots. All patients used Extraneal in the night before the study. Before the study, all patients had a dialysis scheme of four exchanges of glucose-based dialysis fluid (Physioneal© 1.36% to 3.86%) during the day and a dwell of Physioneal© 1.36% to 3.86% or Extraneal during the night. The composition of the AA 1.1% dialysis solution (g/L) was: histidine 0.714, isoleucine 0.850, leucine 1.020, lysine-HCl 0.955, methionine 0.850, phenylalanine 0.570, threonine 0.646, tryptophane 0.270, valine 1.393, arginine 1.071, alanine 0.951, proline 0.595, glycine 0.510, serine 0.510, and tyrosine 0.300. The electrolyte and buffer concentrations (mmol/L) were Na 132, Cl 105, Ca 1.25, Mg 0.25, and lactate 40. The electrolyte and buffer composition (mmol/L) of the 1.36%, 2.27%, or 3.86% G was: Na 132, Cl 95, Ca 1.25, Mg 0.25, bicarbonate 25 mmol/L, and lactate 15 mmol/L.

Experimental Procedures

FSR-albumin and WBPS were determined with a stable isotope technique using a primed continuous intravenous infusion of ^{13}C-leucine. Priming doses of L-[1-^{13}C]leucine (3.8 μmol/kg) and of $NaH^{13}CO_3$ (1.7 μmol/kg) were given to label the leucine and CO_2 pools, followed by a continuous infusion of L-[1-^{13}C]leucine (infusion rate 0.063 μmol/kg/min). In the fasting-state study the 8.5 h dialysis started at 2030 hours (T_0). Continuous leucine infusion was given between 0230 and 0500 hours. Blood samples and expiratory breath samples were collected at 0230 hours (6 h from the start of the dialysis, T_{360}) and 30, 60, 90, 105, 120, 135, and 150 min after priming and starting the tracer infusion at T_{390}, T_{420}, T_{450}, T_{465}, T_{480}, T_{495}, and T_{510} min. The patients were not allowed to eat during the study. In the fed-state study, baseline blood samples and expiratory breath samples were collected at 0800 hours before starting the oral feeding. At 0830 hours oral feeding, consisting of 11 identical portions of a liquid complete nutritional product (Nutridrink Nutricia, Zoetermeer, the Netherlands) was started and was given at regular hourly intervals to meet a steady-state condition, except for the first three portions that were given at half hourly intervals to induce a fed state-steady state. Other food was not allowed. The dialysis started at 0930 hours. (T_0). The constant leucine infusion was administrated during the last 3 h of the 9 h dialysis period, started at 1530 hours and was continued for 180 minutes until the end of the dialysis period at 1830 hours (T_{540}). Blood and expired air samples were simultaneously collected in duplicate at T_{420}, T_{450}, T_{480}, T_{510}, T_{525} and T_{540} min, that is at 60, 90, 120, 150, 165, and 180 min after priming and starting the tracer infusion. In both part of the study noncaloric beverages were permitted.

Analytical Procedures

The plasma-free ^{13}C leucine enrichment was calculated from the ^{13}C enrichment of KIC as described previously.[12,13] In brief, the ^{13}C enrichment was determined by gas chromatography-mass spectrometry by measuring the fragments 259 and 260 of the butyldimethylsilylquinoxalinol derivatives of natural KIC and ^{13}C KIC, respectively. In plasma, the measurement of ^{13}C KIC is representative for free ^{13}C leucine.

The enrichment of ^{13}C leucine incorporated in albumin was analyzed as follows:

Heparinized plasma was deproteinized with TCA 20% and centrifugated. Ethanol absolute was then added to dissolve selectively the albumin, leaving other proteins as precipitate. After centrifugation of this solution, the supernatant containing albumin was dried and the residue was taken up in 0.3 mol/l NaOH at 37 °C. Albumin was precipitated again from this solution with 2 mol/l $HCLO_4$ and the

pellet was hydrolyzed in 6 mol/l HCL for 24 h at 110° C. After drying, the hydrolysate was purified using a cation-exchanger AG 50W-X8, 200-400 mesh, H^+ form. The AA were eluted with 6 mol/l ammonia and converted into the ethoxycarbonyl-ethylester derivate (using ECF ethylchloroformate). The analysis of ^{13}C leucine enrichment in the isolated albumin took place with gas chromatography-combustion interface-isotope ratio mass spectrometry. The AA derivates were converted into the GC (Thermo Electron, Breda, the Netherlands) using PTV splitless injection. The GC contains a CP-Sil-24 CB lowbleed/MS (Varian, Middelburg, the Netherlands) capillary column (30m x 0.25 mm, df = 0.5 um) and subsequently column FACTOR-four (VF-1701MS, 30m x 0.25 mm, df = 1.00 μm, Varian).

The AA derivates leaving the column were combusted online at 940° C and the CO_2 was online introduced into the Delta-XP IRMS (Thermo Finnigan, Bremen, Germany). We measured at masses 44 and 45 for $^{12}CO_2$ and $^{13}CO_2$, respectively.

Blood Chemistries

In the fasting-state study, blood samples for estimation of insulin were taken in the fasting state before the morning exchange before the study protocol. In the daytime study, blood samples were collected after an overnight fast and drainage of dialysate (before starting with oral nutrition) and at the end of the study days. Blood samples were taken during the AA plus G and G only dialysis at the end of the WBPT study to determine plasma AA levels, both in the fasting- and fed- state study.

Insulin was measured by a chemiluminescent immunometric assay (Immulite 2000 Insulin; DPC, Los Angeles, CA, USA). The AA were measured by ion-exchange chromatography on a Biochrome 20 amino acid analyser with ninhydrin detection (Biochrome, Cambridge, UK). Other determinations were performed according to routine laboratory procedures.

Calculations

WBPS was calculated from ^{13}C KIC enrichment, ^{13}C leucine infusion rate and the rate of expired $^{13}CO_2$, as described previously.[12,13] FSR-albumin is the fraction of the intravascular albumin pool, expressed as percentage, synthesized per day and was calculated as shown in the formula: FSR-albumin (%/day) = (^{13}C Leucine^{alb}) x (^{13}C KIC^{ave})$^{-1}$ x 60 x 24 x100.

^{13}C Leucine^{alb} is expressed as mole percent excess (MPE) change per minute of ^{13}C leucine incorporated in albumin. The change in albumin enrichment per min was calculated from the regression line using data between the start and the end of the

time period selected for measurements (t = 390-540 min). The plasma ^{13}C enrichment of free lucine, represented by (^{13}C KIC^{ave}) was found by determining the average ^{13}C KIC enrichment from the period of measurement and is expressed as MPE.

MPE stands for mole percent excess of the isotopic-labeled molecule that was measured. The other numbers convert the data into percentage fractional synthetic rate per day.

Statistical Analyses

Data were analyzed using the statistical program SPSS, version 11.0, for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± SD, or indicated otherwise. The paired *t*-test was used to compare differences between the two treatment regimens (AA plus G *versus* G only dialysis) in the cross-over experiment. The unpaired *t*-test was used to compare differences regarding FSR-albumin between the fasting- and fed-state study. Differences were considered statistically significant when the two-sided P-value was < 0.05.

Results

The liquid food contained on average 0.96 ± 0.2 g protein/kg/day and 22 ± 5.0 kcal/kg/day, that was consumed completely and was well tolerated in all patients.

In the night-time fasting and daytime-fed study, baseline serum C-reactive protein levels ranged from 1 to 33 mg/l (median 9.5 mg/l) and from 1 to 40 mg/l (median 6.0 mg/l), respectively. Baseline serum albumin was 38 ± 3.0 and 38 ± 2.0 g/l, respectively. Baseline levels of serum bicarbonate in the fasting- and fed-state study were 22.8 ± 2.2 and 23.7 ± 3.3 mmol/l, respectively.

As shown in Table 2 in the night-time fasting-state study, FSR-albumin with AA plus G dialysate was not significantly increased as compared with G only dialysate (mean difference: 1.0 ± S.E. 0.7%/day, P = 0.18). In the daytime-fed state study, FSR-albumin was higher using AA plus G dialysate than with G only dialysate, but again, not statistically significant (mean difference: 0.6 ± S.E. 0.4%/day, P = 0.17). FSR-albumin during G dialysis in the fed state was also not significantly higher than that in the fasting state (12.0 ± S.E. 0.5 *versus* 10.3 ± S.E. 0.9%/day, respectively, mean difference 1.7 ± S.E. 0.9, P = 0.07).

WBPS was significantly higher in the fed state compared with the fasting state, both for AA plus G and G only containing solutions (Figure 2). Baseline

serum insulin in the fasting-state study was 32.1 ± 9.9 mU/l. In the fed-state study, serum insulin levels increased significantly with AA plus G dialysate (22 ± 13 mU/l *versus* 73 ± 45 mU/l, P < 0.001) or G dialysate (18 ± 11 mU/l *versus* 62 ± 35 mU/l, P = 0.001) when compared with baseline values. There were no significant differences in insulin levels between the AA plus G and G only dialysis as reported previously.[13] As shown in Table 3, plasma AA levels and in particular the essential AA increased significantly during AA plus G dialysis compared with G only dialysis.

Table 2. Albumin kinetics

	AAG dialysate FSR- albumin %/day	G dialysate FSR- albumin %/day
Fasting patients (N = 8)		
1	11.43	10.40
2	8.11	9.71
3	16.22	13.21
4	12.46	10.23
5	9.64	7.33
6	9.34	7.44
7	11.31	9.99
8	12.00	14.18
Mean ± SD	11.37 ± 2.51	10.25 ± 2.53
Fed patients (N = 12)		
1	11.46	12.26
2	13.40	12.41
3	12.91	12.19
4	9.52	9.92
5	11.83	12.28
6	11.58	12.14
7	9.32	9.74
8	14.21	14.82
9	16.41	14.25
10	12.53	10.08
11	15.43	12.11
12	12.89	12.11
Mean ± SD	12.62 ± 2.11	12.03 ± 1.55

AAG, amino acid plus glucose; G, glucose; FSR, fractional synthesis rate of albumin.

Table 3. Plasma AA during AAG and G dialysis in the fasting- and fed state-study[a]

	Overnight fasting state		Daytime-fed state	
	AAG dialysis	G dialysis	AAG dialysis	G dialysis
Essential				
Threonine	177 ± 38**	121± 28	194 ± 30**	167 ± 28
Valine	240 ± 41**	125 ± 22	357 ± 72**	260 ± 55
Methionine	18 ± 8*	9 ± 8	60 ± 15**	37 ± 14
Isoleucine	85 ± 19**	56 ± 13	110 ± 24**	90 ± 21
Leucine	132 ± 23**	110 ± 23	198 ± 41**	172 ± 37
Phenylalanine	76 ± 12**	66 ± 10	113 ± 24**	93 ± 24
Lysine	174 ± 31**	155 ± 37	197 ± 27	184 ± 33
Histidine	94 ± 10**	72 ± 10	85 ± 15**	77 ± 15
Total essential[b]	995 ± 141**	715 ± 129	1314 ± 144**	1080 ± 152
Semiessential				
Tyrosine	41 ± 9	39 ± 9	96 ± 22*	89 ± 22
Cystine	ND	ND	14 ± 11	11 ± 7
Nonessential				
Taurine	43 ±13	45±20	30 ± 12	35 ± 27
Aspartic acid	ND	ND	37 ± 7	31 ± 14
Asparagine	39 ± 10	36 ± 9	70 ± 13	75 ± 15
Serine	107 ± 48	76 ± 15	90 ± 18	89 ± 19
Glutamic acid	286 ± 112	284 ± 116	93 ± 55	90 ± 48
Glutamine	260 ± 107	224 ± 95	475 ± 57	481 ± 64
Glycine	382 ± 116*	328 ± 104	254 ± 70	260 ± 66
Alanine	413 ± 95*	354 ± 93	542 ± 91*	500 ± 107
Citrulline	105 ± 28**	90 ± 30	90 ± 27	90 ± 22
Ornithine	61 ± 13**	49 ± 15	70 ± 11**	60 ± 12
Arginine	127 ± 29**	101 ± 29	94 ± 16*	84 ± 14
Proline	290 ± 43**	240 ± 42	622 ± 117*	574 ± 112
Total non essential[c]	2111 ± 233**	1827 ± 239	2465 ± 243*	2369 ± 254
Total AA[d]	3148 ± 344**	2581 ± 356	3890 ± 349**	3550 ± 381

AA, amino acid; AAG, amino acid and glucose; G, glucose; ND, not determined.

[a]Data are expressed as mean ± SD in μmol/L.

[b]Calculated as the sum of threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and histidine. [c]Calculated as the sum of taurine, aspartic acid, asparagine, serine, glutamic acid, glutamine, glycine, alanine, citrulline, ornithine, arginine,and proline. [d]Calculated as the sum of total essential AA and total nonessential AA with tyrosine (overnight fasting- state study) and with cystine and tyrosine (daytime fed state study). *P < 0.05 *versus* G dialysis; **P < 0.01 *versus* G dialysis.

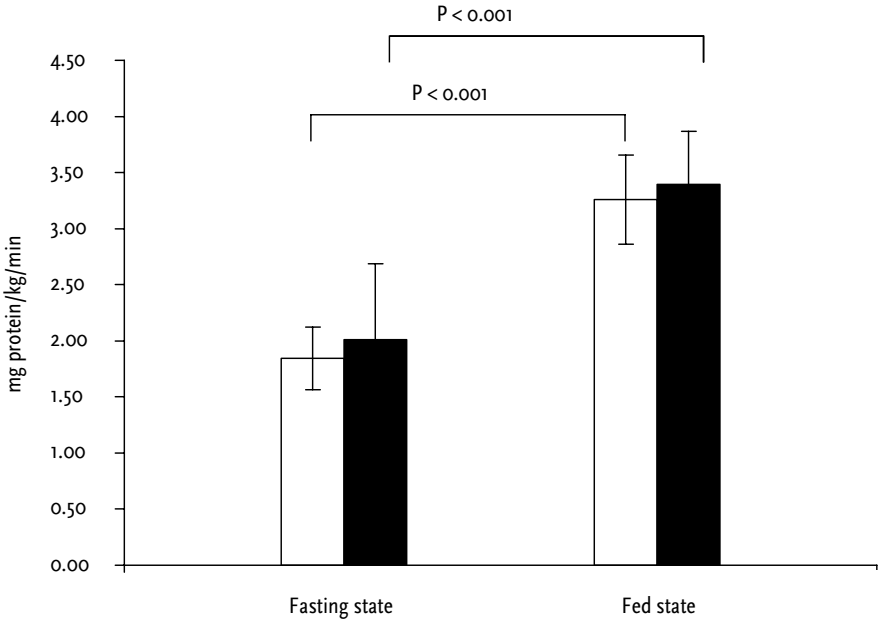

Figure 2. Whole-body protein synthesis during fasting and fed state. □ glucose (G) dialysate and ■ amino acid (AA) plus glucose dialysate.

Discussion

Recently, we showed an acute anabolic effect of AA plus G-based dialysis solutions in PD patients in the fasting and fed state[12,13] In this study, we investigated the influence of such dialysis solutions on the FSR-albumin in PD patients. We found that FSR-albumin values were similar to those obtained in one previous study in Continuous Ambulatory Peritoneal Dialysis patients.[8] We did not find a statistically significant increase in FSR-albumin with combined AA plus G dialysate compared to G only dialysate in either the fasting or in the fed state. In contrast, in several reported experimental and human studies it was found that albumin synthesis increased in response to proteins or AA supplementation.[14,15.] In a recent study in seven hemodialysis patients, FSR-albumin increased in response to Intra Dialytic Parenteral Nutrition.[16] In this study, 11 g of AA were given per hour of dialysis time. In our studies, about 3 g of AA per hour of dialysis

were administered. Taking into account that about 40-50% of the supplied AA was absorbed, 1.6 g AA per hour of dialysis was given. In our study, the failure to demonstrate a significant effect on FSR-albumin may be due to the low amount of AA given. However, even an oral protein intake of 8 g per hour on average did not bring about a statistically significant increase in FSR-albumin, as shown when the corresponding results of the fasting- and the fed-state study are compared. When comparing fasting and fed state in PD patients, it is important to realize that a moderate hyperinsulinemia is present even in fasting PD patients through the continuous flow of glucose via dialysis solutions.[17] Various experimental and human studies provide ample evidence that in addition to substrate availability, insulin has a stimulating effect on hepatic albumin synthesis.[18-20] In previously reported studies, the effects of nutrition were assessed by comparison with a true fasting state.[14,15] This suggests that the increase in albumin synthesis as found in those studies after a meal containing both protein and carbohydrates could, in part be due to increased insulin secretion. One may speculate that in our studies in fasting PD patients using G only dialysate, albumin synthesis was already stimulated via insulin, so that supply of intraperitoneal or oral AA did not induce a significantly further increase of FSR-albumin.

It is certainly possible that an even higher protein intake could have resulted in a significant increase of FSR-albumin. Yet the effects on FSR-albumin contrast markedly with the substantially increase in WBPS, demonstrating a differential effect of food on the synthesis rates of albumin and whole-body proteins. WBPS reflects the sum of protein synthesis rates of all tissues and, in particular, of muscle and liver, which together account for the major part of WBPS. Using the forearm perfusion method it has been shown in PD patients that availability of AA promotes skeletal muscle protein synthesis, whereas insulin has an inhibitory effect on muscle protein breakdown.[17] As muscle protein synthesis contributes substantially to WBPS, the stimulating effect of AA on WBPS may be attributed to a large extent to increased muscle protein synthesis. Protein has also been shown to stimulate hepatic albumin synthesis. In malnutrition and protein-depleted conditions in humans and animals, albumin synthesis was found to be depressed, whereas protein repletion resulted in a prompt recovery.[21-23] In these studies, energy intake was kept constant in the protein-depleted and protein-replenished conditions clearly indicating that proteins and AA modulate albumin synthesis. In postoperative patients, intravenous administration of AA and energy each was found to stimulate albumin synthesis.[24] In many

studies, however, it is not possible to distinguish the effects of insulin and AA. Moreover, the comparison of studies is complicated by marked differences in patient characteristics, such as the presence or absence of inflammatory conditions,[1,25] and in design including the composition and the route of nutrient administration, time frame, and measurement of albumin synthesis rate. In an animal study for example, it was found that the stimulating effect of insulin on albumin synthesis was blunted because of an inhibitory effect on whole-body protein breakdown and AA availability.[26] There is some evidence that protein depletion make albumin synthesis responsive to protein intake, which suggests that the nutritional state may play an important role in the effects of proteins and calories on albumin synthesis.[25,27] On the other and, in healthy subjects with a good nutritional status, albumin synthesis was also found to be stimulated after a meal.[14,15] In another study in healthy humans, albumin synthesis rates were not found to be responsive to short-term intravenous nutrients, suggesting that the route of administration may also play a role.[28]

In this study, albumin synthesis was assessed by estimation of the FSR-albumin using stable isotope infusion technique. With this method, enrichment of α-ketoisocaproic acid (KIC) in plasma is measured after infusion with labeled leucine. Other methods include determination of mRNA or the enrichment of liver aminoacyl-tRNA, the direct precursor of albumin synthesis.[26] For these techniques and for the determination of total liver protein synthesis as well, hepatic tissue samples are required. Plasma KIC enrichment has been proven to be a reliable surrogate measure of leucyl-tRNA in liver.[26] The contribution of albumin synthesis to total liver protein synthesis is estimated at about 15%.[29] Different effects of insulin on the fractional synthesis rates of albumin and another liver protein fibrinogen have been found in several studies.[15,18]

This is the first study in PD patients to compare the effects of supplementation of intraperitoneal AA or food on albumin synthesis with those on whole-body protein metabolism. We found that the significant improvements in WBPS were not accompanied by a parallel increase of albumin synthesis. Such a discrepancy is consistent with the finding that the regulation of albumin synthesis is different from that of skeletal muscles and various other hepatic proteins such as fibrinogen. It is worth mentioning that plasma AA, mainly the essential AA, increased significantly with both intraperitoneal and oral AA supply. It has been reported that essential AA, in particular, the branched chain AA, are responsible for the stimulation of muscle protein anabolism by AA.[30-32] Our

study was conducted in a small, relatively stable and well-nourished population free of liver diseases[33] or acidosis,[34] and without clinical signs of inflammation. In addition, the fact that the fasting patients were not in a true fasting state may have complicated the interpretation of the results. Further work including PD patients in a true fasting state are needed to evaluate the effects of AA plus G containing dialysis solutions in various clinical conditions such as inflammation and malnutrition.

Acknowledgements

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Chapter 6

Whole-body protein turnover in peritoneal dialysis patients: a comparison of the [^{15}N]glycine end-product and the L-[1-^{13}C]leucine precursor methods

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Submitted

Abstract

Two well-described methods for measuring whole body protein turnover (WBPT) are the precursor method using a primed continuous infusion of [1-^{13}C]leucine and the end-product method with a single oral dose of [^{15}N]glycine. We previously measured the effects of amino acid (AA) containing dialysate on protein anabolism in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) using the [1-^{13}C]leucine technique. Here, we examine whether the less invasive [^{15}N]glycine-method could also be appropriate for studying nutritional interventions.

We compared the results of WBPT measurements using a single oral dose of [^{15}N]glycine with those obtained with the primed continuous infusion of [1-^{13}C]leucine during AA plus glucose (G) dialysis and glucose-only dialysis in twelve CAPD patients in the fed state.

The end-product method showed a wide variation for protein synthesis and breakdown measurements. It did not detect a small but significant increase in protein synthesis with AA containing dialysate shown by the precursor method. However, a significant relation was found between both methods for net protein synthesis (*i.e.*, protein synthesis minus breakdown) during AA plus G ($r = 0.75$, $P = 0.005$) or during G only dialysis ($r = 0.86$, $P < 0.001$). The agreement between the two methods for the net protein balance was good (Intracclass correlation coefficient (ICC) = 0.88) with G-only dialysate and moderate (ICC 0.70) with AA plus G dialysate. In conclusion, while the precursor method shows less variation, the more convenient end-product method may be useful in larger groups of selected patients including those on peritoneal dialysis.

Introduction

Stable isotope-labeled tracers have been used extensively to study whole-body protein turnover (WBPT) in humans.[1] Both [1-^{13}C]leucine as primed continuous infusion and [^{15}N]glycine as single oral dose are validated and commonly used techniques for measuring WBPT in humans.[2-6] In recent years, leucine is the most frequently used amino acid (AA). [1-^{13}C]leucine given as a primed continuous infusion has been considered the reference tracer method for measuring WBPT.[7] However, this method requires a complex study protocol, the taking of several blood and breath samples, and cannot be performed in an outpatient setting.[8] Another method to measure WBPT is the end-product method using a single oral dose of [^{15}N]glycine, which was introduced by Waterlow.[9,10] Due to its less invasive character, studies can be repeated in an outpatient setting without much discomfort to the patient, and it has been shown to be suitable for evaluating WBPT in a variety of conditions.[11-18] The end-product method is an attractive method for evaluating the acute and chronic effect of nutritional interventions on WBPT. To our knowledge no studies have been reported comparing the end-product method with the precursor method to measure WBPT in patients on peritoneal dialysis (PD). Therefore we studied WBPT in patients on continuous ambulatory PD (CAPD) in the fed state using the precursor method with a primed continuous infusion of [1-^{13}C]leucine and the end-product method using a single oral dose of [^{15}N]glycine. The two methods were simultaneously applied and were compared during dialysis with amino acids (AA) plus glucose (G) containing solutions (AAG) and during dialysis with G-only solutions. The present report is focused on comparing the results obtained with the two isotope methods. In our previous publications,[19,20] we discussed the clinical implications based on the [1-^{13}C]leucine precursor method, which is considered the 'gold standard.'

Materials and Methods

Patients

Twelve CAPD patients were recruited from the Peritoneal Dialysis Unit of the Erasmus MC. Inclusion criteria called for stable patients, on PD for more than 3 months and a weekly Kt/V above 1.7. Exclusion criteria were peritonitis, other

infectious or inflammatory diseases in the previous 6 weeks, malignancy and a life expectancy of less than 6 months. The Medical Ethics Committee approved the study and written informed consent was obtained from all patients.

Study Design

Previously we performed an open-label, randomized, crossover study on 2 d with a 6 d interval comparing the effect of AAG dialysate (one bag of 2.5 L Nutrineal 1.1%, containing 27 g AA, mixed with 4 bags of 2.5 L Physioneal 1.36% to 3.86%, Baxter BV, Utrecht, the Netherlands, depending on ultrafiltration targets) with a control dialysate containing only G (5 bags of 2.5 L Physioneal 1.36% to 3.86% individualized per patient depending on ultrafiltration targets). WBPT was measured using the precursor method with a primed continuous infusion of [1-^{13}C]leucine.

In the present publication we compared the end-product method using an oral single dose of [^{15}N]glycine with the precursor method to measure WBPT during a 9h dialysis with AAG *versus* G-only dialysis in CAPD patients in the fed state (Figure 1). Frequent exchanges were carried out with an automated cycler, because metabolic steady-state conditions are required for WBPT using [1-^{13}C]leucine, and these are not achieved with a conventional CAPD scheme. The dialysis took place during the day while the patients consumed a liquid complete diet, isonitrogeneous and isocaloric to their habitual diet, based on food records and a dietary interview. The total food intake was divided into 11 identical portions. The first two portions

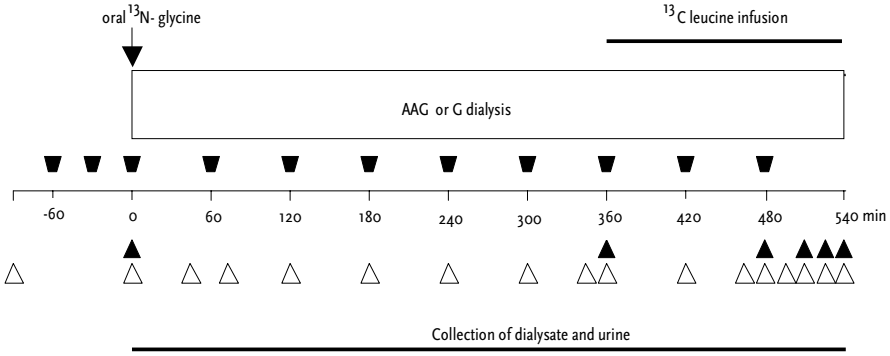

Figure 1. Schematic diagram of the study-day protocol. ▼ denotes portions of the liquid food, the first two portions given half hourly and thereafter hourly. ▲ denotes time points of blood sampling and △ breath sampling.

were given at half hourly intervals and the remaining nine portions at hourly intervals to provide metabolic steady state conditions. Patients were randomized to start with AAG or G on the first day by drawing one of twelve sealed envelopes. On the 2 study days the patients stayed in the Department of Nephrology of the Erasmus MC during the 9 h study period entering at 8.00 a.m. and leaving at the end of the study and after filling the abdomen with their usual nightly dialysate. Patients were not allowed to eat anything except their liquid diet during the whole study, but non-caloric fluids were permitted.

WBPT using a primed continuous infusion of [1-^{13}C]leucine

On the two study days, rates of WBPT during 9 h daytime dialysis and oral feeding were determined with a primed continuous intravenous infusion of [1-^{13}C]leucine, which was carried out during the last three hours of the dialysis period. To measure plateau plasma keto-isocaproic acid (KIC)[21] and CO_2 ^{13}C enrichment, blood and expired air samples were simultaneously collected at appropriate time points. Indirect calorimetry (Deltatrac metabolic monitor, Datex, Finland) was performed to measure CO_2 production (VCO_2).

WBPT using a single oral dose of [^{15}N]glycine

On the two study days, rates of WBPT were studied with a single oral dose of [^{15}N]glycine. At 08.00 a.m. the overnight dialysis fluid was drained and 'dry' body weight was measured using a chair wheel weight (Seca Corp. Scale, USA). At 8.30 a.m. a catheter was inserted into a superficial vein. Blood samples were collected for baseline ^{15}N-urea values. Oral liquid nutrition was then started. After emptying the bladder all voided urine was collected and blood samples were taken. At 9.30 a.m. (T_0) 200 mg ^{15}N of glycine dissolved in 50 ml water was given orally and dialysis was started, which ended at 6.30 p.m. ^{15}N urea was determined in collected drained dialysate, plasma and all voided urine during the 9 h study period.

Analytical Determinations and Calculations

The WBPT based on [^{15}N]glycine as a single oral dose was calculated according to Waterlow.[10] Because of the very low amount of ammonia in the dialysate, only the end-product ^{15}N urea was used for the analysis. Any traces of free ammonia were previously removed. Urea in plasma, dialysate and urine was converted by urease to ammonia and the formed ammonia was trapped in $KHSO_4$ by the Conway diffusion method. Collected ammoniumsulphate was combusted

in an elemental analyzer (Carlo Erba NC1500, Interscience BV, Breda, the Netherlands), and the effluent was led to an Isotope Ratio Mass Spectrometer (ABCA, Sercon LMTD, Crewe, United Kingdom) for ^{15}N determination. Changes in the body urea pool were calculated from blood urea nitrogen measurements before (T_0), and 9 h after the start of the experiment ($T_{540\ min}$) and calculated using total body water as distribution volume. Flux (Q), Synthesis (S), and Breakdown (B) were calculated on the basis of ^{15}N urea excretion disregarding the very low amount of ^{15}N ammonia present in dialysate and the data are expressed as g protein /kg/9 h. The flux (Q) is the rate of nitrogen flux (grams of nitrogen over 9 h) and was calculated using the equation $Q = d\ (E_d+E_u+E_p)/(e_d + e_u+e_p)$; E_d, E_u and E_p are the amount N-urea in the dialysate, urine and the corrected ureapool, respectively. e_d, e_u and e_p are the amount ^{15}N-urea in the dialysate, urine and the corrected ureapool, respectively; d is the amount of isotope administered (grams of ^{15}N). Using calculated nitrogen (N) intake (I) and measured total N loss (E), the Synthesis by urea (Su), the Breakdown to urea (Bu) was calculated using the equation Q = I + B = S + E.

In the precursor method the leucine carbon flux was calculated from the ^{13}C enrichment of ∝-ketoisocaproic acid (KIC), oxidation of L-[1-^{13}C]leucine was determined by measuring breath CO_2 ^{13}C- enrichment, and Flux (Q), Synthesis (S), and Breakdown (B) were calculated as previously reported.[8,21] To convert the results of the ^{15}Nglycine-WBPT (g of N/9 h) to its protein equivalent, it was assumed that 1g of N corresponds to 6.25 g of protein. The results of WBPT using [1-^{13}C]leucine (μmol leucine.kg^{-1}.min^{-1}) are converted to its protein equivalent (g of protein/9 h), assuming a leucine content of 590 umol/gram protein.

Statistical Analyses

Data were analyzed using the statistical program SPSS, version 11.0, for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± SD, or indicated otherwise. The paired *t*-test was used to compare differences between the two treatment regimens (AAG *versus* G dialysis) with regard to leucine and glycine-method. The Pearson's correlation coefficient was used to assess the relations between the two methods for measuring net protein synthesis during the two treatment regimens. Intraclass correlation coefficient (ICC) and the Bland and Altman method were used to assess the agreement between the two methods. Differences were considered statistically significant when the two-sided P value was < 0.05.

Results

The baseline characteristics of the twelve patients are summarized in Table 1. Three patients were anuric, whereas the remaining nine patients had a mean residual renal function of 4.45 ± 2.16 ml.min/1.73 m^2. The study protocol was well tolerated by all patients. The liquid diet was consumed completely. None of the patients dropped out of the study. The baseline weight and the weight at the end of G-only dialysis were 77.1 ± 13 kg and 77.3 ± 13 kg, respectively, P = 0.015. The baseline weight and the weight at the end of AAG dialysis were 77.5 ± 13 kg and 77.6 ± 13 kg, respectively, NS. Serum urea concentration prior to the start and at the end of WBPT during G dialysis were 20.5 ± 7.6 mmol/l and 20.0 ± 6.9 mmol/l, respectively. Serum urea concentration before the start and at the end of WBPT during AAG dialysis were 22.0 ± 7.6 mmol/l and 21.8 ± 6.5 mmol/l, respectively.

Flux and oxidation (excretion) were significantly higher using AAG compared with G only dialysate, both with the precursor method (P < 0.05) and the end-product method (P < 0.05) Using AAG protein synthesis rate was significantly higher with the precursor method (P < 0.03) but with the end-product method significant difference was not attained (P = 0.06) between AAG and G dialysate. There is a larger variation coefficient in the results with the [^{15}N]glycine end-product method compared with the data found with the [1-^{13}C]leucine method measuring protein synthesis and protein breakdown (Table 2). Both methods showed a significant relation for net protein synthesis (*i.e.*, protein synthesis minus breakdown) during AA and G (r = 0.75, P = 0.005) or during G only dialysis (r = 0.86, P = 0.000). Figure 2 shows moderate agreement between both methods (ICC = 0.70) during AAG dialysis. Figure 3 shows good agreement (ICC = 0.88) during G-only dialysis. The Bland and Altman plots show that for individual cases the difference between the two methods can be rather large and the limits of agreement are rather wide.

Table 1. Characteristics of the patients[a]

Number of patients	12
Sex, m/f	8/4
Age, years	54.8 ± 12.2
BMI, wt/ht^2	26.4 ± 3.8
Residual renal function, ml/min/1.73 m^2	4.45 ± 2.16
Study dietary intake	
protein, g/kg/day	0.96 ± 0.2
energy, kJ/kg/day	89 ± 22

[a]Data are expressed as mean ± SD; m, male; f, female; BMI, wt/ht^2, body mass index (weight/height^2).

Table 2. Comparison between [^{15}N]glycine-WBPT and [^{13}C]leucine-WBPT during both dialysis schemes*

	AAG-dialysis						G-dialysis					
	[^{15}N]glycine			[^{13}C]leucine			[^{15}N]glycine			[^{13}C]leucine		
Patient nr.	Synthesis	Breakdown	Net Protein synthesis	Synthesis	Breakdown	Net protein synthesis	Synthesis	Breakdown	Net Protein synthesis	Synthesis	Breakdown	Net protein synthesis
1	1.10	0.96	0.14	1.71	1.52	0.18	0.81	0.66	0.15	1.76	1.68	0.08
2	1.78	1.40	0.38	1.79	1.33	0.47	1.35	1.06	0.30	1.69	1.37	0.32
3	4.42	3.97	0.46	2.40	1.75	0.66	2.16	1.59	0.57	2.28	1.77	0.51
4	1.52	1.13	0.39	1.73	1.35	0.39	1.30	0.91	0.39	1.77	1.46	0.31
5	1.15	0.84	0.31	1.53	1.21	0.32	0.67	0.39	0.28	1.51	1.22	0.29
6	1.24	1.04	0.20	1.46	1.11	0.35	1.09	0.81	0.28	1.50	1.20	0.30
7	1.36	0.95	0.41	1.74	1.33	0.41	1.87	1.48	0.39	1.69	1.36	0.33
8	0.94	0.35	0.59	2.03	1.60	0.43	0.97	0.46	0.51	1.93	1.51	0.42
9	1.24	0.97	0.27	1.96	1.63	0.32	1.30	1.03	0.28	1.96	1.52	0.44
10	1.71	1.41	0.29	1.94	1.54	0.40	1.09	0.66	0.43	1.67	1.25	0.42
11	2.32	1.64	0.68	2.03	1.43	0.60	1.52	0.77	0.75	1.75	1.08	0.68
12	1.31	1.13	0.19	1.69	1.31	0.38	1.26	0.90	0.35	1.61	1.13	0.48
Mean	1.67	1.32	0.36	1.83	1.43	0.41	1.28	0.89	0.39	1.76	1.38	0.38
SD	0.94	0.90	0.16	0.25	0.19	0.13	0.42	0.36	0.16	0.22	0.22	0.15

*Individualized data are expressed in g protein/kg/9 h; WBPT, whole-body protein turnover; AAG, combined amino acids plus glucose dialysis; G, glucose dialysis.

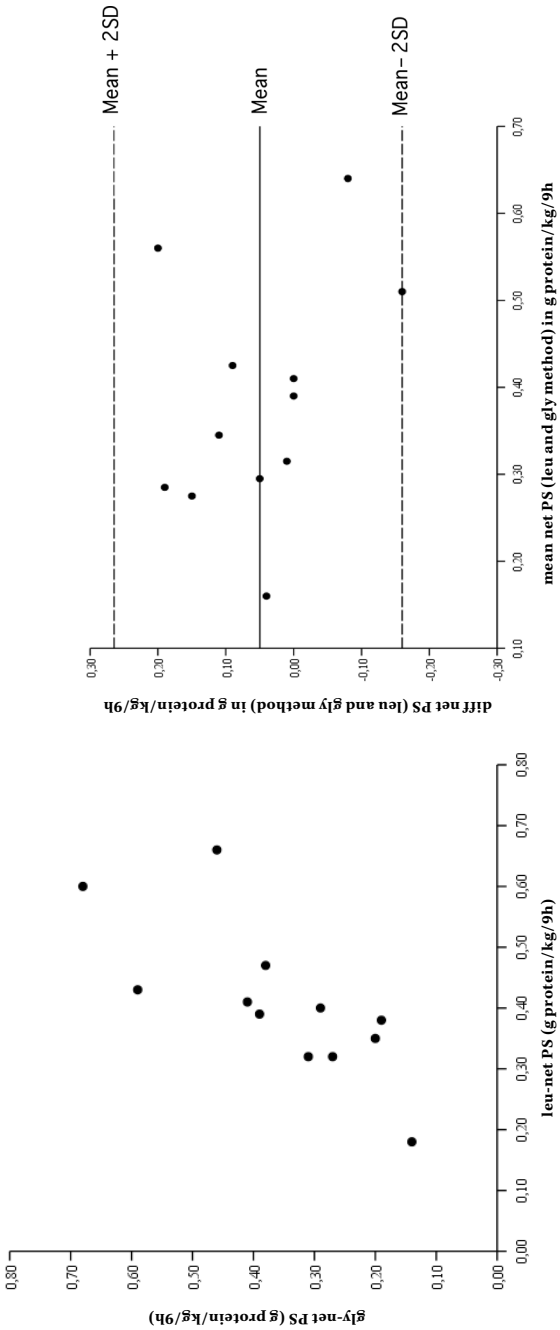

Figure 2. Left panel: scatterplot of net protein synthesis (PS) during AAG dialysis of glycine (gly) method (vertical axis) *versus* leucine (leu) method (horizontal axis). Right panel: Bland and Altman plot for agreement of the two methods. The difference(diff) of the two methods (glycine and leucine) is plotted against the mean of the two methods.

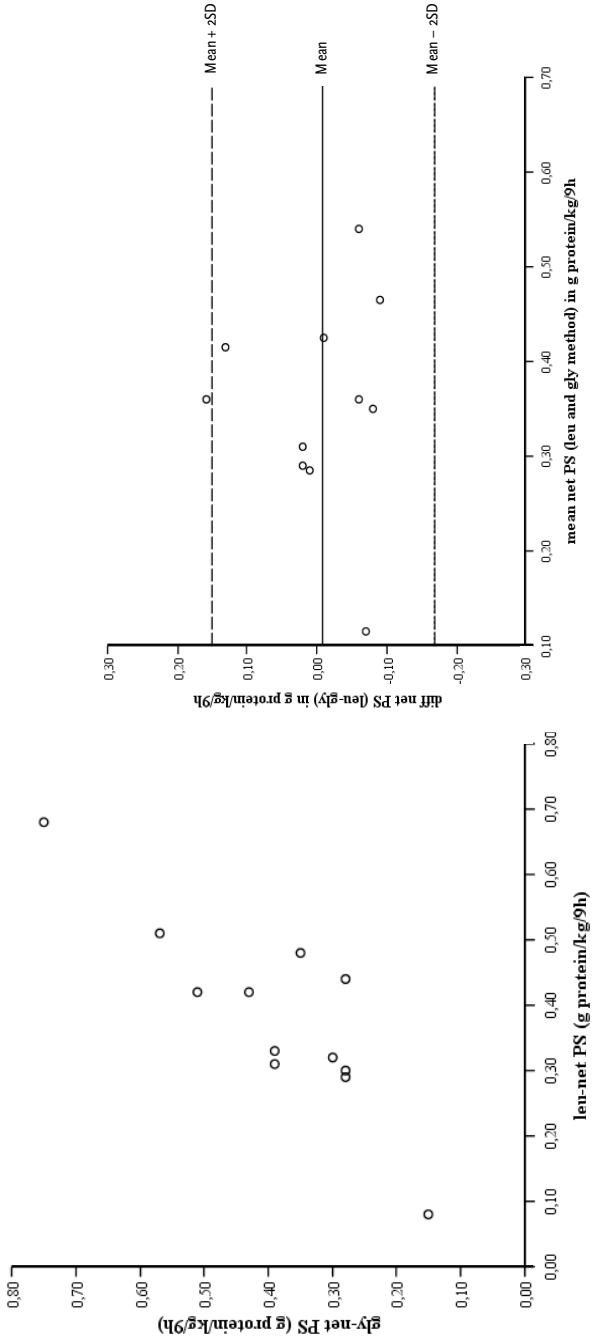

Figure 3. Left panel: scatterplot of net protein synthesis (PS) during G dialysis of glycine (gly) method (vertical axis) *versus* leucine (leu) method (horizontal axis). Right panel: Bland and Altman plot for agreement of the two methods. The difference (diff) of the two methods (glycine and leucine) is plotted against the mean of the two methods.

Discussion

In this study we investigated whether the results of WBPT in patients undergoing CAPD, measured with the end-product method using a single oral dose of [^{15}N]glycine, were comparable with those obtained with the precursor method with a primed continuous infusion of [1-^{3}C]leucine.[20] Both methods were applied simultaneously in the same patients to measure WBPT during dialysis with AAG solutions and during dialysis with G-only solutions. To our knowledge this is the first study comparing the two methods in PD patients.

The two isotope methods used in this study are based on models of complicated metabolic processes. Each of the models makes use of different assumptions and calculations.[7] It has been reported that each of these methods gives an approximation of the true value for the process in question. These methods are, however, suitable for comparative studies and to detect trends in relative magnitude and direction of changes that might have clinical relevance.

The objective of the present publication is to compare data found for synthesis, breakdown, and net protein synthesis obtained with each of the two methods. The data are represented in Table 2. The two methods show a good correlation for measuring the net protein balance (*i.e.* protein synthesis minus protein breakdown) during AAG dialysis and G-only dialysis as well (Figures 2 and 3). There is an acceptable agreement between both methods. The net protein synthesis can be regarded as the actual amount of protein changing in whole-body protein mass. Therefore these figures are most relevant for clinical practice.

Comparisons of the end-product and precursor method performed in the same patients at the same time are scarce and it is difficult to compare our results with previously performed studies in non-dialysis patients.[22,23] In most of the studies in non-dialysis patients, the calculation of WBPT measured with [^{15}N]glycine was based on the average of ^{15}N enrichment in urinary ammonia and urea.[24] Our calculations of WBPT were based on the method of Waterlow et al.[7] However, our patients were treated with peritoneal dialysis because of end-stage renal failure. Therefore we measured ^{15}N enrichment in urea, not in ammonia as end product present in dialysate, plasma and, if any, in urine. Complete collection of urine is essential for the calculations. The ^{15}N enrichment of ammonia was ignored because ammonia was found in minute amounts in dialysate. Some patients produced a small amount of urine, and in them, the analysis was also based on ^{15}N urea. In contrast to the 1-[^{13}C]leucine-precursor method, splanchnic retention is not taken into account in the

calculation of the WBPT with the [^{15}N]glycine method. Although the efficiency of intestinal absorption of [^{15}N]glycine might be less than 100%, we assumed that the absorption was complete because no gastrointestinal problems were present. The end-product method with a single oral dose of [^{15}N]glycine gives results in calculated data (synthesis, breakdown) with wider variation coefficients than the precursor method (Table 2). One of the reasons is that patients with renal failure have a large urea pool in which ^{15}N urea enrichment is measured. This might increase the error in its measurement. Body water content may also be abnormal in renal patients, and indeed more so in patients on dialysis. As body water has to be estimated in order to calculate the N-retention, the error in WBPT results might increase further. Calculations of the WBPT based on measured ^{15}N enrichment of urea as end product, entail a substantial potential error in results for synthesis, breakdown, and net synthesis. In addition to difficulties related to renal failure peritoneal dialysis interferes with the end-product method in that total N(E) must be corrected for N in non-absorbed amino acids left behind in effluent dialysate. This interferes in particular with the comparison of AAG and G-only dialysis. Together these factors may explain why [^{15}N]glycine method failed to detect the subtle increase of WBPS during AAG dialysis as found with the precursor method.

The primed continuous infusion of L-[1-^{13}C]leucine is a widely accepted method for measuring WBPT in all population groups including dialysis patients and is considered to be the reference method. It is, however, an invasive method, which needs frequent blood sampling and admission to a hospital or a metabolic ward. The end-product method using a single oral dose of [^{15}N]glycine is less invasive and more convenient. It can be performed in an outpatient setting, and it is therefore more suitable for population studies.

In conclusion, our results demonstrate that in patients treated with CAPD, the end-product method shows wider variation than the precursor method. Small, but clinically relevant differences may therefore be more difficult to detect with the [^{15}N]glycine method as it has been found in this study. The basic assumptions differ between the two methods; especially peritoneal dialysis complicates the end-product method. It is noteworthy that on average the results with both methods were in the same order of magnitude. Furthermore, the level of agreement between the two methods regarding net protein synthesis is acceptable. While the precursor method is preferable to study whole-body protein turnover in small groups, the more practical end-product method is useful in larger groups of selected patients, including those undergoing PD.

Acknowledgements

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Chapter 7

Peritoneal protein losses and cytokine generation in automated peritoneal dialysis with combined amino acids and glucose solutions

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Abstract

Protein-energy malnutrition as a consequence of a poor appetite occurs frequently in peritoneal dialysis (PD) patients. Previously we showed that peritoneal dialysate containing a mixture of amino acids (AA) and glucose has anabolic effects. However, AA-dialysate has been reported to increase intraperitoneal protein and AA losses and the release of proinflammatory cytokines. We investigated the effect of AA plus glucose (AAG) solutions on peritoneal protein losses and cytokine generation. In six patients on standard automated PD (APD) twelve APD sessions of six cycles each were performed during the night using dialysate containing 1.1 % (AA) plus (glucose) or glucose alone as control. Protein losses and TNFα and IL-6 concentrations were measured in dialysates separately collected from nightly cycling and daytime dwell. The 24 h protein losses with AAG (median 6.7 g, range 4.7 to 9.4 g) were similar to control dialysate (median 6.0 g, range 4.2 to 9.2 g). Daytime dialysate IL-6 levels were higher after nightly AAG dialysis than after control dialysis (142 pg/ml and 82 pg/ml respectively, $P < 0.05$). TNFα concentrations were very low. In conclusion, nightly APD with amino acids containing dialysate was associated with an increase in peritoneal IL-6 generation during the day. The addition of AA to the standard glucose solutions did not induce a significant increase in peritoneal protein losses.

Introduction

One of the untoward effects of peritoneal dialysis (PD) is the loss of proteins and amino acids into the dialysate effluent. Patients undergoing continuous ambulatory PD (CAPD) lose amino acids, 2 to 4 grams a day, and a substantial amount of proteins, varying between 5 and 15 grams per day. The protein and AA losses increase during peritonitis.[1-9] The continuous loss of proteins and amino acids through peritoneal clearance can be compensated by adequate dietary intake, but a poor appetite limits protein intake in many patients.[10] Previously we showed that the gain in protein provided by supplying amino acids (AA) plus glucose (G) intraperitoneally exceeds the usual 24 h protein and AA losses through peritoneal clearance.[11] However, it has been reported that AA-based dialysate may increase intraperitoneal protein losses, as well as stimulating the release of various vasoactive substances including several proinflammatory cytokines.[12-15]

In this study we investigated the protein losses into overnight and daytime dialysate effluents and changes in the levels of the cytokines TNFα and IL-6 in effluent and in plasma, comparing dialysate containing AA plus G (AAG) with glucose (G)-only solutions in patients on APD. Additionally we determined the peritoneal AA losses into the overnight and daytime effluents using standard glucose-based dialysis solution.

Patients and Methods

Patients

Six patients who had been on APD for more than 3 months and had a weekly Kt/V greater than 1.7 were recruited from the Peritoneal Dialysis Unit of the Erasmus MC. Patients who had had peritonitis or other infectious or inflammatory diseases in the previous 6 weeks, a malignancy or a life expectancy < 6 months were excluded. The Medical Ethical Committee of Erasmus MC approved the study and written informed consent was obtained from all patients.

Study Design

This study was a part of our previous open label, randomized, crossover single center study of the effects of dialysate containing AA and G (Nutrineal

1.1% plus Physioneal 1.36 to 3.86% G (Baxter BV, Utrecht, the Netherlands) on whole body protein metabolism, compared with a control solution containing G alone (Physioneal 1.36% to 3.86%). Prior to the study all patients used standard glucose-based dialysis fluid (Dianeal or Physioneal, Baxter BV, Utrecht, the Netherlands). After the patients were randomized to start with either AAG or with G (concentration of 1.36% to 3.86% depending on the ultrafiltration targets) each fluid was used in two consecutive periods of 7 d. The APD schedule for each patient before and during the study was kept constant.

The dialysis procedure during the study was as follows: six nighttime exchanges were performed automatically using a cycler (HomeChoice, Baxter BV, Utrecht, the Netherlands). In the daytime, all patients, except for one patient, used polyglucose-containing dialysate (Extraneal, Baxter BV). Two patients had an additional exchange with glucose dialysate (Physioneal). The APD schedule for each patient was similar to that used before the study in order to meet adequacy and ultrafiltration targets. The cycler regulated the mixing procedure. The composition of AA 1.1% dialysis solution has been described previously.[11] The AA 1.1% solution contained as buffer 40 mmol/L lactate and the 1.36%, 2.27%, or 3.86% G-solutions 25 mmol/L bicarbonate, plus 15 mmol/L lactate.

At the end of each study week after an overnight fast using either AAG or G-only dialysate and before the morning exchange, venous blood samples were taken for determination of the levels of C-reactive protein and the cytokines, interleukine-6 (IL-6) and tumor necrosis factor alpha (TNFα). In all six patients overnight and daytime dialysates were collected separately during the last three days of each week to determine cytokine and protein and amino acid concentrations.

Analytical Determinations

Blood Chemistry

Concentrations of C-reactive protein and dialysate protein were determined by particle-enhanced immunoturbidimetric assay in the central clinical chemistry laboratory of our hospital. The dialysate amino acids were analysed by ion exchange chromatography on a Biochrome 20 aminoacid analyser (Biochrom, Cambridge, UK.) with UV-Vis detection using ninhydrin.

Cytokines

For measurement of TNFα and IL-6, plasma and dialysis fluid samples were diluted 1:1 in appropriate calibrator diluent assay buffer. Cytokine assays were performed following the manufacturers protocol (Pelikine™ human ELISA compact kits for IL-6 (cat. no. M1906) and TNFα (cat. no. M1920), Sanquin, Amsterdam, the Netherlands). The standard curve ranges and mean calculated zero signal plus 3 SD for IL-6 were 0-450 pg/ml and 0.2 pg/ml, respectively; and for TNFα 0-1000 pg/ml and 0.5 pg/ml, respectively. The requested solutions were provided with the ELISA compact kits and additional toolkits (Pelikine-Tool™ set (cat. no. M1980), Sanquin, Amsterdam, the Netherlands). Following the manufacturers assay instruction step-by-step, at the end of the procedure the absorbance per well was measured at 450 nm with a Medgenix ELISA reader. Sample concentrations were calculated using the appropriate standard calibration lines and the Softmax software of the reader.

Statistical Analyses

Data were analyzed using the statistical program SPSS, version 14.0, for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as median with range, or indicated otherwise. Comparisons of outcomes were made using the Wilcoxon Signed Rank test. All tests of significance were two-sided, and differences were considered statistically significant when the P value was < 0.05.

Results

Table 1 shows the baseline characteristics of the patients. Three patients were anuric. Apart from the use of medication regularly taken by PD patients, two patients used prednisone in a maintenance dose. Peritoneal ultrafiltration after administration of AAG based dialysis fluid did not differ from that with G only dialysate (data not shown). Serum C-reactive protein levels ranged from 6 to 65 mg/L (median 7.5 mg/L) with AAG based dialysate and from 1 to 33 mg/L (median 9.5 mg/L) using G only dialysate. The protein losses per 24 h, *i.e.* the sum of daytime dwell and nightly APD with standard G solution ranged from 4.2 to 9.2 g (median 6.0 g) were not significantly different from that occurring with AAG, which ranged from 4.7 to 9.4 g (median 6.7 g). No difference was found between the protein losses in daytime and overnight dwells with both

Table 1. Baseline characteristics of the patients[a]

Patient	Primary diagnose of renal disease	Time on PD (months)	Gender	Age (years)	BMI (wt/ht²)	Kt/V (weekly)	PET
1	Nephrosclerosis	14	M	57	23.9	1.95	HA
2	Reflux nephropathy	8	M	43	25.1	1.85	HA
3	Alport disease	63	M	35	25.9	1.82	H
4	Rapidly progressive glomerulonephitis	45	M	56	22.8	2.04	H
5	Periarteritis nodosa	5	M	47	22.1	1.76	HA
6	Polycystic disease	36	F	45	30.1	1.98	HA
Mean		29		47	25	2	
SD		23		8	3	0.1	

[a]M, male; F, female; BMI (wt/ht²), body mass index (weight/height²); PD, peritoneal dialysis; PET, peritoneal equilibrium test; H, high; HA, high average.

Table 2. Protein and amino acid losses in dialysis effluent in APD patients[a]

	Overnight		Daytime	
	AAG dialysis(1)	G dialysis(2)	After AAG dialysis(3)	After G dialysis(4)
Protein (g/8.5h)	3.06 (2.28-3.51)	3.0 (1.84-3.99)	3.48 (2.44-6.16)	3.03 (2.17-5.54)
Protein (g/h)	0.36 (0.27-0.41)	0.35 (0.22-0.47)[b]	0.22 (0.16-0.40)	0.20 (0.14-0.36)
Total AA (g/8.5h)		1.47 (1.02-1.94)[c]		0.70 (0.62-1.25)
EAA (g/8.5 h)		0.37 (0.25-0.55)[d]		0.18 (0.17-0.37)

[a]Data are expressed as median and range; AAG, amino acid and glucose; G, glucose.
Comparisons were made for columns (2) *vs* (4). These resulted in significant differences for: b,c and d ($P < 0.05$ *versus* daytime after G). No significant differences were found for columns (1) *vs* (2) and ((3) *vs* (4).

type of dialysis schemes. The appearance rate of protein losses in the overnight effluent was significantly higher than that in the daytime (Table 2). The mean total AA losses, *i.e.* essential AA (EAA) and non-essential AA (NEAA) per 24 h, *i.e.* the sum of daytime dwell and nightly APD with standard G solution varied between 1.7 and 3.2 g (median 2.2 g), and 26 ± 1.7% of effluent AA were essential AA. Using standard G only dialysis solution, the total AA losses in the 8.5 h overnight effluents were higher than in the 15.5 h daytime effluents. Likewise, the appearance rate of total AA losses into overnight dwells was significantly

higher than that into daytime dwells (data not shown). The total EAA losses in the overnight dwell were also higher than that in daytime dwell (Table 2). The IL-6 levels in plasma were lower than in the effluents with both AAG and G-only dialysis, in particular as compared with the daytime effluent (Table 3). Dialysate IL-6 concentrations at daytime dwell differed significantly from overnight dwell during both type of dialysis schemes. The IL-6 levels in the daytime effluents after nightly cycling with AAG were significantly higher that that after G. Plasma and both overnight and daytime effluent levels of tumor necrosis factor (TNF)α were low using both AAG and G-only dialysis solutions.

Table 3. IL-6 and TNF-α levels in plasma and dialysate[a]

			Overnight		Daytime	
	Plasma AAG (1)	Plasma G (2)	Dialysate AAG (3)	Dialysate G (4)	After dialysate AAG (5)	After dialysate G (6)
IL-6 (pg/ml)	13 (4-26)	13 (3-48)	21 (16-59)	17 (13-40)	142 (71-599)[b,d,f]	82 (51-338)[c,e]
IL-6 (ng)			247 (206-784)	197 (168-536)	343 (240-1613)	178 (133-872)
TNFα (pg/ml)	2 (1-52)	2 (1-49)	1 (1-1)	1(0-2)	2 (1-39)	2 (1- 5)
TNFα (ng)			15 (9-18)	14 (5-21)	5 (2-93)	5 (2-12)

[a]Data are expressed as median and range. AAG, amino acid and glucose; G, glucose; IL-6, interleukine-6; TNFα, tumor necrosis factor α. Comparisons for IL-6 (pg/ml) were made for columns (1) *vs* (5), (2) *vs* (6), (3) *vs* (4), (5) *vs* (6), (3) *vs* (5), (4) *vs* (6) and (5) *vs* (6).These resulted in significant differences for b ($P < 0.05$ *versus* plasma AAG); c ($P < 0.05$ *versus* plasma G); d ($P < 0.05$ *versus* overnight dialysate AAG); e ($P < 0.05$ *versus* overnight dialysate G); f ($P < 0.05$ *versus* daytime after dialysate G). No significant differences were found for columns (1) *vs* (3) and (2) *vs* (4).

Discussion

As part of previous work on the effects of amino acids containing dialysate on whole body protein metabolism, we investigated in this study the loss of proteins in dialysate in APD patients using either the standard dialysate containing only glucose or a mixture of amino acids plus glucose. In addition we studied the effects of these dialysis fluids on the peritoneal release of the cytokines IL-6 and TNFα and we investigated the losses of amino acids with the use of glucose-based (standard) dialysis solution. Although APD is widely used, the peritoneal protein losses have been studied only occasionally in adult patients undergoing APD.

We found that the mean protein losses per 24 h, *i.e.* the sum of daytime dwell and nightly APD with standard glucose dialysate was on average about 6 grams, which is in the same order of magnitude as previously reported for CAPD patients by various authors.[3,4] The peritoneal protein losses during 8.5 h nightly APD and in the daytime dwell of 15.5 h were similar. By comparison, in a recent study, protein losses during nightly cycling were found to be rather high, exceeding protein losses during the daytime dwell.[16] Yet, also in our patients protein losses per time were higher during cycling with standard glucose solution. This may seem surprising as it is generally accepted that peritoneal protein losses, in contrast to small molecular weight solutes, are linear with time irrespective of the number of dialysate exchanges. It has, however, been shown in patients on intermittent peritoneal dialysis (IPD) that protein loss is greater during the first dwell after a 'dry' period and stabilizes at lower levels after a few exchanges. This finding could be explained by wash out of proteins from the previous dry period.[17,18] Assuming a residual volume of 400-500 ml in our patients, wash out of proteins from the long daytime dwell could account for the difference of protein losses per time between day time dwell and nighttime cycling.

In previous studies we showed that the amino acids plus glucose-containing dialysis fluid has anabolic effects on protein metabolism.[11,19] The current study shows that the presence of amino acids in the dialysate did not increase protein loss in the overnight dialysate effluent as compared with G-only dialysis solutions in APD patients. Others have reported that a 2.6% amino acid peritoneal dialysis solution induced an increased loss of macromolecules including albumin and IgG and the small molecular weight amino acids, which was accompanied by an increased prostanoid generation in the peritoneal cavity.[12] One percent AA solutions were also found to stimulate protein losses and release of prostanoids and several proinflammatory cytokines consistent with an increase in peritoneal blood flow and effective peritoneal surface area.[13,14] In some studies, however, no significant effect on protein losses or release of prostanoids was found.[20,21]

Reports on the peritoneal losses of AA in APD are scarce. In our present study the mean 24 h AA losses, *i.e.*, daytime dwell and nightly APD was similar as earlier reported in patients who are on CAPD and somewhat higher compared with a previous report in APD patients, using standard G dialysis solution.[1,2,16] We found that total AA losses were greater during nightly APD than during the long daytime dwell with standard G-based dialysis solution. This finding is in line with the fact that transperitoneal transport of small molecular weight solutes

is dependent on the dialysate flow rate, *i.e.*, number of dialysate exchanges. Approximately 26% of the effluent AA were essential AA in agreement with previous findings in patients on CAPD.[1]

We also studied the influence of amino acid-based dialysate on cytokines both during the night and during the day. The levels of TNFα and IL-6 as found in the present study in daytime dialysate are on the whole comparable to those described in several studies in CAPD patients with standard glucose dialysis solutions during an infection-free period.[13,22-24] Daytime dialysate IL-6 levels were significantly higher, when during the preceding nightly APD dialysate was used that contained amino acids instead of only glucose. This finding may be somewhat puzzling as during nightly cycling no statistically significant difference in IL-6 levels was found. It cannot be ruled out, however, that any effect of AA on IL-6 during cycling was diluted by rather low IL-6 concentrations as a result of the high dialysate flow rate. It has been shown in several studies in CAPD that amino acid dialysate was accompanied with increased levels of various cytokines including IL-6 and TNFα.[13,25] There is ample evidence that even in the absence of peritonitis IL-6 is produced locally within the peritoneal cavity rather than transported across the peritoneal membrane. Our finding that IL-6 levels in dialysate was higher than in plasma, especially during the long daytime dwell, are consistent with local production and release.[22,23] The main source of peritoneal TNF-α is thought to be the mononuclear phagocyte, whereas both mesothelial cells and macrophages are able to produce and release substantial amounts of IL-6.[23,26] The release of IL-6 from mesothelial cells occurs constitutively and can be stimulated by macrophage-derived TNFα and IL-1β.[13,23,26] In the dialysate of patients with PD-related peritonitis and in ascites of liver cirrhosis patients with spontaneous bacterial peritonitis, high levels of various cytokines including TNFα and IL-6 are found due to increased local production.[23,26-28] In the present study the patients were clinically free of infection, and C-reactive protein levels in most of the patients were normal or only slightly increased. Dialysate TNFα was very low both with the standard glucose and the amino acid and glucose mixture. The clinical implications of increased intraperitoneal IL-6 levels are far from straightforward. Any increases in levels of cytokines in dialysate *in vivo* may be interpreted as a sign of a local inflammatory stimulus or as an improvement of the capacity to synthesize inflammatory factors. It is therefore a matter of debate as to whether any changes in cytokine release should be considered as harmful or as a marker of improved tissue responsiveness.

In conclusion, our study suggests that in APD the use of dialysis fluid containing a mixture of amino acids and glucose induces an increase in peritoneal production of IL-6 without increasing protein losses as compared to dialysate containing only glucose. In contrast, no appreciable amounts of TNFα were found in peritoneal dialysate. This is the first controlled study to investigate the effects of amino acids containing dialysate on cytokines and protein losses. A limitation of this study is the small number of patients. Further studies are required to elucidate the relationship between amino acids containing dialysate and the cytokine network of the peritoneal cavity.

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Chapter 8

Discussion and Conclusions

This thesis was aimed at studying the concept that the nutritional state of patients undergoing peritoneal dialysis can be improved by dialysis with solutions containing amino acids as well as glucose instead of glucose only.

Assessment of the nutritional status of peritoneal dialysis patients

It is crucial to identify peritoneal dialysis (PD) patients who are at risk of or suffering from malnutrition, and to treat these patients in time in order to prevent malnutrition-related complications. At present there is no universally accepted standard method available. The original three-point subjective global assessment (SGA) scale is a simple, reproducible method that can be carried out in daily practice.[1] We assessed the nutritional state of thirty PD patients treated in our center using the SGA as originally described to identify malnourished surgical patients.[2] We found malnutrition in 37% of our patients, and 10% of them were severely malnourished, which is in agreement with the literature. We also compared the SGA with the results of body composition measurements using the body mass index (BMI), four-site skinfold anthropometry (FSA), bioelectrical impedance analysis (BIA), and dual energy x-ray absorptiometry (DEXA). There is no consensus in the literature which method is the best to assess body composition in PD patients and each method has its own limitations. In particular, FSA does not work well in routine clinical practice as this method is not only subject to a high intra-observer and inter-observer variability, but is difficult to perform in obese patients.[3] We found a high intraclass correlation coefficient (ICC) between the different methods in our study (0.90-0.94), in line with what has been reported previously by others.[4] In males we showed a strong correlation between SGA and all measured objective parameters that discriminate the malnourished from the well-nourished patients. In female patients there were no significant correlations between SGA and FFM index measured with all three methods. The reason for this discrepancy is not clear, it may due to the small number of patients. SGA differs from the other methods in that it takes clinical findings into account and reflects changes in the nutritional state as developed in the preceding months, and also includes functional components. We also found that a cut-off value for BMI of $< 23\ kg/m^2$ was suitable for defining malnutrition. This indicates that a normal BMI does not exclude malnutrition in PD patients. In part, this may be due to the higher visceral fat content of PD patients at a given BMI when compared with the normal population.[5] Interestingly, we observed a strong correlation between the

SGA and the residual renal function. This may indicate that a decline in residual renal clearance plays a role in the development of malnutrition in PD patients.[6,7] In conclusion, there is a high prevalence of malnutrition in our PD population. SGA is a practical screening tool to assess malnutrition in PD patients.

Rational use of dialysis solution containing both amino acids and glucose

Protein deficiency is often observed in PD patients and is to a greater or lesser extent due to the combination of reduced intake of nutrients and loss of amino acids and proteins through the peritoneal membrane. Dialysis solutions containing amino acids (AA) have been introduced in an attempt to prevent and treat protein deficiency. Others have shown that simultaneous ingestion of calories is crucial to achieving an optimal utilization of intraperitoneal AA in PD patients.[8] A substantial group of PD patients, however, have not only an inadequate intake of proteins but also of calories due to a poor appetite. The reduced calorie intake is partly compensated by absorption of glucose from the dialysate. Therefore supplying both AA and glucose (calories) in dialysate is a possible way to guarantee adequate nutritional intake in malnourished PD patients and in PD patients with inadequate food intake: a concept we have called 'dialysate as food.' To determine whether a mixture of AA plus glucose does indeed promote protein anabolism, we have studied the effects of this type of dialysate on protein turnover in both fasting and fed conditions in PD patients.

Anabolic effect of combined amino acid and glucose dialysate

In eight patients on automated PD (APD) we examined the metabolic effects of a dialysate containing AA plus glucose by performing whole-body protein turnover (WBPT) studies with a primed continuous infusion of L-[1-^{13}C]leucine according to the precursor. These patients dialyzed with an automated device (HomeChoice) during the night; therefore they were in the fasting state. The dialysis solutions containing both AA plus glucose (AAG) were administered using an automated cycler, as part of their regular dialysis schedule. Although neither the increase in protein synthesis nor the inhibition of protein breakdown attained statistical significance, the net protein synthesis (*i.e.* protein synthesis minus protein breakdown) improved significantly during dialysis with combined AA plus glucose solutions as compared with glucose-only solutions. This improvement in protein anabolism during nocturnal APD is an acute effect in

the fasting state. The results of the nitrogen balance (NB) studies, which reflect the effects of the mixture on a 24 h period, indicate an improvement in nitrogen retention, although this was not statistically significant. The turnover studies with L-[1-^{3}C]leucine showed that virtually all AA absorbed from dialysate were utilized for protein synthesis, and that gain in protein exceeded the daily AA and protein losses into the dialysate. Our results support the concept that giving AA simultaneously with glucose intraperitoneally inhibits protein breakdown and stimulates protein synthesis. Splanchnic tissues may account for a significant fraction of body protein synthesis and degradation,[9] but splanchnic extraction cannot be measured reliably. Little is known about splanchnic retention of peritoneally absorbed AA and a value up to 40% has been reported.[8-11] In this study splanchnic retention of peritoneally absorbed AA was not taken into account. This may have resulted in overestimation of the entry rate of absorbed leucine in the plasma pool and thereby in underestimation of protein breakdown. However, even if a value as high as 40% for splanchnic retention had been used in calculations with AA plus G dialysis, the net protein balance found in this study would still have improved in all patients. The conclusion of this study is that APD with a dialysate composed of a mixture of AA plus glucose increases protein anabolism. This procedure when applied over a long-term could improve the nutritional status of peritoneal dialysis patients whose dietary protein and calorie intake is inadequate.

Considering that a substantial portion of patients undergoing continuous ambulatory PD (CAPD) have insufficient protein and calories intake due to anorexia, and that combined AA plus glucose dialysis fluids could serve as a useful extra supply of nutrients in CAPD patients, we evaluated the metabolic effects of this solution in twelve CAPD patients. In contrast to APD patients, CAPD patients dialyze without an automated cycler, but change their dialysate bags manually during the day, while they consume their regular daily meals. Since a steady-state metabolic condition is required for the WBPT-study with the precursor method, the exchanges of dialysis solutions were carried out using an automated cycler instead of manual exchanges, and a defined liquid complete diet comparable in nitrogen and energy content to their habitual diet was given at regular intervals. Although the amount of AA absorbed from the peritoneal cavity was small in comparison with oral AA intake, there were significant anabolic effects. We found a statistically increased protein synthesis during dialysis with combined AA plus glucose dialysate as compared with glucose-only dialysate. These metabolic

responses are in line with other studies reporting a stimulation of both flux and protein oxidation by an increase in protein supply.[12,13] Supplying combined AA plus glucose intraperitoneally caused a significant increase in protein synthesis, despite increased protein oxidation. Protein breakdown and net protein synthesis were not different between AA plus glucose dialysis and glucose-only dialysis. Some of the absorbed AA derived from the oral feeding is sequestered in the splanchnic tissues. In this study splanchnic retention was taken into account. We assumed a value of 25% for both orally and peritoneally absorbed AA.[10] In contrast to the negative net protein synthesis during fasting in our APD study, the net protein synthesis was found positive in all patients who were taking oral food, a finding in agreement with reported studies in healthy subjects and in hemodialysis patients.[12-14] When we compared the results of our study in the fed state with the results of our APD study in the fasting state, we found a significantly higher protein synthesis rate and net protein synthesis in the fed state as compared with the fasting state, during both combined AA plus glucose and glucose-only dialysis. In both studies protein breakdown did not significantly change in response to feeding, and this was true for both the combined AA plus glucose and glucose-only dialysis schedules. This shows that ingested protein was used mainly for increased protein synthesis. It has been reported that in fasting patients glucose-containing dialysis solutions inhibit protein breakdown as a consequence of moderate hyperinsulinemia.[15] This may explain why, in our study, feeding did not lead to further reduction of protein breakdown. It should also be noted that four out of the twelve CAPD patients in the fed state study were clinically malnourished according to the subjective global assessment (SGA). When we compared the results of the WBPT in these four malnourished patients (SGA-B) with the eight well-nourished subjects (SGA-A), we found a statistically significant increase in net protein synthesis with the combined AA plus glucose dialysate as compared to glucose-only dialysate in the malnourished patients. This finding suggests that the nutritional state plays a role in the metabolic effects of combined AA plus glucose dialysis. It lends further support to the proposition that the use of a dialysis solution containing a mixture of AA plus glucose is capable of supplying both protein and calories, when oral intake of proteins and calories is deficient in CAPD patients with poor appetite. We conclude, that even in a fed state, dialysis solutions that contain AA plus glucose improve protein synthesis in CAPD patients. Such solutions could function as a nutritional supplement and may help to improve the nutritional state in these patients.

Effect of combined amino acid and glucose dialysate on the fractional synthesis rate of albumin and whole-body protein synthesis in PD patients in fasting and fed state

Several previous studies reported an increased albumin synthesis in response to proteins or AA supplementation.[16-18] In contrast, we did not find that the fractional synthesis rate of albumin (FSR-albumin) increased with combined AA plus glucose dialysate as compared to glucose only in twelve CAPD patients either in the fasting or in the fed state, in spite of the significant improvements in whole-body protein synthesis (WBPS). Our FSR-albumin values were similar to those achieved in a previous study in patients on CAPD.[19] When comparing fasting and fed state in PD patients, it should be realized that, due to the continuous glucose absorption from the dialysate, a moderate hyperinsulinemia is present even in the 'fasting' PD patients. Thus they are not truly in the fasting state. Insulin is known to have a stimulating effect on hepatic albumin synthesis additional to that of an increase in substrate availability.[20-22] In previously published studies the effects of nutrition were assessed by comparison with the true fasting state. This implies that the increased albumin synthesis observed in those studies might be induced, at least in part, by an increase in insulin secretion. It is conceivable that, in our study of fasting PD patients, albumin synthesis was already stimulated by insulin, so that supplying intraperitoneal or oral AA could not induce a further increase in FSR-albumin. By conducting our studies both during the night while the patients were in the fasting state (APD) and during the day with the patients in the fed state (CAPD), we were able to examine the effects of oral food. It appeared that even the larger amount of oral protein intake in the fed state as compared to the fasting state, did not lead to a statistically significant increase in FSR-albumin. Thus neither intraperitoneal amino acids nor oral food induced an increase in fractional synthesis rate of albumin, in spite of a substantial increase of WBPS. Whole-body protein synthesis and FSR-albumin apparently respond differently to intraperitoneal and oral amino acids. This difference may well be related to region-or organ-specific kinetics; measured whole-body protein turnover is a composite of all of the visceral and somatic compartments taken together.[23] Others have shown in PD patients that availability of AA promotes skeletal muscle protein synthesis, whereas insulin has an inhibitory effect on muscle protein breakdown.[15] As muscle protein synthesis contributes substantially to WBPS, the stimulating effect of AA on WBPS may to a large extent be attributed to increased muscle

protein synthesis. Our findings are therefore consistent with the notion that the regulation of skeletal muscle protein synthesis is different from that of the synthesis of albumin. It has also been reported that essential AA are mainly responsible for the AA-induced stimulation of muscle protein anabolism.[24] We found that plasma AA, and in particular the essential AA, increased significantly with both intraperitoneal and oral AA supply. We would like to emphasize that our studies were conducted in a small, relatively stable population without liver diseases or acidosis, and without clinical signs of inflammation. The fact that PD patients were not in a true fasting state due to the continuous glucose absorption from the dialysate complicates the interpretation of the results. We conclude that the supply of AA has different effect on whole-body protein synthesis and the fractional synthesis rate of albumin in clinically stable PD patients.

Comparison between end-product and precursor methods for measuring whole-body protein turnover in peritoneal dialysis patients

The precursor method with a primed constant infusion of L-[1-^{13}C]leucine is considered to be the reference standard for protein turnover, but is laborious to perform.[25] There is a need for a validated, less invasive method to evaluate the effect of nutritional intervention on protein metabolism, which can be applied repeatedly in various patient populations, in different situations, and outside the hospital or metabolic ward. The end-product method using a single oral dose of [^{15}N]glycine[26] is less invasive than the L-[1-^{13}C]leucine technique,[27] is more convenient, and is suited to population studies in different circumstances. We compared both methods by applying them simultaneously to measuring WBPT during combined AA plus glucose dialysis and during glucose-only dialysis in twelve CAPD patients in the fed state. In the end-product method we measured the ^{15}N enrichment of urea in dialysate as the end product of nitrogen (N) metabolism. In the precursor method, WBPT was calculated from the ^{13}C enrichment of α-ketoisocaproic acid in plasma and the ^{13}C enrichment in expired CO_2.[27,28] Both methods are based on different assumptions and calculations.[25] We found that the absolute values for rates of protein synthesis and breakdown measured with the two methods were different. However, the calculation of the final outcome, *i.e.* the net protein synthesis (protein synthesis minus breakdown) during both dialysis schemes showed a good correlation between the two methods. In agreement with the literature, the variability within the group is lower with the precursor method, probably due to the more rigidly

standardized protocol of this method.[29] We therefore conclude that the results of the two methods are in agreement but that for studies of relatively small groups of patients, the precursor method with a primed continuous infusion of L-[1-^{13}C]leucine is superior. The precursor method remains the reference method. The choice between the precursor and the end-product method will ultimately depend on the purpose of the study and on practical considerations.

Effect of combined amino acids and glucose-containing dialysate on protein loss and cytokines generation, and amino acid loss with standard glucose solution in APD.

Although APD is commonly used nowadays, peritoneal protein losses have been studied only occasionally in adult patients undergoing APD. We reported that the mean protein loss per 24 h, *i.e.* the sum of daytime dwell and nightly APD with standard G was in the same order of magnitude as previously reported for CAPD patients by other authors.[30,31] No difference was found between the peritoneal protein losses during 8.5 h nightly APD and 15.5 h daytime dwell. In our study all patients had six cycles during APD, and protein losses per time were higher during cycling with standard glucose solutions. Washout of proteins from the long daytime dwell could well account for the difference of protein losses per time between daytime dwell and nighttime cycling as previously suggested in patients on intermittent peritoneal dialysis (IPD).[32,33] Studies of the effects of AA-based dialysis solutions on peritoneal protein losses reported inconsistent results.[34-37] The low amino acid concentrations (0.22%) we used may account for the absence of an effect of AA on protein losses in our study. We found that the mean AA loss per 24 h was similar to those reported in patients who are on CAPD and somewhat higher than found in a previous study in APD patients, using standard G dialysis solution.[38-40] The total AA losses per time were greater during nightly APD than during the long daytime dwell with standard glucose-based dialysis solution in line with the transperitoneal transport of small molecular weight solutes. The levels of TNFα and IL-6 we found in the daytime dialysate with standard glucose dialysis solutions are comparable to those described in other studies in CAPD patients during an infection-free period.[34,41,42] We also found a significant effect of amino acids on the intraperitoneal appearance of IL-6, in particular in the daytime effluent compared to standard glucose-only dialysate. The higher IL-6 levels in dialysate than in plasma indicate local production of IL-6 as previously reported by other studies in CAPD.[34,43] There is evidence that

even in the absence of peritonitis, IL-6 is produced locally within the peritoneal cavity.[41,42,44] Both mesothelial cells and macrophages are able to produce and release substantial amounts of IL-6. The release of IL-6 from mesothelial cells occurs constitutively and can be stimulated by macrophage-derived TNFα and IL-1β.[34,41,44] We found very low levels plasma and dialysate levels of TNFα. As IL-6 is involved in immune and inflammatory responses and also has anti-inflammatory properties, the clinical implications of increased intraperitoneal IL-6 levels are far from straightforward. It is a matter of debate whether any changes in cytokine release should be considered as harmful or as a marker of improved tissue responsiveness. In conclusion, in this study with a small number of patients, nightly APD with amino acids containing dialysate was associated with an increase in peritoneal IL-6 generation during the day. Combined AA plus glucose dialysate did not induce a significant increase in peritoneal protein losses compared to dialysate containing only glucose. Peritoneal losses of amino acids per 24 h with standard glucose solutions were similar to those previously reported in CAPD.

Perspectives for future research

A larger randomized controlled (multi-center) study is warranted to evaluate the long-term effects of dialysis solutions containing AA plus glucose on the nutritional state of PD patients, and to determine whether an improvement in the nutritional status of PD patients is associated with a decrease in their mortality and morbidity and improved quality of life.

The optimal proportions of AA plus glucose in combined diaysate have not yet been determined. As the amount of AA given in the studies of this thesis is relatively modest, the question arises whether higher doses could be given if dietary protein intake is far below target levels. The fact that plasma urea levels did not increase and bicarbonate levels only slightly decreased in our studies suggests that higher amounts of amino acids may be given without the risk of uraemic adverse effects and acidosis. Further studies are needed to investigate how much amino acid can be given safely.

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Chapter 9

Summary (English/Dutch)

Summary

Up to 50% of uremic patients, including those treated with peritoneal dialysis (PD), suffer from protein and energy malnutrition (PEM). Malnutrition is strongly associated with an increased morbidity and mortality, in particular from cardiovascular diseases. Inflammation, low nutrient intake due to anorexia, and peritoneal losses of amino acids and proteins are important factors contributing to malnutrition.

In the **Introduction** to the thesis, the peritoneal dialysis procedures and the pathogenesis of malnutrition in peritoneal dialysis (PD) patients are described. Methods to assess the nutritional state are mentioned. Protein and amino acid (AA) metabolism and the methods to measure nitrogen balance and whole-body protein turnover (WBPT) are outlined. The composition, the characteristics and the previously conducted studies of AA-based PD fluids are described and the aim of the thesis is put forward. The main theme is to determine whether the nutritional state of PD patients can be improved by modulating conventional PD fluid.

In **chapter 2** we report a high prevalence of malnutrition in PD patients treated in the Erasmus MC using a clinical screening tool, the subjective global assessment (SGA). A good correlation was demonstrated between the original SGA and body composition measurements. SGA proved to be a practical screening tool to assess malnutrition in PD patients, BIA and DEXA, however, provide additional information on body composition and may be used to follow up changes in lean body mass or fat mass.

In **chapter 3** we investigate the metabolic effects of solutions containing combined AA plus glucose in 8 patients on APD, given as part of their regular overnight dialysis scheme, as compared with glucose-only dialysate. These effects of the AA plus glucose containing dialysis solutions were evaluated by measuring WBPT with L-[1-^{13}C]leucine. Dialysis solutions containing AA plus glucose improved protein anabolism in the fasting state. A prolonged anabolic effect of the mixture on protein metabolism could not definitely be shown by classical nitrogen balance studies. No intolerance of the solutions was observed. Administering a dialysate composed of a mixture of AA and glucose for a longer period might improve the nutritional status of patients treated with APD, whose dietary protein intake is inadequate, but this needs to be confirmed by prospective studies.

In **chapter 4** we studied the acute effects on the protein metabolism of combined AA plus glucose dialysate compared with glucose-only dialysate by performing WBPT study in CAPD patients in the fed state. Although the amount of AA absorbed from the peritoneal cavity was small in comparison with oral AA intake, there was a significant increase in protein synthesis with AA plus glucose dialysate compared with glucose only. The ingested protein was used mainly for stimulation of protein synthesis, while feeding did not further reduce protein breakdown, which was already inhibited, probably by the moderate hyperinsulinemia induced by glucose-containing dialysis solutions in the fasting state. Thus, AA administered intraperitoneally have similar anabolic effects to oral feeding, which lead us to call this concept 'dialysate as food.' In addition we showed that the anabolic effects of AAG dialysate are more pronounced in malnourished patients. Giving a dialysis solution that contains a mixture of AA plus glucose in addition to their daily food intake might therefore be of benefit to CAPD patients who are unable to meet their requirements due to a poor appetite.

In **chapter 5** we showed that neither intraperitoneal amino acids nor oral feeding induced a significant increase in the fractional synthetis rate of albumin (FSR-albumin), despite of a substantial increase of WBPT. These findings indicate that whole-body protein synthesis (WBPS) and the FSR-albumin respond differently to intraperitoneal and oral AA. As suggested by previous studies, the supplied essential AA are probably mainly used for muscle protein synthesis rather than for hepatic protein synthesis in clinically stable and well-nourished PD patients.

In **chapter 6** we present the results of a comparison of the WBPT data measured with the end-product method with [^{15}N]glycine with those derived from measurements using the precursor method with a primed constant infusion of L-[1-^{13}C]leucine. Although the absolute values of the components of WBPT are different, the calculation of the final outcome, *i.e.* the net protein balance, showed a good correlation between both measurements. As the more strict and standardized protocol, the variability within the group is lower with the precursor method. At present the choice between the precursor and the end-product method will ultimately depend on the purpose of the study and on practical considerations.

In **chapter 7** we reported that APD dialysis fluid containing a mixture of amino acids plus glucose increased intraperitoneal appearance of proinflammatory cytokine, IL-6, as compared with dialysate containing only glucose, but the mixture did not affect peritoneal protein losses. The loss of amino acids into the 24 h dialysate with standard glucose solutions was similar as previously reported in CAPD.

Chapter 8 provides a discussion and the conclusions of our results in a broader context. The assessment of the nutrition state in our PD population and the significance of the tools applied in the nutritional assessment are described. The effects of dialysate containing AA plus glucose on whole-body protein metabolism in PD patients in the fasting as well as in the fed state are outlined. The different response of intraperitoneal and oral AA supply on the albumin synthesis are also discussed. The applicability of the less invasive method to measure WBPT in PD making use of a single oral dose of ^{15}N glycine is discussed. Peritoneal protein losses and cytokines generation during APD with AA plus glucose-containing dialysis solutions, and the peritoneal amino acids losses with standard glucose solution are described.

The addition of AA to glucose-containing dialysis solutions is a promising approach to the long-term prevention and treatment of malnutrition in uremic patients undergoing peritoneal dialysis. Whether such an approach using dialysate as food will indeed have a beneficial effect on morbidity and mortality remains to be seen. Suggestions are given for future research.

Samenvating

Tot 50% van de patiënten met chronische nierinsufficiëntie, inclusief de patiënten die behandeld worden met chronische peritoneale dialyse (PD), is ondervoed. Ondervoeding is sterk geassocieerd met een verhoogd risico op ziekte en overlijden, met name aan hart- en vaatziekten. Verminderde voedselinname als gevolg van een slechte eetlust en het verlies van eiwitten en aminozuren via de dialysevloeistof zijn belangrijke factoren die bijdragen aan het ontstaan van ondervoeding bij PD patiënten.

In de **Introductie** van dit proefschrift worden de vormen van en de procedures bij PD beschreven, alsmede de oorzaken van ondervoeding bij PD patiënten. De toegepaste onderzoeksmethoden om de voedingstoestand te bepalen worden vermeld. De eiwit- en aminozuur stofwisseling en de methodieken om de stikstof- en eiwitbalans te meten worden besproken. De samenstelling en eigenschappen van de aminozuren (AZ)-bevattende PD vloeistoffen worden beschreven, alsmede eerder verrichte studies met deze oplossingen. De doelstellingen van dit onderzoek worden uiteengezet. Hierbij staat het verbeteren van de voedingstoestand van PD patiënten door middel van toevoeging van AZ aan de gebruikelijke glucose-bevattende PD vloeistof centraal.

In **hoofdstuk 2** worden de resultaten van het onderzoek naar de prevalentie van ondervoeding beschreven bij patiënten die in het Erasmus MC met PD worden behandeld. De originele 3-puntsschaal SGA werd gebruikt om de voedingstoestand te beoordelen. Ook in onze PD populatie komt ondervoeding veelvuldig voor namelijk in 37%. Daarnaast werd gebruikgemaakt van de body mass index (BMI), de 4-punts huidplooimeting en van meer geavanceerde onderzoeksmethoden zoals de bioelectrical impedance analysis (BIA) en de dual energy x-ray absorptiometry (DEXA). De resultaten van deze methoden blijken ook in ons onderzoek goed onderling overeen te komen. De SGA is een betrouwbare, snelle en goedkope methode voor de beoordeling van de voedingstoestand van PD patiënten in de klinische praktijk. De overige methoden kunnen als aanvulling dienen om de voedingstoestand nader in kaart te brengen en zijn zinvol voor het vervolgen van de veranderingen in de spier en de vetmassa.

In **hoofdstuk 3** beschrijven wij de resultaten van het onderzoek naar de anabole effecten van een AZ- en glucose bevattend PD-vloeistofmengsel in 8 patienten die behandeld werden door middel van automatische PD (APD). Deze

patiënten werden gedialyseerd met behulp van een geautomatiseerd apparaat voor toediening en drainage van dialysaat. Het AZ-en glucose bevattende PD-vloeistofmengsel werd toegediend tijdens de gebruikelijke peritoneale dialyse procedure 's nachts thuis. De acute effecten op de eiwitstofwisseling werden bestudeerd met eiwitomzetonderzoek middels het met het stabiele isotoop ^{13}C gelabelde aminozuur leucine (L-[1-^{13}C]leucine). Om het effect over een langere periode te kunnen beoordelen hebben we gebruik gemaakt van de klassieke stikstofbalans. Uit de resultaten blijkt dat bij het AZ- en glucose bevattende PD-vloeistofmengsel de netto eiwitbalans (snelheid van eiwit synthese minus snelheid van eiwitafbraak) significant verbetert. Een langer bestaand anabool effect kon op grond van de resultaten van de stikstofbalans niet met zekerheid worden aangetoond. Geconcludeerd wordt dat gebruik van PD-vloeistof bestaande uit een mengsel van AZ en glucose de voedingstoestand van PD patiënten, die ondervoed zijn of slecht eten, zou kunnen verbeteren.

Een aanzienlijk deel van de PD patiënten wordt behandeld met continue ambulante peritoneale dialyse (CAPD). In tegenstelling tot APD-patiënten infunderen deze patiënten de PD-vloeistof zelf via een permanente CAPD katheter in de buik en ze dialyseren overdag. Om te kunnen beoordelen of ook CAPD patiënten baat zouden kunnen hebben van dialyse met AZ- en glucose bevattende PD-vloeistoffen wanneer zij tijdens de dialyse overdag hun maaltijden gebruiken, werd vergelijkbaar onderzoek ook bij deze patiënten verricht. Teneinde een evenwichtssituatie te verkrijgen hetgeen vereist is voor een eiwitomzetstudie, vond de dialyse tijdens deze studie plaats met behulp van een automatisch dialyse apparaat (cycler). Om de gevoede toestand na te bootsen en een evenwichtstoestand daarin te creëren, werden op gezette tijden, identieke porties vloeibare voeding toegediend. De resultaten zijn in **hoofdstuk 4** beschreven. Hieruit blijkt, dat de AZ- en glucose bevattende PD-vloeistof de eiwitsynthese stimuleert ondanks de - in vergelijking met de uit de orale voeding verkregen hoeveelheid - relatief geringe hoeveelheid uit dialysaat geabsorbeerde AZ. De geabsorbeerde AZ werden voornamelijk gebruikt voor de eiwitsynthese. De conclusie is dat de via de dialysevloeistof toegediende AZ een soortgelijk anabool effect hebben op de stofwisseling als normaal voedsel, vandaar de titel van de thesis 'dialysaat als voeding.' De eiwitafbraak werd bij de CAPD-groep niet geremd, dit in tegenstelling tot de APD-patiënten in de nachtelijke gevaste toestand, waarbij de eiwitafbraak werd geremd waarschijnlijk door stimulatie van de insulinesecretie als gevolg van het vanuit het dialysaat geabsorbeerde

glucose. Aangezien de effecten het meest uitgesproken waren bij de ondervoede CAPD patiënten, is de voedingstoestand vermoedelijk van belang voor het verkrijgen van de anabole effecten. Dialyseren met een mengsel bestaande uit AZ- en glucose naast het gebruik van de dagelijkse maaltijden, lijkt zinvol bij ondervoede danwel slecht etende CAPD patiënten.

In **hoofdstuk 5** beschrijven wij, dat noch orale voeding noch de aminozuren die via de dialysevloeistof werden toegediend noch de snelheid van de albuminesynthese stimuleerden, terwijl de totale eiwitsynthese toch duidelijk toenam. Dit wijst er op dat de snelheid van de albumine synthese en die van de totale eiwitsynthese verschillend reageren op toegediende aminozuren. In de literatuur wordt gesuggereerd dat bij klinisch stabiele niet ondervoede PD patiënten de toegediende essentiële AZ veel meer gebruikt worden voor de eiwitsynthese in de spier dan voor de synthese van eiwitten in de lever. Misschien verklaart dit waarom wij geen toename van de albumine-synthese hebben kunnen aantonen.

Hoofdstuk 6 beschrijft de vergelijking tussen de eindproduktmethode met behulp van [^{15}N]glycine en de voorloperproduct-methode met [L- 1-^{13}C]leucine voor het meten van de eiwitomzet. De laatste methode wordt beschouwd als de 'gouden standaard.' Uit de resultaten blijkt dat de [L- 1-^{13}C]leucine gemiddeld nauwkeuriger- dat wil zeggen met minder spreiding binnen de patiëntengroep- de eiwitomzet meet dan de [^{15}N]glycinemethode, vermoedelijk door het strakkere protocol. Echter beide methoden komen goed overeen in de meting van de netto eiwitbalans, waar het uiteindelijk omgaat bij de eiwitomzet studies. Onze resultaten wijzen er op, dat men voor een meer nauwkeurige analyse beter de [L-1-^{13}C]leucine methode kan gebruiken voor het meten van eiwitomzet bij PD patiënten, als men de studie in het ziekenhuis kan uitvoeren, maar dat voor poliklinisch danwel veldonderzoek de glycine-methode valt te overwegen.

In **hoofdstuk 7** beschrijven wij dat tijdens dialyse met de standaard glucosevloeistof het eiwitverlies in het dialysaat per tijdseenheid 's nachts (APD) groter is dan overdag (CAPD). Dit verschil lijkt te wijten aan de uitwas van eiwitten in het overgebleven dialysaat van overdag. Wij hebben geen verschil kunnen aantonen in het eiwitverlies in het dialysaat tijdens het gebruik van het AZ-en glucose bevattende PD-vloeistofmegsel in vergelijking met alleen de glucose bevattende PD-vloeistof. Tijdens dialyse met het AZ bevattende PD-vloeistofmengsel zijn de concentraties van het cytokine IL-6 in het dialysaat hoger dan tijdens dialyse met alleen de glucose bevattende PD-vloeistof, terwijl

de concentraties van het cytokine tumor necrosis factor α (TNFα) laag zijn. De IL-6 spiegels in het dialysaat, met name overdag, zijn hoger dan in het plasma tijdens het gebruik van zowel het PD-vloeistofmengsel als alleen de glucose PD-vloeistof. Dit wijst op lokale productie van IL-6. Echter de klinische betekenis van deze verhoogde IL-6 spiegels in het dialysaat is vooralsnog onduidelijk. In ieder geval heeft de toename in de IL-6 concentraties in het dialysaat niet geleid tot een toegenomen verlies van eiwitten in het dialysaat tijdens dialyse met de AZ-bevattende PD-vloeistof. Het verlies van aminozuren in het dialysaat tijdens APD met standaard glucose PD-vloeistof is in dezelfde orde van groottte zoals beschreven bij CAPD patiënten.

De discussie en de conclusies die uit de onderzoeksresultaten voortvloeien zijn beschreven in **hoofdstuk 8.** Onderzoek naar ondervoeding bij PD patiënten in het Erasmus MC verricht met behulp van de originele subjective global assessment (SGA) wordt beschreven. Verschillende methoden voor het meten van lichaamssamenstelling worden besproken en gerelateeerd aan de SGA-classificatie. Fysiologische mechanismen die betrokken zijn bij de eiwitomzetting in de gevaste en gevoede toestand bij PD patiënten tijdens dialyse met AZ en glucose bevattende PD vloeistofmengsel worden besproken. Op de invloed van de intraperitoneaal en via de voeding toegediende aminozuren op de albuminesynthese en de totale lichaamseiwitsynthese wordt uitgebreid ingegaan. Twee gerenommeerde methoden om met behulp van met stabiele isotopen gemerkte aminozuren de eiwitomzet te meten worden besproken, met name ook de toepasbaarheid van de minder ingrijpende methode met ^{15}N glycine bij PD patiënten. Een plausibele verklaring voor het verschil tussen het nacht (APD) en de dag (CAPD) eiwitverlies per tijdseenheid via het dialysaat tijdens dialyse met de standaard glucose PD-vloeistof wordt gegeven, alsmede voor de produktie van cytokines in het dialysaat. Tevens wordt het verlies van aminozuren in het dialysaat tijdens APD met de standaard glucose beschreven.

Tenslotte worden enkele aanbevelingen gedaan voor toekomstig onderzoek, dat de huidige bevindingen klinische toepasbaarheid zou kunnen geven.

Abbreviations

AA	:	Amino acids
AAG	:	Amino acids and glucose
AZ	:	Aminozuren
APD	:	Automated peritoneal dialysis
BIA	:	Bioelectrical impedance analysis
BMI	:	Body mass index
CAPD	:	Continuous ambulatory peritoneal dialysis
CRF	:	Chronic renal failure
DEXA	:	Dual energy x-ray absorptiometry
FMI	:	Fat mass index
FFMI	:	Fat free mass index
FSA	:	Four skin-fold anthropometry
FSR-alb	:	Fractional synthesis rate of albumin
G	:	Glucose
GFR	:	Glomerular filtration rate
ICC	:	Intraclass correlation coefficient
MIA	:	Malnutrition inflammation, and atheroslerosis
N	:	Nitrogen
PD	:	Peritoneal dialysis
PEM	:	Protein energy malnutrition
SGA	:	Subjective global assessment
WBPT	:	Whole-body protein turnover
WBPS	:	Whole-body protein synthesis

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Now, I would like to express my thanks to those who have played an important role in my medical career.

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Colleagues of the former hypertension group (Dr. F.H.M. Derkx, Dr. G.J. Wenting, Dr. A.J. Man in 't Veld, Dr. F. Boomsma, Dr. A.H. van den Meiracker, and Dr. J. Deinum): Dear all, and Frans Derkx in particular, I enjoyed working with you all in the past. Ton and I still work together. Dear Frans, thanks you for all your help during my first attempts to do research. You were always willing to help me also in other fields – you are a real friend. Thanks for everything.

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Residents in the consultative internal medicine training program: I regret I have had too little time to teach you all. Thank you for your kindness, support, understanding and all your help.

The patients, the residents in internal medicine, and the medical students have all played a very important in my career, that is why I dedicate my PD thesis to them.

Colleagues, nurses, and others, thank you all for the pleasant working relationship we had during my time as supervisor of consultative internal medicine training.

My parents: I am deeply sorry you could not be here with me today. I am grateful to you for the loving, carefree, and protected childhood and youth I had. I cannot imagine having had any parents better than you were. You gave me

every possibility to educate myself further and never prevented me from making my own choices in life. Father, I know you were passionate about knowledge and study, and that you wanted me to obtain my doctoral degree. Unfortunately, the spirit was willing but the flesh was weak. Nevertheless, I have finally fulfilled your desire. Above all, I dedicate this dissertation to your memory, my beloved parents.

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With this doctoral thesis as my symbol of farewell, I will be leaving Dijkzigt Hospital (Erasmus MC), where I have worked with so much pleasure for thirty-seven years. My best wishes to you all.

Curriculum Vitae

Hoey Lan Tjiong was born in the former Dutch East Indies (now Indonesia).

She attended Dutch primary school and Dutch-Indonesian secondary school in Jakarta and graduated in 1962.

From 1962 to 1969 she studied medicine at the University of Cologne, Germany, and received her medical degree in 1969.

From 1971 to 1976 she received her training in internal medicine at the Erasmus MC university hospital, Rotterdam, the Netherlands (Prof.dr. J. Gerbrandy).

From 1976 till 2002 she was supervisor of the 3M-unit of the Department of Internal Medicine I (Prof.dr. J. Gerbrandy from 1976 to 1984, Prof.dr. M.A.D.H. Schalekamp from 1983 to 2000, and Prof.dr. H.A.P. Pols from 2000 to 2002).

In 2002 she began her research in the Section of Nephrology in close collaboration with the Section of Metabolism of the Department of Internal Medicine, which resulted in this dissertation.

From 2002 until the present she has worked as supervisor for the consultative internal medicine training program.

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