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INTRACELLULAR FREE AMINO ACIDS AND NUTRITIONAL STATUS IN CHILDREN WITH CHRONIC RENAL FAILURE ON DIFFERENT TREATMENTS

Alberto Canepa

Stockholm 2001
Dedication

1 DEDICATION
To my wife Federica, my daughter Camilla and my sister Franca
ABSTRACT

Intracellular free amino acids and nutritional status in children with chronic renal failure on different treatments.

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In untreated adult patients with chronic renal failure (CRF), and in chronic ambulatory peritoneal dialysis (CAPD) and kidney-transplanted patients, typical plasma and muscle intracellular amino acid (AA) patterns have been described. Similar AA abnormalities to those seen in adult patients have been observed in plasma and muscle tissue of children with CRF, but no studies have been undertaken to evaluate the AA levels in plasma and muscle in children on CAPD or after kidney transplantation. Polymorphonuclear granulocytes (PMN) are valuable tools for evaluating AA metabolism in nucleated cells but an error in PMN AA concentration may be induced by protease activation during the analytical procedure. Red blood cells (RBC) contain a large proportion of the free AA in the blood and are actively involved in the inter-organ transport of AA. The AA profile in RBC has been reported abnormal in patients with CFR. There are no studies in which AA concentrations in PMN have been compared with muscle or RBC concentrations.

In this thesis anthropometric measurements, plasma and muscle proteins and AA were determined in children with CRF, in children on CAPD and in kidney-transplanted children. The results were compared with data from healthy age-matched children. A reliable method for AA determination in PMN was set up and comparison between intracellular AA in muscle, RBC, PMN and plasma were done in CRF children.

In study I, before the start of dialysis 15 children with CRF were investigated with anthropometric measurements and determination of plasma proteins, muscle alkali-soluble proteins (ASP) and AA. The results were compared with data obtained from 10 age-matched control children. The nutritional parameters were normal in the CRF children, suggesting that a satisfactory nutritional status can be maintained during conservative treatment. However, they exhibited several AA abnormalities in plasma and muscle typical of uremia. In study II, 10 children on CAPD were investigated with anthropometric measurements and determination of plasma and muscle proteins and AA. The results were compared to data obtained from 22 age-matched control children. The findings of a compromised nutritional status, reduced plasma levels of most essential AA and low muscle valine and leucine concentrations suggests that they may potentially benefit from AA supplementation to improve protein malnutrition. In study III, 13 kidney-transplanted children were investigated with anthropometric measurements and plasma and muscle proteins and AA determinations and the results were compared to data obtained from 10 age-matched control children. The transplanted children showed an almost normal muscle AA profile, whereas the plasma AA pattern exhibited minor abnormalities, which seem to be related to the prednisone therapy. In study IV, proteolytic activity and PMN AA were determined in blood samples processed with and without anti-proteolytic agents in 10 adult volunteers. PMN AA concentrations and protease activity in samples treated with anti-proteolytic agents were 8 to 10 times lower than in samples processed without anti-proteolytic agents. Hence, prevention of proteolysis during the sample preparation is necessary for reliable estimation of free AA in PMN. In study V, anthropometric measurements, plasma and muscle proteins, plasma, RBC, PMN and muscle AA were studied in 12 chronically uremic children and the results compared to data obtained from 13 age-matched normal children. The lack of correlation between the concentrations in RBC, PMN and muscle for most of the AA in the same subject may be due to differences in metabolism and function of these cells. In consequence, one should be cautious in assuming that AA concentrations, determined in PMN, reflect the concentrations in muscle cells, as previously suggested, although in groups of subjects the general AA pattern in these nucleated cell types are similar.

In summary, this thesis has demonstrated that 1) children with CRF, on CAPD and kidney transplanted show typical plasma and muscle AA abnormalities, 2) the use of anti-proteolytics during the sample preparation is necessary for reliable estimation of free AA in PMN 3) although in groups of subjects the general AA pattern in PMN and muscle may be similar it is hazardous assuming that AA concentrations, determined in PMN, reflect the concentrations in muscle cells.

Key words: Chronic renal failure, transplantation, nutritional status, children, amino acids, plasma, muscle, polymorphonuclear granulocytes, proteolysis, red blood cells.
List of Publications

2 LIST OF PUBLICATIONS

1. NUTRITIONAL STATUS AND MUSCLE AMINO ACIDS IN CHILDREN WITH END-STAGE CHRONIC RENAL FAILURE Alberto Canepa, José C. Divino Filho, Ann-Marie Forsberg, Francesco Perfumo, Alba Carrea, Rosanna Gusmano, Jonas Bergström Kidney International 1992; 41:1016-1022

2. CHILDREN ON CONTINUOUS AMBULATORY PERITONEAL DIALYSIS: MUSCLE AND PLASMA PROTEINS, AMINO ACIDS AND NUTRITIONAL STATUS Alberto Canepa, José C. Divino Filho, Ann-Marie Forsberg, Francesco Perfumo, Alba Carrea, Enrico Verrina, Emilio Podestá, Rosanna Gusmano, Jonas Bergström Clinical Nephrology 1996; 46, 2:125-131

3. NUTRITIONAL STATUS AND MUSCLE AMINO ACIDS IN KIDNEY-TRANSPLANTED CHILDREN Francesco Perfumo, Alberto Canepa, José C. Divino Filho, Eva Nilsson, Alba Carrea, Enrico Verrina, Rosanna Gusmano, Jonas Bergström Nephrology Dialysis and Transplantation 1994; 9: 1778-1785


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<tr>
<td>AA</td>
<td>Amino acids</td>
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<tr>
<td>AMC</td>
<td>Arm muscle circumference</td>
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<td>ASP</td>
<td>Alkali-soluble protein</td>
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<td>BCAA</td>
<td>Branched chain amino acids</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>BSS</td>
<td>Balanced salt solution</td>
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<td>BW</td>
<td>Body weight</td>
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<td>CAPD</td>
<td>Continuous ambulatory peritoneal dialysis</td>
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<td>CRF</td>
<td>Chronic renal failure</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<td>Cy-A</td>
<td>Cyclosporine-A</td>
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<td>EAA</td>
<td>Essential amino acids</td>
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<td>FFS</td>
<td>Fat free solids</td>
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<td>HD</td>
<td>Hemodialysis</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>ICW</td>
<td>Intracellular water</td>
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<td>MAC</td>
<td>Mid arm circumference</td>
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<td>MAMC</td>
<td>Mid arm muscle circumference</td>
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<td>NEAA</td>
<td>Non essential amino acids</td>
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<tr>
<td>PMN</td>
<td>Polymorphonuclear granulocytes</td>
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<td>PD</td>
<td>Peritoneal dialysis</td>
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<td>RBC</td>
<td>Red blood cells</td>
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<td>RDA</td>
<td>Recommended dietary allowance</td>
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<td>SDS</td>
<td>Standard deviation score</td>
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<td>SGNA</td>
<td>Subjective global nutritional assessment</td>
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<tr>
<td>SSA</td>
<td>Sulphosalicylic acid</td>
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<tr>
<td>TSF</td>
<td>Triceps skinfold thickness</td>
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<tr>
<td>WHI</td>
<td>Weight/height index</td>
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5 INTRODUCTION

5.1 BACKGROUND

Dialysis and kidney transplantation are established methods for treating chronic renal failure (CRF) when conservative management is no longer possible [1]. Kidney transplantation is the novel method for treatment of children with end stage renal disease (ESRD). However, maintenance dialysis treatment is necessary for children waiting to be transplanted or for those who cannot be transplanted. For children requiring dialysis, continuous peritoneal dialysis, either in the form of continuous ambulatory dialysis (CAPD) or continuous cycling peritoneal dialysis is now used at least as commonly (in 50-65% of the cases) as hemodialysis (HD). For example, no infant enrolled in the Italian Pediatric Register was treated with chronic HD during the period 1986-1994 [2], which confirms the tendency in Europe to treat this particular population with PD instead of HD, due to the better clinical and rehabilitation results. Children in Sweden are pre-emptively kidney-transplanted or put on CPD while waiting for transplantation.

In young children, CPD has several advantages over HD. An important advantage is the fact that an arteriovenous fistula, with all its implications and complications, is not needed. Unlike HD, the blood levels of various substances during CPD treatment are steady, since there is a constant removal of waste products from the body. The disequilibrium syndrome, caused by the rather sudden changes in body composition during intermittent HD in children, does not occur during CPD. Since treatment is performed at home, school attendance and family life are closer to normal during PD [3].

5.2 MALNUTRITION IN CHRONIC RENAL FAILURE

Virtually every survey dealing with the nutritional status of chronically uremic and dialysis patients reports a high prevalence of wasting and protein-energy malnutrition [4-10]. The problems of malnutrition and negative nitrogen balance in uremic patients may arise during conservative management and during dialysis treatment [11-15]. Some data show that protein intake is reduced as GFR decreases [16] and that malnutrition is prevalent before starting renal replacement therapy. During the feasibility phase of the Modification of Diet in Renal Disease study early signs of malnutrition, such as reduction in per cent desirable body weight, body mass index and decline in urinary creatinine excretion, were observed when GFR was reduced to 30% of normal and nutritional status further deteriorated when renal function declined [16].

Protein-energy malnutrition plays a major role among the many factors that adversely affect outcome in uremic patient [17-20] and has been shown to be strongly associated with increased morbidity and mortality. In addition, growth rate is affected in pediatric uremic patients [9, 10, 21, 22]. Therefore, the care of infants and children with chronic renal failure requires special attention to nutritional factors and their implications on growth and development due to the difficulties in regaining growth lost during this period.

CPD may have various effects on the nutritional status. On the one hand, the effectiveness in controlling uremia, the glucose absorbed from the dialysate, the fewer dietary restrictions, the possibility of using peritoneal dialysis as a source of nutrients, and the better control of acidosis can lead to a greater intake of nutrients and hence improved nutritional status [23]. On the other hand, the losses of protein and amino acids [23], anorexia related to glucose loading and
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gastrointestinal disturbances, peritonitis, and possible low-grade inflammatory responses induced by particles and chemicals may have adverse consequences [24].

The most extensive evaluation of nutritional status in adult CPD patients was made with the “subjective global nutritional assessment”, using 21 variables derived from historical and clinical examination, anthropometry, and biochemistry [4]. The anthropometric parameters most frequently correlated with subjective global assessment included mid-arm muscle circumference (MAMC), weight loss, and the clinical judgment of muscle wasting and loss of subcutaneous fat [25].

It is known that prolonged administration of pharmacological amounts of glucocorticoids in man causes muscle wasting [26-28] and that kidney-transplanted patients who receive large doses of prednisone for anti-rejection therapy develop a negative nitrogen balance [29]. However, information about the prevalence of malnutrition in kidney-transplanted patients is limited. In a study of 6 patients Williams et al reported that whole-body contents of nitrogen, potassium and calcium were significantly reduced, especially during the first year of transplantation [30]. Miller et al suggested that although several indices of nutrition improve after transplantation, abnormalities in anthropometric measurements could be found in up to 38% of transplanted patients [31]. Horber et al found evidence of myopathy and decreased muscle function in 12 otherwise clinically stable kidney-transplanted patients [32]. Qureshi et al reported that transplanted patients had low muscle ASP/DNA and serum albumin concentrations within the first 2 months after transplantation, suggesting depletion of somatic and visceral protein stores [33]. However, in patients studied 11 to 17 months after transplantation, ASP/DNA was normal and the reduction in serum albumin was less; in patients studied more than 5 years after transplantation ASP/DNA and serum albumin were normal. The results suggest that depleted protein stores in the immediate post-transplant period are probably caused by the surgical trauma and high-dose corticosteroid therapy, and that full recovery takes place along with time after transplantation.

5.3 CAUSES OF MALNUTRITION IN CHRONIC RENAL FAILURE

Malnutrition in patients with CRF may have many causes, including disturbances in protein and energy metabolism, hormonal derangement, as well as low food intake because of anorexia, caused by uremic toxicity, various superimposed illnesses and psychosocial problems [34]. Although some of the catabolic effects of chronic uremia may diminish or disappear after the start of maintenance dialysis therapy, others may persist. To these are added the catabolic effects and the loss of protein and AA by the dialytic procedure, which may increase protein requirements above those of non-dialyzed uremic patients and further aggravate malnutrition [35-37].

Metabolic acidosis is common in CRF patients and is associated with a negative nitrogen balance [38], enhanced muscle protein catabolism [39] and reduced albumin synthesis [40]. Mitch et al have shown that acidosis directly stimulates muscle proteolysis by activation of the ATP-dependent ubiquitin-proteasome pathway [41] and increases oxidation of branched-chain amino acids by activation of branched-chain ketoacid dehydrogenase [42].

Several recent studies have demonstrated that inflammation is associated with malnutrition, and that inflammation per se may play a pivotal role in the development of atherosclerosis and death from ischemic heart and cerebrovascular disease [43-47].

Chronic inflammation, as evidenced by increased levels of pro-inflammatory cytokines and CRP, is common in CRF and dialysis patients [6, 7, 48, 49]. Increased plasma levels are
associated with hypoalbuminemia, malnutrition and cardiovascular disease [6, 7, 50] and with an increased total and cardiovascular mortality [37, 49, 51, 52]. It is well documented that high levels of pro-inflammatory cytokines cause muscle wasting by enhancing protein catabolism via the ubiquitin-proteosome pathway [53], by stimulating branched-chain ketoacid dehydrogenase, leading to greater oxidation of branched-chain AA [54], by reducing albumin synthesis (as part of the acute phase protein response), and inhibiting appetite [55]. It has also been observed that interleukin-1, tumor necrosis factor-alpha and endotoxins may induce net catabolism of muscle protein.

Emerging evidence suggests that there may be at least two fundamentally different types of malnutrition in patients with CRF. In the first type, malnutrition is related to low protein and energy intake, in which case malnutrition may be reversed by adequate nutritional and dialysis support. By contrast, in the second type, malnutrition is associated with inflammation and arteriosclerosis [24] which may be much more difficult to reverse with nutritional support and dialysis therapy, if the underlying co-morbidity and chronic inflammatory response cannot be treated.

5.4 ASSESSMENT OF NUTRITIONAL STATUS

Analysis of body composition by anthropometric measurements, such as absolute and relative body weight (BW), skinfold thickness and mid-arm muscle circumference (MAMC) and arm muscle circumference (AMC) are among the most used method to assess nutritional status. Relatively simple biochemical measurements, such as serum albumin, transferrin, cholesterol, creatinine, blood lymphocyte count, pseudocholinesterase, are also used as indices of nutritional status. Among these parameters, serum albumin may be the most extensively evaluated nutritional index in almost all patient populations, probably because of its easy availability and strong association with clinical outcome [19, 50, 56, 57], especially in ESRD patients.

In ESRD patients, serum albumin is a questionable marker of nutritional status [58]. Indeed albumin concentration may be decreased by a variety of non-nutritional factors such as fluid overload, urinary and peritoneal fluid albumin losses and inflammation. A slight decrease in plasma albumin levels (to just above 3 g/dL) does not always seem to reflect impaired nutritional status in CPD patients [59]. It is notable that serum albumin in general show a weak correlation to other nutritional estimates, whereas significant correlations are found between serum albumin and inflammatory markers (such as CRP). Thus, in dialysis patients serum albumin is more related to inflammation than to nutritional status [60-62].

Several nutritional parameters, other than serum albumin, have also been associated with increased risk of death, including serum transferrin, prealbumin, and IGF-1, as well as total lymphocyte counts [19, 63-66]. However, most of these studies were performed on smaller study populations and their validity, or relationship to serum albumin, remains to be determined.

Skeletal muscle, which in adults represents 40% of the body weight, is the major protein and free AA reserve of the body. The alkali-soluble protein (ASP) in relation to DNA (ASP/DNA ratio) in muscle has been used as a quantitative index for assessing the muscle protein content, since it reflects the amount of protein per cell nucleus [67]. As regards biochemistry, measurement of this ratio is comparatively simple and can be regarded as reliable and reproducible.
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5.5 AMINO ACID ABNORMALITIES IN CHRONIC RENAL FAILURE

The kidney plays a major role in the regulation of body pools of many AA trough synthesis, degradation and/or urinary excretion. The renal handling of AA has been studied by measuring renal clearance, arteriovenous differences, micropuncture, perfusion of isolated kidneys or tubules, studies of renal cortical or medullary slices, and cell culture. In a normal adult, about 70 grams of non-protein-bound AA are filtered by the kidney each day, 97% of which are actively reabsorbed in the proximal tubules [68]. Post-translationally modified amino acids, such as 1-methylhistidine and 3-methylhistidine, are quantitatively excreted in the urine and accumulate when glomerular filtration is reduced. Some amino acids (e.g., citrulline, glycine and glutamine) are metabolized in the kidney and others (e.g., serine and tyrosine) are generated there. Other abnormalities in AA metabolism may be related to several features of chronic uremia, such as low nutritional intakes of protein and energy, disturbances in intermediary metabolism, hyperinsulinemia (insulin lowers AA in plasma and muscle), metabolic acidosis (increased oxidation of branched-chain amino acids) and in dialysis patients, loss of protein and AA by the dialytic procedure.

Determination of free amino acids in plasma is widely used to assess malnutrition and other abnormalities in protein metabolism, and numerous reports concerning abnormal plasma AA concentrations in patients with chronic renal failure have been published [1]. The abnormal plasma AA pattern is characterized by low concentrations of most essential AA, including the branched chain AA valine, isoleucine and leucine, and high concentrations of some non-essential AA [69]. The plasma AA pattern, which is restricted to chronic renal failure, shows many similarities to the AA pattern seen in protein-energy malnutrition [70]. However, the clinical significance of these findings remains unknown, since plasma AA levels can be affected by many factors. The plasma AA pattern tends to reflect recent protein intake rather than body composition or protein mass. Therefore, the use of plasma AA concentrations for evaluation of body nutrition has been of limited value [71].

Intracellular free AA in muscle are the largest pools of free amino acids and are immediate precursors of protein synthesis. Determination of free AA can therefore provide useful information about the total pool of each free AA and about protein metabolism. This is particularly interesting in children since, in early life, the protein synthesis is maximally stimulated. Samples of muscle tissue are usually obtained by needle biopsy, an invasive and sometimes painful procedure, which limits its utilization in children [69, 72-74].

In untreated adult patients with CRF, a typical intracellular muscle AA pattern has been observed, with low concentrations of threonine, valine, lysine and histidine [69, 73, 75]. This pattern may be modified by nutritional intervention and by dialysis treatment [73, 75-77]. Previous studies on muscle AA in adults on CAPD are conflicting: some authors report an almost normal pattern [78], but Graziani et al [79] have reported several muscle AA abnormalities. In the latter study, significantly low muscle valine, leucine and tyrosine concentrations were found in a group of adult CAPD patients. However, samples were obtained with a full peritoneal cavity, indicating that increased glucose absorption and hyperinsulinemia could have affected the muscle AA concentrations. Indeed, it has been reported that acute hyperinsulinemia can induce a significant decline in muscle free AA, especially BCAA, tyrosine and phenylalanine [80].

Scolari et al reported that a successful kidney transplant led to complete normalization of the plasma AA profile six months after surgery [81]. Qureshi et al found that plasma EAA levels were normal after renal transplantation, apart from an elevated phenylalanine, while the plasma
level of some NEAA, among them arginine, asparagine and citrulline were elevated. The concentrations of several EAA and NEAA were also elevated in muscle [33].

Polymorphonuclear granulocytes (PMN) may offer an alternative cell model in which all major metabolic pathways are present [82-84]. PMN have been used to study intracellular metabolism of free amino acids in relation to nutritional factors and in some pathological states [85, 86]. Abnormal AA levels in PMN, which resemble those described in muscle in CRF, have mainly been reported in uremic children [87-89]. However, no studies are reported in which the intracellular AA pools in PMN and muscle were taken from a given subject at the same time.

Red blood cells (RBC) contain a large proportion of the free AA in blood, the intraerythrocyte pool of free AA being actively involved in the inter-organ transport of AA [90, 91]. The RBC posses no nuclei, mitochondria, ribosomes or other organelles and therefore cannot synthesize protein. However, numerous AA transport systems have been found in human RBC similar to those in other cells and the AA profile in RBC has been reported as abnormal in patients with CRF [92, 93]. Although RBC contain a large proportion of the free AA in blood and are actively involved in the interorgan transport of AA, only a few studies have compared the RBC AA profile to plasma AA in metabolic disorders [94-97]. It is far from clear to what extent AA changes in RBC reflect changes in nucleated cells, such as muscle and PMN. There are only two studies in which comparisons have been made between intracellular AA in muscle and RBC [92, 98] and no studies comparing AA in PMN to muscle or RBC concentrations.

5.6 NUTRITIONAL PROBLEMS IN CHILDREN WITH CHRONIC RENAL FAILURE

Children with renal failure grow poorly; the ultimate height of those with congenital renal anomalies and early renal insufficiency being particularly affected [99-102]. Factors during the first year of life contribute most significantly to height loss, since growth velocity in later childhood seems to stabilize at a level close to normal for age [103-105]. Despite remarkable advances in dialysis and transplantation for the treatment of end-stage renal disease in children, these therapeutic modalities are not associated with improved or catch-up growth [99-102, 106]. Moreover, it remains unclear how nutrient requirements for growth are altered in uremic children, making it difficult to predict the effectiveness of various nutritional regimens, particularly in infants [107].

Inadequate energy intake has been implicated as the major cause of short stature in uremic children [10, 108-111]. The two commonest causes of energy deficiency are anorexia and the effect of inter-current infections in accentuating the loss of appetite and increasing the metabolic rate. Protein depletion and negative nitrogen balance are not uncommon, partly because of anorexia, but at times due to the practice of reducing dietary protein intake in patients who have moderately elevated serum urea nitrogen concentrations [10, 112-114]. When either calorie or protein intake is inadequate, growth failure commonly occurs in normal infants and children as well as those with renal failure. Nutritional status in children is assessed using information on dietary intake and anthropometric measurements [21, 108, 110, 115]. The most useful clinical method for monitoring intake is the 3 to 4 day prospective dietary diaries. When the reported intake falls below 80% of the recommended dietary allowance (RDA) for energy or specific nutrients, one should check to see whether the observations are correct and if the reduced intake is transient or sustained. This information, combined with that on changes in body weight and other anthropometric, hematological or biochemical measurements usually permits a sound assessment of nutritional status. In children, the evaluation of various
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anthropometric parameters includes the measurement of length, bodyweight, body mass index (BMI), triceps skinfold thickness (TSF), mid-arm circumference (MAC) and MAMC. The evaluation of nutritional status in chronic renal failure is of paramount importance [115]. Values from patients are compared with the corresponding control values from the table using the standard deviation score (SDS) [116-118]. This score allows for the expression of values in terms of standard deviation above (positive value) or below (negative value) the median (50th percentile) value for normal controls. SDS = Vp - Vn / SDn, where Vp is the value for an individual patient, Vn is value for normal controls, and SDn is the standard deviation for normal controls. Values of patients are compared also to percentiles for normal girls and boys of the same age [116-118].

Analysis of anthropometric and biochemical parameters have shown deterioration of nutritional status during advanced renal disease in children [21, 119, 120] but it has been reported by Orejas et al. as normal in 15 children with moderate CRF [121]. Recently, Norman et al [122] found that all anthropometric indices and mean total energy intake deteriorated with worsening of renal function.

In children less than ten years old on CAPD, the SDS evaluated for chronological and height-age have been reported as negative for weight, TSF, and MAC but weight, MAC, MAMC, and TSF SDS were not reduced in those at least ten years old or more, when compared to normal children of the same height age [21].

MAC and TSF at CAPD/CCPD initiation and after one year in children have been reported in the CAPD Register's pediatric patient population in the United States [123]. They found that only a few children were near the median value for MAC and TSF at start of treatment, but the distribution showed hardly any change after one year of PD.

In another study height, weight, TSF, subscapular skin fold thickness, MAC, MAMC, arm muscle area (AMA), and arm fat area have been evaluated in two groups of children on CAPD [119]. The first group comprised 20 children who had been on CAPD for periods ranging from 6 to 12 months and the second group comprised 15 who had been on CPD for more than 24 months. The percentage of patients in the lowest percentiles (<5th, 5th and 15th) for MAC, MAMC, AMA, and arm fat area were lower in the group of children on CPD for more than 24 months than in those on CPD for 6 to 12 months. These data, although the limited number of patients does not permit to draw any conclusions, do not accord with the finding of the progression of malnutrition reported in adult PD patients[124] and show an improvement not only of fat store indicators, but also of muscle mass indices.

There are few studies that have been done on nutritional status in transplanted children. Ghio et al reported, in a prospective study of intentional cessation of methyl-prednisolone 6 months after transplantation in 29 pediatric renal transplant recipients, which linear growth significantly improved after stopping this drug [125]. The mean catch-up growths were for pre-pubertal children 1.38 height SDS and for pubertal children 1.6 height SDS. Bone age did not increase more rapidly than chronological age, and the weight/height index (WHI) also improved. Similarly, Offner et al showed that the immunosuppressive regimen of cyclosporine A and low-dose prednisolone was followed by significantly better growth rates than azathioprine plus high-dose prednisolone [106]. Thus, when graft survival is good, stopping corticosteroids corrects the major handicap of children with irreversible uremia, poor linear growth, and improves the WHI.

There is one study by Delaporte et al in which muscle ASP content was determined in children on maintenance HD [126]. The authors found marked protein depletion and suggested that it resulted chiefly from an inadequate protein intake.
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Plasma and muscle intracellular AA abnormalities similar to those in adult patients have also been reported in children with CRF [72, 74, 127]. Broyer et al found lower levels in muscle cells of valine and alanine and higher levels of glutamine in 20 children with CRF than in healthy children [72]. Delaporte et al observed that muscle pools of essential and non-essential AA were increased in eight children with CRF [74].

Intracellular AA have also been measured in PMN of uremic children [88, 128] and of children on CAPD and HD [129, 130]. Although comparisons of results are hard to make, due to differences in laboratory techniques and ways of expressing the data, the trend is the same: most EAA in the PMN (leucine, isoleucine, valine, phenylalanine, methionine, lysine) are significantly lower than in healthy subjects.
6 AIMS OF THE THESIS

1. To investigate plasma and muscle AA concentrations and anthropometric, biochemical and muscle nutritional parameters in children with ESRD.

2. To investigate plasma and muscle AA concentrations and anthropometric, biochemical and muscle nutritional parameters in children on CAPD.

3. To investigate plasma and muscle AA concentrations and anthropometric, biochemical and muscle nutritional parameters in kidney-transplanted children.

4. To evaluate if some of the free AA measured in PMN granulocytes suspensions could originate from proteolysis other than from the physiological metabolic pathways and transport systems.

5. To explore relationships between AA concentrations in plasma, RBC, PMN and muscle obtained at the same time and from the same individual in normal and uremic children in order to evaluate to what extent they reflect each other and how these concentrations are influenced by uremia.


7 SUBJECTS

The Tanner stages of sexual development in patient and control groups were:

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</table>

Most children in both patient and control groups were prepubertal. Completed development was present in a small number of patients.

7.1 CONTROL GROUPS

The controls comprised 22 children (3 girls and 19 boys) with a mean age of 8.0±3.2 years (range 2.6-13 years), who underwent elective surgery for hernia correction or uretero-pelvic junction stenosis. They were all in good health, except for the minor disabilities that required elective surgery, and had normal renal function (GFR >100 ml/min/1.73 m² evaluated by Schwartz formula) and no sign of metabolic or renal disease.

7.2 PATIENTS

The patients studied were children with ESRD who were just going to start dialysis (study I and V), who had been on CAPD for 2-31 months (study II and V), or who were kidney-transplanted children (study III). None of them had diabetes mellitus, systemic disease or chronic infection. They were treated with diuretics, antihypertensive drugs, calcium carbonate, Shohl's solution, vitamin D analogues and supplements of water-soluble vitamins (B vitamins and ascorbic acid). None of the children were treated with androgenic steroids, erythropoietin or growth hormone.
Methods

8 METHODS

8.1 SAMPLING

Venous blood and muscle samples for AA analysis were obtained simultaneously after an overnight fast, in patients as well as in controls. The muscle biopsy specimens were taken from the rectus abdominis muscle during peritoneal catheter insertion in children with ESRD, during surgery for hernia correction or catheter replacement in CAPD children, during catheter removal in transplanted children and during elective surgery for hernia or uretero-pelvic junction stenosis correction in controls.

8.2 ASSESSMENT OF NUTRITIONAL STATUS

Height, body weight, TSF, mid-arm circumference (MAC) were recorded. Skin fold thickness was measured with a Harpenden skin fold caliper (British Indicators Ltd., St Albans, Herts, UK).

The mid-arm muscle circumference (MAMC) was derived from MAC and TSF as follows: MAMC = MAC - π x TSF. The weight for height index (WHI) is calculated as: observed weight / ideal weight for the height.

The body mass index (BMI) was derived from the height and weight as follows: BMI = body weight in kg / (height in m²).

Values from patients have been compared with the corresponding control values from standard tables using the standard deviation score (SDS) [116-118]. Values of patients have been compared also to percentiles for normal girls and boys of the same age [116-118].

8.3 ASSESSMENT OF DIETARY INTAKES

The protein and energy intakes were estimated from dietary recall records that were taken over a three-day period by a dietitian. The protein intake was also estimated from the 24 hours urea excretion according to Maroni & Mitch [131].

8.4 ANALYTICAL PROCEDURES

Serum electrolytes, urea, creatinine, bicarbonate, total protein and albumin levels were evaluated by routine methods. A heparinized blood sample was centrifuged at 4000 rpm for 10 minutes at +4°C to obtain plasma that was then deproteinized with sulfosalicylic acid (SSA) and centrifuged. The supernatant was stored at -70°C until AA analyses were performed.

The muscle specimen was carefully and rapidly dissected to remove visible fat and connective tissue. The specimen was weighed repeatedly on a Cahn electromagnetic balance and the initial wet weight was calculated by extrapolating the weight curve to zero time. Immediately afterwards, each specimen was frozen in liquid nitrogen. The frozen material was placed in sodium-free glass tubes that had previously been rinsed with nitric acid (1mol/liter), freeze-dried and re-weighted. The dried, fat-extracted specimen was powdered in an agate mortar and carefully dissected under a magnifying glass to remove remaining flakes of connective tissue. About 2.5 mg of the powder for chloride analysis was dried at 80°C for 30
minutes and re-weighted. This procedure reduced the water content by approximately 5%. Chloride was extracted using 1M nitric acid (1 mol/liter) and determined by electrometric titration, as described earlier [132]. The true dry weight of the remaining powder was calculated as 95% of the observed weight after powdering at room temperature and humidity. Alkali-soluble protein (ASP), which is, non-collagen protein, and DNA were determined in 3 to 4 mg of the powder after precipitation with 4% SSA. The precipitate was incubated for one hour in KOH (0.3 mol/liter) and ASP was determined in an aliquot using the Lowry method [133]. DNA analysis of the residues was based on the Schmidt and Tanhauzer technique [134]. For DNA extraction the precipitate was hydrolyzed by adding 0.25 ml perchloric acid (1 mol/liter) and incubated for one hour at 70° C. The tube was weighed again to obtain a dilution volume for DNA. DNA was estimated by the diphenylamide reaction [135]. The calculations of extra- and intracellular water and intracellular AA concentrations in muscle, which are based on the chloride method, have been described earlier by Bergström et al [132]. Total, extra and intracellular water, fat, DNA and ASP are expressed per kg of fat-free solids (FFS). The ASP/DNA ratio is presented as an indicator of the amount of cell protein per cell unit.

For measurement of RBC AA, white cells and platelets were carefully removed and 1 g of packed red cells was rapidly hemolysed by adding 1.0 ml of 1% Saponin (Sigma, St. Louis, MO, U.S.A.). The sample was then extracted with 0.3 ml 50% SSA, mixed and centrifuged at 1700 g for 20 minutes at 4° C. The supernatant was filtered using 0.45 m HA filter (Millipore) and frozen at -70° C pending analyzed. We calculated the intracellular AA concentrations in RBC by taking the water content as 66% of RBC weight in all samples, as described by Fligel-Link et al [136].

The polymorphonuclear leukocytes were separated from blood by gradient centrifugation on Mono-Poly Resolving Medium. After separation, the cells were washed in Ca++ and Mg++ free Hank’s balanced salt solution, and the red cells lysed by adding distilled water. After centrifugation, the pellet was suspended in 0.5 ml of 0.16 M potassium chloride. Apart from centrifugation, all subsequent procedures were performed with the sample kept in crushed ice. A combination of fresh protease inhibitors was added to the M.PRM, Hank’s solution, distilled water and potassium chloride. The mixture of protease inhibitors consisted of: leupeptin (1 M/L), pepstatin (1 M/L), sulphonyl fluoride Phenylmethane-sulphonyl fluoride (200 M/L) and ethylenediamide tetraacetate (EDTA) (100 M/L). The cell suspension was lysed by three cycles of freezing and thawing. The cells were frozen at -80° for 15 min. and thawed at 4° for 60 min. The suspension was deproteinized by adding SSA (7 mg of SSA per ml of suspension). DNA analysis was based on the Schmidt Thannhauser technique [134]. The intracellular water content (ICW) in PMN correlated with the DNA content that is, ICW µL=1.93+0.055 DNA µg (P=0.0001), as described by Metcalf et al [87]. This relationship was used to calculate cell water since minimal differences were reported in direct measurements of PMN water for several uremic patients and controls.

Free AA were analyzed in the supernatants after SSA acid precipitation by reversed phase HPLC (Beckman Instruments, Fullerton, California, USA), using precolumn derivatization with orthophalldialdehyde and an internal standard (homoserine) as described by Qureshi et al. [137], Taurine, alanine, and 1-methylhistidine and 3-methylhistidine co-eluted in the muscle and in some of the plasma samples. The combined concentrations of these pairs of were used to calculate the sum of AA.
9 STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation (SD). A p-value <0.05 was considered to be significant. Differences between the groups were in general assessed by analysis of variance. When the analysis of variance was significant, an appropriate test was used to compare the differences between the control and patient groups. Comparisons between two groups of continuous variables were made by the Student’s t-test or with the Mann-Whitney test for variables, which were not normally distributed. Spearman’s rank correlation was used to calculate the relation between two variables.

The protocol of the studies was approved by the Ethics Committee of G. Gaslini Institute, Genova, Italy. Parents’ informed consent was obtained.
10 RESULTS AND DISCUSSION

10.1 STUDY I: PATIENTS IN THE PRE-DIALYSIS STUDY

15 children with CRF (nine girls and six boys) with a mean age of 8.9 ±4.6 years (range 1.3-15.5 years) were studied at the time of PD catheter insertion. They all had a GFR < 5 ml/min/1.73 m². They were prescribed a diet with a protein content which was 75% and an energy content which was 100% of the RDA for statural age. The control groups comprised 10 children (two girls and eight boys) with a mean age of 8.8±3.1 years (range 2.7-13 years) who had no diet restrictions.

10.1.1 Results

Height and weight (Table II, Study I) were lower in the uremic children than in controls but the weight for height index was not significantly different from normal. The subscapular and biceps skinfold thickness and the arm muscle circumferences in the uremic children varied from the 5th to the >75th percentiles for height and age (Fig. 1).

Serum proteins (total protein, albumin, transferrin and pseudocholinesterase), blood lymphocyte count; serum bicarbonate, muscle ASP and ASP/DNA ratio were not significantly different in the uremic children compared to the controls (Table II, Study I).

Taken together the anthropometric and biochemical data indicate that the uremic children were not suffering from protein-energy malnutrition. Only two children had slight acidosis (s-bicarbonate 20.0 and 20.6 mmol/l, respectively).

Total water, extracellular water and intracellular water in muscle were also normal in the uremic children (Table II, Study I).

Plasma and muscle intracellular amino acid concentrations in the children with ESRD and in the controls are presented in Table III and IV, Study I. The concentrations of amino acids in percentage of controls are given in Fig. 2 and 3.
Results and discussion

The plasma concentrations of serine and most of the essential AA (Table III, Study I and Fig. 2) were significantly lower in the uremic children than in the controls. The plasma levels of citrulline and 1-methyl-histidine and 3-methyl-histidine were significantly increased. The intracellular concentrations of valine and isoleucine in muscle were significantly lower than in the controls (Table IV, Study I and Fig. 3).

Among the non-essential AA only 1-methyl-histidine, 3-methyl-histidine and glycine showed increased intracellular concentrations.

The intracellular/extracellular gradient for the AA in the uremic children was not significantly different from that in the controls.

The ratio of tyrosine to phenylalanine was significantly decreased in plasma whereas the ratio of glycine to serine was significantly increased (Table III, Study I). In muscle, the ratio of valine to glycine was significantly decreased whereas the ratio of glycine to serine was significantly increased (Table IV, Study I)

10.1.2 Discussion
Results and discussion

![Graph showing relationship of ASP/DNA ratio in muscle to age in 15 CRF children and in 10 controls. The regression equation is $y = 270.8 + 1.473 x; r = 0.75; P < 0.001$.]

The methods for processing muscle samples, which were used in this study, make it possible to determine several different constituents in one muscle specimen. Various tissue components are usually evaluated in relation to one another and some of them serve as references and represent the cell mass or the number of cell units in the sample. Hence, by performing the analyses on aliquots from the same muscle specimen, sampling variability can be minimized. The ability to perform many different determinations in one small muscle sample is of special value in studies of small children.

There was a linear correlation between the ASP/DNA ratio in muscle and age, and no difference between the controls and the uremic children (Fig. 4).

Thus, the intracellular protein content "per unit cell" increases with the maturation of the individual, presumably because of an age-dependent increase in cell size. The normal ASP/DNA ratio for age in the children with CRF is further evidence that they did not suffer from protein malnutrition.

Most studies in adult patients with CRF have shown that the total and extracellular water levels in muscle are increased [132, 138]. It is therefore of interest that the children with CRF in this study had no increase in total and extracellular water although they were in such an advanced stage of renal failure that dialysis had to be started very soon. It is obvious that even in severely uremic children tissue overhydration can be prevented by careful conservative therapy.

In the present study, muscle samples were taken from the rectus abdominis muscle and plasma and muscle AA were determined by HPLC, whereas in earlier studies of healthy controls and uremic patients samples were taken from the quadriceps femoris muscle and the AA were determined by ion exchange chromatography. In spite of these differences in methodology, the plasma and muscle levels of most AA in the controls were similar to those reported previously [75]. Compared to the controls the uremic patients had several significant AA abnormalities in plasma and muscle, although they had no clinical, anthropometric or laboratory signs of protein malnutrition.

In conclusion, non-dialyzed children with end-stage chronic renal failure exhibit AA abnormalities in plasma and muscle that may be caused by uremia per se or by the loss of metabolic and excretory functions in the kidneys. Despite these abnormalities, it is possible to maintain a satisfactory nutritional status in such patients, as demonstrated by our results.

10.2 STUDY II: PATIENTS ON CAPD
Results and discussion

10 children (nine males, one female) with a mean age of 6.4 ± 5.6 years (range 1.2-17 years) were studied. The mean time of treatment was 17.8 ± 13.5 months (range 2-31 months) at the moment of sample collection. CAPD was performed with 4 daily exchanges (40-45 ml/kg body weight) of dialysis fluid (Viaflex, Baxter-Travenol, Rome, Italy) containing (in mmol/liter) sodium 137, calcium 1.75, magnesium 0.5, chloride 101, lactate 40 and anhydrous dextrose either 13.6, 22.7 or 38.6 grams/liter. The dextrose concentration varied depending on the patient's weight and blood pressure. They were prescribed a diet with a protein content that was 110% and an energy content that was 100% of the RDA for chronological age. The control groups comprised 22 children (three girls and 19 boys) with a mean age of 8.0 ± 3.2 years (range 2.6-13 years).

10.2.1 Results

Height and weight evaluated as SDS were reduced in the CAPD children (Tab. 1, Study II) and the BMI varied from the 3rd to the 75th percentile for chronological age (Fig. 5) [116].

In the uremic children, serum levels of total proteins and albumin were reduced; serum transferrin, pseudo-cholinesterase, serum bicarbonate and muscle ASP/DNA ratio were not significantly different when compared to controls (Tab 2 and 3, Study II). The ASP/DNA ratio was significantly correlated to age (Fig. 8).

Total and intracellular muscle water were increased in the CAPD children (Tab 3, Study II).

Plasma and muscle intracellular amino acid concentrations in the patients and in the controls are presented in Table 4 and 5, Study II.

The plasma concentration of most of the essential AA and the valine/glycine and tyrosine/phenylalanine ratios were significantly lower in the patients than in the controls (Tab. 4, Study II and Fig. 6).

The plasma levels of citrulline, glycine, 1-Methylhistidine + 3-Methylhistidine and taurine + alanine were significantly increased as the glycine/serine ratio (Table 4, Study II and Fig. 7). The intracellular muscle concentration of leucine and valine were significantly lower than in the controls (Table 5, Study II and Fig. 6).
Results and discussion

Fig. 8 Relationship between ASP/DNA ratio in muscle and age in 10 children on CAPD (□) and in 22 control children (O). Regression equation for the CAPD children: \( y = 302.49 + 0.77 \times (r=0.71; p<0.05) \). Regression equation for the control children \( y = 330.7 + 0.944 \times (r=0.47; p<0.05) \).

Fig. 9. Mid-arm muscle circumference (A) and skinfold thickness (B) in 11 transplanted children, expressed as percentiles in comparison to material from the literature. Symbols are (a) triceps; (b) subscapular.

Among the NEAA, aspartic and glutamic acids showed increased intracellular concentrations while taurine+alanine was decreased when compared to controls (Tab 5, Study II and Fig.7).

The phenylalanine intracellular to extracellular gradient was reduced (1.74 ± 0.48 versus 2.24 ± 0.7, p<0.05) and the lysine gradient increased (17.5 ± 7.2 versus 12.7 ± 5.0, p<0.05) in the CAPD children compared to the controls. No other significant intracellular to extracellular gradient differences were observed between groups.

10.2.2 Discussion

The lowered total protein and albumin plasma levels may be related to the continuous peritoneal losses of plasma proteins. Broyer et al. have shown a correlation \( r=0.62, p<0.05 \) between plasma albumin and peritoneal protein loss in children on CAPD [9]. Another possible explanation is the lower energy intake, in spite of adequate protein intake, in this group of children.

CAPD children showed a reduction of the muscle ASP/DNA ratio that was marginally significant. In Study I paper we have shown a linear correlation between the muscle ASP/DNA ratio and age and no difference between patients and controls. The regression line in Figure 8,
Results and discussion

Fig. 10 Essential amino-acid concentrations in plasma (A) and muscle (B) of 11 transplanted children and children with ESRF (Study I). The data (mean±SE) are given as percentages of the normal concentrations in the age-matched control group. a, P=0.05, b, P=0.01, c, P=0.001 Tpx and ESRF versus Controls, d, P=0.05, e, P=0.01, f, P=0.001 Tpx and versus ESRF.

Fig. 11 Non-essential amino-acid concentrations in plasma (A) and muscle (B) of 11 transplanted children and children with ESRF (Study I). The data (mean±SE) are given as percentages of the normal concentrations in the age-matched control group. a, P=0.05, b, P=0.01, c, P=0.001 Tpx and ESRF versus Controls, d, P=0.05, e, P=0.01, f, P=0.001 Tpx and versus ESRF.

demonstrates that the relationship between age and muscle ASP/DNA ratio in CAPD children lies below that for the controls, suggesting that the ASP/DNA ratio is still age-related but at a lower level in this group of patients; the slope of regression lines was not significantly different.

The children on CAPD in this study showed an increase in total and intracellular water not observed in Study I paper in children with pre terminal renal failure and transplanted children Study III paper. Lindholm et al [139] have also found increased intracellular water in adults CAPD patients and Bergström et al [138] have reported the same findings in hemodialyzed and ESRD patients. The extracellular water in CAPD children was normal, indicating no extracellular overhydration in contrast to the findings reported in adults on CAPD [139] [124].

The finding of significantly reduced plasma levels of methionine, lysine, leucine, isoleucine, tyrosine and valine with normal concentration of threonine, phenylalanine and histidine are in
Results and discussion

general agreement with several previous studies in adults and children on CAPD [9, 76, 78, 79, 140, 141].

The low plasma AA levels may be the effect of continuous AA losses into peritoneal dialysate and perhaps also an effect of hyperinsulinemia related to uremia [80]. The glucose load may have stimulated insulin release, although this might be of little, if any, importance 12-14 hours after the last exchange.

The present results are very similar to those reported in adults on CAPD by Lindholm et al [78] under the same study conditions (empty abdomen and post absorptive state), except that they did not find reduced muscle leucine and valine levels.

The finding of growth retardation, reduced plasma levels of most essential AA and of low muscle valine and leucine concentrations suggests that they may potentially benefit from AA supplementation. The clinical results from the application of AA solutions in adults and children on CAPD are conflicting [129, 142-145]. According to recent reports, AA containing dialysate is in fact able to improve protein malnutrition and nitrogen balance in adults on low protein diets receiving AA containing dialysate [146] and in children on Automatized Peritoneal Dialysis [147, 148].

10.3 Study III: Kidney-Transplanted Children

13 renal transplant children (six girls and seven boys) with a mean age of 11.6±4.6 years (range 2.5-18.3 years) were studied. All patients received corticosteroids and other immunosuppressive drugs (Table II, Study III). The mean time from transplantation to the study time was 97±14 days (range 72-114). All patients, except four, had one rejection episode at least 30 days before the study and the rejection was treated with 3 to 5 pulses of methylprednisolone. They were prescribed a diet with a protein content that was 106% and an energy content that was 100% of the RDA. The control groups comprised: 10 children (2 girls and 8 boys) with a mean age of 8.8±3.1 years (range 2.7-13 years).

10.3.1 Results

Height (Table I, Study III) was lower in the transplanted children than in controls but the weight for height index was not significantly different from normal. The subscapular and biceps skin fold thickness and the arm muscle circumferences in the transplanted children varied from the 13th to the > 97th percentiles for height and age (Fig. 9).
Results and discussion

Serum proteins (total protein, albumin, transferrin and pseudocholinesterase), blood lymphocyte count; serum bicarbonate, muscle ASP and ASP/DNA ratio were not significantly different in the transplanted children compared to the controls (Table I and III, Study III). Taken together the anthropometric and biochemical data indicate that the transplanted children were not suffering from protein-energy malnutrition.

Total water, extracellular water and intracellular water in muscle were also normal in the transplanted children (Table III, Study III).

Plasma and muscle intracellular amino acid concentrations in the transplanted children and in the controls are presented in Table IV and V, Study III. The concentrations of amino acids in percentage of controls are given in Fig. 10 and 11.

The plasma concentrations of the essential AA were comparable in both groups, except for leucine, which was significantly reduced (Table IV, Study III and Fig. 10). The plasma levels of citrulline, ornithine and alanine+taurine were significantly increased (Table IV, Study III and Fig. 11).

The intracellular concentrations lysine in muscle was significantly lower than in the controls (Table V, Study III and Fig. 10). Among the non-essential AA only glycine showed increased intracellular concentrations (Table V, Study III and Fig. 11). The ratio of glycine to serine was significantly increased in plasma (Table IV, Study III) and muscle (Table V, Study III).

10.3.2 Discussion

It has been reported that successful renal transplantation leads to complete normalization of the plasma AA profile six months after surgery [81]. However, the finding of normal AA plasma values does not mean complete tissue recovery, taking into account the different factors that can influence protein metabolism, especially the steroid immunosuppressive therapy [26, 27].

The anthropometric measurements, the source of muscle specimens and the methods to processing muscle and blood samples applied in this study are the same as the ones used in our previous paper (Study I), which makes it possible to compare two different clinical conditions: ESRD and renal transplantation.
Results and discussion

The anthropometric data, except for height SDS, and the plasma nutritional biochemistry were not different from those of normal children, indicating that the nutritional status was well maintained in spite of these children having a previous history of uremia (range 13-156 months) and were receiving prednisone (0.42 ± 0.16 mg/kg BW/day) according to the immunosuppression protocol. We have previously demonstrated that there is a correlation between ASP/DNA ratio and age in normal and uremic children (Study I). Such a relationship was also observed in the present study, transplanted and control children showing overlapping values (Fig. 12).

These findings indicate that the diet followed by these patients, about 125% and 103% of the RDA respectively for protein and energy, supplied an adequate amount of AA and energy to preserve the nutritional status.

Our finding of a high significant correlation (r= 0.864) between the prednisone dose (mg/kg BW/day) and the ASP/DNA ratio in muscle (Fig 13) can be considered a further confirmation that corticosteroid influences the muscle protein content, at least to some extent, although the ASP/DNA ratio, ASP and DNA content of muscle were not significantly reduced in comparison with controls.

Our patients showed a highly significant correlation (r=0.859) between plasma alanine + taurine and prednisone dose. Alanine + taurine values are mostly due to alanine, as taurine values in plasma are low (39±7 umol/l in 5 patients and 54 ±13 umol/l in 10 controls). Such observation seems to confirm the role of prednisone in increasing plasma alanine levels. The elevation of citrulline and ornithine levels in plasma of transplanted children could be related to enhanced activity of the urea cycle enzymes. In fact, we have observed a moderate increase of the plasma urea nitrogen levels in relation to the prednisone dose used (Fig 13).

To our knowledge there is no data concerning muscle AA composition in renal transplanted children. The analysis of muscle tissue can give important information on the metabolic effects of a normally functioning kidney graft and immunosuppression therapy in previously uremic children.

The muscle AA concentration in transplanted children was comparable to controls, except for a reduction in lysine and an increase in glycine. Evidently, slight changes in plasma AA do not reflect alterations in intracellular AA levels.

We have shown in Study 1 paper that AA pattern in uremic non-dialysed children with a satisfactory nutritional status presented several abnormalities in the form of decreased BCAA and increased glycine, 1-methyl-histidine, 3-methyl-histidine in muscle and decreased BCAA, tyrosine, serine and increased citrulline, 1-3-methyl-histidine in plasma. It's remarkable to observe that just three months after transplantation it is possible to evidenciate an almost normal muscle AA pattern while there are plasma AA abnormalities, which can be related to the immunosuppression therapy. In conclusion, transplanted children with normal renal function and good nutritional status (as assessed by anthropometric, biochemical and muscle composition parameters) show an almost normal muscle free AA profile and a plasma AA pattern that can be mostly related to the prednisone therapy. This can be considered a further expression of the fundamental role of uremia itself in the genesis of the plasma and muscle AA abnormalities observed in chronic renal failure and dialysed patients.
Results and discussion

10.4 STUDY IV: PROTEOLYTIC ACTIVITY AND FREE AA CONC IN PMN

We measured PMN protease activities and AA concentrations in cell suspensions, from the same blood sample collected in 10 healthy volunteers after an overnight fast.

10.4.1 Results

Table I, Study IV lists the elution order of AA OPA derivatives, the retention times and the fluorescence intensities compared with those of homoserine, which was used as an internal standard. The mean variation in retention times for amino acids in standard plasma and PMN suspension was ± 0.2-2 % (n = 18). Retention times as the mean values after five runs under similar conditions are reported in Table 1, Study IV. Orthogonal regression analysis of the area of each amino acid peak versus concentration over the range 2.5 to 125 pmol per 20 μL injected onto the column gave correlation coefficients ranging from 0.953 to 0.966 for the 23 amino acids analyzed, with a coefficient of variation for the peak areas in the range 1-3 %. The absolute analytical recoveries calculated with the pure AA standard (10 μmol/L) added to cell suspension before deproteinization are also shown in Table 1, Study IV. It was not possible to calculate the analytical recovery for taurine and alanine because the presence of very high levels of taurine and (probably) of hypotaurine and cysteinsulfonic acid in cell suspensions do not allow the separation of the two peaks. The detection limit of the assay was 10 pmol of each AA injected onto the column, at a signal-to-noise ratio of two; this corresponds to a concentration of 2 μmol/L in the PMN suspension.

The proteolytic activity in the PMN suspension from samples processed with and without the anti-proteolytic mixture is shown in Fig. 14.
### Results and discussion

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<tr>
<td>EAA</td>
<td>936.7 (342-3)</td>
<td>13858 (4486)</td>
</tr>
<tr>
<td>NEAA</td>
<td>2966 (626)</td>
<td>43943 (6341)</td>
</tr>
<tr>
<td>BCAA</td>
<td>466 (161)</td>
<td>6890 (2072)</td>
</tr>
</tbody>
</table>

** = \( P < 0.001 \); * = \( P < 0.01 \). AA = Amino acid; ICW = intracellular water; EAA = essential amino acids; NEAA = non essential amino acids; BCAA = branched chain amino acids.

Protease activity, assessed by the Azocoll method, was detectable in samples processed as usual, whereas it was almost completely inhibited in the samples with the addition of the anti-proteolytic cocktails.

No peak that could interfere with AA analysis was detected in a chromatographic analysis of the anti-proteolytic mixture.

It has been reported that the intracellular water (ICW) content correlates with the DNA content according to ICW (µL) = 1.93±0.005 DNA (µg) (\( P=0.0001 \)) [87]. This relationship has been used to calculate cell water. Concentrations of PMN amino acids are expressed both as nmol/mg DNA and as derived nmol/mL ICW in order to compare our results with previous data from the literature. PMN amino acid concentrations from samples processed with and without the addition of the anti-proteolytic substances are shown in Table 1.

Concentrations of threonine, methionine, valine, phenylalanine, isoleucine, leucine and tyrosine were 8 to 15 times lower in samples treated with anti-proteolytics. Concentrations of arginine and lysine in treated samples were 22 to 32 times lower.

Possible leakage of AA from the leucocytes during treatment with anti-proteolytic agents was investigated. Chromatographic analysis of the washing buffers showed negligible concentrations of free AA.
Results and discussion

10.4.2 Discussion

PMN contain a variety of proteolytic enzymes, including elastase, cathepsins G, B and D and collagenase [149]. An important function of proteases is the degradation of endogenous protein within cells, particularly in the lysosomes [150-153].

Separating, washing and lysing of cells may represent potential activatory stimuli of proteases and an activated proteolytic activity could in turn result in the release of a large amount of AA from intracellular protein. The use of protease inhibitors throughout the procedure of preparation of PMN can lead to a reliable analysis of free AA in PMN, overcoming the problem of AA release from proteins. We decided to use a cocktail of leupeptin and phenylmethane-sulphonyl fluoride for serine and thiol proteases like elastase, cathepsin G, cathepsin B and pepstatin for acid protease such as cathepsin D and EDTA for metalloproteases such as collagenase. This combination of protease inhibitors can effectively eliminate almost all protease activity and gives an effective protection against proteolysis (Fig. 14).

PMN AA from samples processed with and without the anti-proteolytic cocktail show important differences (Table 1).

PMN AA levels from samples not treated with the anti-proteolytic agents, expressed in nmol/ml ICW are partially comparable with previous reports by McMenammy [154], Wells [155] and Soupart [156]. A very high intracellular/extracellular gradient for most of the AAs has been observed, confirming the previous reports of intracellular AA concentrations 4-60 times higher than in the surrounding plasma. The high levels of serine, glycine and BCAA observed in the untreated samples could be related to the activation of elastase. Indeed this enzyme hydrolyzes peptide bonds to the C terminal side of AA containing uncharged non-aromatic side chains such as alanine, valine, isoleucine, glycine and serine. PMN AA concentrations from samples treated with the anti-proteolytic cocktail show comparable values with those of Metcalf et al [87] although these workers do not report the use of anti-proteolytic agents.

The precision of AA measurements in PMN is similar to that found in other tissues, e.g. muscle in Study I. This observation supports the hypothesis that the variability is not affected by the anti-proteolytic treatment but probably related to inter-individual variation.

We conclude that the use of protease inhibitors throughout the sample preparation procedure is necessary for reliable determination of free AA in PMN.

10.5 STUDY V: FREE AA CONCENTRATIONS SIMULTANEOUSLY COLLECTED IN PLASMA RBC, PMN AND MUSCLE IN NORMAL AND UREMIC CHILDREN

12 uremic children (five female, seven males) with a mean of 9.4±4.8 years (range 1.7-17.7 years) were studied. Eight of the children had ESRD before start of dialysis and four were on CAPD. The ESRD children were prescribed a diet with a protein content, which was 75%, and energy content, which was 100% of the RDA for statural age. The CAPD children were prescribed a diet with a protein an energy content that was 100 % of the RDA for statural age. The protein and energy intakes were estimated from the dietary recall records that were taken over a three-day period by a dietitian. The control group comprised 13 children (3 girls) having a mean age of 9.1±3.9 (range 2.8-16.5) years.
Results and discussion

<table>
<thead>
<tr>
<th></th>
<th>RBC/PLASMA</th>
<th>PMN/PLASMA</th>
<th>MUSCLE/PLASMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Patients</td>
<td>Controls</td>
</tr>
<tr>
<td><strong>ESSENTIAL AA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>1.6±0.4</td>
<td>1.5±0.5</td>
<td>4.6±2.9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.8±0.3</td>
<td>0.7±0.4</td>
<td>2.1±1.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.7±0.3</td>
<td>1.0±0.7</td>
<td>2.3±1.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.3±0.5</td>
<td>1.5±0.9</td>
<td>1.6±1.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.7±0.7</td>
<td>0.3±0.2</td>
<td>2.3±1.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.7±0.2</td>
<td>0.8±0.3</td>
<td>2.6±1.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.5±0.2</td>
<td>1.3±0.5</td>
<td>2.4±1.4</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.6±1.4</td>
<td>1.6±0.5</td>
<td>3.2±1.8</td>
</tr>
<tr>
<td>Valine</td>
<td>0.8±0.3</td>
<td>0.7±0.3</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td><strong>NON-ESSENTIAL AA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>1.4±2</td>
<td>0.9±0.6</td>
<td>2.3±2.1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>3.4±3.2</td>
<td>2.7±2.3</td>
<td>2.3±1.5</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>258.3±159.0</td>
<td>183.8±104.7</td>
<td>163.1±96.3</td>
</tr>
<tr>
<td>Citrulline</td>
<td>2.1±1.4</td>
<td>1.4±0.6</td>
<td>1.6±1.0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>23.0±12.1</td>
<td>16.4±17.1</td>
<td>46.5±31.1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.1±0.8</td>
<td>1.7±0.9</td>
<td>1.5±0.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.8±0.2</td>
<td>0.6±0.4 a</td>
<td>6.2±3.9</td>
</tr>
<tr>
<td>Ornithine</td>
<td>5.8±2.3</td>
<td>6.2±4.7</td>
<td>12.9±8.4</td>
</tr>
<tr>
<td>Serine</td>
<td>2.9±0.9</td>
<td>3.4±2.4</td>
<td>4.8±2.3</td>
</tr>
<tr>
<td>1 MH + 3 MH</td>
<td>3.6±3.2</td>
<td>0.8±1.5 a</td>
<td>ND</td>
</tr>
<tr>
<td>Taurine+Alanine</td>
<td>1.4±0.5</td>
<td>1.2±0.7</td>
<td>128.7±74</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD: a) p<0.05, b) p<0.01; histidine and tyrosine are regarded as EAA

10.5.1 Results

Heights and weights, evaluated as SDS, were lower in the uremic children than in controls, but the body mass index did not differ significantly from normal (Table 1, Study V).

The protein intake in the uremic children, estimated from the dietary recall records, was, on average, 1.4 g/Kg BW/day; the intake estimated from the 24-hour urea excretion was in close agreement (Table 1, Study V). Their estimated energy intake was, on average, 87 % of RDA (Table 1, Study V).

In the uremic children, serum levels of total proteins, albumin, transferrin, pseudocholinesterase and serum bicarbonate were about the same as in the controls (Table 2, Study V). The muscle ASP contents and ASP/DNA ratios were significantly lower in the uremic children than in the controls (Table 3, Study V). Total water, extra- and intracellular water in muscle were similar (Table 3, Study V).

Plasma, RBC, PMN and muscle AA concentrations in patients and controls are shown in Table 4, Study V.

10.5.1.1 Plasma

The concentrations of leucine, tyrosine, valine, serine and the tyrosine/phenylalanine and EAA/NEAA ratios were significantly lower in patients than in controls (Table 4, Study V). The plasma levels of citrulline, glycine, 1-methylhistidine+3-methylhistidine, alanine+taurine and the glycine/serine ratios were significantly increased.
Results and discussion

10.5.1.2 RBC
The RBC concentrations of isoleucine, tyrosine, valine, arginine and glutamic acid and the valine/glycine and tyrosine/phenylalanine ratios were significantly lower in patients than in controls. The RBC levels of citrulline, 1-methylhistidine+3-methylhistidine and the glycine/serine ratios were significantly increased (Table 4, Study V).

10.5.1.3 PMN
The intracellular concentrations of methionine, phenylalanine, tyrosine and valine in the PMN were significantly lower than in the controls as were the valine/glycine ratios. Citrulline levels and the glycine/serine ratio were higher and serine lower in PMN of uremic children (Table 4, Study V).

![Graphs showing correlation between valine concentrations in different compartments.](image)

*Fig. 15.* Correlation between valine concentrations in the four compartments. Controls (●), Patients (▲). Plasma vs RBC (p<0.001), plasma vs PMN (p<0.0001), plasma vs muscle (p<0.001), PMN vs muscle (p<0.001). Not shown in the figure: RBC vs PMN (r=0.45, p<0.05), RBC vs muscle (r=0.51, p<0.05).

10.5.1.4 Muscle
The intracellular concentrations of isoleucine, leucine, tyrosine, valine, serine and the valine/glycine ratio in muscle were significantly lower in the patients than in the controls and the citrulline concentration and the glycine/serine ratio were significantly higher (Table 4, Study V).
10.5.1.5 Gradients

In the uremic children, the RBC/plasma concentration gradients of glycine and 1+3-methylhistidine were significantly lower than in the controls (Table 2). The PMN/plasma concentration gradients of methionine, valine, asparagine, glycine, serine, taurine+alanine were also significantly lower as also were the muscle/plasma concentration gradients of isoleucine and phenylalanine.

10.5.1.6 Correlations

The data from controls and patients have been plotted together to assess correlations between individual AA in plasma, RBC, PMN and muscle by univariate analysis.

Significant correlations were found between plasma and RBC concentrations of isoleucine (p<0.05), phenylalanine (p<0.01), lysine (p<0.01), histidine (p<0.05), citrulline (p<0.01) and arginine (p<0.01).

Regarding tyrosine, a significant correlation was seen between plasma and RBC levels (p<0.001), between plasma and muscle levels (p<0.001) and between RBC and muscle levels (p<0.001).

As for leucine, significant correlations were found between plasma and RBC (p<0.05), plasma and PMN (p<0.05) and plasma and muscle (p<0.05) levels and between RBC and PMN levels (p<0.05).

The most striking result concerned valine, of which concentrations in the four compartments were all significantly correlated: plasma and RBC (p<0.001), plasma and PMN (p<0.0001), plasma and muscle (p<0.001), RBC and PMN (p<0.05), RBC and muscle (p<0.05), PMN and muscle (p<0.001) (Fig. 15).

There were no correlations between nutritional parameters (BMI, s-albumin, s-transferrin, muscle ASP/DNA) and concentrations of individual AA in plasma, RBC, PMN and muscle.

10.5.2 Discussion

This report presents the first data on AA concentrations obtained simultaneously from four compartments (plasma, RBC, PMN and muscle) in 12 uremic and 13 healthy age-matched children.

The ages of the controls were 2.8 to 16.5 years and of the patients 1.7 to 17.7 years, implying that the children were at different stages of development and had wide variations in growth rate and endocrine status, which may have affected the results. Indeed, we found significant correlations between age and plasma glutamine (p<0.01), plasma isoleucine (p<0.05), plasma leucine (p<0.05), muscle glycine (p<0.05) and muscle threonine (p<0.05).

Our study shows that the free AA concentrations of the various AA in plasma, RBC, PMN and muscle bear little relation to the average composition of tissue proteins, the AA pattern of dietary protein or the requirements of essential AA [157]. The intracellular AA patterns in the three cellular compartments were qualitatively similar, but the absolute intracellular concentrations (Table 4, Study V) and the intra- and extracellular gradients (Table 5, Study V), which varied considerably among the AA, were higher in muscle than in PMN, which had higher values than in RBC, presumably reflecting different levels of metabolic activity and differences in membrane transport characteristics.
Results and discussion

In general, it appears that the AA patterns in plasma, RBC, PMN and muscle in the uremic children have many similarities, typical features being low BCAA, tyrosine and serine concentrations and variably high concentrations of some NEAA.

One aim of the present study was to evaluate the extent to which the concentrations of AA in plasma, RBC, PMN and muscle are associated in the same person. To analyze this, we determined by univariate analysis for each amino acid the correlation between the concentrations in each compartment with that in the other compartments.

There were significant correlations between plasma and RBC amino acid concentrations regarding most essential amino acids and also for arginine and citrulline. The relatively close association between the concentrations in both compartments may be related to the fact that they are in direct contact and involved in the interorgan transport of AA.

Among the NEAA, there was no correlation between their concentrations in the three cell compartments (RBC, PMN, muscle) and among the EAA, there were only a few significant correlations between the concentrations in these compartments. A noticeable exception was valine, for which there were significant correlations between the concentrations in all three compartments and between these concentrations and that in plasma (Fig. 15). The lack of correlation between the concentrations of most of the amino acids in RBC, PMN and muscle indicates that there is no close association in the same subject between individual free amino acid concentrations in various types of cells, presumably because they differ as regards metabolism and function. Consequently, one should be cautious in assuming that in individual patients, amino acid concentrations, determined in PMN, reflect the concentrations in muscle cells, as has been suggested [87], although in groups of subjects, the general AA patterns may be similar.
11 SUMMARY AND CONCLUSIONS

The aim of these studies was to evaluate nutritional status and intracellular free amino acid (AA) concentrations in children with chronic renal failure (CRF) immediately before the start of CAPD, in children stabilized on continuous ambulatory peritoneal dialysis (CAPD) and in kidney-transplanted children, comparing the results with values obtained in healthy, age-matched children.

Samples were taken from the rectus abdominis muscle and prepared by a method that enables the analysis of several different constituents in the same small muscle specimen, thus minimizing sample size and sampling variability; this is of special value in studies of small children. Water, electrolytes, alkali-soluble protein (ASP), DNA and free amino acids (AA) were determined in the muscle specimens. Free AA were also determined in simultaneously collected plasma samples.

Water content and electrolyte concentrations in muscle in the non-dialyzed CRF children (study I) were not different from the values in the controls, suggesting that normal fluid and electrolyte balance can be maintained by conservative treatment, taking risks of fluid overload into consideration. Muscle water content was increased in the children on CAPD (studies II and V), which agrees with findings in adult CAPD patients, suggesting that they were fluid overloaded.

The non-dialyzed children with CRF, treated conservatively (study I), and the children on CAPD (study III and V) were growth-retarded but they had no clinical, anthropometric or laboratory signs of protein-energy malnutrition, except that the children on CAPD had a marginally low (Study II) or low (Study V) ASP/DNA ratio in muscle, which may be a sign of subclinical protein malnutrition. The ASP/DNA ratio was positively correlated with age both in the nondialyzed and CAPD patients. The observation that all nutritional parameters were normal in the non-dialyzed children demonstrates that it is possible to maintain a satisfactory nutritional status by conservative treatment in this group of patients.

The non-dialyzed CRF children (study I) as well as the CAPD children (study II and V) had abnormally low plasma concentrations of most essential AA and low muscle intracellular concentrations of valine and leucine, despite essentially normal nutritional status, suggesting that these amino acid abnormalities per se do not necessarily reflect clinical malnutrition but may have other causes, related to loss of renal function, uremia and its treatment. The findings of growth retardation, reduced plasma levels of most essential AA, low muscle intracellular concentration of valine and leucine and low ASP/DNA ratio (in the children on CAPD patients) suggest that children with CRF may benefit from AA supplementation.

In the kidney-transplanted children (study III) studied 72-114 days after transplantation, the nutritional status, as assessed by anthropometric and biochemical parameters, including ASP/DNA ratio, was normal in spite of a previous long history of uremia and medication with prednisone for immunosuppression. Moreover, the plasma and muscle essential AA concentrations were normal, except for a low plasma concentration of leucine, possibly related to the immunosuppressive therapy, and high muscle concentration of threonine. The observation that most of the AA abnormalities found in CRF had disappeared as shortly as
Summary and Conclusions

about 3 months after kidney transplantation, lends further support to the conclusion that these abnormalities are the consequence of uremia and its treatment.

In one study (Study V), free amino acids were also determined in red blood cells and in polymorphonuclear granulocytes (PMN). PMN may offer a cell model, in which all metabolic pathways are present, as an alternative to muscle tissue, which is generally obtained by needle biopsy, an invasive and sometimes uncomfortable procedure that limits its utilization in children.

Since PMN contain a variety of proteolytic enzymes that may degrade protein and increase free AA levels, antiproteolytic agents should be added during sample preparation to prevent protein breakdown, as demonstrated in study IV.

In general, the AA patterns in plasma, RBC, PMN and muscle in the uremic children have many similarities, typical features being low branched-chain amino acids, tyrosine and serine concentrations and variably high concentrations of some non-essential amino acids. The lack of correlation between the concentrations in RBC, PMN and muscle for most of the amino acids in the same subject indicates that these cells differ regarding amino acid transport, metabolism and function. In consequence, one should be cautious in assuming that amino acid concentrations, determined in PMN, reflect the concentrations in muscle cells, as previously suggested, although in groups of subjects the general AA pattern may be similar.

The AA concentrations in plasma, RBC, PMN and muscle in normal children and children with CRF were determined in samples collected at the same time to evaluate whether these concentrations are similar in various compartments and to what extent they are affected by uremia. In general, it appears that the AA patterns in plasma, RBC, PMN and muscle in the uremic children had many similarities, typical features being low branched-chain amino acids, tyrosine and serine concentrations and variably high concentrations of some NEAA.

The lack of correlation between the concentrations in RBC, PMN and muscle for most of the amino acids in the same subject indicates that these different types of cells presumably differ regarding metabolism and function. In consequence, one should be cautious in assuming that amino acid concentrations, determined in PMN, reflect the concentrations in muscle cells, as previously suggested, although in groups of subjects the general AA pattern in nucleated cells may be similar. Water content and electrolyte concentrations in muscle in the non-dialyzed CRF children (study I) were not different from the values in the controls, suggesting that normal fluid and electrolyte balance can be maintained by conservative treatment, taking risks of fluid overload into consideration. Muscle water content was increased in the children on CAPD (studies II and V), which agrees with findings in adult CAPD patients, suggesting that they were fluid overloaded.
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13 REFERENCES

References


28. Tomas EM, Munro HN, Young VR: effect of glucocorticoid administration on the rate of muscle protein breakdown in vivo rats, as measured by urinary excretion of N-methylhistidine. Biochem J 178:139-146, 1979


References

References


57. Herrmann FR, Saffan C, Levkoff SE, Minaker KL: Serum albumin level on admission as a predictor of death, length of stay, and readmission [see comments]. Arch Intern Med 152:125-130, 1992


References


References


References


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References


