IMPROVEMENT IN ACID/BASE STATUS AND NUTRITION IN PERITONEAL DIALYSIS PATIENTS

A Thesis

Submitted for the degree of MD

To the University of Leicester

1999

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ABSTRACT

Short-term correction of metabolic acidosis (MA) has been shown to decrease protein degradation in animals and humans, both normal and uraemic. But the long-term effects of better correction of MA are unknown. The aim of this study was to assess the possible benefits, in terms of nutritional state and morbidity, of improved correction of MA in the first year of treatment with peritoneal dialysis (PD).

Two hundred consecutive new PD patients were randomised, in a single-blind fashion, to receive a high (HA: lactate 40 mmol/L) or low (LA: lactate 35 mmol/L) alkali dialysate, and studied for one year. Calcium carbonate and sodium bicarbonate were also used to further correct acidosis in the HA group. The four key nutritional endpoints were body weight, midarm circumference, triceps skin-fold thickness and serum albumin. The two principle morbidity endpoints were the number of admissions per year, and days spent in hospital per year.

At one year, the venous serum bicarbonate and arterial pH were 7.44 ± 0.004 and 27.2 ± 0.3 mmol/L in the HA group, and 23.0 ± 0.3 mmol/L and 7.4 ± 0.004 in the LA group (both $p<0.001$).

At one year, the increase in body weight in the HA group (6.1 ± 0.66 kg) was higher than in the LA group (3.71 ± 0.56 kg) ($p<0.05$). The increase in midarm circumference in the HA patients (1.26 ± 0.16 cm) was significantly higher than the increase in the LA patients (0.61 ± 0.16 cm) ($p<0.05$). The increases in triceps skinfold thickness were not significantly different (HA: 2.5 ± 0.41 mm vs LA: 1.24 ± 0.38 mm; ($p = 0.1$). Serum albumin was 37.8 ± 0.4 g/dl at one year in the HA group, and 38.2 ± 0.5 g/dl in the LA group (NS). Dietary protein intake at one year (HA: 0.9 ± 0.2 g/kg/day vs LA: 1.0 ± 0.1 g/kg/day) was not significantly different.

There were less hospital admissions in the HA group (1.13 ± 0.16 per patient) compared to the LA group (1.71 ± 0.22 per patient) ($p<0.05$). The HA patients spent

less days in hospital than the LA patients (16.4 ± 1.4 days vs 21.2 ± 1.9 days; $p<0.05$).

It is concluded that better correction of MA leads to greater increases in body weight and midarm circumference, but not triceps skinfold thickness, in the first year of PD. The improvement in morbidity, in terms of number of admissions and days in hospital per year, may be associated with the improvement in nutritional state.

"Imagination is more important than knowledge"

Albert Einstein (1879-1955)

PERSONAL INVOLVEMENT

The work presented in this thesis was carried out during my tenure as a Research Registrar in the Department of Nephrology at Leicester General Hospital, from February 1992 to July 1995.

The work was carried out under the supervision of Professor John Walls, Professor of Nephrology at Leicester General Hospital.

In view of the complexity of running a long-term clinical study on 200 PD patients, a multidisciplinary approach was required.

My responsibilities included:

1. The design of the study and the original power analysis
2. The recruitment and consenting of the patients
3. Assessing all 200 patients at upto 6 different time points
4. Being responsible for all aspects of their clinical care during their first year of dialysis
5. Recording all the data
6. Calculating the kinetic modelling parameters
7. Carrying out all the statistical analysis and interpreting the data.

All the simple haematological and biochemical analyses were carried out at the Leicester General Hospital. The parathyroid hormone levels were measured at the Leicester Royal Infirmary. The aluminium assays were carried out at the Northern General Hospital, Sheffield. The urinary and dialysate urea and creatinine measurements (on which the kinetic modelling parameters were based) were performed by Mr Frease Baker, Research Scientist, in the Department of Nephrology, Leicester General Hospital.

The nutritional assessments (including measurements of dietary protein intake) were made by four experienced renal dietitians: Gavin James, Jane Johnstone, Gemma Bircher and Jaqui Troughton.

Statistical advice was obtained from Dr Nick Taub, Lecturer in Medical Statistics in the Department of Epidemiology and Public Health, University of Leicester.

The primary concern of this work was the clinical significance of the nutritional hypothesis being tested. The methodology of the assays and techniques used in the study will therefore not be examined in great detail, but the problems of interpretation of the results will be explored.

ACKNOWLEDGEMENTS

I would like to thank Professor John Walls for being my supervisor, and mentor. He provided me with the time, space and money to carry out this work. He, above all, gave me the opportunity to think laterally and use my imagination. Whenever I came up with a new idea (eg, kinetic modelling or muscle strength measurements, neither of which had been done in Leicester when the study started), his response was always his favourite phrase ... "just do it".

Jenny Moorhouse, a research nurse at Leicester General Hospital, helped me co-ordinate the study. Towards the end of the study, her work was taken over by another research nurse, Heather Iles-Smith. I would like to thank them, the dietitians, Frease Baker and Nick Taub, whose roles are mentioned in the previous section. Dr Olof Heimburger, a nephrologist in Stockholm, gave me advice at various times through the study, especially relating to kinetic modelling. I would also like to thank Dr Steve Kardasz, Dr Judith Stevens, Dr Alice Allen and Dr Alan Bevington for their help and advice.

The library staff of Leicester General Hospital obtained all the papers concerning the 'early' literature on acidosis, via the British Library. The Wellcome Library in London allowed me to read Richard Bright's 1827 book. Both libraries have my thanks.

Two hundred patients, already having to cope with the fear of starting dialysis, had the courage to take part in this study. I thank and respect them.

I would also like to thank all the people, especially my mother, for nagging me into writing it up.

DEDICATION

This work is dedicated to the two hundred peritoneal dialysis patients who agreed to take part. Some died before the end of the research. I hope that this work has improved the lives of the survivors.

PUBLICATIONS

The following publications have been derived from this work:

Editorials

1. **STEIN A**, WALLS J. The correlation between Kt/V and PCR - a self-fulfilling prophecy. Nephrol Dial Transplant; 9(7): 743-745, 1994.

2. **STEIN A**, BEVINGTON A, WALLS J. Metabolic acidosis, protein intake and malnutrition in renal failure; 60 years of progress? Rev Port Nefrol Hipert; 8(2): 87-93, 1994.

Papers

3. **STEIN A**, BAKER F, BENNETT S, LARRATT C, HARRIS K, FEEHALLY J, WALLS J. Correction of metabolic acidosis and the protein catabolic rate in peritoneal dialysis patients. Perit Dial Int; 14: 187-189, 1994.

4. **STEIN A**, BAKER F, MOORHOUSE J, WALLS J. Peritonitis rate - traditional versus low calcium dialysate. Am J Kid Dis; 26(4): 632-633, 1995.

5. **STEIN A**, MOORHOUSE J, ILES-SMITH H, BAKER F, JOHNSTONE J, JAMES G, TROUGHTON J, BIRCHER G, WALLS J. Role of an improvement in acid-base status and nutrition in CAPD patients. Kidney Int; 52: 1089-1095, 1997.

This is the main paper summarising this work

6. **STEIN A** and WALLS J. What is the relationship between metabolic acidosis and nutritional status in dialysis patients? Am J Kid Dis; 31(5): 884-889, 1998.

Letters

7. **STEIN A**, BENNETT S, FEEHALLY J, WALLS J. Does low calcium dialysate improve the nutritional status of CAPD patients? Perit Dial Int; 13(1): 69, 1993.

Abstracts

8. **STEIN A** and WALLS J. The reason why Kt/V and PCR correlate so well. Nephrol Dial Transplant; 9(2): 209, 1994 (Autumn meeting of the Renal Association, London, 1992).

9. **STEIN A**, PRICE S, FEEHALLY J, WALLS J. Low calcium dialysate in CAPD patients. Perit Dial Int; 12 (suppl 2): S79, 1992 (VIth Congress of the International Society for Peritoneal Dialysis in Thessaloniki, Greece, October 1992).

10. **STEIN A**, HARRIS K, FEEHALLY J, WALLS J. Two year's experience with low calcium dialysate in CAPD patients - another trade-off? Nephrol Dial Transplant; 8(12): 1415, 1993 (Spring meeting of the Renal Association, Leicester, 1993).

11. **STEIN A**, BENNETT S, LARRATT C, BAKER F, FEEHALLY J, WALLS J. The effect of correction of acidosis on peritoneal dialysis patients. Clin Sci; 85(1): 4P, 1993 (Spring meeting of the Medical Research Society, London, 1993).

12. **STEIN A**, BAKER F, MOORHOUSE J, WALLS J. Peritonitis rate - traditional versus low calcium dialysate. J Am Soc Neph; 5(3): 478, 1994 (Spring meeting of the Renal Association, London, 1993; and the American Society of Nephrology, Orlando, October 1994).

13. **STEIN A**, MOORHOUSE J, BAKER F, JOHNSTONE J, JAMES G, TROUGHTON J, BIRCHER G, WALLS J. Correction of acidosis and nutrition in CAPD patients: A randomized controlled trial of low versus high alkali dialysate. J Am Soc Neph; 6: 588, 1995 (American Society of Nephrology, San Diego, 1995).

ABBREVIATIONS

Alk Phos	Alkaline phosphatase
$CaCO_3$	Calcium carbonate
CrCl	Creatinine clearance
CRF	Chronic renal failure
ESRF	End stage renal failure
HCO_3	Bicarbonate
HA	High alkali (treatment group)
HD	Haemodialysis
LA	Low alkali (control group)
MA	Metabolic acidosis
PCR	Protein catabolic rate
PD	Peritoneal dialysis
PTH	Parathyroid hormone
U-P	Ubiquitin-proteasome

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CHAPTER ONE. INTRODUCTION

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IMPROVEMENT IN ACID/BASE STATUS AND NUTRITION IN PERITONEAL DIALYSIS PATIENTS

CHAPTER ONE

And now remains

That we find out the cause of this effect,

Or rather say, the cause of this defect

Hamlet II, 2

Polonius, being facetious

about Hamlet's supposed madness

CHAPTER ONE

INTRODUCTION

1.1. Background

Protein malnutrition is a salient feature of chronic renal failure (CRF) in man[1]. A link between metabolic acidosis (MA) and albuminuria was first suggested by Richard Bright in 1827, 170 years ago (**1**).

There was interest in this area in 1920's and 30's, culminating in the pioneering work of Lyon in 1931 (**2**). In this study, it was found that in two patients with CRF, the blood urea was greater when the patients received an acid rather than alkali generating diet.

In the last 15 years there has been a resurgence of interest in the effects of the MA on protein metabolism in renal failure. The reason for this resurgence is not obvious. It may have been, in part, driven by the interests of three research groups, Leicester and Newcastle in the United Kingdom, and Boston, Massachusetts in the United States of America. More importantly, there has been a growing awareness, throughout the nephrological world, of the increasing numbers of patients with End Stage Renal Failure (ESRF) taken on to dialysis programmes, and the increasing morbidity and mortality of this patient group.

The reason for this increasing morbidity and mortality would seem 'obvious'. These 'new' patients are largely derived from a less 'well' patient group, not previously dialysed. They have more serious underlying diseases (eg, myeloma, diabetes mellitus and renovascular disease) and greater co-morbid problems at the start of renal replacement therapy (ischaemic heart disease, cerebrovascular and peripheral vascular disease). Few of these diseases are amenable to curative treatment.

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[1]This fundamental tenet of renal nutritional research may not be true. Current renal nutritional dogma dictates that malnutrition is very common in ESRF. However, few studies (including Young's study, described above) compare ESRF patients to age-sex matched controls. This is discussed in more detail on pages 140-143 in the Discussion.

Malnutrition has been suggested as another major factor in this greater morbidity and mortality. Protein malnutrition seems to be the most important nutritional deficit, especially as there is evidence that the increased body water (per kg of body weight) of patients with CRF is caused by a loss of lean body mass (Coles, 1972, **3**). In the last five years in particular, the extent and importance of malnutrition in patients with ESRF has been fully appreciated. For example, in 1991, in a multicentre international study of 224 patients, Young et al (**4**), found that,

> "eighteen patients (8%) were severely malnourished, 73 (32.6%) were mildly malnourished, and 133 (59%) did not show evidence of malnutrition".

The possible effects of malnutrition were highlighted in the study of Lowrie and Lew, published in 1990 (**5**). In this study of over 12,000 HD patients in the USA, those starting dialysis with a serum albumin of 2.5 g/dl, were 17 times more likely to die than those starting dialysis with an albumin of 3.5-4.0 g/dl[2].

The causes of malnutrition in CRF are unknown but probably multifactorial. Possible contributory factors include: reduced food arising from the symptoms of uraemia itself; 'uraemia' and anaemia leading to poor mobility, and therefore reduced food and energy need; the necessity to take multiple medications, which may directly reduce appetite, or if meals are associated with taking tablets, eating may be avoided.

In recent years, research has focused on another possible factor in the aetiology of malnutrition - namely, inadequate dialysis[3]. This may be related to the use of shorter hours and more effective membranes in HD patients. In PD patients, it may be related to an inability to increase the dialysis dose above a certain (as yet unknown) maximum dose - either because patients cannot tolerate larger or more frequent, dialysis exchanges, or because they refuse to do them. In larger patients, even if larger doses

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[2]Even this (second) fundamental tenet of renal nutritional dogma may not be true. Patients that die *with* a low serum albumin do not necessarily die *of it* - ie, the link may not be causative.

[3]Gotch and Sargent's National Cooperative Dialysis Study, written up in 1983 (**147**), remains the only controlled study that provides evidence for this assumption. Certainly, there is little evidence that by improving dialysis adequacy, mortality can be improved.

are tolerated and used, the technique may be unable to provide an 'adequate' dialysis dose.

Nephrologists world-wide have felt a duty to search for other factors that might 'cause' malnutrition. One such factor is metabolic acidosis (MA). Why should MA be a candidate factor, contributing to the malnutrition, and perhaps therefore for the increased morbidity and mortality of ESRF?

There is considerable circumstantial evidence that MA plays a dominant role in the malnutrition associated with CRF. Retrospective studies of children with CRF have shown that they have a reduced growth velocity which can be improved by better correction of acidosis alone (Chantler, 1988, **6**). In 1972 Nash et al showed that correcting MA in patients with non-uraemic renal tubular acidosis improves growth (**7**). So, it seems likely that the 'benefit' of correcting acidosis is not specific to uraemia as a cause of MA. Whether this improved growth is due to reversing the effects of MA on bone or muscle metabolism is uncertain. The effects of MA on bone metabolism will be described in more detail later in the Introduction.

As well as such circumstantial evidence, much direct evidence exists of a link between MA and protein metabolism. The majority of the Introduction to this thesis will concentrate on this evidence.

1.2. Causes, and 'other'[4] metabolic consequences of MA

In normal humans, approximately 60 mmol of hydrogen ions are excreted by the kidney per day - 40 mmol (two-thirds) bound to ammonia, and 20 mmol (one third), called 'titratable acid', bound to phosphate and other anions (including sulphate and organic anions). So, the dominant mechanism, in the MA of renal failure, by which acid is eliminated is by its binding to ammonia (Tanner et al, 1978, **8**). In this way, ammonia and these other substances act as buffers. Only 0.1% of acid is excreted as free H^+ ions.

Ammonia is largely derived from the amino acid glutamine, which is converted into ammonia in the proximal tubule of the kidney. "Titratable acids" received their name because they have long been measured in terms of the amount (in mmol) of sodium hydroxide that must be added to a litre of urine to return its pH to 7.4. This value, plus the ammonia content minus the bicarbonate content of the urine, is the quantitative measure of the acid excretion in the urine.

Why do humans, and all mammals, have this elaborate buffering system? Why not excrete free H^+ ions? The answer relates to the energy required to sustain the pH gradient across the kidney. Normally the pH of the blood is approximately 7.4 and the urine is 5.0. For this to occur without buffering, the H^+ concentration gradient would have to be very high across the kidney, as the concentration of H^+ ions in the urine is 250 times that of the blood. If the urinary pH was 3.0, the H^+ concentration in the urine would be 25,000 times that of the blood. The energy requirement to maintain even a 250 fold difference is prohibitive. So, H^+ ions are 'masked' by being attached to buffers, such as ammonia and phosphate.

The renal compensatory mechanisms are capable of excreting the increased acid load in mild to moderate CRF, principally by increasing ammonia production. In fact, the rate of ammonia excretion can increase up to 500 mmol per day, ie over 10 times normal. In severe renal failure hydrogen ion excretion decreases (to around 20 mmol per day);

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[4]ie, other than effects of MA on protein metabolism

mainly due to an inability to excrete ammonia, with the development of metabolic acidosis. Hence MA only appears when the GFR has fallen to roughly 20-30% of normal.

Why does the kidney reach a point whereby it can no longer excrete enough ammonia? It may be due to a defect in its production or concentration.

According to one hypothesis regarding the pathophysiology of MA in renal failure, there is an adaptive increase in ammonia production by the remaining renal parenchyma, as functional renal mass is reduced by disease. However, an indefinite increase in the induction of enzymes responsible for ammonia production is not possible. Eventually, a plateau in ammonia production (per functioning nephron) is reached (Tizianello et al, 1980, **9**). Further diminution of renal mass results in an absolute decrease in ammonia excretion, and MA supervenes.

The decrease in ammonia excretion is greater than the decrease in titratable acid excretion; and has been demonstrated in animals, to show a linear decline with inulin clearance (Walls et al, 1972, **10**). This is another reason that the diminution in ammonia excretion can be thought of as the major 'cause' of the MA associated with severe CRF.

Conversely, others believe that ammonia production in the proximal tubule is normal in experimental renal insufficiency.

Renal excretion of phosphate (and H^+ ions bound to phosphate, ie titratable acid) is initially normal in CRF. Two factors are responsible for the continued delivery of phosphate to the distal nephron. Firstly, there is a reduction in the reabsorption of phosphate in renal disease, mainly due to secondary hyperparathyroidism. Secondly, there is a gradual elevation of blood phosphate as GFR falls. Thus, distal phosphate delivery, and hence titratable acid formation, are relatively well maintained in CRF; at the expense of higher plasma phosphate concentrations and hyperparathyroidism. As GFR declines, titratable acid excretion eventually rises, to a level 2-3 times normal (Hamm et al, 1987, **11**). With severe renal failure the excretion of titratable acid (like ammonia) decreases, which then also becomes a factor in the MA of renal failure.

One of the other main functions of the kidney - in terms of acid/base balance - is the reabsorption of bicarbonate. This is one of the vital 'housekeeping' functions of the kidney. A normal adult with a plasma bicarbonate level of 26 mmol/L and a GFR of 180 L/day filters approximately 4700 mmol of bicarbonate (equivalent to nearly 395g of sodium bicarbonate) each day. To maintain status quo, almost all of it is reabsorbed in the proximal tubule. So, bicarbonate behaves in this system like glucose, as a 'threshold substance'; in that it only appears in the urine if the plasma concentration exceeds 28 mmol/L.

It is not clear whether a significant reduction in bicarbonate reabsorption occurs in patients with CRF. Certainly, overt bicarbonate wasting is unusual in renal failure; though it has been described after the administration of alkali (Schwartz et al, 1959, **12**). Other investigators have found the opposite, ie bicarbonate reabsorption (per surviving nephron) is increased rather than decreased (Schmidt et al, 1976, **13**). The cause of this observation is uncertain. Nonetheless, decreased bicarbonate reabsorption is probably not a factor in the MA of chronic renal failure.

Metabolic acidosis has several important consequences in patients with CRF. When severe, uraemic acidosis causes hyperventilation, and can increase serum potassium levels. This is as a consequence of intracellular buffering of hydrogen ions in exchange for potassium. Indeed, intravenous bicarbonate is a long-established emergency treatment for hyperkalaemia. Furthermore, MA causes the oxygen dissociation curve to shift to the right. It is also likely that MA plays a role in the insulin resistance seen in uraemia (Reaich et al, 1995, **14**).

One of the most important (non-protein) metabolic consequences of MA in man, and perhaps in this study in particular, is its effect on bone. Renal tubular acidosis (RTA) in the absence of renal failure, is a rare but well-recognised cause of short stature in children and osteomalacia in adults. In 1978 McSherry and Morris demonstrated a 2-3 fold increase in growth velocity of children with RTA by correcting MA (**15**). The natural history of five such children has been described in detail by Rodriguez-Soriano et al in 1982 (**16**). It seems unlikely that this extra growth is secondary to changes in

protein metabolism alone - ie, bone metabolism is also affected. Certainly, in adults it is possible to treat the osteomalacia of RTA with oral bicarbonate alone (ie, without vitamin D analogues), with resolution of the bone pain. Exactly how MA influences bone metabolism remains unclear.

Influence of MA on bone metabolism

It has been shown that MA has effects on calcium and phosphate, vitamin D and PTH:

(1) Calcium/phosphate. In 1967 Litzow et al showed that treating an acidotic patient in renal failure with alkali, can reduce urinary and faecal calcium loss (**17**).

However, in 1974, Burnell et al showed no correlation between the degree of MA and the calcium or phosphate content of bone (**18**). But, in a study of autopsy material in 1977, Pellegrino et al (**19**) found a decrease in the calcium content of bone and an increase in the phosphate content, which was proportional to the duration of uraemia. Furthermore, Kraut et al, in 1986, showed that bone formation was inhibited and bone resorption diminished by MA in the rat (**20**).

Later studies have indicated that the effects of MA on bone are not just 'negative' - they have a useful homeostatic role. Acidosis leads to the physicochemical dissolution of bone, causing release of HCO_3 together with sodium, potassium and calcium[5] (Bushinsky et al, 1995, **21**). The HCO_3 so released is part of the body's buffering response to MA; though the glutamine/ammonia and titratable acid responses are more important. The net result is another 'trade-off' - ie, in CRF/ESRF blood pH and HCO_3 concentration are protected at the expense of bone mineral content.

The mechanisms of these effects are uncertain, and may not solely relate to systemic pH.

(2) Vitamin D. Both osteomalacia and osteopenia were shown to be associated with MA in a study by Mora Parma et al in 1983 (**22**). It was thought initially that this was due to MA reducing 1,25-vitamin D levels (Lee et al, 1976, **23**) by *decreasing* the activity of renal 1α-hydroxylase activity (Kawashima et al, 1982, **24**). More recently this hypothesis has been challenged. It has been suggested by Krapf et al in 1992, that MA *increases* 1,25-vitamin D levels (**25**).

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[5]The hypercalciuria so produced by MA may be part of the reason why patients with renal tubular acidosis develop renal stones.

Confusingly, there is also evidence that MA *correction* raises vitamin D levels. In 1996 Lu et al investigated the effect of rapid correction of MA on serum 1,25-vitamin D levels in predialysis patients (**26**). They used a free calcium clamp to negate any changes in calcium induced by altering acid/base balance. Vitamin D levels increased significantly (from 38.6 to 47.0 pmol/L) after rapid correction of MA.

(3) Parathyroid hormone. MA may increase the sensitivity of bone to PTH. In 1980, Martin et al, using an isolated bone perfusion technique, demonstrated increased PTH uptake by bone during acidosis (**27**).

Bichara et al, in 1990, showed that acute MA can increase PTH levels in the normal rat (**28**). It has also been shown by St John et al, in 1992, that PTH correlates independently with the serum bicarbonate level (**29**). More recently, Graham et al (1996) showed that correction of MA reduces PTH levels in haemodialysis patients, probably by increasing the sensitivity of the parathyroid glands to calcium (**30**).

Even though MA clearly affects all aspects of renal bone disease, how much it is involved in the pathogenesis of the problem is controversial. Furthermore, the clinical relevance of MA, in terms of treatment of renal bone disease, is not obvious.

For example, in 1968, Stanbury showed that alkali therapy for patients with overt renal bone disease failed to produce healing of skeletal lesions, whereas vitamin D produced healing even in the presence of acidosis (**31**). Conversely, in a later study (1989), Lefebvre et al, investigating 21 haemodialysis patients who were dialysed against different bicarbonate concentrations for six months, came to a different conclusion (**32**). Effective correction of MA occurred in the treatment group with a pre-dialysis plasma bicarbonate level of 24 mmol/L, compared to 15.6 mmol/L in the control group. Osteoid and osteoblastic surfaces increased in the more acidotic group, but not in the 'alkalotic' group. Furthermore parathyroid hormone levels increased in the control but not in the treatment group.

In summary, MA causes calcium release from bone, probably increases PTH levels and possibly inhibits vitamin D activation by the kidney. Some of these responses may be part of a useful homeostatic response to MA. The relative importance of MA -

compared to hypocalcaemia, hyperphosphataemia, vitamin D deficiency and hyperparathyroidism (some of which it may contribute to) - in the pathogenesis of renal bone disease is less clear. Nonetheless, if the effects of MA on bone metabolism are seen in the long-term, they are probably not helpful. If so, better correction of MA may have beneficial consequences for renal bone disease in patients with ESRF.

1.2. Summary: causes, and 'other' metabolic consequences of MA

(1) Ammonia. The dominant mechanism, in mild-to-moderate renal failure, by which acid is eliminated is by the increased excretion of ammonia.

(2) In severe renal failure hydrogen ion excretion decreases, mainly due to an inability to excrete ammonia - with the development of metabolic acidosis.

(3) As well as the effects of MA on protein metabolism (to be discussed), the 'other' metabolic consequences of MA are significant, particularly the effects of MA on bone metabolism. MA can influence bone metabolism by a variety of different mechanisms.

1.3. Early literature

Galen the Greek physician (AD 129-199) was aware of the acidic nature of various body fluids, including urine (**33**):

> "The urinary bladder continues to collect urine up to the time that it becomes uncomfortable through the increasing quantity of urine or the irritation caused by its acidity".

Strong acids and bases were first refined in India in the 8th and 9th Centuries (Gottschalk et al, 1987, **34**). The strong bases were made by heating lime or by burning wood, the *ash* of which contained Potash, plus potassium carbonate. In fact, the term 'alkali' is derived from the Arabic word for ash, *al qali.* The alkalis were given as dilute solutions for the treatment of ascites and renal calculi in these societies (Astrup, 1986, **35**).

As mentioned previously, a link between the MA and albuminuria was first suggested by Bright, 170 years ago (**1**):

> "I may remark further, that in the natural albuminous fluids *(presumably normal urine)* ... we always meet with an excess of alkali".

However, Bright doubted the significance of acid/base balance in the disease that was to bear his name:

> "But the deficiency of acid or the presence of alkali, although separately or conjointly they may produce some effects, cannot be considered the principle cause".

Even so, one of his most well-used treatments for renal disease was "Supertartrate of Potash":

(whilst discussing diuretics) "Amongst these, I have found the Supertartrate of Potash the most efficacious".

He may not have realised that the salts of most heavy metals are usually weak alkalis; or that Potash - by a nice coincidence - was one of the first bases ever refined by the Human Race.

However, most of the early studies on MA (including Bright's) focused their attention on the effects of acidosis on the kidney (especially albuminuria) rather than on muscle. In other words, the capacity of MA to induce renal injury was the main area of interest. For example, Von Hoesslin in 1909, noticed a relationship between the acidity of the urine, and the amount of albumin and number of casts present (**36**). Albuminuria decreased when sodium bicarbonate was given.

In 1917, Sellards "obtained most favourable results by the administration of alkalies" in cases of acute nephritis associated with Asiatic cholera (**37**). MacNider, in 1918, observed that animals with CRF could be protected from the acidotic effect of an anaesthetic by the use of alkali (**38**).

Sansum et al investigated, in 1923, the effects of a "basic diet" , given for 3 weeks to 5 months, on 10 patients with "nephritis" (**39**). They found:

> "Under such conditions, patients afflicted with arterial hypertension and chronic interstitial nephritis appear to improve, as evidenced by a fall in blood pressure, a reduction in urinary casts and albumin, and other symptoms referable to this combination of diseases, in 90 per cent. of the cases."[6]

[6]This work was clearly going on at an exciting time. The last sentence of the discussion reads, "this work was temporarily interrupted by our laboratory work following the discovery of insulin by Banting"! This study may also have been the first controlled prospective study in the MA/protein metabolism area.

In 1925, Wells found that experimental nephritis in animals was largely prevented by the administration of alkali (**40**). In 1927, "The Prevention of Scarlatinal Nephritis" was published by Carter and Osman (**41**). In this study 620 patients (all but 51 cases were 15 yrs or under) with scarlet fever were treated with at least 21 days with an oral alkaline mixture (sodium bicarbonate and potassium citrate), the aim being to prevent the onset of nephritis. Of these, no cases developed albuminuria. Since 1.6% (5/316) of cases of scarlet fever not treated with alkali (in another ward at the same hospital), and 3.5% (12/336) of cases not treated with alkali in another hospital, developed albuminuria, the authors considered benefit may have accrued. Even though not a randomised controlled trial, the authors clearly tried to compare their treatment group to contemporary 'controls'.

In the same year, Osman (**42**) published another paper; this was a 49 page article which summarised the literature to date, and provided detailed case reports of 16 patients all treated with oral or intravenous alkali. They had a wide range of renal diseases, including acute and chronic nephritis, and anuria. A variety of responses to alkali therapy were seen. Overall, as most patients seemed to 'improve' on alkali therapy and experience a reduction in albuminuria, the treatment was thought to be of benefit in many cases of renal disease.

In two further studies (Newburgh et al, 1928 (**43**), and Sansum et al, 1930 (**44**)), it was noted that when animals were fed on a highly acidic diet, they gradually developed albuminuria. In 1930, Newburgh et al followed up their animal study with a similar human study; on a normal man whose urine contained no albumin and a less than average number of casts (**45**). After six months on a high protein (beef) diet, he developed albuminuria and a twenty-fold increase in casts.

Finally, in 1931 Lyon et al (**2**) published "The Alkaline Treatment of Chronic Nephritis". This paper consisted of a pair of lengthy case reports, again showing the ability of an alkaline diet to reduce albuminuria. What made this study different was that it also looked at muscle metabolism. There was an increase in urinary urea and

creatinine excretion, and therefore presumably muscle breakdown, when the patients consumed an acidic diet. All effects were reversed by an alkaline diet.

1.3 Summary: early literature

Renal injury, usually as judged by albuminuria, was the primary endpoint in most, but not all, of these early studies. Some used oral or intravenous alkali to correct acidosis, others manipulated the diet. Most were uncontrolled and all lacked statistical analysis. Nonetheless, considering the lack of technology available to early workers, much was discovered in this period: principally, that correction of MA reduces urinary albumin and urea excretion.

Long before the resurgence of interest in this area, there was ample evidence that the manipulation of acid/base balance had profound effects on renal and muscle metabolism in man.

1.4. Effects of MA on protein and amino acid metabolism in the normal (non-uraemic) state

1.4(i). Normal animals

In 1982, Hannaford et al (**46**), investigated the effects on nitrogen balance of chronic MA in normal rats and rabbits. Nitrogen balance was significantly more negative during acidosis in the rat but not in the rabbit. The changes in nitrogen excretion were due to an increase in ammonium generation with no detectable difference in urea excretion. In a controlled study, May et al (**47**), showed that MA induced by ammonium chloride led to growth retardation in normal rats:

> "Acidosis did not change protein synthesis; hence, the increase in net protein degradation was caused by stimulation of proteolysis"

This study also showed that the impaired growth rate of the acidotic animals was glucocorticoid dependent.

In 1988, Welbourne et al (**48**) further investigated the role of glucocorticoids on protein metabolism in normal rats, examining their effects on the mobilisation and renal utilisation of the amino acid glutamine. In this study, in adrenal-intact animals, MA increased the flow of glutamine from the hindquarters to the kidneys. Adrenalectomy prevented this effect; providing further evidence for the glucocorticoid-dependent nature of this effect.

As previously mentioned, increased renal excretion of ammonia (as a urinary pH buffer), has long been known to be a central component of the compensatory increase in acid excretion in humans and animals (Tanner, 1978, **8**). Wherever the origin, glutamine is the principle substrate for renal ammoniagenesis, though other amino acids contribute (Pitts, 1961, **49**). MA is said by some to increase the release of glutamine from skeletal muscle (Schrock et al, 1980, **50**). Others suggest the liver may produce

the increased quantities of glutamine (Lueck et al, 1970, **51**). Glutamine is converted in the liver to urea, a process that itself consumes bicarbonate. These and other observations have led Mitch (1994, **52**) and others to propose a 'trade-off hypothesis' for the MA/protein metabolism effect.

'Trade-off hypothesis': MA leads to a wasteful (acid-generating) cycle of protein degradation in CRF

MA increases the production of hormones (including glucocorticoids) that stimulate protein degradation. This response is beneficial in humans without CRF because it leads to the synthesis of glutamine, either from the liver or skeletal muscle, which permits more ammonia-bound acid to be excreted. Also, glutamine can be used as a substrate for gluconeogenesis and energy. The urea generated by the use of glutamine can be excreted by the kidney.

Why have a homeostatic system whereby MA can cause protein degradation? In some stressful states - fasting is the most obvious example - it may be a useful survival adaptation for acidosis to cause muscle breakdown, to provide the animal with another source of glucose or energy. This system requires good renal function to get rid of excess ammonia so produced.

However, in CRF, this response is maladaptive. The combination of acidosis and glucocorticoid leads to glutamine production which produces ammonia-bound acid that cannot be excreted. Furthermore, the conversion of glutamine to urea in the liver uses up bicarbonate, worsening acidosis further. Neither can the urea so produced be excreted. This effect that has led to the suggestion that MA causes a wasteful (acid generating) cycle of protein degradation - ie, MA causes protein degradation which worsens acidosis, causing more protein degradation.

The following diagram summarises the 'trade-off hypothesis':

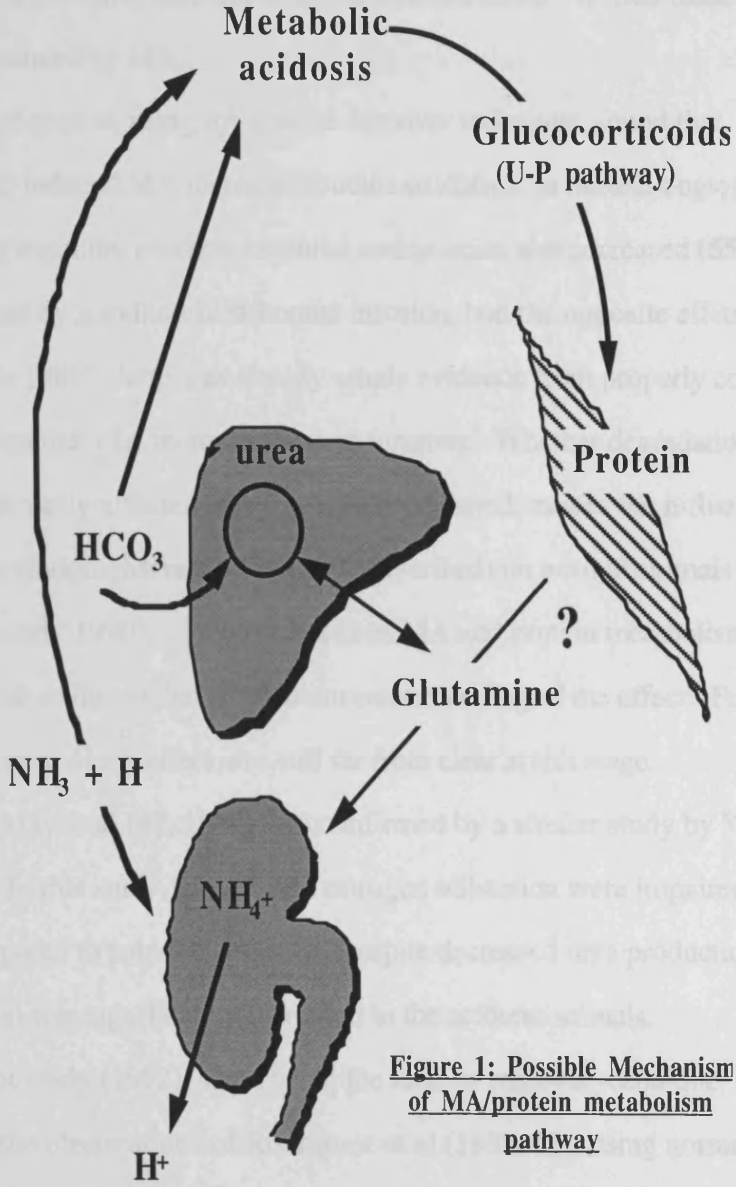

Figure 1: Possible Mechanism of MA/protein metabolism pathway

How specific is the effect of MA on protein metabolism effect, either in terms of the cause of the acidosis or the tissue affected? In 1988, Preedy et al investigated the effects of acute *respiratory* acidosis on protein metabolism in normal rats; and found that respiratory acidosis induced a *decrease* in protein synthesis (**53**). Similarly, Fuller in 1989, showed that decreasing pH d*ecreased* protein synthesis in the working perfused rat heart (**54**). In this way, the effect seems quite specific. The cause of MA, the tissues affected by it, and possibly even, the species investigated - as suggested by Hannaford's 1982 study (**46**) - may influence *how* protein metabolism is affected. Furthermore, as will be described in the human data following this section, the type of

protein affected by MA may also not be specific to this effect - ie, non-muscle proteins may also be influenced by MA.

In 1989, Rodriguez et al, using the leucine turnover technique, found that hydrochloric acid-induced MA increased leucine oxidation, in normal dogs; plasma concentrations of essential and non-essential amino acids also increased (**55**). Alkalosis, induced by a sodium bicarbonate infusion, had the opposite effect.

By this stage (in 1989), there was already ample evidence from properly controlled prospective studies that MA increases protein turnover. Whether degradation or synthesis was primarily affected was less well established, as was the influence of MA on amino acid oxidation. More studies (to be described) on normal animals were published in the early 1990's. The link between MA and protein metabolism was confirmed, but the studies added little to our understanding of the effect. Furthermore, the clinical relevance of the effect was still far from clear at this stage.

The study by May et al (**47**, 1986) was confirmed by a similar study by Williams et al in 1991 (**56**). In this study, growth and nitrogen utilisation were impaired in acidotic normal rats compared to pair-fed controls. Despite decreased urea production, total nitrogen excretion was significantly increased in the acidotic animals.

In a more recent study (1992), again using the leucine turnover technique, May et al (**57**) confirmed the observations of Rodriguez et al (1989, **55**), using normal rats rather than dogs; they showed that MA was associated with an increase in the catabolism of protein and essential amino acids, both *in vivo* and in isolated muscle. Whole body protein turnover was accelerated by acidosis; as reflected in a 70% increase in proteolysis and a 55% increase in protein synthesis and a 145% increase in amino acid oxidation.

As well as protein breakdown, amino acid degradation seems to be affected by MA. The activity of liver branched-chain ketoacid dehydrogenase (BCKAD), which controls the first step in BCAA breakdown, was increased by 104% in the acidotic rats in the same study. In contrast, kidney BCKAD activity was decreased 38% by acidosis. The authors felt that these differences illustrated the "tissue-specificity of the changes that

were present". The increase in liver BCKAD activity in *normal* rats was similar to the increase in skeletal muscle BCKAD activity caused by acidosis in *uraemic* rats, in a study by the same group (Hara et al, 1987, **58**).

The tissue-specificity of the effect of MA on amino acid breakdown as suggested by these two studies (**57, 58**) is analogous to the tissue-specificity of the effect of MA on protein breakdown, as proposed by Preedy et al (**53**) and Fuller et al (**54**).

The mechanism of the MA/protein metabolism effect is still far from clear. Challa et al in 1993 found that growth retardation in normal rats with MA was associated with impairment of growth hormone secretion (**59**). In 1994 Mitch et al (**60**), also investigating the mechanism of this effect, proposed a different mechanism. They demonstrated that MA stimulated muscle proteolysis in normal rats, by activating a ATP-dependent ubiquitin-proteasome proteolytic pathway. Later that year, this mechanism was shown by the same group to be partially glucocorticoid dependent (Price et al, **61**). The authors concluded that:

> "Glucocorticoids are required but not sufficient to produce the co-ordinated increase in mRNAs encoding ubiquitin and proteasome subunits occurring in muscles of acidotic rats"

The ubiquitin-proteasome mechanism will be discussed in more detail in section 1.7. of the Introduction.

In 1993, Cupisti et al (**62**) investigated the effects of MA on arterial amino acid profiles in normal rats. The results tended to confirm Rodriguez' study (1989, **55**) - in that, the MA rats showed increased levels of threonine, histidine, proline, serine and glycine, but decreased levels of tryptophan. Intracellular amino acid levels in muscle tissue partly reflected the changes in extracellular levels. Acidotic animals also gained less body weight:

> "In conclusion, the results show that acidosis *per se* modifies the circulating amino acid profile in fed rats, resulting in a pattern similar to the post-prandial amino acid changes

described in uraemic patients. These abnormalities occur together with impaired growth of skeletal muscle cells".

In 1995, Throssell et al assessed whether prolonged MA caused chronic renal injury in a controlled study on normal rats (**63**). They found that MA did not cause any renal injury in terms of GFR or serum creatinine. However, it did induce renal hypertrophy (and increased albuminuria), as judged by greater kidney:body and kidney:heart weight ratios in the acidotic group of rats. This study confirmed the effects of MA on renal tissue seen in another animal study, by Lotspeich et al in 1965 (**64**). Both these studies further demonstrate the specificity of the effect, ie different tissues respond in different ways to MA.

The MA-induced worsening of albuminuria seen in the study of Throssell et al (1995, **63**) is consistent with the improvement in albuminuria caused by better correction of MA in the human studies from the 'early literature'.

In a leucine turnover study in 1996, May et al (**65**) further investigated the glucocorticoid-dependent nature of the effect. In adrenalectomised normal rats receiving no glucocorticoids, there was no difference in leucine oxidation, protein degradation or synthesis, whether or not the rats had acidosis. Conversely, chronically acidotic, adrenalectomised rats receiving glucocorticoids showed accelerated whole body protein turnover with a 84% increase in amino acid oxidation and a 26% increase in protein degradation, compared to rats not receiving glucocorticoids, or those given glucocorticoids but without acidosis:

"We conclude that MA accelerates amino acid oxidation and protein degradation *in vivo*, and that glucocorticoids are necessary but not sufficient to mediate the catabolic effects of metabolic acidosis".

1.4(ii). Normal humans

Compared to normal animals, surprisingly little is known about the MA/protein metabolism effect in normal humans.

Pre-term infants are born with a degree of MA. Paediatric researchers, aware of the MA/protein metabolism effect, have investigated the possibly 'pro-nutritional' effects of the correction of acidosis - many years before the resurgence of interest from nephrologists[7]. For the purposes of this review, pre-term infants will be considered 'normal' humans, born with less ability to control acid/base balance than infants born at term. In 1976, Sulyok investigated nitrogen balance and acid/base balance in 15 male pre-term infants with low birth weights, in the first six weeks of life, in an uncontrolled study (**66**). MA was most pronounced in the second and third week, thereafter it improved until the end of the study. During the first three weeks of life there was a significant positive correlation between MA, and nitrogen intake and urinary nitrogen excretion.

Also investigating pre-term infants, Zilleruelo et al, in 1986, studied the fractional excretion of bicarbonate in 10 similar children (**67**). In this uncontrolled study, all the infants were given sodium bicarbonate. After HCO_3 therapy, all infants corrected their negative base excess, and plasma HCO_3 increased significantly.

Kalhoff et al, in 1993, carried out the first prospective randomised controlled trial of the MA/protein metabolism effect in humans that used 'hard' endpoints (**68**). Fifty-two premature infants were randomised to receive oral alkali therapy with sodium bicarbonate or no therapy for seven days. Infants in the control group, without alkali therapy, gained weight less well than those in the treatment group. This is the first study that showed the beneficial effects, in terms of hard endpoints, of the better correction of MA in humans - albeit for a non-renal cause of MA. For this reason, it should be considered a 'landmark' study.

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[7]It seems unlikely that these three 'paediatric' researchers were (or are) aware of the 'early' literature in this field. Certainly, their studies do not cite any of the 'renal' studies.

Studies in adults are few. In 1992, using the leucine turnover technique, the effects of MA on protein metabolism in normal humans, were reported by Straumann et al (**69**) in acute acidosis, and by Reaich et al (**70**) in chronic acidosis.

In Straumann's study, an increase in protein degradation and oxidation was seen after only three hours of the onset of MA. Reaich et al investigated the effects of the induction of MA on protein and amino acid metabolism in seven healthy subjects, over a five day period. They discovered that MA induced an increase in protein degradation and synthesis, and amino acid oxidation. Plasma amino acid levels also increased.

All the human studies from the modern era so far described, have investigated the effects of MA on *muscle* proteins and the products of muscle breakdown. Few studies, however, has investigated the effects of MA on the metabolism of *non-muscle* protein, including albumin. In 1995, Ballmer et al showed that induction of MA in normal humans led to a *decrease* in albumin synthesis - ie, opposite to the effects of MA on muscle protein metabolism (**71**). Clearly, as with renal tissue, MA can affect different proteins in different ways.

1.4. Summary: effects of MA on protein and amino acid metabolism in the normal (non-uraemic) state

(1) MA increases protein and amino acid degradation, and probably protein synthesis. There is now ample evidence, from properly controlled prospective studies, that MA increases protein turnover in the non-uraemic state, probably by increasing protein degradation and oxidation, at least in the short-term. Its effects on protein synthesis are less certain. But MA probably increases protein synthesis, in humans at least. In animals MA has similar effects on amino acid breakdown.

Better correction of MA led to an increase in body weight in neonates in a controlled study by Kalhoff et al in 1993 (**68**). This should be considered a 'landmark' study.

(2) Specificity of effect - different effects on kidney and muscle. The effects of MA seem quite specific, both in terms of the tissue affected and the cause of the acidosis. For example, May's 1992 study (**57**) showed that *kidney* BCKAD activity in animals was decreased by MA. Furthermore, in the study of Throssell et al (1995, **63**) and others, it has been shown that MA induces renal hypertrophy in normal rats.

Thus, it appears that in the non-uraemic state, MA has opposite effects on muscle and renal tissue. For example, it causes an increase in protein degradation (causing muscle wasting) in muscle tissue, and a decrease in protein degradation (causing renal hypertrophy) in renal tissue.

In humans, Preedy's study indicated that *respiratory* acidosis decreased protein synthesis - ie, in the 'opposite' direction to MA (1988, **53**). Furthermore, Fuller's study (1989, **54**) suggests that cardiac as well as skeletal muscle is affected by MA, also in the opposite way. Ballmer's 1995 study indicated that the synthesis of a non-muscle protein was decreased by MA - ie, (again) opposite to the effects of MA on muscle metabolism (**71**).

(3) Mechanism of effect. The effect seems to be glucocorticoid dependent, possibly mediated by MA activating an ATP-dependent ubiquitin-proteasome proteolytic pathway.

Clearly, in the *non-uraemic* state, MA has profound effects on protein and amino acid metabolism. The literature concerning the influence of MA on protein metabolism in the *uraemic* state will be now be reviewed.

1.5. Effects of MA on protein and amino acid metabolism in the uraemic state

1.5(i). Uraemic animals

1.5(i).A. Effects of CRF alone on protein metabolism

The effects of CRF *per se* on protein metabolism in animals have been established since the early 1930's. In 1932, Chanutin and Ferris demonstrated impaired growth (and, by inference, abnormal muscle protein turnover) in rats with CRF (**72**). In 1969, Shear et al, using the leucine turnover technique, suggested that protein synthesis was decreased in rats with ARF (**73**).

The effects of CRF on protein metabolism were investigated in more detail by various workers in the 1980's. Li et al carried out a controlled study in 1981 (**74**) in which a comparison was made between the fed and fasted states in rats with CRF, without focusing on MA. Protein degradation was measured using urinary 3-methylhistidine excretion, in moderately uraemic and sham-operated control rats. The rats were studied in the fed state or after 24 and 48 hours of fasting. They showed, during fasting, that degradation rates increased in both sham and uraemic rats with a larger increase being seen in the uraemic group. During 48 hours of fasting, the uraemic animals lost more weight (17.1%) than the shams (13.2%). When fed, both uraemic and control rats gained weight at the same rate.

In another rat study, Flugel-Link (1982, **75**) came to a similar conclusion concerning protein degradation but a different conclusion to Shear et al (1969, **73**) concerning protein synthesis. Uraemia shown again to increase protein degradation but protein synthesis was unaffected.

Studies investigating the effect of CRF 'alone' on protein metabolism should be interpreted with caution. The uraemic state incorporates many metabolic abnormalities

known to affect protein metabolism: anorexia, muscle disuse through anaemia and uraemia, impaired glucose tolerance and growth hormone disturbance.

Precisely how each of these and other abnormalities affect protein metabolism in uraemia is beyond the scope of this review. They are summarised in an excellent review by Mitch and Clark in 1983 (**76**). Therefore, before effects on protein metabolism can be attributed to 'uraemia', methodology that controls for these factors must be developed. This has, so far, proven to be difficult in humans.

In summary, in animals, both uraemia and MA increase protein degradation. Their effects on protein synthesis are less clear.

1.5(i).B. Effects of MA and CRF on protein metabolism

Just as studies investigating CRF alone should be interpreted with caution, so should studies examining MA and CRF. This is because uraemia itself influences protein metabolism, and CRF is almost always associated with a degree of MA.

In their 1986 paper (**47**) described above, May et al showed that MA *per se* stimulated muscle protein degradation in normal rats, by a glucocorticoid-dependent mechanism. In 1987, May et al repeated this study using partially nephrectomised rats (**77**). These rats experienced a 90% increase in net protein degradation, and a 34% *lower* rate of protein synthesis. However, only the former was corrected by alkali.

Also in 1987, Hara et al investigated the influence of MA on branched-chain, amino acid metabolism in the muscles of both uraemic and non-uraemic rats (**58**). The result of the study can best be described by the title of the paper, "Acidosis, not azotemia, stimulates branched-chain, amino acid catabolism in uremic rats"; or, as they concluded:

> "... in CRF, chronic MA stimulates BCAA decarboxylation in skeletal muscle and this could contribute to the reduced intra- and extracellular concentrations of BCAA. Correction of acidosis should be a goal of therapy in CRF, especially when dietary regimens restrict the intake of BCAA"

This was an extremely good study; properly controlled for the influences of both uraemia *and* acidosis. Even so, at this stage in the history of this research area, the evidence for the conclusion of the second sentence of this quote was non-existent.

The effect of MA on protein metabolism during a low protein diet (LPD), has been investigated by various workers, both in animals (Gretz et al, 1990, **78**) and humans (Goodship et al, 1990 (**79**), and Williams et al, 1991 (**80**)). The human studies are described in Section 1.5(ii).B.

In 1990, Gretz et al carried out an animal study (**78**). The effect of MA on protein metabolism was assessed in 5/6-nephrectomised rats, given either a normal or low protein diet. The animals were then given additional acid or alkali. Food intake, body weight and urinary urea excretion declined during MA in both groups, but more in the group receiving the normal diet. This principle finding was summarised in the title of the study, "A LPD protects uremic rats against the negative sequelae of MA".

In 1992 and 1994, Maniar et al (**81, 82**) repeated May et al's 1987 study (**77**) and also found that protein degradation increased under the influence of MA in rats with CRF. Protein synthesis, however, stayed the same. This lack of effect of MA on protein synthesis contradicts May's findings' and most of the human studies described below.

Also in 1992, Maniar et al (**83**) investigated the *level* of MA that affects growth in CRF; using uraemic rats, made acidotic by receiving a 30% protein diet, separated into five groups by increasing severity of acidosis. They found a reduction in weight gain only with severe acidosis (with a pH around 7.20 or less); but a reduction in length gain with less severe degrees of acidosis.

The effect of better correction of MA on *renal* tissue and the progression of CRF has been investigated in uraemic animals. In 1985 Nath et al found that both proteinuria and tubulointerstitial injury were reduced by dietary supplementation with sodium bicarbonate (**84**). The findings of this study are contrary to those of a similar rat remnant model study, carried out by Throssell et al in 1995 (**63**). This study was

discussed in Section 1.4(i), on non-uraemic animals. As well as a group of normal rats, there was also a group of uraemic animals investigated. Rats who had undergone 5/6 nephrectomy were given sodium bicarbonate leading to an arterial pH of 7.27, or sodium chloride (pH 6.95). Proteinuria, GFR, histological injury and the time to terminal uraemia were the same in the two groups.

So, better correction of MA did not seem to affect albuminuria, contrary to the animal study by Nath, and the studies of the 'early literature' in humans. Why should there be such a discrepancy? The main difference between the studies of Throssell and Nath was the duration of the two studies. Nath's study lasted 6 weeks, whilst Throssell's was up to 14 weeks, and this was felt to be more analogous to the duration of CRF in humans. It should be pointed, however, out that both groups of rats in Throssell's study were markedly acidotic. How analogous either group is to patients with CRF/ESRF, who usually have a pH in the low normal range, is uncertain. For this reason, there must *still be* a possibility that better correction of MA (within the normal range) in humans with CRF could slow the progression of the disease. This remains to be proven.

1.5(ii). Uraemic humans

1.5(ii).A. Effects of CRF alone on protein metabolism

The literature on this subject is also summarised by Mitch and Clark in their review of 1983 (**76**). There has been considerably less work done on uraemic humans than animals.

In 1970, Coles et al demonstrated lower rates of albumin synthesis in uraemic humans compared to controls (**85**). In 1982, in an *in vitro* study, it was found that human uraemic serum decreased muscle protein synthesis (Cernacek et al, 1982, **86**).

Garibotto et al, in 1992, came to different conclusions. They investigated muscle protein turnover and amino acid metabolism in patients with CRF and normal humans,

using a 3H-Phenylalanine forearm perfusion technique (**87**). An increase in muscle protein breakdown *and synthesis* was found in the CRF patients compared to the controls. Furthermore, protein breakdown was inversely related to arterial bicarbonate. The release of total amino acids, glutamine and alanine was not different from controls; whereas the release of valine and leucine was reduced, with decreased serine uptake.

In 1994, Carr et al studied plasma amino acid profiles in 7 control subjects and 7 elderly patients with CRF (**88**). Uraemic patients had significantly reduced plasma levels of valine, tyrosine, phenylalanine, tryptophan and elevated histidine when compared to controls. No correlation between arterial pH or bicarbonate and plasma amino acid levels was found.

In summary, in humans, as with animals, uraemia and MA have been shown to have a similar effect on protein degradation (an increase). Again, their effects on protein synthesis are less clear.

1.5(ii).B. Effects of MA and CRF on protein metabolism

In 1984, Papadoyannakis et al (**89**) investigated nitrogen balance in six nondialysed uraemic patients. Each patient was investigated before and after supplementation with sodium bicarbonate and sodium chloride. There was a 36% decrease in blood urea during bicarbonate supplementation, with no improvement during sodium chloride administration:

> "indicating that the effect of bicarbonate was the result of the correction of MA and not of the expansion of the extracellular volume".

In the following year, Papadoyannakis et al, carried out a similar study on 11 HD patients (**90**); using calcium carbonate rather than sodium bicarbonate as the alkali. They found a decrease in protein catabolic rate (reflecting urea excretion) and increase in serum albumin after 8 weeks of calcium carbonate administration.

Jenkins et al (**91**), in 1989, carried out a similar study to that of Papadoyannakis in 1984 (**89**). Eleven non-dialysed patients with CRF, on low protein diets, were given sodium bicarbonate for 8 weeks. This resulted in a reduction in serum urea and uric acid levels.

In 1990, Bergstrom et al found a strong positive correlation between plasma bicarbonate levels and the free valine levels in the muscles of HD patients (**92**).

In 1991, Mochizuki et al (**93**) examined the effects of MA on amino acid and keto acid metabolism in fourteen patients with CRF on a low protein diet. Each patient was investigated before and after correction of MA with sodium bicarbonate. The correction of MA improved nitrogen balance and elevated plasma branched-chain amino acids, keto acids, glutamine and alanine concentrations.

The inter-relationship between MA and LPD in uraemic humans has also been investigated, with conflicting results. The 'normal' response to LPD in the non-uraemic humans involves a decrease in protein turnover, achieved by a small decrease in protein synthesis and a larger decrease in protein degradation (Motil et al, 1981, **94**). In 1990, Goodship et al showed that in humans with moderate CRF (creatinine approximately 500 μmol/L), the same adaptive responses to a LPD exist (**79**).

However, these results may not be representative of the response to LPD in more advanced CRF or ESRF, when MA is more severe. Therefore in 1991, Williams et al, investigated the effects of correcting MA in humans with CRF complicated by MA (**80**). Unlike Goodship's study (**79**), LPD led to an increase in protein degradation. Correction of acidosis led to a decrease in protein degradation to a level below that measured prior to protein restriction. The authors concluded:

> "... that MA can override the expected metabolic adaptive response to a LPD."

Thus, Williams' study (**80**) contradicted both the study of Gretz in the equivalent animal study (**78**), and that of Goodship (**79**).

In 1993, two studies on PD patients were carried out by Stein et al - one retrospective (**95**), one prospective (**96**) - examining the effects of better correction of MA on protein metabolism. In the prospective study (**96**), eleven PD patients, were given sodium bicarbonate for two weeks. A fall in urea production, as judged by the protein catabolic rate, was observed. In the retrospective project (**95**), a much larger group of PD patients were studied for one year; there was a significant improvement in their nutritional state, as judged by body weight and midarm circumference, when serum bicarbonate increased from 23.7 to 31.5 mmol/L[8].

In 1993, Reaich et al investigated the effects of correction of acidosis in nine non-dialysed patients with CRF, using the leucine turnover technique (**97**). Correction of acidosis decreased protein degradation (and synthesis, to a lesser degree) and protein oxidation. Plasma amino acid concentrations did not change.

In the same year, Lim et al (**98**) also using the leucine turnover technique, demonstrated that giving sodium bicarbonate to a small group of haemodialysis patients caused the opposite effect, ie an *increase* in protein synthesis. Other workers have not been able to duplicate these results. For example, in 1997 Graham et al (**99**) carried a similar study to that of Reaich et al on predialysis patients (1993, **97**), also using the leucine turnover technique - this time in haemodialysis patients. They showed correction of MA decreases both protein degradation *and synthesis* in haemodialysis patients.

In 1996, in another study by Graham et al, this time using PD patients (**100**), correction of acidosis decreased protein degradation and synthesis but had no effect on leucine oxidation. They also found no change in plasma amino acid concentrations.

[8]It was the findings of these two studies that led me to believe that a large scale prospective randomised controlled trial would have a chance of producing a positive result.

Garibotto et al (**101**), also in 1996, also investigated the effects of MA on muscle protein metabolism in patients with CRF, using the 3H-phenylalanine forearm perfusion method. Nine acidotic patients were compared to 4 patients with normal acid-base balance. In patients with MA, the rates of phenylalanine synthesis and degradation were increased as compared to controls.

In the same year, Roberts et al (**102**) examined the hypothesis that, if dietary protein intake was reduced in acidosis, that this might be a cause of the increased protein degradation, at least in part. In fact, they found that MA in CRF patients did not affect dietary protein intake, concluding therefore:

> "... and that dietary changes therefore do not contribute significantly to the changes in protein metabolism seen in acidosis".

Finally, in 1996, Bastani et al (**103**), in a retrospective study of 70 HD patients, found a correlation between serum bicarbonate levels and protein catabolic rate. As expected, the better the correction of MA, the lower was the urea excretion, and therefore the lower the protein catabolic rate.

Most of the studies, both human and animal, carried out so far in the non-uraemic state are scientifically valid. Conversely, many of the studies carried out in the uraemic state are of less good quality, especially the human studies. There are several areas of concern, which will now be discussed. Some of the difficulties are unavoidable.

Difficulties with studies performed in the uraemic state

(1) Short-term studies. Most of the human studies have been short-term studies, using 'soft' endpoints - such as urea (Papadoyannakis et al, 1985, **90**) or 3-methylhistidine (Williams et al, 1991, **80**) excretion, or more latterly, leucine turnover studies (Reaich et al, 1993, **97**) - as markers of protein degradation.

(2) Invalid assumptions. Certain assumptions are made by virtually all of the studies in the uraemic state. Most studies investigate the effects of MA (and not other causes of acidosis) on skeletal muscle protein metabolism. Since, skeletal muscle is the principle repository of protein in the body, it is assumed that the findings of these studies reflect whole body protein metabolism. This may not be true.

There is very little information in the literature about the influence of uraemic MA on the metabolism of other (non-muscle) proteins, such as the liver-produced secretory proteins. Furthermore, data on the influence of other forms of acidosis (eg, respiratory acidosis, diabetic ketoacidosis, and lactic acidosis) on protein turnover, is sparse.

Most dynamic studies of protein metabolism, in this and other areas of protein metabolism research, are carried out in the fasted state. It is assumed that changes in the fasted state reflect real life, ie the fed state. This assumption may be false.

(3) Non-specific endpoints. Of the various endpoints used, some (including urea and urinary 3-methylhistidine excretion) are only able to non-specifically reflect protein turnover. In many of these studies, the authors assume that the endpoint used reflects protein degradation.

Other techniques (including leucine turnover) are said to be able to distinguish protein degradation, from protein synthesis and oxidation. Even these more 'advanced' techniques are based on possibly false assumptions. For instance, it is assumed that protein synthesis, degradation and oxidation are truly independent.

(4) Lack of proper control groups. The lack of control groups occurs at three levels:

a) Few incorporate control groups for the state of uraemia. In other words, in most studies in which uraemic animals or humans are made acidotic (or have their MA corrected), two further control groups of non-uraemic animals or humans are *not* investigated at the same time.

b) Most human studies are based solely on the reversal of MA, with little or no information obtained on the induction of MA in the uraemic state; though ethical approval of such a human study would be hard to obtain.

c) Worse still, some studies are completely uncontrolled - ie, they include neither animals nor patients that did not have MA induced or corrected.

(5) Difficulties in separation of effects of MA and CRF. These difficulties are intrinsic to the disease process and are unavoidable. They relate to the complexity of the state of 'uraemia', and the effects of uraemia *per se* on protein metabolism. In the non-uraemic state, it is quite easy to prove 'cause and effect' as it is easier to alter one factor, ie acidosis. Conversely, CRF is associated with other many other metabolic abnormalities that affect protein metabolism.

1.5. Summary: effects of MA on protein and amino acid metabolism in the uraemic state

(1) MA increases protein and amino acid degradation, and probably synthesis. Despite the difficulties outlines above, much has been learnt about the effect of MA on protein metabolism in the uraemic state. As in the non-uraemic state, MA somehow causes or facilitates protein and amino acid breakdown, in humans and animals with renal failure. As in the non-uraemic state, its effects on protein synthesis, are less clear. However, there is now growing evidence, in humans at least that MA in CRF also increases protein synthesis and oxidation, but to a lesser extent than the increase in protein degradation. The addition of alkali somehow reverses all these effects.

(2) Effect of MA on albuminuria and progression of CRF. The effect of better long-term correction of MA on albuminuria and the progression of CRF is uncertain in uraemic animals and unknown in humans.

(3) MA and CRF have similar effects on protein metabolism. CRF *per se* increases protein degradation. The effects of CRF on protein synthesis are less certain. In other words, comparing the 'CRF alone' to the 'MA and CRF' studies, it appears that MA and CRF have similar effects on protein metabolism. The relative importance of their influences is harder to deduce. From the published literature, however, it would appear that the effects of MA are *over and above* those of CRF alone. It is difficult to say whether the influences of each are synergistic or additive.

CRF, being associated with chronic MA, is therefore only a model in which this effect can be investigated in humans. It is probably a very good model, as the muscles may already be in a 'vulnerable' state (ie, already near to catabolism). Their 'vulnerability' might be partly due to muscle disuse (due to the tiredness and general malaise associated with CRF, and its associated anaemia); and partly, because of the non-

prescribed 'LPD' (induced by anorexia and vomiting), especially if Williams et al are right - ie, MA can override the influence of a LPD (1991, **80**).

1.6. Cellular studies

Relatively few studies have been carried out concerning the effects of acidosis on cells.

Proximal tubular cells have been shown to hypertrophy in response to ammonia by Golchini et al in 1989 (**104**) and to low pH by Bevington et al in 1994 (**105**). Rabkin et al (**106**), in 1996, studied protein turnover in proximal renal tubular cells isolated from rats with ammonium chloride-induced MA. They found that in chronic acidosis the normal balance in protein turnover was altered, due to decreased protein degradation and increased protein synthesis. These findings in cellular studies, support the animal studies carried out by Lotspeich et al (1965, **64**) and Throssell et al (1995, **63**), in which MA induced renal hypertrophy in normal rats.

England et al (**107**) in 1992, investigated mechanisms of pH-induced changes in protein metabolism, using BC3Hl myocytes and LLC-PK1 renal epithelial cells. Low pH increased protein degradation in myocytes, but had no effect on the LLC-PK1 cells. Exogenous glucocorticoids did not alter protein degradation in either cell line, but inhibited protein synthesis in the myocytes. They concluded that,

> "Since extracellular pH stimulates PD (protein degradation) only in BC3Hl myocytes and since LLC-PK1 cells may not possess GC (glucocorticoid) receptors, we can compare and contrast the effects of pH and GC on protein metabolism to study the role of GC in acid-stimulated proteolysis".

Whether this is true or not, again a difference in the effects of MA on different cells was demonstrated. The previously discussed animal study by Fuller et al (1989, **54**) had a different conclusion to this cellular study, ie MA *decreased* protein breakdown on the hearts of normal rats. Also in 1992, Jurkovitz (**108**), published a very similar study to that of England et al (1992, **107**), with similar conclusions.

In another part of the study by Cupisti et al (1993, **62**) described in the section on normal animals, the protein:DNA ratio in gastrocnemius muscle cells from acidotic and control rats was compared. There was evidence of impaired growth in the skeletal muscle cells from the acidotic animals.

In 1996, Isozaki et al (**109**) evaluated the importance of glucocorticoids in acidosis-associated proteolysis, by measuring protein degradation in BC3H1 myocytes in a variety of conditions. They found that acidification alone increased ubiquitin-proteasome complex mRNA expression, but both acidosis and glucocorticoids were required to stimulate protein degradation,

> "Since these changes occur without adding cytokines or other hormones,
>
> we conclude that the proteolytic response to acidification requires glucocorticoids".

1.6. Summary: cellular studies

The few cellular studies carried out have added relatively little to our understanding of the effect of MA on protein metabolism.

What studies have been done, show that MA has different effects on different cells. The cellular studies have largely confirmed what was known from animal and human studies. In other words, MA has opposite effects on muscle and renal tissue; causing an increase in protein degradation (and therefore muscle wasting), in cardiac and non-cardiac muscle cells; and a decrease in protein degradation (and therefore renal hypertrophy) in kidney cells.

There no seems little doubt that the MA/protein metabolism effect exists. Less clear is the mechanism behind the effect.

1.7. Possible mechanisms of the MA/protein metabolism effect - the Ubiquitin-Proteasome (U-P) Pathway

In mammalian cells all proteins are continuously degraded to constituent amino acids, and rebuilt from amino acids.

About 280 g of protein are synthesised and degraded each day in a normal 70 kg adult human; most of these proteins are intracellular. This amount is 200 g greater than the average intake (about 80 g per day), emphasising the large contribution made by the dynamic state of the body proteins to the free amino acid pool. It is also large when compared to the size of the amino acid pool, approximately 100 g. The size of this pool is small compared to the 10 kg of protein present in an average 70 kg adult.

The turnover is high in infancy and decreases with age. The *amount* of protein turnover varies between organs. Skeletal muscle, which accounts for 50% of the body's protein content (and is therefore the body's main repository for protein) is responsible for about 25% of the turnover. Whereas the gut and liver, which account for 10% of the protein, contribute 50% of the turnover; emphasising the key metabolic role played by the gut and liver in terms of protein synthesis and degradation.

The *rate* of protein turnover is also different in different organs. Rat hepatocytes are replaced every few days; but muscle or brain cells are replaced every one to two weeks. At the other end of the spectrum is haemoglobin which is stable for the 120 days of the life of an erythrocyte. Furthermore, different proteins are degraded in different parts of the cell (cytosol, nucleus or organelle), by different proteolytic mechanisms, at widely different rates.

The continual destruction of cell proteins may appear to be wasteful, but it serves several important homeostatic functions. The rapid removal of rate-limiting enzymes and regulatory proteins is essential for the control of growth and metabolism. Selective protein degradation is almost always a component of regulatory mechanisms that involve timing controls, such as circadian rhythms. Mistargeting of essential proteins or failure to degrade other proteins at essential times would wreak havoc within the cell.

Protein breakdown also provides quality-control by selectively eliminating abnormally folded proteins, that may have arisen through mutation.

Finally - and of relevance for discussions of MA in renal failure - in the first few days of inadequate intake or disease (which often leads to the former), cell protein breakdown increases, especially in muscle. This provides amino acids for hepatic gluconeogenesis and some (especially leucine, isoleucine and valine) for energy. After that, protein breakdown decreases and fatty acids from adipose tissue become the chief source of energy, reducing the need for gluconeogenesis. If an animal is to tolerate periods of fast or disease, clearly having a proteolytic system that is adaptable and can respond *quickly* to new demands, will have survival advantages.

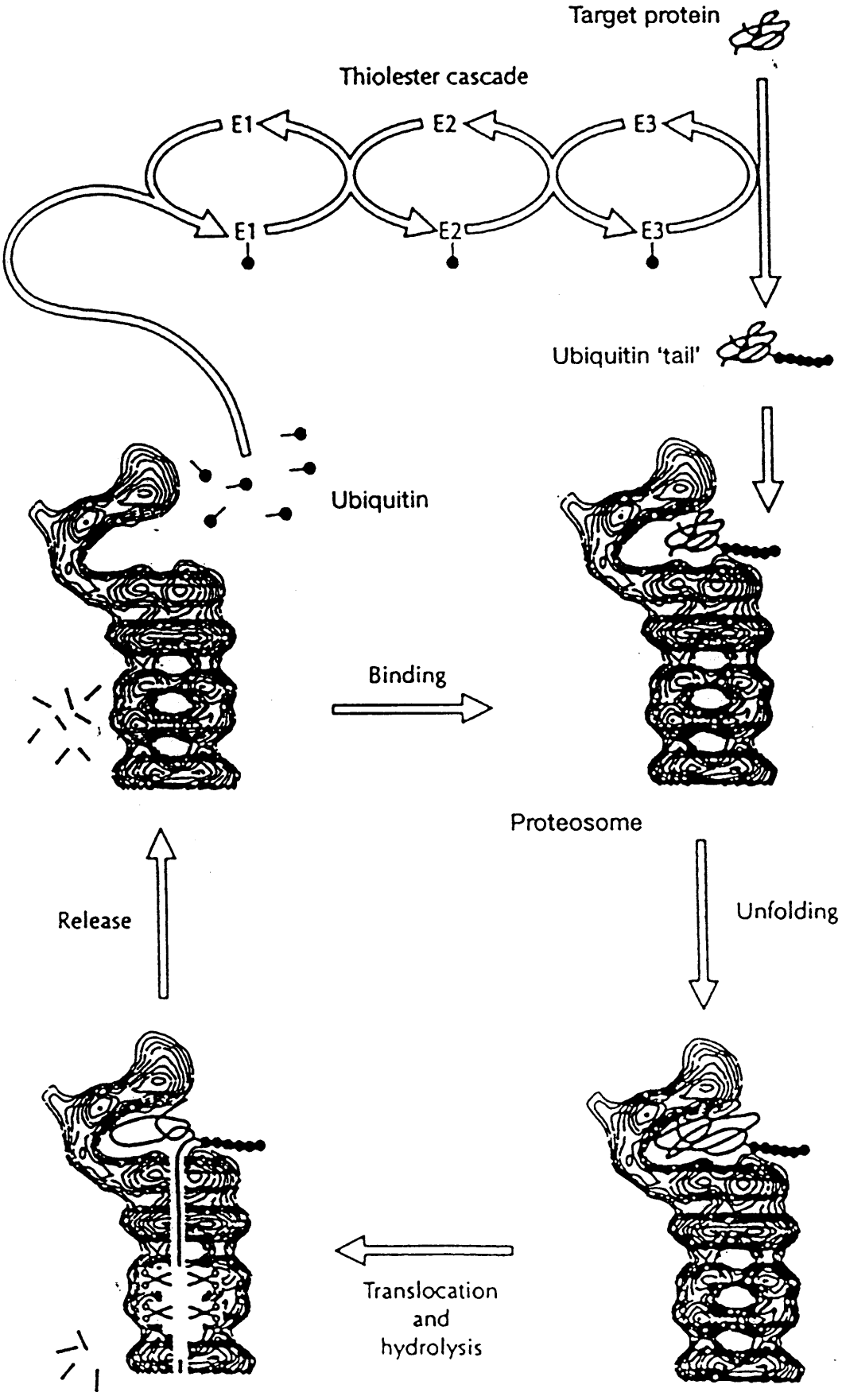
As has been emphasised, protein is in a dynamic state, being continuously formed and undergoing proteolysis. Some proteolysis is performed by cytosolic calcium-activated proteases. However, much of it takes place in the lysosome, where many extracellular proteins (eg plasma proteins, lipoproteins and hormones), phagocytosed bacteria and some intracellular proteins are degraded. But lysosomal proteolysis is not quantitatively important in the normal turnover of most intracellular proteins, or in accelerated breakdown of muscle proteins in catabolic states.

Eukaryotic cells have evolved another system for carrying out protein breakdown, one that is ATP dependent. At the heart of this process is a small (8.5 kD) 76-amino acid cytosolic protein, *ubiquitin*. Its intracellular concentration is very high, and it seems to play a vital role in the 'tagging' of proteins for destruction. Ubiquitin is one of the most highly conserved proteins in evolution: yeast and human ubiquitin differ at only 3 out of 76 residues. There is only one amino acid difference between the ubiquitin found in humans and that of the nematode worm - two taxa believed to have diverged 1.1 billion years ago. That ubiquitin is so highly conserved through evolution implies: (1) that small mutations in the system are lethal 'because' (2) it is a fundamental biochemical system in all eukaryocytic cells.

Ubiquitin 'tags' several key proteins that control the cell cycle, and major histocompatibility class (MHC) I antigens. Three enzymes (E_1, E_2 and E_3) participate in

the conjugation of ubiquitin to proteins. They sequentially attach themselves to the ubiquitin protein, thereby activating it. The final protein in this system is able to attach multiple molecules of ubiquitin to make a 'ubiquitin tail'. This long molecule can attach itself to proteins, such as partially denatured proteins, marking (or 'tagging') them as 'ready for destruction'. Proteins with a ubiquitin tail are 'engulfed' into a large proteolytic complex, the *26S proteasome*, also present in the cytosol - and ATP-dependent hydrolysis of the protein follows. The 26S proteasome is a barrel-shaped structure, which tagged proteins pass through, being unfolded and progressively cut into smaller peptides (of 6-12 amino acids); until they are degraded completely into individual amino acids, which are recycled. The proteasome spares the polyubiquitin molecule, which is then hydrolysed into monomeric units that are also recycled.

The U-P pathway is summarised in the following diagram, Figure 2:



What determines whether a protein becomes ubiquitinylated? One signal turns out to be surprisingly simple. The half-life of a protein is largely determined by its amino (N)-terminal residue. For example, a protein with a methionone at its N-terminus typically has a half-life of more than 20 hours. With arginine at this position it has a half-life of 2 minutes. The E_3 enzyme, as part of the activated ubiquitin molecule, is the reader of the N-terminal residues. Whether N-terminal residues are stabilising or destabilising is similar for bacteria, yeast and mammals; supporting the very early (and presumably therefore, successful) evolutionary role of this system.

As with all homeostatic systems, a 'feedback' mechanism exists. To prevent uncontrolled protein degradation via the U-P pathway, complex pathways of deubiquitination are in place; by which U-P complexes are deconjugated or ubiquitin itself is degraded. A discussion of deubiquitination is beyond the scope of this Introduction. It is summarised in a review article by Hochstrasser, published in 1995 (**110**).

How is this important for the MA/protein metabolism effect, and patients with CRF? In response to acidosis, and other catabolic stimuli such as infection or certain tumours, there is a preferential loss of protein from skeletal muscle and probably skin. But visceral organs (eg, kidneys and liver) lose little or no protein, and the brain is unaffected. In rats in the fasting state, and in those with diabetes or tumours, protein synthesis also decreases, contributing to muscle wasting. The muscle wasting that occurs in catabolic stress results largely from accelerated breakdown of normally long-lived myofibrillar proteins (actin and myosin) that make up 60-70% of skeletal muscle protein. Unlike other proteins, actin and myosin contain a unique amino acid, 3-methylhistidine, produced by the post-translational modification of histidine. The breakdown of myofibrillar proteins can be monitored by measuring the release of 3-methylhistidine; this technique has been used successfully by workers investigating the MA/protein metabolism effect (eg, Williams et al, 1991, **56**).

The U-P pathway has recently been recognised, using studies of isolated rat muscle, as responsible for the muscle wasting associated with various catabolic states -

including fasting (Wing et al, 1993, **111**), diabetes (Price et al, 1996, **112**) and acidosis (Mitch et al, 1994, **60**). The following table lists the conditions that are now known to alter muscle-protein degradation through the U-P pathway (Mitch et al, 1996, **113**):

Rats	**Humans**
increased protein degradation	
Fasting	Eating disorders
MA	Renal tubular defects
Renal failure	ARF and CRF
Muscle denervation	Neuromuscular disease
	Immobility
Diabetes	Diabetes
Thermal injury	Burns
Endotoxaemia	Sepsis
Tumour implantation	Cancer
Glucocorticoid treatment	Cushing's Syndrome
Hyperthyroidism	
decreased protein degradation	
Dietary protein deficiency	Low protein diet
	Malnutrition
Hypothyroidism	Hypothyroidism
	Hypopituitarism

This list, unsurprisingly, looks like the list of the causes of increased or decreased protein degradation in older review articles (Mitch et al, 1983, **76**). Even though these and other diseases have been associated with dysfunction of the U-P system, whether it is as cause or effect is less clear. For example, 'spheroid' bodies containing precipitates of proteins 'tagged' with ubiquitin have been shown to accumulate in the cell bodies and the proximal axons of the motor neurones of patients with motor neurone disease.

A significant absence from the 'human' list on the right is metabolic acidosis. Nonetheless data on the involvement of the U-P pathway in rats with MA is accumulating. For example, *normal* rats with experimentally induced MA grow poorly and lose muscle protein through a non-lysosomal pathway that requires ATP (May et al, 1986, **47**). MA also causes an increase in the levels of mRNA encoding ubiquitin and two subunits of the 20S proteasome (the core of the 26S proteasome), despite a sharp reduction in muscle RNA (Mitch et al, 1994, **60**). Similarly, in *uraemic* rats with MA,

the correction of the acidosis not only reverses the proteolysis but also prevents the increase in mRNA encoding ubiquitin and proteasome subunits (Bailey et al, 1996, **114**).

Factors that activate MA associated changes in protein degradation via the U-P pathway are also becoming known. Again, these factors are the ones that were known to increase protein degradation, before the importance of this pathway was recognised. In studies with rats that have undergone adrenalectomy and then been maintained in the fasting state or given ammonium chloride to induce acidosis, muscle proteolysis does not increase unless glucocorticoids are administered (May et al, 1986, **47**). Glucocorticoids are required, together with other signals, for the increase in mRNA encoding ubiquitin and proteasome subunits and in muscles from rats in the fasting state (Wing et al, 1993, **111**).

Many of the physiological responses to sepsis, cancer and burns result from the release of tumour necrosis factor, and interleukins from activated macrophages and endothelial cells. Together with glucocorticoids, these cytokines are now known to stimulate the U-P pathway in muscle tissue in various catabolic states (Costelli et al, 1993 (**115**), and Zamir et al, 1994 (**116**)). In other words, the *mechanism* by which glucocorticoids and other factors stimulate protein degradation in a variety of catabolic states (including MA) is now known to be the U-P pathway, in animals at least.

Unfortunately there is no evidence that MA stimulates protein degradation via the U-P pathway in humans with CRF. On the contrary, there is now evidence *against* its involvement in humans. In 1996, Roberts et al showed that pH alone had no influence on ubiquitin mRNA expression in either the L6 rat myoblast line or human skeletal muscle cells (**117**). In 1997 Roberts et al (personal communication) investigated ubiquitin mRNA expression in the muscle biopsies of CRF patients, before and after one month or oral bicarbonate. No significant changes in gene expression were observed.

Why might there be this discrepancy between normal/uraemic rats with MA and humans with CRF? Three possible explanations should be considered:

(1) Inappropriate animal model. Most of the animal experiments described in this section of the Introduction involve the 5/6 nephrectomy model of 'CRF' and/or the induction of MA via ammonium chloride. This 'CRF' model rapidly induces severe renal failure, with all its metabolic and catabolic effects. In this way, it is not really analogous to the stable patient with CRF (or ESRF on dialysis), in whom various compensatory mechanisms have had time to respond to (and control) such catabolic influences - including the compensatory mechanisms to MA.

The model may be more analogous to the ARF patient, in whom catabolism induced by sepsis, starvation and hypoxia almost always accompanies uraemia and acidosis. Furthermore, induction of acidosis by ammonium chloride often leaves the animal profoundly acidotic, with a pH value of 7.1 or less. Patients with CRF (or ESRF on dialysis) are not profoundly acidotic, and usually have a pH in the low normal range. ARF patients can (and do) become this acidotic, but again the acidosis is usually accompanied by multiple other catabolic stimuli.

As previously suggested, even if there was evidence for the involvement of the U-P system in the MA/protein metabolism effect in humans with CRF, it is unclear whether it would be as a 'cause' or 'effect' of muscle wasting (which could be due to other factors).

(2) An underestimation of the complexity of the causes of muscle wasting in catabolic diseases may have happened. Insights into the relative importance of the U-P and other proteolytic systems have been gained by Attaix and co-workers (**118, 119**) in France, in the last few years. This group have shown, in a rat model of chronic sepsis, that lysosomal and calcium-activated proteolytic pathways as well as the U-P system, are responsible for muscle wasting (Voisin et al, 1996, **118**). Furthermore, they have found similar results in humans with head trauma (Mansoor et al, 1996, **119**). Their data supports a role for multiple proteolytic pathways in situations where increased

muscle proteolysis occurs. Clearly, the relative importance of the U-P pathway in the acidosis of CRF remains to be determined.

(3) Proteolytic system 'blocking' experiments have so far been employed, especially by Mitch and workers, to investigate the U-P pathway. In other words, to investigate the U-P pathway, other proteolytic systems (lysosomal and calcium-activated, for example) are usually inhibited during the experiment. If the protein degradation system is more complex than currently thought, and different proteolytic systems are involved (and indeed interact), then blocking any one pathway may have important consequences on the other two. More subtle investigative tools may have to be developed to fully investigate the role of the U-P pathway in the MA/protein metabolism effect.

1.7. Summary: possible mechanisms of the MA/protein metabolism effect

(1) The Ubiquitin-Proteasome pathway has recently been recognised, in animals. as responsible for the muscle wasting associated with various catabolic states - including fasting, diabetes and MA.

(2) Glucocorticoids are required, together with other signals, for the increase in activity of the U-P pathway.

(3) In humans with the MA of CRF, the role of the U-P pathway is unclear.

(4) More subtle investigative tools may have to be developed to fully investigate the possible role of the U-P pathway in the MA/protein metabolism effect in CRF.

1.8. Overall summary of the literature

The following table summarises the studies presented in sections 1.3 to 1.6, on the effects of MA (and CRF) on (muscle) protein metabolism.

Effects of MA and CRF on (muscle) protein metabolism

	CRF				MA			
	PS	PD	PO	PT	PS	PD	PO	PT
Normal								
Animals					→ (47) ↑ (57)	↑ (47) (57) (65)	↑ (55) (57) (65)	↑ (46)
Humans					↑ (70)	↑ (69) (70)	↑ (69) (70)	↑ (66)
Uraemia								
	CRF				MA			
	PS	PD	PO	PT	PS	PD	PO	PT
Animals	↑ (74) → (75) ↓ (73)	↑ (72) (75)			↓ (77) → (81, 82)	↑ (77) (81, 82)		
Humans	↑ (87) ↓ (86)	↑ (87)			↑ (97) (100) (101) (69) ↓ (98)	↑ (97) (100) (70) (69)	↑ (97)	↑ (89) (90) (91) (93) (96) → (100)

Key
PS = protein synthesis, PD = protein degradation, PO = protein oxidation
PT = protein turnover (where endpoint used could not distinguish PS, PD and PO)
↑ = increase; ↓ = decrease; → = no change

1.9. Summary of Introduction

(1) Role of ammonia. The dominant mechanism, in mild-to-moderate renal failure, by which acid is eliminated is by the increased excretion of ammonia. In severe renal failure hydrogen ion excretion decreases, mainly due to an inability to excrete ammonia - with the development of metabolic acidosis.

(2) MA causes protein degradation. MA in the non-uraemic and uraemic states, in some way, increases muscle (and non-muscle) protein and amino acid degradation; and, may increase, to a lesser degree, muscle protein synthesis (in humans at least).

If the 'trade-off hypothesis' is correct, the effect may be 'beneficial' in the non-uraemic state. But, in CRF, protein degradation may be counterproductive, causing increased and 'wasteful' acid-cycling - ie, acidosis stimulates protein degradation which leads to additional acid generation, which in turn, stimulates further protein degradation.

(3) Significance of uraemia. The similarity of the influence of MA in the two states leads to a simple conclusion - ie, the state of uraemia has *little to do with* the MA/protein metabolism effect. CRF and MA have similar influences on protein metabolism. Therefore separating out their individual effects is difficult.

(4) Specificity of effect. MA has different effects on different tissues. The effect is quite specific, in terms of the cause of acidosis, the tissues and cells affected; and perhaps, the species in which it occurs.

(5) Mechanism. There is growing evidence, in short-term studies of severe acidosis in animals at least, that increased activity of the ubiquitin-proteasome pathway may be responsible for the increased protein degradation associated with MA. This mechanism

seems to be glucocorticoid dependent. Whether this has any long-term relevance for humans with the mild MA associated with CRF/ESRF is less clear.

1.10. Aim of this study

Hypothesis. Better correction of MA improves the nutritional state of patients with CRF/ESRF.

This section will be divided into four sub-sections: a discussion of the hypothesis, acidosis correction in PD, the reasons for using PD patients in this study, and the limitations of the study.

(1) The hypothesis. The clinical significance of short-term studies using soft endpoints is uncertain. Whether this effect, or more particularly the reversal of this effect, has any clinical relevance for patients with CRF/ESRF in the long-term is unknown. Indeed, whether the short-term influence of MA on soft endpoints, such as plasma urea or urea excretion, would be seen at all in the long-term is unknown.

Clearly, *if* MA is a *significant* factor in the protein malnutrition of renal failure, reversing it might lead to an improvement in the nutritional state of ESRF patients. Taken further, if malnutrition is a factor in the high morbidity and mortality of ESRF, improving nutrition may be of benefit.

Clearly, the important question is ... "does improved correction of acidosis improve the nutritional state of patients with CRF/ESRF in the long-term*?*". *The aim of this study was to answer this question.* A large randomised controlled trial - comparing two dialysate/drug regimes designed to correct MA to varying degrees - in PD patients, in the first year of treatment was therefore carried out.

(2) Acidosis correction in PD. As previously mentioned, the kidney not only excretes H^+ ions (bound to ammonia and phosphate), but reabsorbs bicarbonate. It is therefore necessary, in all forms of dialysis, to replace bicarbonate as well as absorb and remove H^+ ions. These are the basic requirements for an effective dialysis buffer.

In the 1960's acetate was used as the buffer in PD. Its use was abandoned when it was suspected that acetate was responsible for peritoneal ultrafiltration failure (Faller et al, 1984, **120**) and sclerosing peritonitis (Slingmeyer et al, 1984, **121**).

Lactate is the buffer currently used in peritoneal dialysis. In nature, two stereoisomers of lactate exist, D-lactate and L-lactate. Commercially available PD fluids contain either L-lactate or a mixture of the two forms.

When inside the peritoneum, both forms of lactate are converted to pyruvate, and both forms are excreted by the liver. Lactate, after conversion to pyruvate, is either oxidised (80-85%) or converted to glucose (15-20%). Both pathways consume H^+ ions and generate bicarbonate. Blood lactate levels are slightly higher than in normal subjects, but no different from predialysis patients (Heaton et al, 1985, **122**). The levels stay constant through the day.

The use of mixed solutions is not physiological because D-lactate is not a product of human metabolism (Brin et al, 1965, **123**). Furthermore, the absorption of D-lactate is slower than L-lactate, and it is metabolised to pyruvate by D-2 hydroxyacid-dehydrogenase. L-lactate is easily metabolised to pyruvate by lactic dehydrogenase. Patients with blind loop syndrome and abnormal gut flora, who produce sufficient D-isomer to elevate blood D-lactate to greater than 3 mmol/L, can develop encephalopathy (Oh et al, 1979, **124**).

Commercially available PD solutions contain 35 or 40 mmol/L lactate. That many units still use 35 mmol/L lactate is surprising, as it is well established that this concentration of lactate inadequately corrects acidosis. In 1983, Nolph et al reported a mean venous TCO_2 of 23.8 mmol/L in 78 patients (163 measurements) using 35 mmol/L (**125**). 38% of the values were below 22 mmol/L.

What is more surprising is that in studies of 40 mmol/L lactate, this higher concentration of lactate corrects acidosis no better than the 35 mmol/L solution. In 1992 Yamamoto et al (**126**) found a mean arterial bicarbonate of 21.6 mmol/L in 8 stable patients on 40 mmol/L lactate dialysate.

(3) Reasons for using PD patients in this study. This study could have been performed in the other groups of patients with ESRF: predialysis, haemodialysis and transplant. Why were PD patients chosen?

Predialysis patients could have been used as a model to test the above hypothesis. For example, a simple double-blind study of oral sodium bicarbonate versus sodium chloride would have been possible, and simple. But, as protein malnutrition seems to become a clinical problem in *end-stage* renal failure, it was felt that predialysis patients with CRF would not be an appropriate model. Furthermore, the possibility of either (or both) of these sodium salts precipitating fluid overload was a concern, without the ability to remove fluid through dialysis. An extra variable, the 'natural' decline of in the predialysis period, would also have had to be incorporated into the analysis.

This should not have been too great a problem, as the progression of CRF should have been similar in the two groups[9].

On the other hand, using this patient group would have had advantages. Such a study may have been able to test the hypothesis above. It may have been simpler to co-ordinate. Being double-blind, it may have been more convincing. It may also have been able to answer the question of whether better correction of MA could slow the progression of CRF, or even delay dialysis. This possibility is discussed in more detail in Section 4.10. of the Discussion.

Haemodialysis patients would have been the major alternative patient group. The two groups could have been randomised to different levels of acidosis correction by varying the bicarbonate concentration of the dialysate. However, it would have been much harder to 'blind' the patients. Being dialysed by so many different nurses, they would have been more able to find out which group they were in, and why; and that they were in a nutritional study.

The correction of MA in haemodialysis is obviously not stable, with venous bicarbonate following a 'saw-tooth' pattern. It is also uncertain how cytokine release during haemodialysis sessions would have affected any nutritional benefit gained. Furthermore, it would have been more difficult to describe the 'acidosis experience' of haemodialysis patients. Would the pre or post-dialysis bicarbonate level have been more relevant? Should an average bicarbonate level have been used? If so, how should it have been calculated?

In some ways, however, haemodialysis patients would have been a 'better' group to study. In the United Kingdom, they are usually a 'less well' patient group. Hence they might have been more malnourished than the PD patients used, with more to gain from better correction of acidosis. Furthermore, the correction of acidosis in haemodialysis may be less effective than in PD. Even with a 38 mmol/L acetate dialysate, predialysis bicarbonate is usually in the 16-20 mmol/L range (Gennari et al, 1990, **127**). So, again, an actively treated group might have had 'more to gain'. They would have been

[9]In fact, in the study carried out, this variable did have to be incorporated into the analysis, as residual

logistically easier to study, as the majority of haemodialysis patients in Leicester receive their treatment within the renal unit.

Transplant patients could have been used for this study; especially suitable would have been those with failing grafts. As they are a 'selected group' of the fitter patients, it was felt that they would be least likely to show evidence of significant protein malnutrition.

All the advantages and disadvantages of the above alternative patient groups were considered before the final protocol involving PD patients was devised. In the end, it was felt that PD patients were best group to study, for three reasons:

a) they are relevant to the clinical problem;

b) their acidosis correction is stable;

c) they were easiest to 'blind' from the knowledge of which treatment group they were in.

(4) Limitations of the study. When the study was designed in early 1991, a statistical power analysis was performed, based on a predicted 2kg difference in weight gain in the two groups at one year (if the study was positive). The "2kg" figure was a calculated guess, based on an earlier retrospective study (1993, (95)). This power analysis suggested that 200 patients should be studied - if two groups of patients were to be compared, ie 100 in each group.

It was clear that the study would *not* have the statistical power to show a benefit in terms of mortality, even if a positive effect was seen - ie, better correction of MA *did* improve nutrition. It was hoped if there was that a positive effect on nutrition, it would also show itself in one or more of the morbidity markers. At the time, this was far from certain. There was no basis, looking at the published literature, to think that improving nutrition would have a 'knock-on' effect on morbidity.

Neither was this a mechanism study. The mechanism of the MA/protein metabolism effect was, and is, unknown. Nonetheless, as part of the protocol, cortisol was

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renal function declined in the first year of dialysis.

measured and kinetic modelling data was obtained. Perhaps, if the effect was confirmed in the long-term, some marker of protein metabolism would be altered.

CHAPTER TWO

A surfeit of the sweetest things
The deepest loathing to the stomach brings

A Midsummer Night's Dream II, 2
Lysander to Hermia, asleep

CHAPTER TWO

METHODS

Note: The core of this chapter, and the results and discussion that follow will follow a similar 7-part structure:

Matching

Haematology

Acid/base balance

Nutrition

Dialysis dose / kinetic modelling data

Renal bone disease

Outcomes

2.1. Introduction: basic protocol

Permission for the study was granted by the Ethical Committee of Leicestershire Health Authority. Consent was obtained from 200 consecutive new PD who were randomised, in a single-blind fashion, to receive a high alkali (HA; lactate 40 mmol/L) or low alkali (LA; lactate 35 mmol/L) dialysate, and studied for one year. The HA dialysate contained less calcium than the LA dialysate (HA: ionised calcium concentration 1.25 mmol/L vs LA: 1.75 mmol/L). The contents of the two dialysis fluid are as follows:

Constituents (in mmol/L) of two dialysis fluids used in study

	LA (control) gp	HA (treatment) gp
Lactate	35	40
Calcium	1.75	1.25
Magnesium	0.75	0.25
Chloride	102	95
Sodium	132	132

All patients were treated by PD from the start of renal replacement therapy, and were recruited between December 1992 and December 1994.

Baseline (time 0) was defined as the day before PD catheter insertion (or the day before the first haemodialysis, if appropriate). Patients were assessed at 0, 1, 3, 6, 9 and 12 months. The 'key' assessment times were 0 or 1 month (depending on the parameter), 6 months and 12 months. Most of the data that will be presented in the results section come from those time points. At the majority of outpatient appointments, the patients were seen by one physician (AS).

Six 'key' endpoints were decided upon before the start of the study: four nutritional (body weight, midarm circumference, triceps skin-fold thickness and serum albumin), and two morbidity parameters (number of admissions and days spent in hospital). The following page summarises the basic protocol.

Summary of basic protocol

100 HA dialysate

200 new PD pts

100 LA dialysate

Months	**0**	**1**	**3**	**6**	**9**	**12**
Matching						
Demography	+					
Causes ESRF	+					
Co-morbidity	+					
Haematology						
Haemoglobin	+	+	+	+	+	+
EPO use						+
Acid/base balance						
Biochemistry	+	+	+	+	+	+
Serum bicarbonate	+	+	+	+	+	+
Arterial blood gases						+
Nutrition						
Body weight	+	+	+	+	+	+
Midarm circ		+		+		+
Triceps skinfold		+		+		+
Serum albumin	+	+	+	+	+	+
Muscle strength		+		+		+
Protein intake						+
Blood pressure		+		+		+
Cortisol		+				+
Dialysis						
Dialysis dose						+
Kinetic modelling		+		+		+
Renal Bone Disease						
Bone biochemistry	+	+	+	+	+	+
Parathyroid hormone		+		+		+
Aluminium		+		+		+
Hypercalcaemia						+
Outcomes						
Renal modality						+
Hospitalisation						+
Peritonitis/exit site infections						+
Death/technique failure						+
Months	**0**	**1**	**3**	**6**	**9**	**12**

2.2. Matching

The two groups were compared in terms of demographic data, causes of renal failure, biochemistry and haemoglobin, nutritional state and bone biochemistry.

In terms of the demographic data, a patient was said to have ischaemic heart disease if they could give a definite history of angina or myocardial infarction. Diabetes mellitus was recorded, even if it was not the cause of the patients' renal failure. Data was recorded at baseline or at one month (indicated in results section).

At baseline, a comorbidity score (maximum = 7) was calculated for each patient. One 'point' was scored for the presence of diabetes mellitus ischaemic heart disease, cerebrovascular disease, peripheral vascular disease, serious malignancy, left ventricular failure and any other serious comorbid disease. This score has no proven validity. It was created as there is no internationally accepted way of describing the co-morbidity of an ESRF patient.

2.3. Haematology

A target haemoglobin of >11 g/dl was set for both groups. Where possible erythropoeitin (EPO) alone was used to raise the haemoglobin to the target level. Most patients were started on 4000 units subcutaneously twice per week. When the target haemoglobin was achieved, this was cut back to 4000 units once per week. Blood transfusion, if required, was used in some patients.

The haemoglobin was recorded at baseline, 1, 3, 6, 9 and 12 months. The number of patients requiring EPO at one year (or at the point of leaving the study) was recorded; as was the dose of EPO at that time. The number of patients requiring one or more blood transfusion was recorded; as were the number of units given - up to one year, or the point of leaving the study.

2.4. Acid/base balance

Definitions

Acidosis is defined as a disturbance which tends to add acid or remove alkali from body fluids, while alkalosis is any disturbance which tends to remove acid or add base. Acidaemia and alkalaemia are descriptive of the instantaneous [H^+] that is detected in body fluids at the time of testing. In this study venous bicarbonate was used as a surrogate for these concepts.

The target venous bicarbonate level in the HA group was 30 mmol/L. If this was not achieved with HA dialysate and calcium carbonate alone, oral sodium bicarbonate was added at a dose of 0.6g three times per day, and increased to a maximum of 1.8g tds. In the LA group the venous bicarbonate was measured, but discounted from a therapeutic point of view, unless a patient was symptomatic of acidosis.

In this study, calcium carbonate was used primarily as an alkali, rather than a phosphate binder. How it was prescribed is described in Section 2.7.

The number of patients requiring either calcium carbonate, aluminium hydroxide or alfacalcidol at one year (or at the point of leaving the study) was recorded; as were the doses of each drug. The number of patients requiring sodium bicarbonate at one year (or at the point of leaving the study) was recorded; as was the dose of the drug.

Venous bicarbonate was measured at the six designated time points; using a phosphoenolpyruvate assay, in a Vitros 700XR C Series Analyser

Arterial pH was measured from a radial artery sample at one year; using a pH electrode in a Ciba Corning 238pH Blood Gas Analyser.

2.5. Nutrition

The patients were advised on a regular basis to adhere to a dietary protein intake of greater than 1.0 g/kg/day with an adequate calorie intake. No dietary restrictions were put on the patients. The dietitians were not aware of which dialysate/drug regime was being used.

The four key nutritional parameters were: body weight, midarm circumference, triceps skinfold thickness and serum albumin.

Body weight, body mass index (BMI) and anthropometric measurements. Body weight (with clothes on, shoes off) was assessed without PD fluid in the abdomen at baseline, and thereafter with PD fluid in the abdomen if the patient was still on PD.

Body mass index (BMI) was derived from a standard formula:

BMI = weight (kg) / height (metres)^2.

Midarm circumference (cm) and triceps skinfold thickness (mm) were assessed by experienced renal dietitians, at 0, 1, 6 and 12 months.

The midarm circumference was measured with a tape measure at the midpoint of the dominant arm (from the acromial process of the scapula to the olecranon process of the ulna), with the arm hanging loosely by the side. The triceps skinfold thickness was measured with Holtain Skinfold Callipers, using the same anatomical markers and arm position.

Midarm muscle circumference (MAMC) and midarm muscle area (MAMA) were derived from standard formulae:

MAMC (cm) = MAC - (TSF (in cm) x 0.314),

MAMA (cm^2) = MAMC^2 / 12.56.

Even though the four dieticians were all experienced and trained in renal dietetics, a formal validation of their measurements was not attempted. This was partly due to the fact that over the 3-4 year period of the study, different staff were employed in the department - ie, all four were not there at the same time.

Serum albumin was measured at the six designated time points; using a bromocresol based colormetric assay.

Muscle strength. Hand grip strength was assessed at 1, 6 and 12 months, using the Harpenden hand-grip dynamometer (British Indicators, UK). The best of three 'pulls' was recorded, using the dominant hand, with the arm held next to the body.

Leg muscle endurance was assessed at one year. The methods used were devised for this study, and were based on methods invented by Naomi Clyne, a British-Swedish nephrologist. She has carried out extensive studies into the effects of exercise training on patients with CRF/ESRF (Clyne et al, 1991, **128**).

The patient was asked to sit on the couch, and a 5 kg weight was placed on the patients' ankle on the dominant leg. The trunk was vertical, with 90^0 flexion at the hips and knees. The hands were kept on the lap. There was no support for the back or thigh. A marker was placed just above the extended leg. Then the patient underwent two tests:

(1) Static endurance. The patient was asked to keep their weighted leg horizontal (the other leg had to remain vertical) by touching a card for as long as possible. This time was recorded in seconds.

(2) Dynamic endurance. After five minutes rest, the patient was then asked to alternately flex (to a vertical position) and extend the knee of the weighted leg (to touch the card) at a rate of one per second. The patient was 'paced' by counting seconds aloud, in the form of "one thousand, two thousand ... etc". The number of repetitions were recorded.

Dietary protein intake was measured at one year, using a 7 day record, obtained and analysed by experienced renal dietitians. The food weights were estimated, and the data analysed with the Microdiet computer programme.

Blood pressure was recorded at 1, 6 and 12 months, by myself, using a mercury sphygmomanometer on the left arm; measurements were taken between 9am and 12pm.

Serum cortisol was measured at 1 and 12 months, using a solid phase competitive assay on a Chiron ACS180. Mid-morning blood samples were obtained.

2.6. Dialysis dose / kinetic modelling data

2.6(i). Dialysis dose

The majority of patients were started on a standard CAPD regime of four 2 litre exchanges per day. PD was delivered through a 'disconnect' system, for most patients.

Dialysis adequacy was assessed using 'conventional' monitoring of serum urea and creatinine levels. The dialysis dose was increased if the patient recorded *either* a serum urea of over 30mmol/L or creatinine over 1200μmol/L. The dialysis dose was decreased if *both* the serum urea fell to below 15mmol/L, and the creatinine to below 500μmol/L, in the absence of clinical evidence of malnutrition.

Changes to the dialysis dose were recorded. All increases or decreases were recorded.

The volume of the 'standard' dialysis exchange used by each patient at one year (or at the point of leaving the study) was recorded.

The glucose concentration of the 'standard' dialysis exchange used by each patient at one year (or at the point of leaving the study) was recorded.

The dialysis dose (defined as the number of bags multiplied by the volume) for each patient was recorded at one year, or at the point of leaving the study.

2.6(ii). Kinetic modelling data

Kinetic modelling was performed at 1, 6 and 12 months. Treatment was *not* adjusted according to the results. Three parameters were assessed:

(1) Protein catabolic rate (g/kg/day):

$$\frac{0.213\ (UV^{urea} + DV^{urea}) + 19.3}{BW}$$

(2) Kt/V^{urea} (per week):

$$\frac{3.88\ (UV^{urea} + DV^{urea})}{BW \times P}$$

Both urea kinetic parameters equations were described by Lindholm in 1990 (**129**).

(3) Creatinine clearance (L/week):

$$\frac{UV^{creat}+DV^{creat}}{P}$$

Key

UV = amount of substance in urine (volume x concentration)

DV = amount of substance in 4 consecutive used PD dialysate exchanges

P = plasma concentration of a substance

BW = body weight

2.7. Renal bone disease

2.7(i). Renal bone disease protocol

A target serum calcium of 2.5mmol/L was used for both groups. In order to achieve maximal correction of acidosis in the HA group and minimal safe correction of acidosis in the LA group, the method of achieving the target serum phosphate was different in the two groups.

In the HA group, if possible, calcium carbonate alone was used to maintain serum calcium. From PD catheter insertion, patients in this group received high dose calcium carbonate (3g per day, in the form of Calcichew Forte® 1 tds). The dose of calcium carbonate was increased if possible to a maximum of 6g per day, in order to achieve maximal correction of acidosis[10]. If HA dialysate and 6g/day of calcium carbonate was not able to maintain the serum calcium at 2.5 mmol/L or above, alfacalcidol at a dose of 0.25-1.0 mcg once per day was added. If possible, aluminium hydroxide was not used in the HA group.

In the LA group, no phosphate binder was started at baseline. If the serum calcium did not reach 2.5 mmol/L by 3 months, alfacalcidol was added, in the same dose range as the HA group. If the serum phosphate reached over 2.0 mmol/L, a small dose of calcium carbonate (0.5-1.0 g/day; in the form of 1-2 Calcichew® tablets per day) or aluminium hydroxide (950 mg per day, in the form of 2 Alucaps® per day), or both, were started.

2.7(ii). Bone biochemistry

[10] This part of the protocol was intended to maximise the alkalinising properties of the drug, rather than the phosphate binding properties.

Serum calcium, phosphate and total alkaline phosphatase were measured at 0, 1, 6 and 12 months, using standard laboratory techniques.

Hypercalcaemic episodes. The number and magnitude of hypercalcaemic episodes were recorded in the two groups. A hypercalcaemic episode was recorded if a patient experienced two or more consecutive serum calcium concentrations of 2.7 mmol/L or above, separated from a previous 'episode' by at least one month; with at least one 'normal' calcium reading between episodes.

The serum parathyroid hormone and aluminium concentrations were measured at 1, 6 and 12 months. The PTH measurements were made with a two-site assay, using a Nicholls Allegro kit. The aluminium measurements were made using the Pie-Unicam 9200 Series Atomic Absorption Spectrophotometer.

2.8. Outcomes

2.8(i). Renal modality

Seven 'renal modality' states were recognised at one year, and recorded:

1. PD (whether or not the patient had received a failed transplant, a temporary period of haemodialysis or intermittent PD, or temporary recovery of renal function),
2. Functioning renal transplant,
3. Death,
4. Haemodialysis, where this was considered the 'permanent' form of renal replacement therapy at that time,
5. Intermittent PD (IPD), where this was considered the 'permanent' form of renal replacement therapy at that time. It took the form of 48 hours of continuous automated PD, delivered as an inpatient over the weekend,
6. Recovered renal function,
7. Transferred out of unit.

2.8(ii). Hospitalisation

The two key morbidity parameters were the number of admissions, and the days spent in hospital per year. The number of admissions included only those after the insertion of PD catheter. The days spent in hospital included all admissions for one year after discharge from the admission that incorporated the PD catheter insertion, any admission(s) immediately before the PD catheter insertion and the admission for the insertion of the PD catheter. These days were also expressed as days per at risk months - ie, the number of days admitted expressed per time (in months) in which admission was possible.

The number of visits by a community dialysis nurse and to the renal outpatient department were recorded. The total number of days 'in contact' with the renal unit

was calculated, by adding these two numbers to the days spent in hospital. This number will be called the "total contact days".

2.8(iii). Peritonitis / exit site infections

A 'probable' peritonitis episode was said to have occurred if a patient complained of typical symptoms, a PD bag was sent off for culture and the patient was treated *as if* he/she had peritonitis; whether or not the white count of the PD fluid was measured, and whether or not a causative organism was grown.

A 'proven' episode was said to have occurred if an organism was identified. A peritonitis episode (probable or proven) had to be separated from a previous episode by at least one month; with the used dialysate 'clearing' in between.

Comparing the probable and proven peritonitis rates produced a 'culture negative' percentage for each group - ie, the percentage of probable episodes that were *not* proven, in terms of an organism being identified.

The peritonitis rate was determined by relating the number of probable episodes to the number of months in which the patients were at risk of peritonitis.

The unit policy was to remove the PD catheter, even if the patient was 'well', if the patient experienced three episodes in rapid (not defined) succession, caused by the same organism[11].

An exit site infection was much harder to define. In the end, no attempt was made to define it. Part of the problem with definition related to the fact that exit site infections were treated by a range of health professionals: nephrologists in an 'out' and 'in'-patient setting, general practitioners and community dialysis nurses.

So, the patients or their relatives were asked, at one year (or the point of leaving the study), whether they considered that the patient had been treated for an exit site infection, and how many they had experienced. It was to prove very difficult to obtain reliable data on all the patients.

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[11] As can be seen from Table 12, this did not always occur.

2.8(iv). Death / technique failure

The numbers and causes of death were compared in the two groups. Most of the deaths occurred inside the hospital. When a death occurred outside the hospital, the patient's general practitioner was contacted to ascertain the cause of death.

The numbers and causes of permanent technique failures were recorded in the two groups. 'Permanent' technique failure was defined as requiring permanent HD or IPD at one year; or where the decision had been made, at the point of leaving the study, to convert the patient permanently to HD or IPD (eg, before death, transplantation, transfer out or recovery of renal function).

The numbers and causes of temporary technique failures were recorded in the two groups. 'Temporary technique failure' usually meant an period of time (initially planned to be short) in which PD was halted, either by 'resting' on haemodialysis, or by temporarily stopping dialysis. This was usually due:

(1) a leak of PD fluid through the exit site, or into the skin around the exit site,

(2) PD catheter replacement (either at the same time as catheter removal or at a later date), or,

(3) a hernia repair.

2.9. Statistical analysis

Where possible (and appropriate) data was included from patients who 'left' the study, to haemodialysis or transplantation for example. In fact, only the data on body weight and serum albumin incorporates patients who had left the study. Results were analysed on an 'intention-to-treat' basis, ie patients remained in the group into which they were first randomised. Data after a patient had been transferred out of the unit was not included in the analysis.

It was decided to use an unpaired Student's 't' test to compare the two groups at *one time point alone, ie one year*; principally in terms of the key six parameters (four nutritional, two morbidity). It was also decided:

(1) *not* to compare (statistically) the matching of the two groups at baseline;

(2) *not* to analyse changes within either group over time;

(3) *not* to analyse data where numbers were either unreliable or too small;

(4) *not* to analyse discontinuous data;

(5) to compare some of the other parameters, if it the data produced clinically useful information, remembering that these analyses constituted a *post hoc* analysis.

The reasons for these decisions will be explained in Sections 4.1 (ii-iv) of the Discussion.

The symbol "*" indicates that the p value was less than 0.05 (significant), ** = less than 0.01 (highly significant) and *** = less than 0.001 (very highly significant).

If *no* statistical analysis was performed, it will be indicated by a superscript comment of "NSA".

CHAPTER THREE

He and his physicians

Are of a mind;

he, that they cannot help him,

They, that they cannot help

All's Well That Ends Well I, 3

Countess Rousillon to Helena,

concerning the King's illness

CHAPTER THREE

RESULTS

3.1. Introduction

Unless otherwise indicated, it can be assumed that reliable data was obtained on all patients in both treatment groups. Furthermore, unless indicated, the data provided reflects the status of the patients at one year, or at the point of leaving the study. So, for example, the "EPO: LA 47%" comment on Figure 3 means that 47% of LA patients were receiving EPO at one year, or at the point of leaving the study.

Otherwise, the data and graphs presented include all data from the patients, still on PD, who had reached that time point. There were two exceptions to this rule. Body weight and serum albumin data includes all patients who had reached that time point, irrespective of whether they were on PD or not - ie, including patients on haemodialysis (post-dialysis weight and albumin was used), those transplanted and those who had regained renal function. These two parameters were also analysed for those patients still on PD. There were no differences in the results when this was done.

All results are expressed as means plus or minus the standard error from the mean (SEM).

The 'x' axis of all the graphs is time (in months). The units of the 'y' axis is in the title of the graph. LA data is to the left in all tables and graphs, and is in grey in the graphs. HA data is to the right in all tables and graphs, and is in dark grey in the graphs.

3.2. Matching[NSA]

The matching of the two groups at baseline is summarised in the following tables: Table 1 - Demography, Table 2 - Causes of Renal Failure, Table 3 - Biochemistry and Haemoglobin, Table 4 - Nutrition, and Table 7 - Bone Biochemistry.

Even though the two groups were reasonably well matched at baseline, there were some discrepancies. In terms of demographic data, there were differences between the two groups. In the HA group, there were 22 patients with known ischaemic heart disease (ie, a past or present history of angina or myocardial infarction) at baseline, compared to 27 in the LA group. However, there was a larger number of diabetics in the HA group (22 vs 12) but the co-morbidity scores of the two groups were the same.

The causes of renal failure, biochemistry, haemoglobin and bone biochemistry were similar at baseline. However, there was a difference in nutritional state at baseline; with the HA patients being, on average 1.1 kg lighter (HA: 68.0kg vs LA: 69.1kg; Table 4).

Table 1: Demography (values being numbers of patients unless indicated)

	LA	HA
Number	100	100
Age (yrs)	56.5 ± 1.6	56 ± 1.4
Sex (M/F)	67/ 33	64/ 36
IHD[12]	27	22
Diabetes	12	22
Comorbidity score[13]	0.95 ± 0.11	0.92 ± 0.12
Previously known to renal unit[14]	66	70

Table 2: Causes of Renal Failure (values being numbers of patients)

	LA	HA
Unknown	39	32
Diabetes	10	18
Inherited	8	13
GN[15]**/vasculitis**	13	10
Tubulointerstitial	6	9
Myeloma/amyloid	4	7
Renovascular[16]	6	6
Obstruction/stones	11	5
Other	3	0

(2 single kidney, 1 pre-eclampsia)

[12]IHD = ischaemic heart disease
[13]maximum = 7
[14]ie, patient had been reviewed in renal outpatients for at least 3/12 before starting dialysis
[15]GN = glomerulonephritis
[16]includes probable hypertensive nephropathy

Table 3: Haemoglobin, Biochemistry, Acid-base Balance and Cortisol (all values are means ± SEM; all analyses are LA vs HA at one year)

	LA				HA			
	0	**6**	**12**	**mths**	**0**	**6**	**12**	**p value**
Haematology								
Hb	**8.9**	**11.5**	**11.5**		**9.0**	**11.2**	**11.2**	**0.42**
(g/dl)	± 0.2	± 0.2	± 0.2		± 0.2	± 0.2	± 0.2	
min =	*4.2*	*5.8*	*7.8*		*5.4*	*6.9*	*7.2*	
max =	*13.1*	*16.4*	*16.1*		*16.4*	*15.6*	*14.9*	
n =	*93*	*78*	*59*		*96*	*82*	*67*	
Biochemistry								
Sodium	**138.1**		**137.2**		**137.2**		**137.2**	**0.95**
(mmol/L)	± 0.3		± 0.3		± 0.3		± 0.4	
min =	*127*		*128*		*128*		*121*	
max =	*144*		*143*		*143*		*144*	
n =	*96*		*58*		*96*		*67*	
Potassium	**4.4**	**4.2**	**4.0**		**4.6**	**4.2**	**4.2**	**0.26**
(mmol/L)	± 0.1	± 0.1	± 0.1		± 0.1	± 0.1	± 0.1	
min =	*2.7*	*2.7*	*2.7*		*3*	*3*	*2.9*	
max =	*6.6*	*7.6*	*6*		*6.5*	*5.6*	*5.7*	
n =	*93*	*78*	*59*		*97*	*82*	*68*	
Urea	**40.9**	**20.7**	**19.1**		**39.7**	**20.9**	**19.1**	**0.98**
(mmol/L)	± 1.6	± 0.6	± 0.5		± 1.0	± 0.6	± 0.5	
min =	*20.9*	*10.3*	*6.5*		*20.5*	*9.5*	*9.1*	
max =	*117.5*	*48.4*	*33.4*		*69.2*	*37.1*	*35.4*	
n =	*93*	*78*	*59*		*97*	*82*	*68*	

Creatinine	**1106**	**855**	**889**	**1077**	**820**	**852**	**0.37**
(µmol/L)	± 37	± 30	± 27	± 30	± 20	± 19	
n =	*93*	*78*	*59*	*97*	*82*	*68*	
min =	*363*	*305*	*436*	*490*	*395*	*450*	
max =	*2837*	*1983*	*1835*	*1972*	*1428*	*1644*	

Acid/base balance

HCO_3	**20.4**	**24.0**	**23.0**	**20.1**	**28.2**	**27.2**	**<0.001**
(mmol/L)	± 0.6	± 0.3	± 0.3	± 0.5	± 0.3	± 0.3	
min =	*7*	*15*	*15*	*6*	*20*	*19*	
max =	*55*	*31*	*28*	*32*	*35*	*34*	
n =	*88*	*78*	*59*	*93*	*82*	*68*	
pH[17]	**-**	**-**	**7.40**	**-**	**-**	**7.44**	**<0.001**
			± 0.004			± 0.004	
min =			*7.31*			*7.38*	
max =			*7.49*			*7.53*	
n =			*40*			*45*	

Cortisol

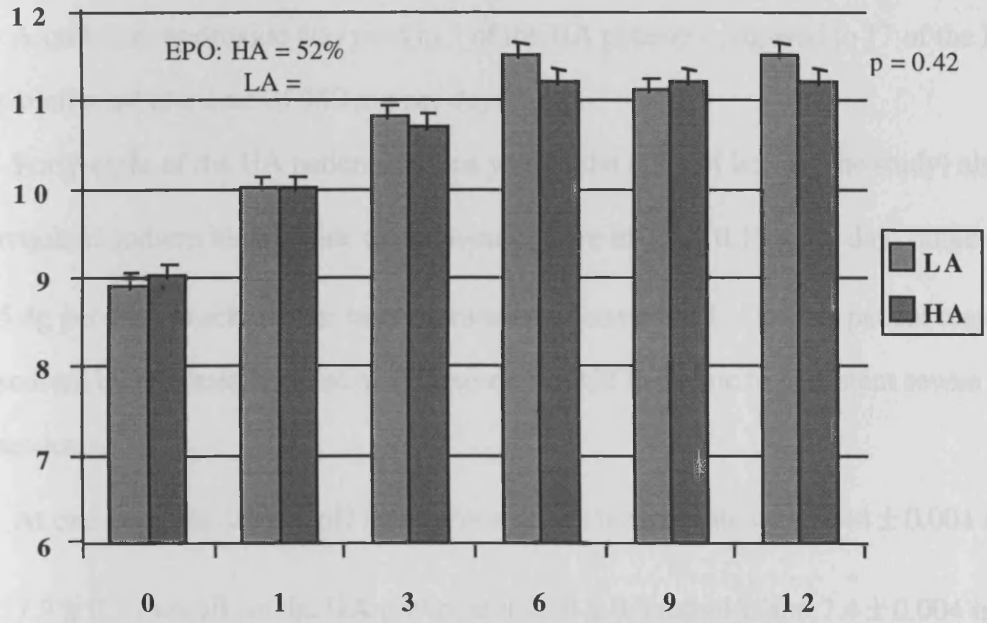
Cortisol	**435**	**-**	**442.5**	**431**	**-**	**544.6**	**<0.05**
(nmol/L)	± 7.1		± 8.8	± 10.8		± 23	

[17]no baseline pH values - explanation in text

3. 3. Haematology

The haemoglobin reached the target level (>11g/dl) by six months in both groups (Table 3 and Figure 3). The haemoglobin was similar in the two groups at one year (HA: 11.2 ± 0.2 vs LA: 11.5 ± 0.2 g/dl; p = 0.42).

Figure 3: Haemoglobin (g/dl)



Fifty-two (52%) of the HA patients were on erythropoeitin (EPO) at one year, or the point of leaving the study, compared to 47% of the LA patients. The average EPO dose was similar in the two groups (HA: 5173 ± 245 units/week vs LA: 5320 ± 237 units/week; p = 0.76). The EPO dose range was 2,000-10,000 units per week in both groups.

Where possible EPO alone was used to raise the haemoglobin to the target level. Blood transfusion also was necessary in some cases. Sixty-one (61%) HA patients required one or more blood transfusion, compared to 64 LA patients. The average number of units of blood transfused was the same (HA: 2.6 ± 0.43, range = 0-35; vs LA: 3.0 ± 0.35, range = 0-20; p = 0.5).

3.4. Acid/base balance

To achieve the target serum bicarbonate level of 30 mmol/L and target serum calcium of 2.5 mmol/L, 92 of the HA patients (at one year or the point of leaving the study) required calcium carbonate (at an average dose of 2.91 ± 0.16 g per day; range = 0-7.2g per day) as well as HA dialysate. Thirty-six of the LA patients required calcium carbonate (0.63 ± 0.11 g per day; range = 0-5.4g per day) ($p<0.001$).

Aluminium hydroxide was used in 3 of the HA patients compared to 17 of the LA patients, all at a dose of 950 mg per day.

Forty-eight of the HA patients (at one year or the point of leaving the study) also required sodium bicarbonate (at an average dose of 1.4 ± 0.17 g per day; range = 0-5.4g per day) to achieve the target serum bicarbonate level. One LA patient required sodium bicarbonate because of symptoms thought to be due to persistent severe acidosis.

At one year, the arterial pH and venous serum bicarbonate were 7.44 ± 0.004 and 27.2 ± 0.3 mmol/L in the HA group, and 23.0 ± 0.3 mmol/L and 7.4 ± 0.004 in the LA group (both $p<0.001$). Serum bicarbonate levels are shown in Table 3 and Figure 4.

Figure 4: Serum Bicarbonate (mmol/L); *** = p < 0.001

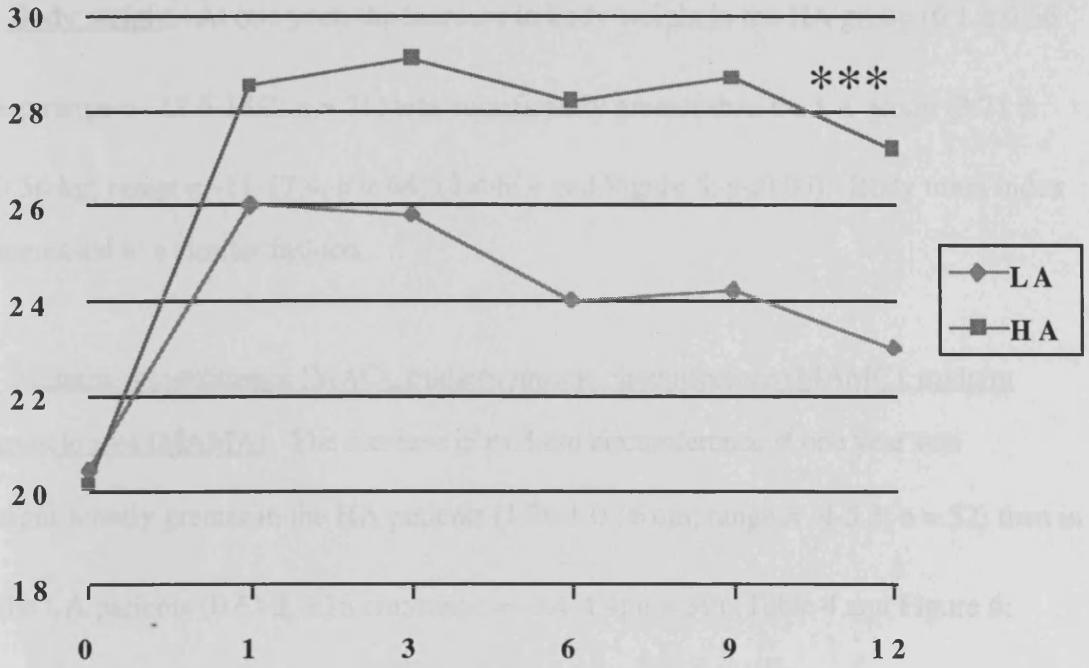
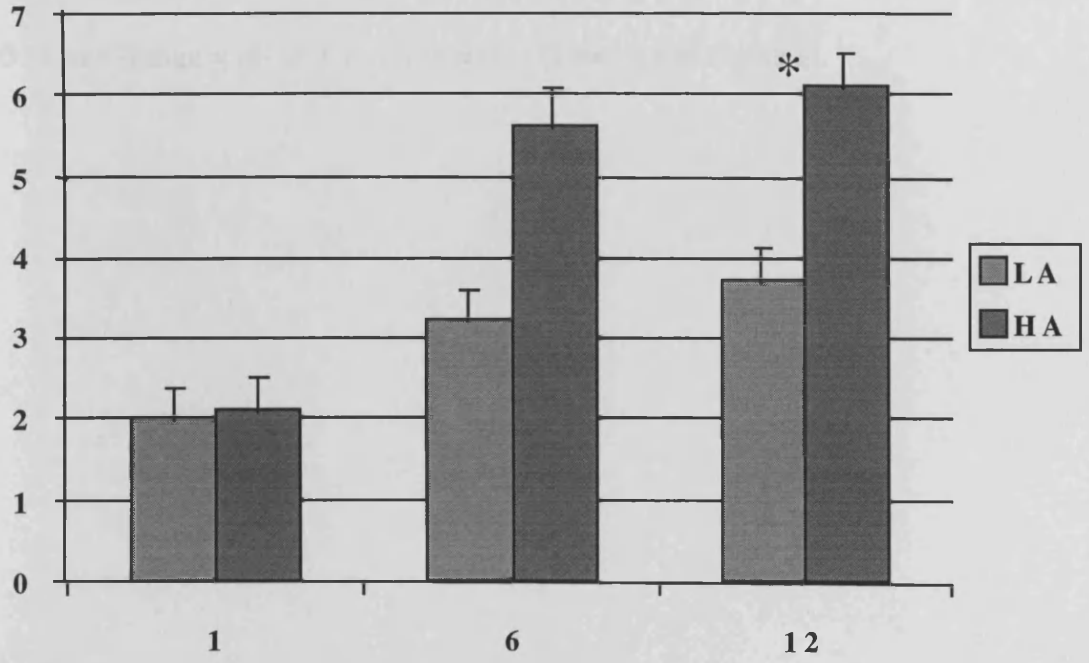


Figure 5: Weight Gain (kg); * = p <0.05



3.5. Nutrition

Body weight. At one year, the increase in body weight in the HA group (6.1 ± 0.66 kg; range = -13.6-18.2; n = 71) was significantly greater than the LA group (3.71 ± 0.56 kg; range = -11-17.4; n = 64) (Table 4 and Figure 5; $p<0.05$). Body mass index increased in a similar fashion.

Midarm circumference (MAC), midarm muscle circumference (MAMC), midarm muscle area (MAMA). The increase in midarm circumference at one year was significantly greater in the HA patients (1.26 ± 0.16 cm; range = -4-5.3; n = 52) than in the LA patients (0.61 ± 0.16 cm; range = -3.4-4.4; n = 39) (Table 4 and Figure 6; $p<0.05$). Similar results were seen with the MAMC and MAMA.

Triceps skinfold thickness. The increases in triceps skinfold thickness were not significantly different (HA: 2.5 ± 0.41 mm (range = -5.8-11.2; n = 39) vs LA: 1.24 ± 0.38 mm (range = -8-15; n = 37) ($p = 0.1$) (Table 4 and Figure 7).

Table 4: Nutrition (all values are means ± SEM; all analyses are LA vs HA at one year)

	LA				HA			
	0	**6**	**12**	**mths**	**0**	**6**	**12**	**p value**
Weight	**69.1**	**72.6**	**72.9**		**68.0**	**74.5**	**74.8**	**0.35**
(kg)	± 1.4	± 1.5	± 1.5		± 1.3	± 1.4	± 1.3	
min =	*44.3*	*49.9*	*47.4*		*41*	*46.9*	*49*	
max =	*124.5*	*132.2*	*126.9*		*106.4*	*119.4*	*118.8*	
no =	*100*	*78*	*83*		*100*	*82*	*86*	
BMI	**24.6**	**25.9**	**26.0**		**24.5**	**26.7**	**26.9**	**0.14**
(kg/m^2)	± 0.4	± 0.4	± 0.4		± 0.4	± 0.4	± 0.4	
min =	*17.4*	*19.7*	*18.1*		*16.2*	*17.2*	*18.5*	
max =	*36.8*	*37.8*	*37.3*		*38.2*	*38.2*	*39.0*	
no =	*100*	*78*	*83*		*100*	*82*	*86*	
Height	**167.2**				**166.5**			
(cm)	± 1.0				± 0.9			
min =	*142*				*146*			
max =	*187*				*184*			
no =	*100*				*100*			
MAC	**28.3**[18]	**28.5**	**28.7**		**28.2**	**29.5**	**29.8**	**0.12**
(cm)	± 0.4	± 0.4	± 0.4		± 0.4	± 0.4	± 0.4	
min =	*22*	*22.7*	*22.3*		*20.4*	*20.8*	*21*	
max =	*43.8*	*45.9*	*44.2*		*40*	*41.5*	*41.6*	
no =	*93*	*74*	*59*		*91*	*78*	*69*	
MAMC	**27.9**	**28.1**	**28.2**		**27.8**	**29.1**	**29.3**	**0.11**
(cm)	± 0.4	± 0.4	± 0.4		± 0.4	± 0.4	± 0.4	
min =	*21.8*	*22.3*	*21.9*		*20.3*	*20.6*	*20.8*	
max =	*42.5*	*44.6*	*43.0*		*40*	*41.5*	*41.6*	
no =	*93*	*74*	*59*		*91*	*78*	*69*	

[18] 1 month value

MAMA	**68.9**	**63.9**	**64.7**	**62.6**	**67.8**	**69.5**	**0.15**
(cm^2)	± 1.7	± 1.8	± 1.9	± 1.8	± 1.7	± 1.8	
min =	*37.8*	*39.6*	*38.3*	*32.7*	*29.9*	*34.6*	
max =	*144*	*158.7*	*146.9*	*127.4*	*137.1*	*137.8*	
no =	*93*	*74*	*59*	*91*	*78*	*69*	
TSF	**13.6**[19]	**14.3**	**14.3**	**14.5**	**15.4**	**16.1**	**0.25**
(mm)	± 0.8	± 0.8	± 0.8	± 0.8	± 0.8	± 0.9	
min =	*4*	*4.6*	*4.4*	*3*	*5*	*4*	
max =	*40*	*40*	*39.6*	*38*	*34.4*	*36*	
no =	*89*	*74*	*58*	*84*	*68*	*64*	
Albumin	**37.2**	**37.3**	**38.2**	**37.5**	**37.2**	**37.8**	**0.57**
(g/L)	± 0.4	± 0.3	± 0.5	± 0.5	± 0.4	± 0.4	
min =	*26*	*29*	*26*	*21*	*27*	*27*	
max =	*47*	*47*	*49*	*48*	*46*	*46*	
no =	*89*	*78*	*82*	*94*	*82*	*84*	
Handgrip	**28.7**	**29.0**	**28.1**	**27.5**	**28.9**	**30.0**	**0.63**
(kg)	± 1.2	± 1.1	± 1.0	± 1.0	± 1.0	± 1.0	
min =	*9*	*9*	*10*	*9*	*10*	*11*	
max =	*62*	*57*	*54*	*58*	*48*	*49*	
no =	*85*	*71*	*53*	*88*	*72*	*63*	

[19] 1 month value

Figure 6: Midarm Circumference Gain (cm); * = p < 0.05

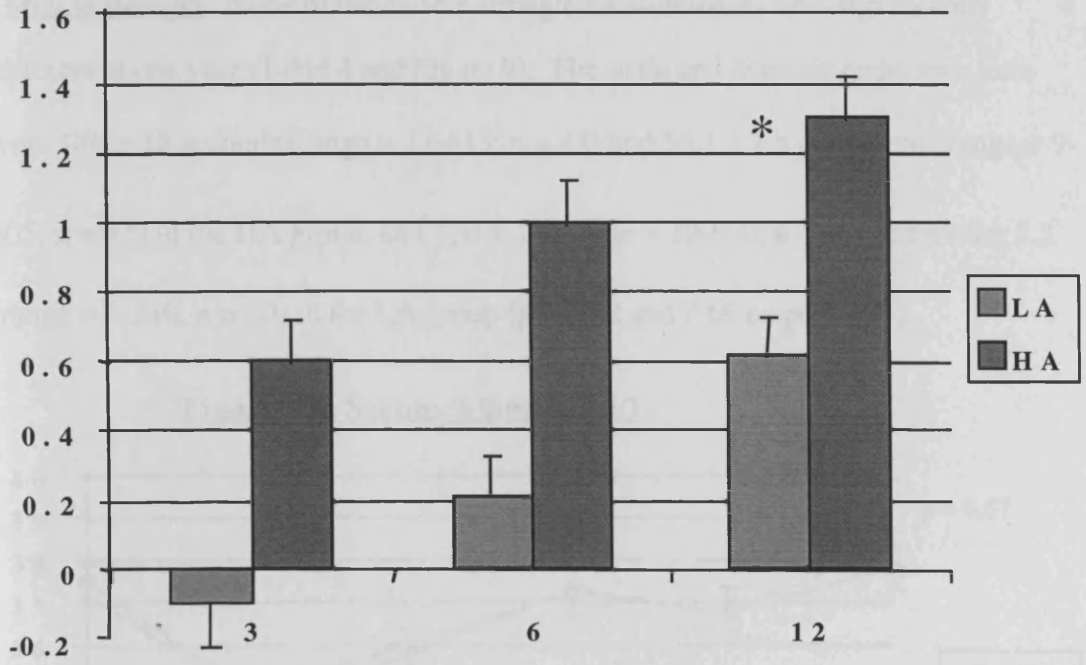
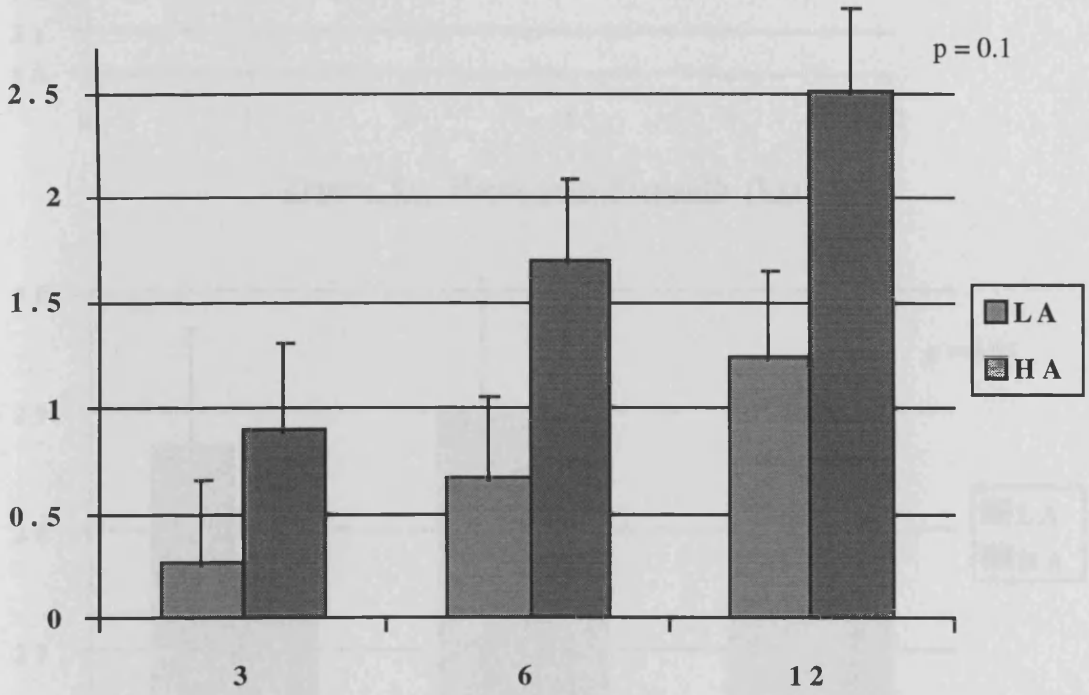


Figure 7: Triceps Skinfold Thickness Gain (mm)



Albumin. Serum albumin was not different at one year (Table 4 and Figure 8).

Muscle strength. None of the muscle strength measurements were significantly different at one year (Table 4 and Figure 9). The static and dynamic endurance tests were 186 ± 16 seconds (range = 11-615; n = 42) and 56.1 ± 7.5 repetitions (range = 9-505; n = 42) in the HA group, and 130 ± 13 (range = 10-660; n = 30) and 49.6 ± 5.3 (range = 3-210, n = 30) in the LA group (p = 0.12 and 0.69 respectively).

Figure 8: Serum Albumin (g/L)

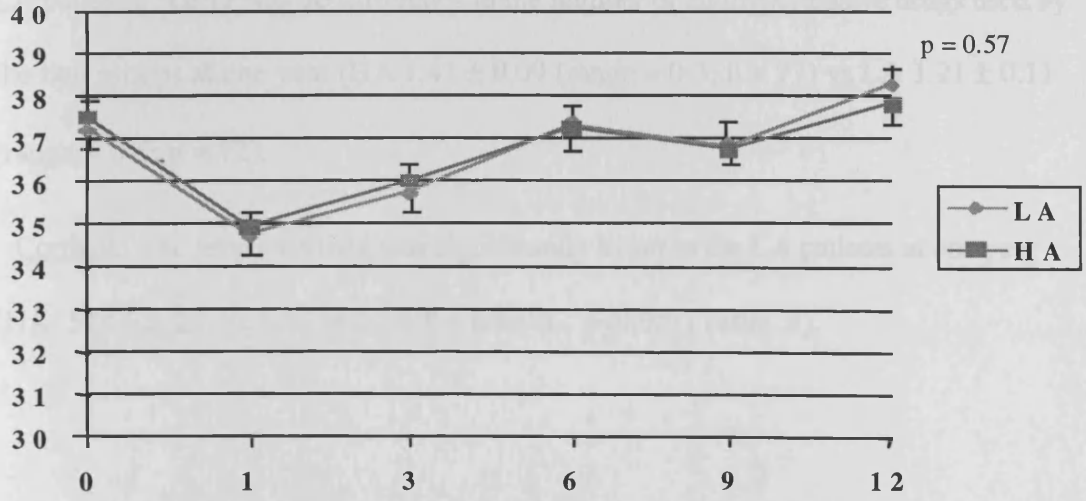
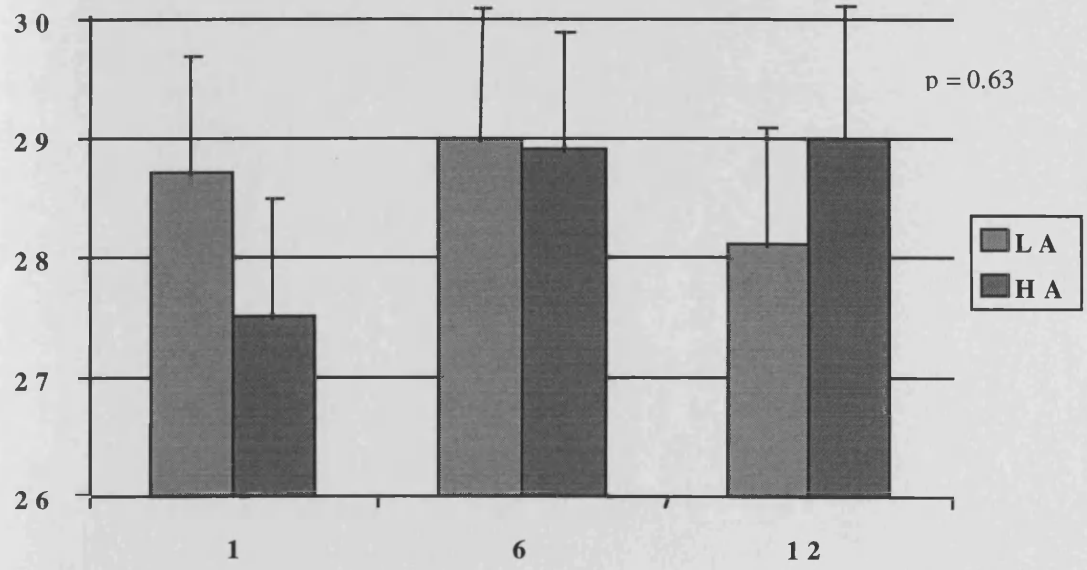


Figure 9: Hand-grip Strength (kg)



Dietary protein intake. Dietary protein intake at one year (HA: 0.9 ± 0.2 g/kg/day vs LA: 1.0 ± 0.1 g/kg/day) was not significantly different.

Blood pressure. There was no difference in systolic or diastolic blood pressure at one year between the two groups; 130 ± 2 / 78 ± 1 mmHg (range = 60-180 (n = 67) / 50-100 (n = 66)) in the HA group vs 129 ± 3 / 76 ± 1 mmHg (range = 80-180 (n = 59) / 50-120 (n = 58)) the LA group (systolic: p = 0.85, diastolic: p = 0.33). Seventy-seven (77%) of the HA patients required anti-hypertensive agents, compared to 72% of the LA patients. There was no difference in the number of antihypertensive drugs used by the two groups at one year (HA 1.41 ± 0.09 (range = 0-3; n = 77) vs LA 1.21 ± 0.11 (range = 0-5; n = 72).

Cortisol. The serum cortisol was significantly lower in the LA patients at one year (HA: 544.6 ± 23, vs LA: 442.5 ± 8.8 nmol/L; $p<0.05$) (Table 3).

3.6. Dialysis dose / kinetic modelling data

3.6(i). Dialysis dose

Dialysis dose changes. Before one year, there were 17 increases and 6 decreases in dialysis dose in the HA group, compared to 18 and 9 in the LA group (Table 5).

Average dialysis dose. Dialysis dose, at one year or at the point of leaving the study (HA: 8.0 ± 0.1 litres/day (range = 6-12.5) vs LA: 8.5 ± 0.3 litres/day (range = 4-30) was not significantly different (p = 0.18) (Table 5).

Volume of exchange. At one year or at the point of leaving the study, out of the HA group, eleven patients were using 1.5 litre exchanges, seventy-four 2-litre, fourteen 2.5-litre and one 3-litre exchanges. In the LA group, eleven patients were using 1.5 litre exchanges, seventy-one 2-litre, thirteen 2.5-litre and five 3-litre exchanges (Table 5).

Glucose concentration of exchange. Forty-nine (49%) of the HA patients used one or more 'strong' bags (3.86% glucose) per day, compared to 52% of the LA patients (Table 5). The average number of 'strong' bags used per day was not significantly different (HA: 0.61 ± 0.07, vs LA: 0.72 ± 0.08). There were only two strengths of bag available to the patients, 'weak' (1.36% glucose) and 'strong' (3.86%).

Table 5: Dialysis Dose (all values are numbers of patients except for the dialysis dose which is a mean ± SEM; with the analysis of the dialysis dose being LA vs HA at one year)

	LA	HA	
Changes			**p value**
Increases	18	17	
Decreases	9	6	
Average dialysis dose	8.45	8.0	**0.18**
(litres)	± 0.32	± 0.13	
Volume of exchange (litres)			
1.5	11	11	
2	71	74	
2.5	13	14	
3	5	1	
Glucose concentration of exchange			
Using 1 or more 3.86% exchange	52	49	

3.6(ii). Kinetic modelling data

None of the three kinetic modelling parameters were significantly different at one year. But, as can be seen from Table 6 and Figures 10, 11 and 12 (summarising the kinetic modelling data) below there was a *decrease* in all three parameters in the HA patients, and *increase (or stability)* in the LA patients.

Table 6: Kinetic Modelling Data (all values are means ± SEM; all analyses are LA vs HA at one year)

	LA				HA			
	1	**6**	**12**	**mths**	**1**	**6**	**12**	**p value**
PCR[20]	**0.88**	**0.98**	**0.94**		**0.92**	**0.93**	**0.89**	**0.24**
(g/kg/day)	± 0.02	± 0.02	± 0.02		± 0.02	± 0.02	± 0.02	
min =	*0.37*	*0.46*	*0.54*		*0.51*	*0.51*	*0.55*	
max =	*1.57*	*1.63*	*1.77*		*1.66*	*1.62*	*1.62*	
no =	*86*	*70*	*47*		*86*	*71*	*58*	
Kt/V	**1.92**	**1.89**	**1.92**		**1.86**	**1.8**	**1.83**	**0.52**
(weekly)	± 0.06	± 0.06	± 0.06		± 0.03	± 0.03	± 0.06	
min =	*0.25*	*0.34*	*0.36*		*0.34*	*0.31*	*0.35*	
max =	*1.14*	*1.35*	*1.14*		*1*	*0.84*	*1.04*	
no =	*86*	*70*	*47*		*86*	*71*	*57*	
CrCl[21]	**78.2**	**80.1**	**80.1**		**76.9**	**76.3**	**76.3**	**0.51**
(L/week)	± 3.0	± 3.2	± 3.3		± 3.3	± 2.6	± 2.5	
min =	*2.35*	*2.65*	*2.64*		*1.76*	*2.9*	*3.09*	
max =	*20.66*	*22.05*	*18.13*		*20.94*	*17.25*	*14.08*	
no =	*86*	*70*	*47*		*86*	*71*	*58*	

[20]PCR = protein catabolic rate
[21]CrCl = creatinine clearance

Figure 10: Protein Catabolic Rate (g/kg/day)

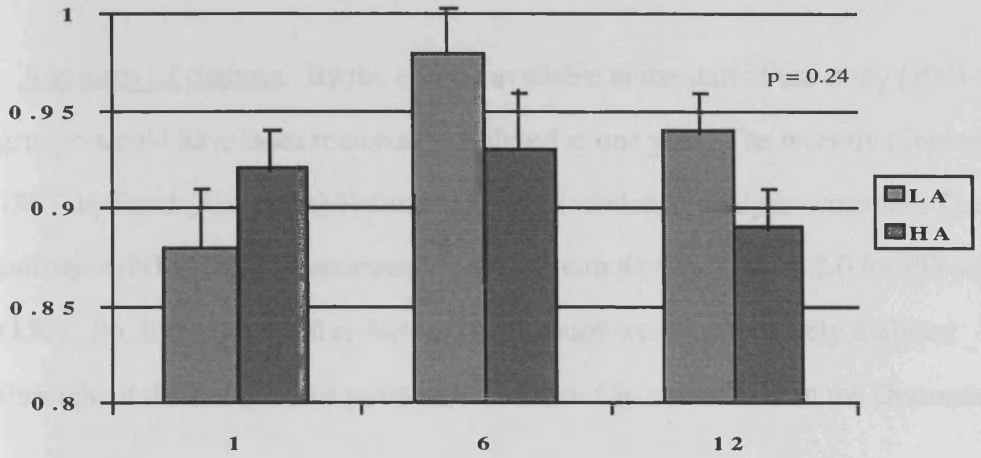


Figure 11: Kt/V (weekly)

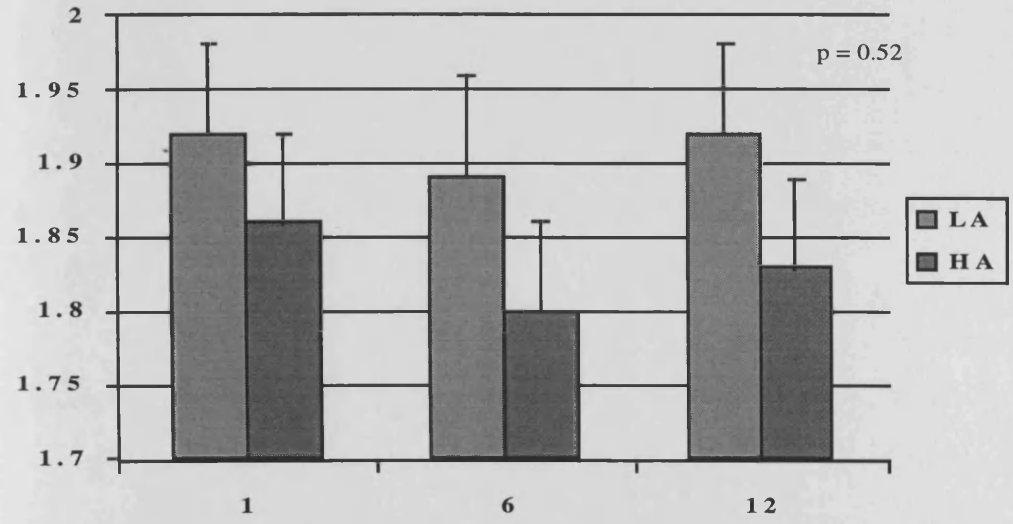
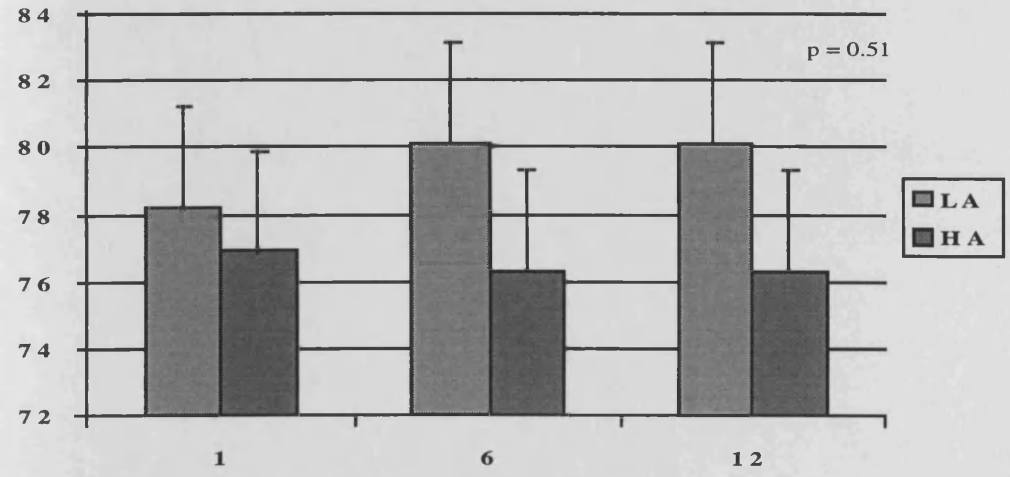


Figure 12: Creatinine Clearance (L/week)



Adequacy of dialysis. By the criteria available at the start of the study (1991-92) both groups would have been reasonably dialysed at one year. The recently (September 1997) updated (American) National Kidney Foundation-Dialysis Outcomes Quality Initiative (NKF-DOQI) recommends a minimum weekly Kt/V of 2.0 for PD patients (**130**). So, by this current criterion, both groups were inadequately dialysed throughout the study. This point will be debated in more detail in the Discussion.

3.7. Renal bone disease

3.7(i). Renal bone disease protocol

The use of calcium carbonate and aluminium hydroxide in the two groups was summarised in Section 3.4. Alfacalcidol was used in 29 HA patients compared to 46 LA patients (average dose 0.13 ± 0.02 μg (range = 0-1) per day vs 0.22 ± 0.03 (range 0-1.5) μg per day; $p<0.05$).

3.7(ii). Bone biochemistry

Calcium. The target adjusted serum calcium of 2.5 mmol/L was achieved by six months in both groups (Table 7 and Figure 13). At one year, the calcium was not significantly different in the two groups ($p = 0.92$).

Hypercalcaemic episodes. Two LA patients had to be changed to HA dialysate because of persistent hypercalcaemia, despite withdrawal of calcium containing or sustaining agents. No other changes from one dialysate to the other occurred for any other reason. There was no difference in the number ($p = 0.7$) or magnitude ($p = 0.99$) of hypercalcaemic episodes between the two groups (Table 8).

Phosphate. Serum phosphate was lower in the HA patients at one year, when compared to the LA patients (HA: 1.66 ± 0.05 vs LA: 1.87 ± 0.05 mmol/L; $p<0.05$) (Table 7 and Figure 14).

Table 7: Bone Biochemistry (all values are means ± SEM; all analyses are LA vs HA at one year)

	LA				HA			
	0	**6**	**12**	**mths**	**0**	**6**	**12**	**p value**
Calcium	**2.25**	**2.47**	**2.51**		**2.27**	**2.49**	**2.52**	**0.92**
(mmol/L)	± 0.04	± 0.02	± 0.02		± 0.03	± 0.02	± 0.02	
min =	*1.35*	*2.07*	*2.02*		*1.31*	*1.67*	*2.19*	
max =	*5.32*	*3.07*	*3.85*		*3.04*	*3.14*	*3.15*	
no =	*89*	*78*	*59*		*94*	*82*	*68*	
Phosphate	**2.36**	**1.78**	**1.87**		**2.33**	**1.64**	**1.66**	**<0.05**
(mmol/L)	± 0.07	± 0.06	± 0.05		± 0.07	± 0.06	± 0.05	
min =	*1.08*	*0.72*	*0.5*		*0.74*	*0.55*	*0.84*	
max =	*5*	*4.54*	*3.06*		*4.78*	*4.15*	*2.89*	
no =	*89*	*78*	*59*		*93*	*82*	*68*	
Alk Phos	**100**	**101**	**103**		**98**	**90**	**84**	**<0.01**
(iu/L)	± 4	± 4	± 5		± 4.9	± 5	± 3	
min =	*38*	*38*	*45*		*33*	*46*	*40*	
max =	*291*	*228*	*389*		*265*	*412*	*163*	
no =	*89*	*78*	*59*		*93*	*82*	*68*	

PTH	**21.2**[22]	**14.5**	**15.7**	**23.6**	**17.9**	**17.0**	**0.77**
(pmol/L)	± 2.4	± 2.3	± 3.0	± 2.5	± 1.9	± 1.9	
min =	*0.5*	*0.5*	*0.7*	*0.5*	*0.5*	*0.5*	
max =	*125*	*152*	*150*	*134.2*	*86*	*90*	
no =	*92*	*75*	*56*	*88*	*76*	*63*	
Aluminium	**6.2**[23]	**10.2**	**11.4**	**6.7**	**8.2**	**9.2**	**0.29**
(mcg/L)	± 0.5	± 2.6	± 1.2	± 0.6	± 1.1	± 1.0	
min =	*1*	*1*	*1*	*1*	*1*	*1*	
max =	*20*	*220*	*68*	*36*	*90*	*55*	
no =	*82*	*77*	*52*	*79*	*77*	*57*	

Table 8: Hypercalcaemic Episodes

	LA	HA	p value
Number	1.28 ± 0.1	1.34 ± 0.12	**0.7**
min =	*0*	*0*	
max =	*5*	*5*	
no =	*100*	*100*	
Maximum Ca^{++} (mmol/L)	2.95 ± 0.03	2.95 ± 0.03	**0.99**
no =	*100*	*100*	

[22]value at 1 month
[23]value at 1 month

Figure 13: Serum Calcium (mmol/L)

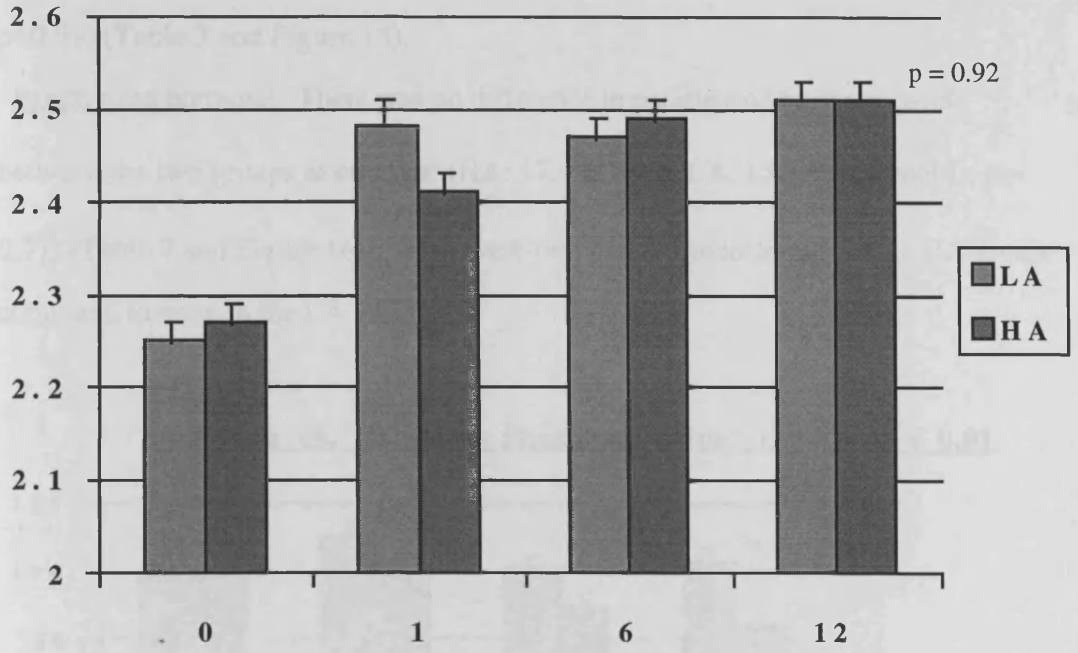
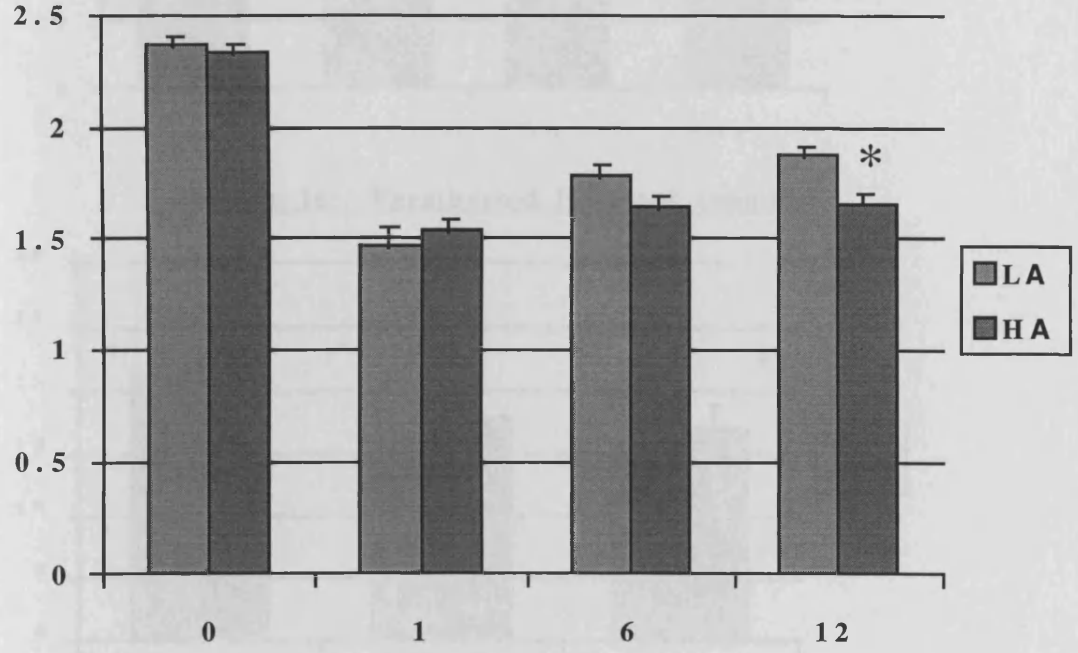


Figure 14: Serum Phosphate (mmol/L); * = p < 0.05



Alkaline phosphatase. The serum alkaline phosphatase was very significantly lower at one year in the HA group compared to the LA group (HA: 84 ± 3 vs 103 ± 5 iu/L; $p<0.01$) (Table 7 and Figure 15).

Parathyroid hormone. There was no difference in parathyroid hormone levels between the two groups at one year (HA: 17.0 ± 1.9 vs LA: 15.7 ± 3.0 pmol/L; $p = 0.77$) (Table 7 and Figure 16). There were two parathyroidectomies in the HA group, compared to none in the LA group.

Figure 15: Alkaline Phosphatase (iu/L); ** = p < 0.01

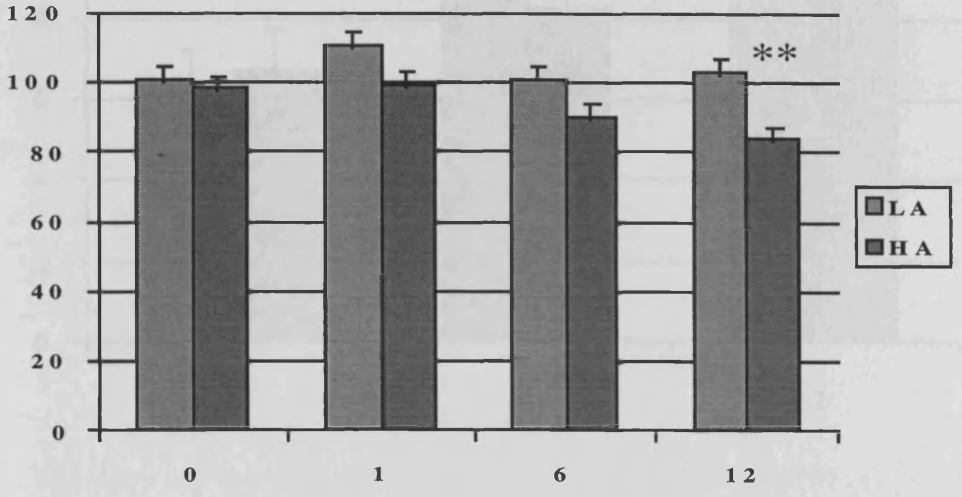
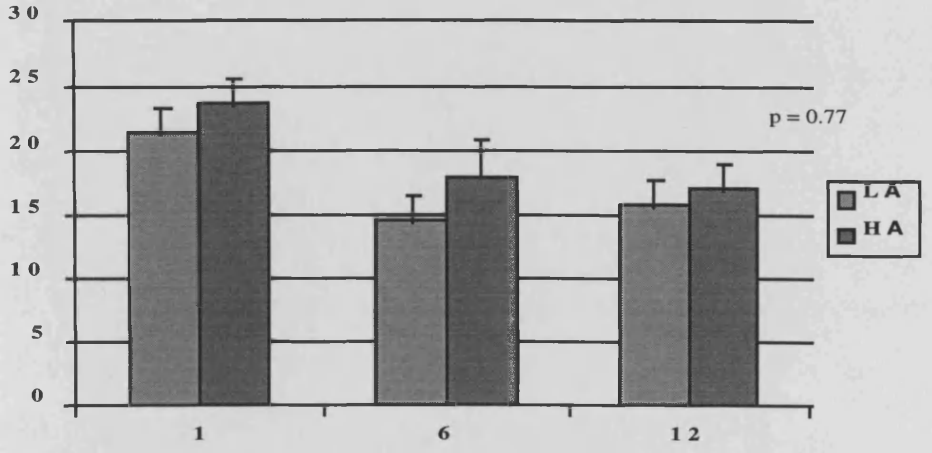
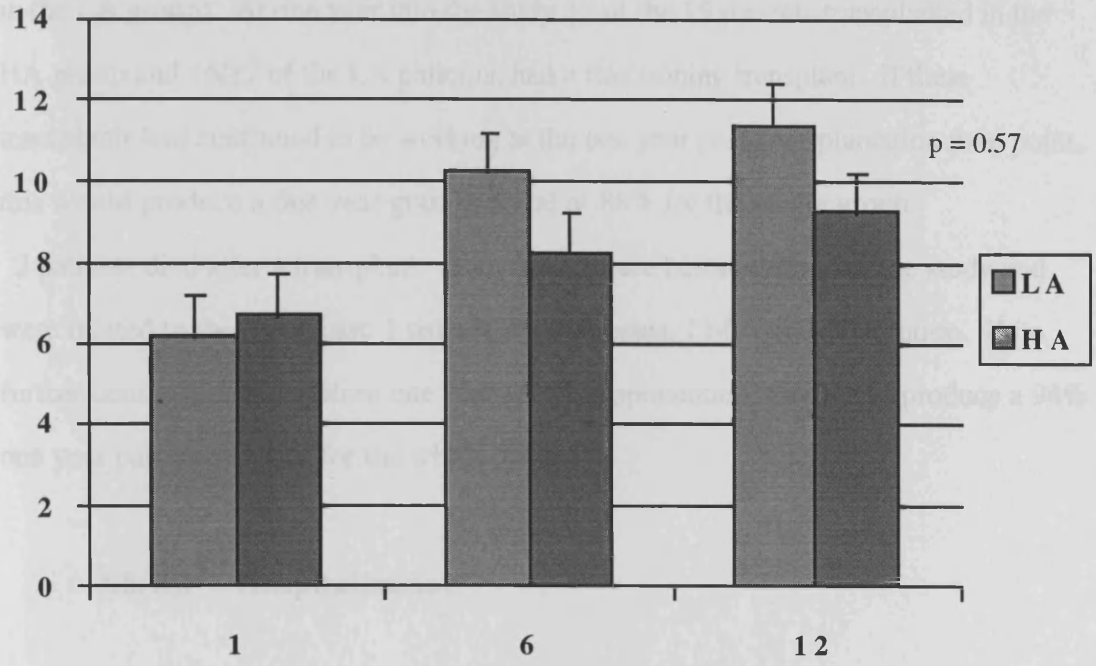


Figure 16: Parathyroid Hormone (pmol/L)



Aluminium. There was a progressive rise in serum aluminium in the two groups over the year, the levels staying within the normal range (0-20 mcg/L). There was no difference in the serum aluminium level at one year between the two groups ($p = 0.57$; Table 7 and Figure 17).

Figure 17: Serum Aluminium (mcg/L)



3.8. Outcomes

3.8(i). Renal modality[NSA]

The treatment modality of the two groups at one year is summarised in Table 9. A similar number of patients were transplanted in the two groups (15 in the HA group, 17 in the LA group). At one year into the study 12 of the 15 patients transplanted in the HA group and 16/17 of the LA patients, had a functioning transplant. If these transplants had continued to be working at the one year post-transplantation time point, this would produce a one year graft survival of 88% for the whole group.

2 patients died after a transplant. Both deaths were before the end of the study and were related to the transplant: 1 patient died of sepsis, 1 of bowel perforation. If no further deaths occurred before one year post-transplantation, this would produce a 94% one year patient survival for the whole group.

3.8(ii). Hospitalisation

There were less hospital admissions in the HA group (1.13 ± 0.16 per patient per year) compared to the LA group (1.71 ± 0.22 per patient per year; $p<0.05$) (Table 10). The HA patients spent less days in hospital per year than the LA patients (16.4 ± 1.4 days/year vs 21.2 ± 1.9 days/year; $p<0.05$) (Table 10).

There was no difference between the two groups, in terms of:

(1) the days spent in hospital for the original admission(s) - ie, the number of inpatient days for an ARF admission (if the patient had one) added to the number of inpatient days for the catheter insertion admission;

(2) the days spent in hospital after the original admission(s) expressed as days per at risk months;

(3) the number of visits by a community dialysis nurse;

(4) the number of visits to the renal outpatient clinic; and,

(5) the total contact days - ie, the total number of inpatient days combined with the number of contacts with a community dialysis nurse or the outpatient department.

Hospitalisation is summarised in Table 10.

Table 9: Renal Modality at One Year (values are numbers of patients)

	LA	HA
CAPD	59	69
Transplant	16	12
Death	15	12
HD	5	5
Intermittent PD	1	0
Recovery of renal function	3	0
Transferred out of unit	1	2

Table 10: Hospitalisation (analyses compare LA to HA groups; all n = 100)

	LA	HA	p value
Admissions	1.71 ± 0.22	1.13 ± 0.16	**<0.05**
min =	*0*	*0*	
max =	*13*	*9*	
no admitted at some time =	*61*	*61*	
Inpatient days/admission	6.4 ± 0.7	6.1 ± 0.4	**0.77**
'Early' inpatient days[24]	10.8 ± 0.7	9.8 ± 0.7	**0.37**
min =	*3*	*3*	
max =	*34*	*45*	
Inpatient days[25]	10.4 ± 1.6	6.7 ± 1.1	**0.05**
min =	*0*	*0*	
max =	*87*	*60*	

[24]= inpatient days for ARF admission (if the patient had one) + inpatient days for catheter insertion admission

[25]= inpatient days after catheter insertion

Inpatient days / at risk month	1.48 ± 0.3	0.87 ± 0.16	**0.09**
Total inpatient days	21.2 ± 1.9	16.4 ± 1.4	**0.05**
min =	*3*	*3*	
max =	*104*	*67*	
Outpatient visits	5.8 ± 0.3	5.9 ± 0.3	**0.73**
min =	*0*	*0*	
max =	*16*	*12*	
Community nurse visits	4.8 ± 0.3	5.0 ± 0.2	**0.5**
min =	*0*	*0*	
max =	*13*	*14*	
no =			
Total contact days	31.8 ± 2.0	27.4 ± 1.5	**0.07**
min =	*6*	*8*	
max =	*112*	*81*	

3.8(iii). Peritonitis / exit site infections[NSA]

The peritonitis rate was similar in the two groups. The data is summarised below.

Table 11: Peritonitis

	LA	HA
Probable episodes[26]	86	75
Proven episodes[27]	53	44
Culture -ve rate[28]	38%	41%
At risk period	976	1014 months
Peritonitis rate[29]	1/11.3	1/13.5 patient-months

Table 12: Peritonitis: Number of Episodes (values are numbers of patients)

	LA		HA	
Number of Episodes				
0	55		65	
1	24	(24)	18	(18)
2	12	(24)	6	(12)
3	3	(9)	4	(12)
4	2	(8)	4	(16)
5	3	(25)	1	(5)
6	1	(6)	2	(12)
		86		75

[26]= typical symptoms + treated *as if* patient had peritonitis
[27]= above *and* organism identified
[28]= (1- (proven / probable episodes)) x 100
[29]using probable episodes

Exit site infections. This data is unreliable. Amongst the 87 HA and 86 LA patients where some (unreliable) data was obtained, there were similar numbers of patients that had experienced one or more exit site infections (32 HA vs 29 LA patients) (Table 13).

Table 13: Exit Site Infections

	LA	HA
Number patients with 1 or more episodes[30]	29 (n = 86)	32 (n = 87)
min =	*0*	*0*
max =	*4*	*5*
Number episodes	55	61

3.8(iv). Death / technique failure[NSA]

Twelve patients died in the HA group, compared to 15 in the LA group (Table 14). Six patients in each group experienced permanent technique failure (Table 15). There were 40 episodes of temporary technique failure in the LA patients, compared to 27 in the HA group. The causes of the temporary technique failures are summarised in Table 16.

[30]incomplete data - the number of patients where data was available is indicated

	LA	HA
Table 14: Causes of Death		
Cardiovascular	6	4
Malignancy	5	4
Stopped dialysis	2	1
Post-transplantation	1 (sepsis)	1 (bowel perforation)
Other	1 (stroke)	2 (amyloid[31])
Total	**15**	**12**

Table 15: Technique Failure (Permanent)		
Peritonitis	2	2
Unable to cope	2	2
Underdialysis	1	1
Other	1 (1.5 litres too big[32])	1 (rectal prolapse[33])
Total	**6**	**6**

Table 16: Technique Failure (Temporary)		
Leak[34]	16 (one pt twice, one 3x)	10
Catheter replacement	12	9
Hernia	12 (1 pt twice)	8
Total	**40**	**27**

[31]amyloid was the cause of renal failure. Towards the end, liver failure occurred, presumed secondary to the widespread amyloidosis.

[32]even though 1.5 litres was 'too much' for this 'small' (50 kg) lady, she had not had previous abdominal surgery, and a problem had not been predicted. Indeed, many other similar sized ladies had no problem with this volume.

[33]combined with a recurrent catheter tunnel infection; the rectal prolapse was the more significant reason for permanent technique failure

[34]two leaks were scrotal leaks (both in the LA group), the others being 'abdominal', ie around the exit site

CHAPTER FOUR

"Art thou so bare and full of wretchedness,
And fear'st to die? famine is in thy cheeks,
Need and oppression starveth in thy eyes,
Contempt and beggary hangs upon thy back"

Romeo and Juliet V, 1
Romeo to Apothecary,
attempting to buy some
poison

CHAPTER FOUR

DISCUSSION

4.1. Introduction

4.1(i). Reliability of data

Every effort was made to include all reliable data available at every time point, in order to produce valid information. Nonetheless, knowing which data to include (and where to put it) in long-term studies such as this, is not obvious. This is especially the case, when, as in this study:

(1) attempts needed to be made to place patients into groups (such as their renal modality, which can change regularly),

(2) patients are changed from one treatment group to another, and,

(3) large numbers of patients are involved.

Almost all of the parameters assessed in this study - including the original six 'key' parameters: four nutritional (body weight, midarm circumference, triceps skin-fold thickness and serum albumin), and two morbidity (number of admissions and days spent in hospital) - were not as simple to record (or analyse) as would first appear.

For example, how do you decide upon the renal modality of a 'PD patient' who had a transplant that failed, then started haemodialysis due to personal preference, all before the end of the study? Was this a 'technique failure' or not? Should you use any of the biochemical data on that patient through that period?

Problems (1) and (2) are largely caused by a need to categorise ESRF patients into groups: 'PD patients', 'haemodialysis patients' etc. In reality, they are all in a CRF/ESRF spectrum, passing from one treatment modality to another while remaining in CRF/ESRF. A patient may pass from being an 'acute HD' to a 'stable PD' to a

'temporary HD' patient in a period of a few weeks. All these descriptions are, of course, arbitrary.

4.1(ii). 'Intention-to-treat' principle

It was decided to attempt to follow an intention-to-treat principle before the start of the study. In some ways, this policy was successful. For example, the patients were analysed according to the group into which they had been originally randomised. In other ways the policy was less successful.

For example, of the four key nutritional parameters, unfortunately *only* body weight and serum albumin were recorded in patients who had 'left' the study, to haemodialysis or transplantation for example. The anthropometric and muscle strength data does not include information from patients after they had left the study, as these measurements were not made. In retrospect, the measurement of these parameters should have been continued, and the data incorporated into the analysis.

Apart from statistical purity, there are good biological reasons for following this principle. It is important to include patients lost to transplantation or haemodialysis as they may, by definition, be the 'better' or 'worse' patients. So, if one group lost more patients to transplantation or haemodialysis, any nutritional parameter measured at one year could be biased. In fact, this could have happened in this study, if the patients 'lost' from the study had not been included in the body weight analysis. Without them, the body weight analysis at one year would have compared 69 HA to 59 LA patients. Part of the difference in proportion of patients still on PD at one year cannot be attributed to the 'better' nutritional state of the HA patients - it was due to a slightly greater number of successful transplants in the LA group (16 compared to 12 in the HA group[35]). These observations make it even more galling that anthropometric data was not obtained from the patients lost from the study.

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[35]Leicester has a relatively low transplant rate for its size, with approximately 40 transplants taking place per year, despite a very large dialysis population (approximately 500 at present). This discrepancy was an advantage in this study, as it meant that good numbers of patients made it through to one year.

It is also possible that patients who had less acidosis correction *could* have had some strange nutritional advantage when transplanted that *could* have outweighed their nutritional 'disadvantage' when on PD. Even though this scenario is clearly unlikely, it needed to be incorporated in the analysis.

Carrying out an 'intention-to-treat' principle is theoretically pure, in statistical terms. However, it can lead to incorporating meaningless data that may 'cover up' real effects. Therefore, when it was felt that it would confuse the answer to an important clinical question, it was not followed. So, for example, the "number of hypercalcaemic episodes" presented in the results section does not include those episodes experienced on haemodialysis - as the reason for counting the number of episodes was to determine the extent of the problem *while on PD*. Similarly, for the hospitalisation analysis, the number of days spent in hospital for PD related events was felt to be the clinically relevant issue.

The acid/base, renal bone disease and kinetic modelling data also does not include data from patients who had left the study. Again, it was felt that including data from these patients was not relevant to the questions being posed.

4.1(iii). Statistical analysis

The only statistical analysis performed in this study was an unpaired Student's 't' test on the one year data. This was a deliberate policy of the study.

The six key parameters were assessed at one year. Some of the other parameters were also analysed, if (1) they could be expressed as continuous variables, and, (2) their analysis was an attempt to answer a clinical question.

Of the six key parameters decided upon before the start of the study, body weight was felt to be biologically the most important. For this reason, it was the parameter that was used in the original power analysis.

Given that the clinical question was "does better correction of acidosis improve long-term nutritional state?", a variety of tests could have been carried out to describe the

'nutritional experience' of the patients. More specifically - given that body weight was thought to be the most important of the four nutritional parameters - the test chosen, above all, had to be suitable to describe the '*weight* experience' of the two groups. Various ways of analysing the weight experience of the patients were considered.

It would have been possible, for example, to compare the weight curves of the two groups, with an analysis of variance for repeated measures. The mathematical assumptions for such measures are both extensive and debatable. Alternatively, the slope of the weight curve could have been analysed by linear regression. Myriad other derived numbers could have been compared - eg, time to maximum value, maximum value, difference from baseline to maximum - by a variety of different methods. All these methods were considered but rejected.

Instead, it was felt that a simple summary statistical test such as a 't' test, at a fixed time point (one year) was both the simplest, and the most appropriate test. Body weight was analysed for it's suitability for a 't' test, since it was the parameter put into the power analysis before the study started. Furthermore, it was felt to be the 'hardest' and most meaningful of the four key nutritional parameters. The two requirements for a t test were checked - ie, the parameter to be analysed (body weight): (1) had a similar standard deviation in the two groups, at baseline, and (2) was derived from a normal distribution.

It was decided to analyse the change in body weight, rather than the actual body weight. This decision was made *not* just because the differences in actual body weight at one year did not reach statistical significance; but because it was felt body weight change was more discriminatory, and hence more clinically relevant.

To compare parameters other than the six key parameters at one year might be considered statistically 'impure'. However, it was felt that this was a reasonable thing to do, as long as it was *remembered* that these analyses: (1) constituted a *post hoc* analysis; and, (2) that they should therefore be considered 'interesting trends' rather than facts.

The main danger of analysing multiple parameters in clinical research - especially when a relatively low 'p' value (<0.05) is used as the arbiter of truth - is that it may lead to a 'stamp collecting' exercise. In other words, multiple analyses will produce statistically significant results by virtue of the numbers of analyses performed.

On the other hand, important parameters not directly relevant to the study, such as bone biochemistry *were* measured; and the results obtained produced important information regarding patient care. So, not to analyse those parameters seemed overly pedantic, and possibly unethical.

4.1(iv). "NSA" = no statistical analysis

As the statistical policy of the study was to carry out an unpaired 't' test at one year:
(1) non-continuous data was not analysed. A 't' test is not suitable to analyse non-continuous data (eg, treatment modality at one year, mortality, whether or not a patient had peritonitis);
(2) data from time points other than one year (eg, at baseline) was not analysed. *Not* to compare (statistically) the matching of the two groups at baseline might seem a strange decision. But it is in keeping with modern statistical techniques in randomised controlled trials - since, if randomisation is fair, any differences between groups could only have arisen by chance.

Furthermore, to compare the two groups at all six designated time points would also have increased the likelihood of arriving at significant results by chance, even if it was restricted to the six key parameters;
(3) data within a group was not analysed over time - to answer such questions as "did the bicarbonate rise significantly in either group over time?" Again, carrying out such analyses would have led to further possibilities of discovering 'significant' results by chance.

4.1(v). Patient blinding

This was a single blind study, which was one of the strengths of the study. Ideally a double-blind study would have been performed. An attempt was made to blind the dietitians carrying out the key anthropometric measurements. In reality, this was not possible. They could tell easily which group the patient was in, by the tablets they were taking.

The patients were not told that they were in a nutritional study, unless they asked specifically. In this way, every attempt was made to encourage the patients to continue their normal eating habits (with some 'protein pushing' in both groups).

4.2. Matching

Hopefully, the discrepancy in demography (27 patients with IHD in the LA group, vs 22 in the HA group) was 'counterbalanced' by the larger number of diabetics in the HA group (22 vs 12). It was gratifying that the co-morbidity scores of the two groups were the same.

It is also fortunate that the difference in nutritional state was the 'right way round' - ie, with the control group possibly starting at a slight nutritional advantage. If there was any bias in the nutritional matching of the study, chance caused that bias to be in favour of the control group[36].

4.3. Haematology

An attempt was made to remove anaemia as a possible factor in the nutritional state of the two groups, using a very liberal EPO and blood transfusion policy. It was hoped that by setting a target haemoglobin (>11 g/dl) similar haematological control could be achieved in the two groups. It was pleasing that the haemoglobin reached the target level by six months in both groups, and stayed at this level. To achieve this, EPO was used as soon as the haemoglobin fell below 10 g/dl. As the same haemoglobin/EPO policy was used for all the patients, it was not surprising that a similar proportion of patients in each group used EPO (HA: 52%, LA: 47%). In 1992, when this study was started, such liberal use of EPO was not the norm. The average doses of EPO, and units of blood transfused were also similar in the two groups.

[36]The nutritional gain of the HA group is even more remarkable when one considers that they started off, on average, 1 kg lighter than the LA group; and ended up 2 kg heavier.

4.4. Acid/base balance

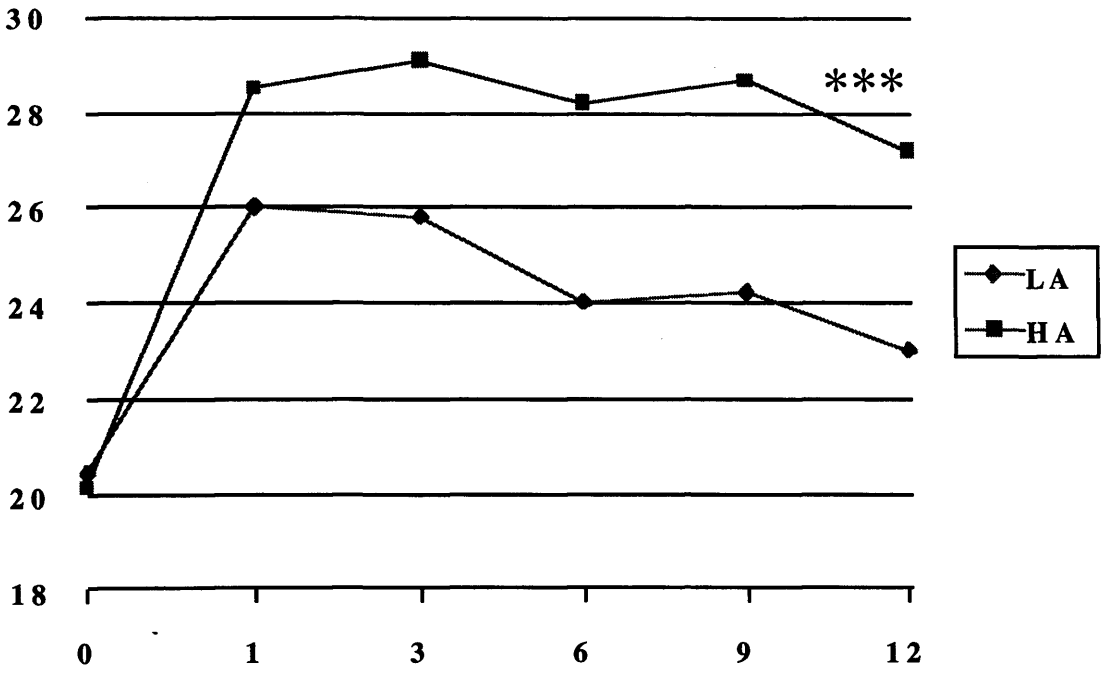
In an attempt to achieve the target serum bicarbonate level (30 mmol/L), 92% of the HA patients were treated with calcium carbonate as well as HA dialysate. The dose of calcium carbonate was increased as much as possible, to correct acidosis maximally - the only limitations being hypercalcaemia and patient compliance. Attempts were made to reduce the well-known poor compliance with calcium carbonate by: (1) regularly reminding the patients to take the drug, and, (2) the use of a preparation called Calcichew Forte®, which has twice the calcium content of 'ordinary' Calcichew®.

The combination of calcium carbonate and HA dialysate was not sufficient to drive the venous bicarbonate anywhere near the target value (>30 mmol/L) in the HA patients. So, 48% of the HA patients also required sodium bicarbonate. Clearly, sodium bicarbonate was to be avoided in the LA patients. Indeed, only one LA patient required sodium bicarbonate.

Even though the different dialysate/medication regimes *were* able to produce differences in the control of acid/base balance, the target venous bicarbonate of 30 mmol/L in the HA group, was surprisingly difficult to achieve.

Despite the measures described above, at one year, only 17 out of 69 HA patients (24.6%) who reached that time point, had a venous bicarbonate of 30 mmol/L or over, compared to none in the LA group. Part of this difficulty can be explained by examining Figure 4 (see below). It can be seen that after 3 months, there was a progressive decline in venous bicarbonate in both groups, presumably as a result of loss of residual renal function.

Figure 4 (repeated): venous bicarbonate concentration (mmol/L) in the two groups over year of study (* = p<0.001 at one year)**



Furthermore, the amount of lactate in HA dialysate (40 mmol/L), even in combination with high dose calcium carbonate and/or sodium bicarbonate, was not sufficient to achieve the target level. Not surprisingly, it was more difficult to improve acidosis correction in the larger patients, especially those losing residual renal function. Also, it is doubtful that when large doses of calcium carbonate and/or sodium bicarbonate were prescribed, compliance with either drug was optimal.

As mentioned in the Introduction, in 1992 Yamamoto et al (**126**) showed that the use of 40 mmol/L lactate alone leads to an average venous bicarbonate of 21.6 mmol/L - ie, it does not fully correct acidosis. Indeed, the acidosis correction is no better than when 35 mmol/L lactate is used. So, perhaps it is not surprising that it was so difficult to fully correct acidosis in the HA group. Presumably, the average venous bicarbonate concentration of 27.2 mmol/L in the HA patients was principally due to the extra calcium carbonate and sodium bicarbonate they received.

It is interesting that the effects seen in this study were observed when neither group was alkalotic or acidotic. Indeed, the nutritional effects occurred at the ends of the

normal range of venous bicarbonate. At one year, arterial pH was different but within the normal range in the two groups (7.44 in the HA group vs 7.40 in the LA group; $p<0.001$)[37]. Clearly acidosis was corrected in both groups, though more so in the HA group. In this way, this study did not compare 'inadequate to adequate' correction of acidosis, which was one of the original intentions. The inability to carry out such a study was due to:

(1) an inability to 'over-correct' acidosis in the HA group, and,

(2) better than expected acidosis control in the LA group.

In the majority of the animal studies carried out so far, in which acidosis has been shown to increase protein degradation, the animals were made profoundly acidotic; for example, in one rat study, an arterial pH of 7.2 was achieved (Williams et al, **56**). It is unlikely that such models are analogous to this study.

It is conceivable, however, that even though neither group was truly acidotic or alkalotic, variation in venous bicarbonate (and/or pH) within the normal range of acid/base balance is relevant for this effect. This possibility will now be discussed in more detail.

[37]The arterial pH was only measured at one year, as it was felt that if a potentially painful investigation had been carried out at baseline, it would have affected the likelihood of patients consenting to take part in the study.

Possible mechanisms of the MA/protein metabolism effect - role of bicarbonate vs pH, and the intracellular pH debate

Assumptions. A series of assumptions underlie this argument, and the MA/protein metabolism effect in general:

(1) the mild extracellular acidosis of CRF is associated with intracellular acidosis,

(2) extracellular pH is the principle determinant of intracellular pH,

(3) changing extracellular pH can alter intracellular pH,

(4) as the majority of the body's proteins are intracellular, any apparent effect of extracellular pH is due to the influence of intracellular pH on intracellular protein metabolism,

(5) changes in intracellular pH/protein metabolism show themselves in the products of extracellular protein metabolism, which are more easy to measure.

If these assumptions are true, for the MA/protein metabolism effect to occur, it is presumed that reducing extracellular pH reduces intracellular pH and thereby increases protein degradation. However, some or all of these assumptions may not be true. In particular, assumptions (1), (2) and (3) may not be true:

Assumption (1). In fact there is *little* evidence that intracellular pH is low in chronic uraemia. Durozard et al, in 1993, investigated intracellular pH in haemodialysis patients and controls, using ^{31}P NMR spectroscopy (**131**). They found no difference, at rest, between the two groups in terms of intracellular pH. Furthermore, in 1996 Bailey et al (**132**) demonstrated that in rats with chronic uraemia (with its associated mild MA) no intracellular acidosis occurs.

Assumption (2). It is possible that extracellular pH is *not* the principle determinant of intracellular pH. If so, the MA/protein metabolism effect may not be dependent on extracellular pH.

There are other possible (non-pH dependent) explanations for the effect. The importance of a sodium/hydrogen/bicarbonate/chloride cellular exchange mechanism

has been stressed by Ng et al (1993, **133**) and others. Part of this mechanism can exchange hydrogen for bicarbonate. It could account for the changes seen in this and other human studies. Intracellular processes produce H^+ ions that need to be extruded from the cells. If a hydrogen/bicarbonate exchanger is important in human cells, a high extracellular bicarbonate concentration could stimulate H^+ ions to pass out of the cell - *independently* of the extracellular H^+ ion concentration (ie, pH). So, in this study, the high extracellular bicarbonate concentration could have reduced intracellular acidosis, by exchanging extracellular bicarbonate for intracellular H^+ ions. This could have reduced intracellular protein degradation, which eventually showed itself in body weight changes.

So, either reducing extracellular pH or increasing the extracellular bicarbonate concentration could be the stimulus for reducing intracellular acidosis, and thereby reducing intracellular protein degradation.

Assumption (3). Changing extracellular pH may not alter intracellular pH. In the previously mentioned study by Bailey et al (1996, **132**), they found that acute MA did *not* reduce intracellular pH. A possible reason for this anomaly was put forward by the authors, "the lack of an acute change in intracellular pH suggests that intracellular buffering capacity changes over time". Though chronic acid loading did reduce intracellular pH.

Despite these anomalies, it seems highly unlikely that acidity (either extracellular or intracellular) has *nothing* to do with the effect. Too many studies have demonstrated that MA *does* affect protein metabolism. Quite how it does so remains to be determined.

4.5. Nutrition

Methods of measuring nutritional state

There are many methods of measuring nutritional state. No one of them is adequate. No one method (or group of methods) has been accepted, nationally or internationally, as a 'gold standard' across all disciplines. The currently available options will now be critically analysed.

The close association between malnutrition and disease contributes to the difficulty in demonstrating that malnutrition is common in ESRF. All the current nutritional parameters (including weight, anthropometric data, serum protein levels and delayed hypersensitivity reaction) may be affected by underlying illness or functional capacity as well as by nutritional status.

A common example is serum albumin. As a negative acute phase protein, its serum concentration is reduced in response to stress and inflammation, reflecting disease severity rather than nutritional deficiency. It is also insensitive to change in protein intake, partly due to its prolonged half life (Ikizler, 1997, **149**; Klein et al, 1997, **150**). Serum proteins with shorter half-lives such as prealbumin and retinol binding protein may be more sensitive to change in nutritional status; but levels decrease with infection and hepatic disease and may be falsely elevated in renal failure (Dwyer et al, 1993, **151**).

Methods such as weight changes, body mass index (BMI) and anthropometry are useful and practical in the clinical setting. However, they may be relatively insensitive to change in nutritional status and results will be affected by fluid abnormalities such as dehydration and oedema (Collins et al, 1979, **152**; Jeejeebhoy et al, 1990, **153**). Activity levels, functional ability and disease may influence results; and the selection of suitable standard reference values is also problematic (Klein et al, 1997, **150**).

Tests of immunocompetence have been proposed as a functional index of nutritional status (Chandra and Scrimshaw, 1980, **154**). Immunological changes correlate with

poor outcome both in medical and surgical patients in terms of complications, duration of hospital stay and mortality (Chandra, 1991, **155**). However these changes are not nutrition-specific and may, for example, occur with liver failure, renal failure and HIV infection in the absence of malnutrition (Jeejeebhoy et al, 1990, **153**).

Subjective global assessment (SGA) was developed to try to overcome the disadvantages of using objective biochemical or anthropometric measurements (Detsky et al, 1987, **156**). Nutritional status is categorised from a medical and dietetic history and physical examination. It is simple and reproducible but time consuming and relatively insensitive to short-term changes. It is not independent of disease severity, but may be the best available method of defining the signs of malnutrition in relation to clinical parameters (Jeejeebhoy et al, 1990, **153**).

Methods such as multifrequency bioelectrical impedance analysis (BIA) of body composition and muscle function testing need further evaluation but may be important tools of nutritional assessment in the future. Handgrip dynamometry has also been shown to be more sensitive than weight for height, weight loss, serum albumin and upper-arm anthropometry in predicting serious postoperative complications (Klidjian et al, 1980, **157**).

However, none of these methods described, either individually or together, adequately 'measure' nutritional state. There is no serum creatinine for nutrition. As in most renal nutritional studies, this work will use a combination of the above, accepting that none really define nutritional state.

What is malnutrition? Are dialysis patients malnourished?

Malnutrition is impossible to define. It is hard enough to define good nutrition, or indeed, nutritional status. In fact, there is no internationally accepted definition of nutritional status. One definition (Krause and Mahan, 1979, **158**) is as follows:

"... the degree to which the individual's physiological need for nutrients is being met by the foods he/she is eating. It is the state of balance in the individual between nutrient intake and the nutrient expenditure or need".

Others have sought to make a distinction between the malnutrition which is an inevitable consequence of disease, and malnutrition which is remediable with nutrition support (Allison, 1995, **159**). Jeejeebhoy has defined malnutrition as:

"the presence, in a body system, of abnormalities observed upon withdrawal of nutrients and correctable by refeeding" (Jeejeebhoy, 1988, **160**)".

This, and most definitions, excludes any precise numerical values.

Whether the patients in this study were malnourished is impossible to say. One method of defining malnutrition is by comparing the percentage differences between 'nutritional data' (eg, weight, midarm circumference, serum albumin) from renal patients to age-sex matched 'normal' controls. One American renal dietetic handbook defines 'mild malnutrition' as data being 80-90% of controls, 'moderate' as 70-79% and 'severe' as <70% (McCann, 1997, **161**). Whether comparing Leicester renal patients - who are a far from typical group (age middle 50s, male preponderance, 30-40% with diabetes, Asian minority) - to 'standard' controls is highly debatable. For this reason it was not done on this study.

In summary, there are several reasons for the inability to define nutrition/malnutrition, and the difficulty in deciding whether our patients were malnourished:

(1) nutrition cannot be defined, nor can an absence or 'reduction' of nutrition, namely malnutrition;

(2) nutrition and malnutrition are part of a continuous spectrum;

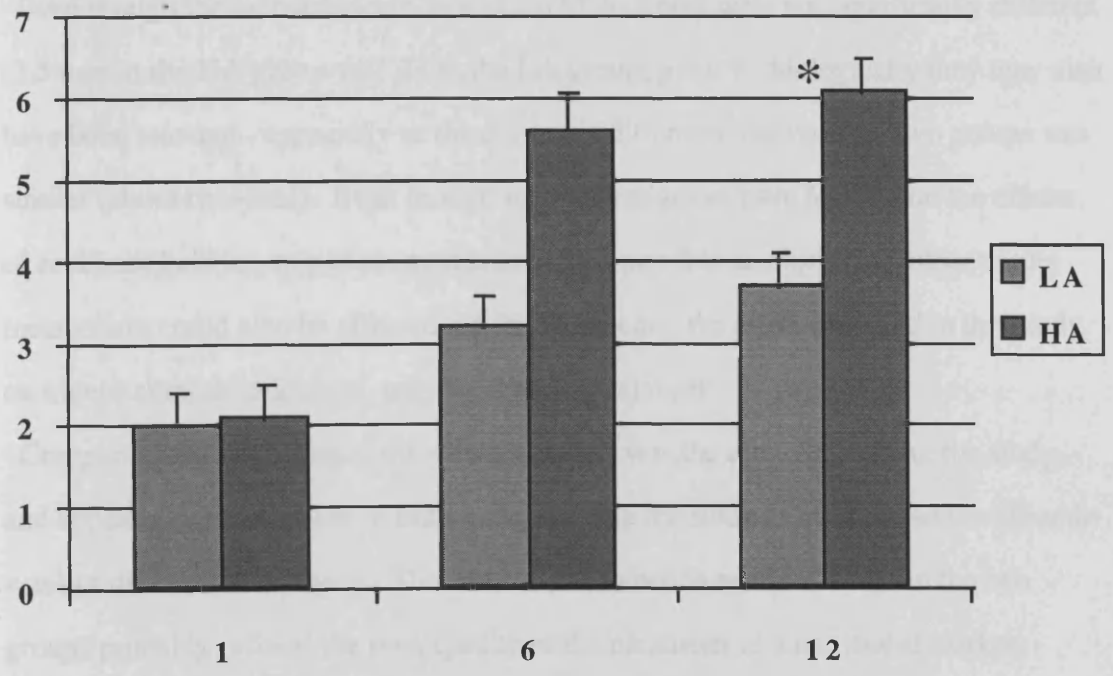
(3) there is no single parameter (or group of parameters) that can be used to adequately describe nutrition or malnutrition;

(4) what parameters are used, are surrogates for nutritional state.

Most importantly, as suggested by the title of the thesis, it was a study of changes in nutritional state, not a study defining or comparing levels of malnutrition.

Despite these difficulties, in terms of nutritional state, this was a very positive study. At one year, the increase in body weight in the HA group (6.1 kg) was significantly greater than the LA group (3.71 kg) (see Figure 5, repeated below). The increase in midarm circumference in the HA group (1.26 cm) was also greater than that of the LA group (0.61 cm); the size of the effect being of similar proportion to that of the weight gain. These are probably the two most important findings of this study.

Figure 5 (repeated): weight gain (kg) of the two groups over year of study (* = p<0.05 at one year)



When the 2kg of the weight gain that can be apportioned to the PD fluid in the abdomen is 'removed' from the analysis, the size of this effect can be appreciated even more. In other words, the 4.1 kg of *true* body weight gain in the HA patients is nearly 2.5 times the 1.7 kg weight gain in the LA group.

Interestingly, there was no significant relationship between starting weight and weight gain in the HA group ($r=0.11$; $p>0.35$); indicating that the positive effect seen is apparent over the whole range of patient size, not just those 'with most to gain' ie, the malnourished patients.

It was decided to present the patients' weight as their actual body weight (in clothes, without shoes on) on the day of measurement - ie, rather than their 'dry body weight' (with PD fluid 'removed'). There were three reasons for this decision:

(1) ease of data transfer - especially when transferring data from the unit computer to the study database;

(2) it was felt that to subtract the weight of PD fluid would be unnecessarily complex as the volume of PD fluid was changing through the year;

(3) it could not be guaranteed that the patients would attend the clinic with fluid in (they usually did).

Even though the increases in triceps skinfold thickness were not significantly different (2.5 mm in the HA group vs 1.24 in the LA group; $p = 0.1$), biologically they may also have been relevant - especially as the *degree* of difference between the two groups was similar (about two-fold). Even though most investigators have focused on the effects of acid/base balance on protein metabolism, it is possible that lipid (and other tissue) metabolism could also be affected. If this is the case, the effect observed in this study on triceps skinfold thickness, may be clinically relevant.

Comparing the two groups, the serum albumin was the same throughout the study, and appeared to rise slowly in both groups during the study. Certainly, serum albumin was not different at one year. That lack of difference in serum albumin in the two groups probably reflects the poor quality of the parameter as a nutritional marker.

The muscle strength/endurance tests were not significantly different at one year. The techniques used were crude, and were largely determined by what could be done easily inside an outpatient room. Nonetheless it is interesting that all three measurements were greater in the HA patients at one year. Indeed, the static leg muscle endurance was not far off significance (HA: 186 seconds vs LA: 130 seconds, ie *almost a minute*

difference; p = 0.12) in favour of the HA group. It was also interesting that the hand-grip measurements declined over the year in the LA patients but increased in the HA patients. These observations should be interpreted with caution, but perhaps show something that is worth investigating in the future.

The serum cortisol was significantly lower in the LA patients at one year (544.6 nmol/L in the HA group vs 442.5 nmol/L in the LA group; $p<0.05$). This finding is difficult to interpret. It is contrary to current ideas on the mechanism of the MA/protein metabolism effect; since the U-P pathway, which may be the mechanism behind the effect (at least in severe MA in animal models), has been shown to be activated by *increased* glucocorticoid levels.

Dietary protein intake at one year was also not significantly different. Whatever the mechanism of the effect, it seems likely that a true metabolic change occurred, rather than the HA patients were simply eating more. This finding was confirmed by Roberts et al in 1996 (**102**).

The greater weight gain experienced by the HA group could have several explanations. These will now be discussed.

Possible explanations of greater weight gain in the HA patients

(1) Decreased protein degradation in the HA patients. As it is thought that the primary metabolic effect of altering acid/base balance concerns protein degradation, it is all too easy to presume that any weight/nutritional change (as seen in this study) was due to a decrease in protein degradation. In other words, it is presumed that the HA patients' greater weight gain was due to an increase in muscle mass. However, the only evidence for this presumption is circumstantial. Firstly, midarm circumference, presumably mainly influenced by muscle volume, increased to a similar extent to weight gain. And secondly, as will be discussed in the next section (4.6.) urea and creatinine excretion was less in the HA group, possibly indicating reduced muscle breakdown in

that group. For this to have happened, it is likely that protein metabolism was altered in some way - especially as dietary protein intake was the same in the two groups.

(2) Increased protein synthesis in the HA patients. The effect of MA on protein synthesis in humans with ESRF is unclear. However, there is some evidence that better correction of acidosis can *increase* protein synthesis in patients with ESRF (Lim et al, 1993, **98**). If Lim's findings are correct, the results obtained in this study could be explained by increased protein synthesis in the 'alkalotic' group.

(3) Fluid overload. Sodium bicarbonate was used to further correct MA in 48% of the HA group. This could have led to fluid overload secondary to sodium and water retention. This is the major criticism that can be levelled against this study. There are four arguments against the possibility of fluid overload:

a) firstly, and most importantly, there was an increase in midarm circumference of a similar degree to the increase in body weight. 'Pure' sodium and water retention would be unlikely to affect midarm circumference;

b) the use of 'hypertonic' PD exchanges was the same in the two groups. Forty-nine per cent of the HA patients used one or more 'strong' (3.86% glucose) bags per day, compared to 52% of the LA patients, and the average number of 'strong' bags used per day was not significantly different;

c) one might expect blood pressure control to be different in the two groups, if one group was significantly fluid overloaded. But there was no difference in systolic or diastolic blood pressure at one year between the two groups; 130/78 mmHg in the HA group vs 129/76 mmHg in the LA group. Furthermore, the number of patients using anti-hypertensive agents was similar, 77 HA and 72 LA patients;

d) one might also have expected more hospital admissions due to fluid overload in the HA group. In fact, there were less admissions in the HA group.

(4) Changes in fat metabolism. The non-significant increase in triceps skinfold thickness may be relevant, if altering acid/base balance can affect fat metabolism. In other words, perhaps the HA patients' greater weight gain was due to fat. Interestingly, in a recent study of haemodialysis patients by (AJ) Williams et al (1997, **134**), it was found that better correction of MA caused a significant increase in triceps skinfold thickness. Williams' study was similar to this study except for the fact that it used a different ESRF group, and had a cross-over design.

(5) Changes in bone metabolism. It is not known that the extra weight gain was not due to a change in bone metabolism.

Certainly, studies in children with renal tubular acidosis favour a positive effect on better correction of MA on bone metabolism. Whether MA has a significant role on the pathogenesis of renal bone disease or not, there are several mechanisms by which better correction of MA *could* have affected bone metabolism. For example, the increase in vitamin D levels shown by Lu et al (1995, **26**) or the decrease in PTH levels shown by Lefebvre et al (1989, **32**) and Graham et al (1997, **30**) might persist with better long-term correction of MA. If so, the effects of better correction of MA on bone metabolism may explain, at least in part, the extra weight gain.

Even if bone metabolism was improved by better correction of MA in this study, this would not explain the changes in midarm circumference, dialysis dose and kinetic modelling data. So, if changes in bone metabolism were a factor in findings of this study, they could not explain all the observations.

Conclusion. The real cause of the extra weight gain may never be known, perhaps it was due to a combination of the above mechanisms. The cause may not matter, if the extra weight gain leads to an improvement in morbidity.

4.6. Dialysis dose / kinetic modelling data

Before one year, there was a similar number of increases and decreases in dialysis dose in the two groups; 17 increases and 6 decreases in dialysis dose in the HA group, compared to 18 and 9 in the LA group.

However, there was a tendency to a greater dialysis dose in the LA patients. The average dialysis dose (number of exchanges multiplied by the volume) was 8.5 litres/day in the LA patients, compared to 8.0 litres/day in the HA patients ($p = 0.18$). Even though this difference did not reach statistical significance, it may be an important observation. This point is further demonstrated by analysing the size of exchanges used by the two groups. In the LA group, eleven patients were using 1.5 litre exchanges, seventy-one 2-litre, thirteen 2.5-litre, and *five* used 3-litre exchanges. Whereas, in the HA group, eleven patients were using 1.5 litre exchanges, seventy-four 2-litre, fourteen 2.5-litre, and only *one* used 3-litre exchanges.

It is possible that, if there was a lower level of muscle breakdown in the HA patients, that they required less dialysis. If this is confirmed by future studies, it is probably one of the most unexpected findings of the study.

There may be other unexpected benefits of less dialysis in PD patients, in terms of cost and lifestyle, if this tendency is confirmed. Clearly less dialysis is cheaper. Furthermore, most patients prefer smaller (and/or fewer) bags, and this might make them more likely to carry out all their prescribed exchanges[38]. There is also now some evidence that patients using larger bags eat less than patients using smaller bags, presumably because the greater abdominal distension suppresses appetite (Harty et al, 1995, **135**).

[38]Of course, as PD patients can carry out their own exchanges at home, it can never be certain that the dialysis dose prescribed is actually carried out. In one study by Warren et al (1994, **148**), based on returns of PD fluid, upto 22% of exchanges were not done. For the purposes of this study, one would hope that a similar number of exchanges were not done in the two groups.

Ideas on mechanism of MA/protein metabolism effect in this study

This study was not designed to investigate the mechanism of any beneficial effect seen. Nonetheless, some information can be gained from the kinetic modelling data collected, accepting that this data did not reach statistical significance.

Even though the protein catabolic rate, weekly Kt/V and weekly creatinine clearance were not significantly different at one year, when the *direction of change* in values is examined, interesting trends can be seen. All three parameters (which are proportional to urea or creatinine excretion, and thereby reflect muscle breakdown as well as dialysis dose) *decreased* (from 0.92 g/kg/day, 1.89 and 76.9 litres per week at one month to 0.89, 1.85 and 76.3 respectively at twelve months) in the HA group, despite an overall tendency to an *increase* (though less than the LA group) in dialysis dose over the year.

This suggests that there may have been a decrease in muscle breakdown in the HA patients over the year of study. Whereas, in the LA group, two out of three parameters *increased* (from 0.88 g/kg/day, 1.93 and 78.2 litres per week at one month to 0.94, 1.91 and 80.1 at twelve months). Indeed, by as early as one month, there was a trend towards greater kinetic modelling parameters in the LA group (see Table 6). As with the dialysis dose data, this may indicate that there was an increase in muscle breakdown in the LA group.

Combined with the dietary protein intake data, the (non-significant) differences in dialysis dose and kinetic modelling parameters between the two groups, may favour the hypothesis that a metabolic change did occur during the study.

So, nutritional gain occurred despite a tendency to less dialysis in the 'alkalotic' patients compared to the 'acidotic' patients. It is possible, therefore, that the reduction in muscle breakdown was so profound as to cause a *reduction* in the production of muscle-derived 'toxins' that needed to be removed by dialysis[39], despite an overall *increase* in muscle bulk.

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[39]Presumably, if the Trade-off Hypothesis is correct, less glutamine would have been created in the HA patients, and therefore less urea would have been synthesised in the liver.

There is no evidence that greater PD doses can lead to an improvement in nutritional state. Conversely, as has been mentioned, Harty et al (1995, **135**) have shown that larger PD exchanges may lead to a reduction in appetite due to increased abdominal distension.

This dilemma might be, at least in part, solved by better correction of MA. Less muscle breakdown may lead to 'better' health on dialysis as 'lower' doses of dialysis can be used, encouraging patients to eat more.

Advocating lower dialysis doses may be sacrilege to the 'pro-kinetic modelling lobby'. But at this stage there is still no controlled evidence for the use of greater dialysis doses in PD, and little in haemodialysis. What evidence there is, is uncontrolled. According to current criteria, based on this uncontrolled data, it is said that PD patients should receive a weekly Kt/V of greater than 2.0. All Kt/V values for the patients in both groups were below this level throughout the study - ie, the patients in this study were 'inadequately' dialysed.

Were the patients adequately dialysed?

For kinetic indices to be validated as clinically useful tools in PD, they must satisfy two criteria:

(1) they must be predictive of important clinical outcomes, ie a 'bad' number reflects a poor outcome - and, more importantly,

(2) there must be evidence that *changing* the number improves outcome.

With regard to PCR, there is no good evidence that excess mortality is associated with a low PCR, and only weak evidence for a link with hospitalisation (Teehan et al, 1990, **136**). There is no accepted PCR level against which the 'adequacy' of our patients' PCR values could have been assessed. Interestingly, two recent studies - by Goodship et al in 1993 (**137**) and Harty et al in 1994 (**138**) - have found no correlation between PCR and other nutritional indices.

Until the CANUSA study was published in 1996 (Churchill et al, **139**), the best evidence for the relevance of Kt/V came from Teehan's study. This showed a better 3 year survival for patients with an average Kt/V over 1.89/week. But, due to drop out, the numbers after 3 years were very small and Kt/V became a less powerful predictor of mortality. In 1992, Lameire et al, in a study of a select group of patients who had been on PD for 5 years, found hospitalisation (and, surprisingly peritonitis), to be better in patients with a 'high' Kt/V (**140**). In 1993 Selgas et al reported similar findings but only studied patients who had been on PD for more than 3 years (**141**). Conversely, in 1991 Blake et al found no correlation between Kt/V and morbidity, but did detect an excess mortality when Kt/V was less than 1.5/week (**142**).

These pre-CANUSA "Kt/V studies", which presented arguments for and against the validity of Kt/V (ie, criterion 1), were all imperfect. Some were cross-sectional, others had only short follow-up periods, while others used 'soft' endpoints. The CANUSA study now seems to have 'settled the debate'.

In the CANUSA study (680 PD patients) there were definite links between Kt/V and survival. There was a 5% decrease in survival associated with every 0.1 decrease in total weekly Kt/V, for Kt/V levels between 1.5 and 2.3. It should be noted that there was no association between Kt/V and technique failure or hospitalisation. Based on this and other evidence, the most recent recommendation (September 1997) of the American-based National Kidney Foundation - published as the National Kidney Foundation-Dialysis Outcomes Quality Initiative, the NKF-DOQI - is a minimum weekly Kt/V of 2.0 (1997, **130**).

As previously stated, the evidence for such guidelines is uncontrolled. In other words, there is no evidence that by *raising* the Kt/V to this or any other level that survival can be improved. Studies are in progress in North America in an attempt to address this question. Whether these studies will provide any evidence for criterion 2 (ie, *changing* Kt/V improves clinical outcome), only time will tell.

A patient's Kt/V may well lie within a 'pre-ordained' small range of numbers. This range is largely determined by the patient's size and residual renal function, neither of

which can be changed easily. Furthermore, mortality in PD (and ESRF in general) is so tightly related to co-morbid disease, and hospitalisation is related primarily to access problems. So it is possible that changing Kt/V will have no measurable benefits.

Whether the patients in this study were really underdialysed is difficult to say. At the time of starting the study, the Kt/V levels were 'adequate'. This was in part because the patients were in the first year of dialysis (and so had significant residual renal function), and partly because the Kt/V standards at that time (">1.8/week") were lower than those of today. Nonetheless, according to today's standards, the patients in both groups were underdialysed.

4.7. Renal bone disease

Even though this was primarily a nutritional study, analysis of the effects of the two treatment regimes on renal bone disease did produce useful clinical information.

Most notably, some interesting conclusions can be drawn about the use of 'low calcium dialysate'. The HA dialysate used in this study is often called 'low calcium dialysate'. It has an ionised calcium concentration of 1.25 mmol/L, compared to the 1.75 mmol/L in traditional high calcium / LA dialysate. When it was developed in the late 1980's, it was hoped that low calcium dialysate would:

(1) allow for aluminium containing phosphate binding (ACPB) agents to be abandoned;

(2) allow for the use of large doses of calcium carbonate, which would act as the substitute for ACPB agents. This would improve phosphate and therefore PTH control;

(3) lead to fewer hypercalcaemic episodes - which were a problem with the combination of traditional dialysate and calcium carbonate - as it contained less calcium.

The target adjusted serum calcium of 2.5 mmol/L was achieved by six months in both groups. At one year, the calcium was the same in the two groups (2.51 mmol/L in both groups). That there was no difference in serum calcium at one year was, in part, related to the design of the study. A serum adjusted calcium of 2.5 mmol/L was one of the *target* parameters used in the study, in an attempt to control for variables other than acid/base balance.

The calcium of 2.5 mmol/L was achieved in the two groups in different ways. In the LA group, it was achieved by:

(1) the high calcium content of the dialysate (ionised calcium concentration 1.75 mmol/L), combined with,

(2) relatively little use of calcium carbonate; 36 of the LA patients required calcium carbonate (at a low dose of 0.63 g per day).

(3) relatively more use of alfacalcidol; 46 of the LA patients required alfacalcidol (at an average dose of 0.22 μg per day).

Whereas, in the HA group, the calcium of 2.5 mmol/L was achieved by:
(1) a low dialysate calcium content (1.25 mmol/L), combined with,
(2) liberal use of calcium carbonate; 92 of the HA required calcium carbonate (at an average dose of 2.91g per day; $p<0.001$), and,
(3) relatively less use of alfacalcidol; 29 of the HA patients required alfacalcidol (at an average dose of 0.13 μg per day; $p<0.05$).

So, even though calcium carbonate use was minimised in the LA group, 36% of the LA patients still required calcium carbonate, at a 1/5th of the dose of the HA patients. To achieve the target calcium in that group, not surprisingly, more LA patients required alfacalcidol (46 vs 29 HA patients), at nearly twice the dose.

There was a progressive rise in phosphate levels in both groups over the year; perhaps due to a combination of improving appetite and loss of residual renal function. Serum phosphate was lower in the HA patients at one year, when compared to the LA patients (1.66 in the HA group vs 1.87 mmol/L in the LA group; $p<0.05$). It should be noted that even though the HA patients had better phosphate control, that of the LA patients was quite adequate.

As *five* times the dose of calcium carbonate (on average) was prescribed in the HA patients (in an attempt to make them more alkalotic), it was not surprising that the phosphate control was better in that group. What was surprising was that the phosphate control in the HA patients was not better by a larger degree. This may be because the compliance with the larger doses of calcium carbonate prescribed was poor. Alternatively it may confirm what is known already about calcium carbonate - ie, that it is not a very good phosphate binder.

The effect of MA on phosphate metabolism is not certain. But, as stated in the Introduction, there is some evidence to suggest MA can increase the phosphate content of bone. If MA and phosphate metabolism are linked in this way, better MA control

may have had some effect on phosphate metabolism in the HA group. However, the most important factor in the better phosphate control of the HA group must have been the much higher doses of calcium carbonate used.

The serum alkaline phosphatase was very significantly lower at one year in the HA group compared to the LA group (84 iu/L in the HA group vs103 ± 5 iu/L in the LA group; $p<0.01$). Even so, the parameter that is now considered the 'gold standard' of renal bone disease activity, PTH, was the same in the two groups at one year (HA: 17.0 pmol/L, vs LA: 15.7 pmol/L; $p = 0.77$). Indeed, two parathyroidectomies were performed in the HA group, compared to none in the LA group. In other words, the 'benefit' of better phosphate and MA control did *not* lead to better parathyroid hormone control, contrary to one of the claimed advantages of low calcium dialysate.

It is also interesting that there was no difference in the number or magnitude of hypercalcaemic episodes between the two groups; contrary to another of the claimed advantages of low calcium dialysate.

As calcium carbonate was largely avoided in the LA patients, aluminium hydroxide (950 mg per day) was required in 17 of these patients, compared to three of the HA patients. Such low doses of the drug did not lead to (biochemical) aluminium toxicity in the LA group. Indeed, there was no difference in the serum aluminium level at one year between the two groups; and both one year values were in the normal range (0-20 µg/L). That there was a progressive rise in serum aluminium in the two groups over the year is strange. Certainly the HA group received little or no aluminium through their dialysate or medications. Perhaps the slow rise in aluminium levels was (like the rise seen in phosphate levels) due to a combination of improving appetite and loss of residual renal function.

It cannot be excluded that better correction of MA did *not* have some effect on the control of renal bone disease in this study. Certainly, as outlined in the Introduction, there are several mechanisms by which MA can affect renal bone disease. However, that PTH control was not significantly different in the two groups does (again) bring

into question the *clinical significance* of MA in the pathogenesis of secondary hyperparathyroidism.

It is more likely that the differences in control of renal bone disease were due to a *combination* of the different dialysate/drug regimes and levels of acidosis correction. And the similar (good) PTH control in the two groups occurred because the serum calcium was kept at a similar high-normal level in the two groups.

Most current clinical research into renal bone disease focuses on the possible roles of hyperphosphataemia and vitamin D deficiency in the pathogenesis of secondary hyperparathroidism. As argued above, the combination of better phosphate control and better control of MA did not lead to better PTH control, or prevent parathyroidectomy. The importance of maintaining the serum calcium in the high-normal range to control secondary hyperparathyroidism in ESRF, has been submersed in the drive to improve phosphate control and use as much vitamin D as possible

The low calcium dialysate debate

When 'low calcium dialysate' was first developed in the late 1980's, initial studies were interpreted as confirming its 'advantages' over traditional 'high calcium dialysate'. For example, a *non-randomised* study by Hutchinson et al in 1992 demonstrated that low calcium dialysate did: (1) allow for the use of large doses of calcium carbonate, which improved phosphate and therefore PTH control, and, (2) lead to fewer hypercalcaemic episodes (**143**). In this study patients who agreed to take part in the research - who would, presumably, have been more compliant with phosphate binding agents - received low calcium dialysate. Patients that declined received traditional calcium dialysate.

The results of this study confirm that the combination of low calcium dialysate and high dose calcium carbonate *does* improve phosphate control; however PTH control and hypercalcaemic episodes were *not* improved. A more recent (1996) *randomised* study of low calcium dialysate drew a similar conclusion concerning PTH control

(Weinreich et al, **144**). The discrepancy between early and later studies (including this study) is probably explained by the different study designs. In other words, when randomised studies are carried out, the bias introduced by giving 'keen' patients a new dialysis/phosphate binder regime is negated.

The use of low calcium dialysate *requires* that the patient also takes high dose calcium carbonate (or alfacalcidol) to maintain serum calcium. In other words, it puts the onus on the patient to maintain his/her serum calcium by taking tablets. In this way, low calcium dialysate may not be suitable for the many non-compliant patients in an average ESRF population. In a retrospective study carried out in this department, a widespread change to low calcium dialysate in a large non-selected group of PD patients, led to a doubling of PTH levels in a two year period (Stein et al, 1993, **145**).

The phrase 'low calcium dialysate' is a misnomer, as its ionised calcium concentration is in the normal range. Commercial companies have advocated the alternative title of "physiological calcium dialysate". It may be 'better' to continue to use the older name, to remind clinicians that calcium containing or stimulating medications are necessary to augment its effects.

Alternatively, it may be 'safer' to raise the ionised calcium content of 'standard' PD fluid to 1.5 mmol/L. In the meantime, it is important that despite the widespread transfer of PD patients to low calcium dialysate in the UK, commercial companies continue to manufacture traditional calcium dialysate, especially for non-compliant patients.

Conclusions.

(1) Low calcium dialysate 'advantages'. Low calcium dialysate allowed for the use of greater doses of $CaCO_3$, leading to better phosphate control.

(2) Low calcium dialysate disadvantages. Two further postulated 'advantages' of low calcium dialysate - better PTH control and fewer hypercalcaemic episodes - were not confirmed. Low calcium dialysate puts the onus on the patients to take high dose

calcium carbonate (or alfacalcidol) to maintain serum calcium, and so may not be suitable for non-compliant patients.

(3) The clinical significance of MA in the pathogenesis of secondary hyperparathyroidism is questioned by the fact that better MA control did not improve PTH control in the HA group.

(4) The importance of the serum calcium in the aetiology of secondary hyperparathyroidism has been confirmed by this study.

4.8. Outcomes

In terms of hospitalisation, the HA patients also fared better than the LA group. There were less hospital admissions in the HA group (1.13 per patient per year) compared to the LA group (1.71 per patient; $p<0.05$). Hence, not surprisingly, the HA patients spent less days in hospital per year than the LA patients (HA: 16.4 days/year, vs LA: 21.2 days; $p<0.05$). In other words, the HA patients spent, on average, 5 days less in hospital per year than the LA patients. It is tempting to postulate that this improvement in morbidity was associated with the improvement in nutritional state in the HA group.

Whether or not this is true, this apparently small improvement in hospitalisation could have major economic benefits (in terms of the cost of PD per year), as well as lifestyle benefits to the patient, if reproduced in large dialysis populations. There was no difference between the two groups, in terms of the number of days spent in hospital for the original admission(s). This number includes the 'ARF/Acute-on-CRF' admission (if the patient had one), and the admission for the insertion of the PD catheter (this may have been the same as the 'ARF admission'). The number of visits by a community dialysis nurse and the number of visits to the renal outpatient clinic were the same in the two groups. These similarities suggest that the original inpatient care, and later outpatient care, was no 'better' for either group. This is as one would expect.

There was a tendency to less peritonitis in HA group (HA:75 vs LA: 86 episodes). Considering that the HA patients had 40 *more* at risk months (HA: 1014 vs LA: 976 months) - partly because more were on PD at one year (HA: 69 vs LA: 59 patients) - and *less* peritonitis, this difference is even more surprising. Perhaps their 'better' nutritional state had some influence on their immune system, thereby improving the peritonitis rate[40].

The peritonitis rates were poor in both groups (HA: 1 in 13.5 vs LA: 1 in 11.3 patient-months), especially when compared to the recommended minimum of 1 in 24 patient-

[40]It could be argued that this was a 'peritonitis study' - ie, less peritonitis in the HA group (for some reason, not necessarily MA) was the *cause* of all the results seen, rather than a consequence of a better

months (current Renal Association guidelines). This was partly because a 'liberal' definition of peritonitis was used (ie, an intention-to-treat basis). It was decided to use such a definition as the culture-ve rate was very high (approximately 40% in each group). So, it was not thought 'fair' to use a definition based on episodes with a positive culture. When the study was carried out, the white count of PD fluid was not routinely measured. So it also could not be a part of the definition.

There were 12 deaths in the HA group compared to 15 in the LA group. The statistical power of this study was never great enough for it to be a mortality study - ie, it was never 'designed' to show an improvement in mortality, even if a better nutritional state could produce such a gain.

Few definite conclusions can be drawn from the other outcome parameters. This was either because the parameters measured produced numbers too small to be analysed (eg, permanent technique failure) or because the data was unreliable (exit site infections). Nonetheless, looking at the data, there are some interesting observations to be made. There was a tendency to less temporary technique failure in the HA group (HA: 27 vs LA: 40 episodes). This was in part due to fewer PD catheter replacements (HA: 9 vs LA: 12) in the HA group. Clearly as less peritonitis was experienced in the HA patients, fewer catheters would have to be replaced. If better correction of MA led to the better peritonitis rate in the HA group, this could be part of the explanation for the better hospitalisation in that group. Lower peritonitis rates have far reaching consequences, not just because peritonitis is an unpleasant illness. For example, fewer episodes lead to fewer admissions and fewer catheter replacements.

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nutritional state. Less peritonitis would mean less admissions and less 'bursts' of catabolism leading to less weight gain. In this way, the findings of the study could have nothing to do with MA.

4.9. Ways in which the study could have been improved

In retrospect, there are several ways in which the study could have been improved:

(1) Sodium bicarbonate. The major criticism of this study concerns the possibility that the liberal use of sodium bicarbonate caused fluid overload, which caused the extra weight gain in the HA group. At the start of the study, I did not realise that it would be so difficult to 'over-correct' MA in the HA group, and so need oral sodium bicarbonate. It might have been better to start all the HA patients on sodium bicarbonate at the beginning of the study, and the LA patients on an equivalent dose of sodium chloride. This manoeuvre should have enabled the importance of fluid overload to be assessed.

No attempt was made to look at total body water through the study. If a technique like bioelectrical impedance had been used, it would have been easier to confirm or refute the possibility that sodium loading was a factor in the study.

(2) Mechanism of effect. This was never designed to be a mechanism study. So, relatively little effort was made to examine the mechanism of any effect seen. Nonetheless, measuring serum cortisol and performing kinetic modelling was an attempt to look at mechanism. In fact, the data obtained from these measurements *did* provide useful information - even if it was, in the case of the cortisol data, contrary to current dogma.

In retrospect, more effort could have been made to look at possible mechanisms of the effect; especially as it could have been predicted that the study would 'produce' two large groups of patients with chronic, stable but *different* levels of acidosis correction. So, it would have been an ideal opportunity to investigate the mechanism of the MA/protein metabolism effect in a 'real' context, rather than in a two week study in a rat. I feel that this was an opportunity missed.

Perhaps, useful physiological information could have been gained if leucine turnover studies had been carried out - as the leucine turnover technique can distinguish protein

degradation, from synthesis and oxidation. However, this technique is invasive, moderately expensive, and both manpower and time consuming - each study lasting upto four hours. It is best suited to small scale physiological work, rather than large randomised controlled trials. Furthermore, there are large variations in leucine kinetics between patients. This means that leucine turnover data is best analysed by using patients as their own controls. In other words, leucine turnover studies are more suited for cross-over studies, which this study was not. Carrying out leucine turnover studies on all 200 patients in this study would have been impractical unless much larger resources had been available. It might have been possible, however, to carry out leucine studies on a small representative sample of patients from each group - at a fixed time point in the study, perhaps one year.

At the molecular/genetic level, useful information could also have been gained. For example, ubiquitin-proteasome gene expression in muscle biopsies could have been measured. Similar logistic arguments exist against such a proposal. Furthermore, in 1991, when the protocol was being devised, the possible importance of the U-P pathway was only just becoming apparent. So, it is doubtful that, at that time, I would have thought that assessing the U-P pathway would have been a fruitful line of investigation.

(3) Size of study. This study was of reasonable size, and did have the statistical power to answer the question posed. Changes in body weight and midarm circumference were clearly different in the two groups. However, the absolute measurements of body weight and midarm circumference at one year were *not* statistically different. If the study had been larger (perhaps by involving other centres) then differences in absolute measurements might have been found, making the study more convincing.

A bigger study would also have had a better chance of showing an improvement in mortality if one was to be seen. If the better survival in the HA group (12 deaths compared to 15 in the LA group) had been seen in 400 patients, statistical comparison of the 24 vs 30 (projected) deaths might have been possible.

(4) Study design. In retrospect, the design of the study could have been improved: the study *could* have been double-blind, or even triple-blind (doctor, patient and dietitian). The key anthropometric measurements could have continued after patients left the study. And a cross-over design might have produced more convincing results.

(5) Muscle strength. Relatively little effort was made to look at muscle strength in this study. What measurements that were made were crude. There are accepted methods of measuring muscle strength, most of which involve specialist (and quite large) machinery. Nonetheless, these techniques *could* have been employed.

4.10. Future research

Even though this study has largely answered the question of whether the MA/protein metabolism effect has any clinical relevance, many questions remain unanswered. Future research might investigate the following areas:

(1) Repeat this study in other groups of CRF/ESRF patients. The reasons for choosing PD patients in this study have been outlined in the Introduction.

These reasons do not mean that the study could *not* have been performed in other groups of patients with CRF: either predialysis, haemodialysis or transplant patients. In fact, a similar study has already been carried out by (AJ) Williams et al in haemodialysis patients (1997, **134**). Some of the findings were similar to this study. In Williams' study, 46 haemodialysis patients were dialysed in a single-blind crossover trial for 2 six-month periods, using 30 mmol/L and 40 mmol/L bicarbonate dialysate. Triceps skinfold thickness decreased significantly (from 14.8 to 11.8 mm) in the low bicarbonate period, and increased (from 14.9 to 15.8) during the high bicarbonate period. There was a non-significant tendency to lower urea excretion in the high bicarbonate period, as seen in the 'alkalotic' group in this study. Midarm circumference and body weight did not change.

It is not clear why it was not a more positive study. It was a good study but probably not big enough or long enough to fully demonstrate any nutritional benefit that could have been induced by better correction of MA. Furthermore, it only included stable patients who had been on dialysis for at least 6 months. For this reason, it may have excluded the more malnourished patients, ie those with 'most to gain'.

Nonetheless, the cross-over design meant that the 'positive' effects of better correction of MA could be seen to 'go away' during the acidotic period. In some ways, this design made its findings more convincing than those of this study, which lacked its cross-over design.

It would be interesting to repeat our study in predialysis patients. It could be hypothesised that if the 'beneficial' reduction in protein degradation were duplicated, this might lead to less urea generation (for a given level of GFR). If this led to less uraemic symptoms for that GFR level, it is possible that dialysis could be delayed.

Furthermore, and perhaps more importantly, there are two reasons that the *progression* of CRF could be slowed by better correction of MA:

a) proteinuria may itself be toxic to the kidney, and not just a consequence of renal disease. If this is true, and if better correction of MA did reduce urinary albumin excretion in predialysis patients in the long-term (confirming the short-term studies in the 1920's), the progression of CRF might be slowed. However, the study by Ballmer et al in 1995 showed that MA reduces albumin synthesis in normal humans (**71**).

So, if a predialysis study was carried out, it is not clear whether better correction of MA would be a 'good' or 'bad' thing. Would it 'help' by reducing proteinuria, or 'hinder' by increasing albumin synthesis and thereby exacerbating proteinuria?

b) if better correction of MA produced renal hypertrophy in predialysis patients in the long-term - as shown by Throssell et al (1995, **63**) and others, in animals in the short-term - this might 'prolong' the compensatory mechanisms of the failing kidney. Conversely, it could accelerate the decline of renal function.

(2) Muscle strength. It could be argued that there is no point in being heavier or having larger muscles, if there is no 'knock-on' effect on muscle strength. Almost nothing is known about the effect of better correction of MA on muscle strength - either in terms of acute or chronic acidosis correction. This study showed a non-significant tendency to greater muscle strength and endurance in the HA patients. This finding needs to be further investigated with more formal muscle strength/endurance testing.

Neither is it known whether a better nutritional state improves quality of life.

(3) Role of muscle synthesis. It has been argued that protein *degradation* was decreased by better control of acidosis in the HA group in this study. However, the

findings could have been caused by an *increase* in protein synthesis; though the decrease in urea and creatinine excretion in the HA group is against this possibility. The ability to distinguish protein synthesis from degradation was beyond the scope of this study. The relative importance of alterations in protein synthesis and degradation should be investigated in long-term studies of MA correction in ESRF patients.

(4) Mechanism of MA/protein metabolism effect. The mechanism of the effect is still far from clear in humans with CRF. Specifically, what is the role of the ubiquitin-proteasome pathway?:

a) It is unclear whether the U-P pathway is involved in humans with CRF.

b) It is also not certain, even if the U-P pathway is involved, that it is the *most important* proteolytic pathway. The MA/protein metabolism effect could involve a more complex proteolytic system.

These possibilities need to be investigated.

(5) New therapeutic options - even more oral bicarbonate?, even better control of MA?, bicarbonate-based dialysate? As has been discussed, the beneficial effect of better control of 'acidosis' was seen by manipulating acid/base balance *within* the normal range. This may be, as has been suggested, because the effect was mediated by bicarbonate loading, rather than by changes in extracellular pH. Perhaps giving dialysis patients oral sodium bicarbonate should be investigated.

If, on the other hand, pH is important to the effect, other therapeutic options to increase pH into the alkalotic range may be useful. The findings of this study would support the study of Yamamoto et al (1992, **126**) which showed that 40 mmol/L lactate alone does not fully correct acidosis. The average bicarbonate of 27.2 mmol/L in the HA group (at one year) was achieved only because the patients were *also* taking very large doses of calcium carbonate and/or sodium bicarbonate. So, for patients not in studies such as this, where researchers have a vested interest to maintain compliance by emphasising the need to take medications, it may be better for commercial companies to

manufacture PD dialysate with higher lactate concentrations, perhaps 45 mmol/L. It is possible that by administering even larger amounts of lactate, and increasing pH further, further nutritional benefit might accrue. This hypothesis should be tested.

The disadvantages of lactate as a buffer have been outlined in the Introduction. Over the last ten years, bicarbonate has been investigated as an alternative buffer for PD. Clearly, it is 'more physiological', being one of the body's natural buffers. The main problem with a bicarbonate-based solution is 'chemical'. It is difficult to prepare, sterilise and store; and during autoclaving, calcium and magnesium precipitate out as carbonate salts. Hence bicarbonate-based solutions have been developed with some form of 'twin-bag' system, whereby the bicarbonate is released into the dialysate just before use. Even though they have been shown to be 'safe' in PD, there is little evidence in humans with ESRF to show that they are any 'better'. Large muticentre studies designed to address this question are in progress.

There are also ongoing studies investigating PD solutions containing mixtures of bicarbonate and lactate (Coles et al, 1997, **146**). Such solutions may have the advantages of each buffer. More work needs to be done in this area, before lactate can be displaced as the standard PD buffer. Despite its largely theoretical disadvantages, lactate has the benefit of a good safety record generated by many years of use.

(6) 'Bicarbonate loading theory' / intracellular acidosis debate. It is far from clear that extracellular pH is responsible for the MA/protein metabolism effect. The exact role of the extracellular bicarbonate concentration needs to be elucidated. Also, the inability to demonstrate that changes in intracellular pH occur in CRF needs to be explained.

(7) Other new therapeutic options - manipulation of the U-P pathway? If the U-P pathway is involved in the effect, this might lead to new treatment possibilities. Theoretically, if ubiquitination could be suppressed, or deubiquitination augmented, protein degradation could be decreased. However the very *ubiquitous* nature of the molecule may mean that inhibiting it could prove to be a 'double-edged sword'. As it is

so highly conserved in nature, small mutations must be lethal. So, presumably, manipulating its expression (or that of its inhibitors) might lead to intracellular havoc - with, perhaps, uncontrolled protein synthesis leading to cancer.

So, even if non-specific ubiquitin manipulation is possible in humans, it is doubtful that it would ever be safe. However, more protein-specific manipulation (eg, preventing muscle protein breakdown alone) might one day be possible. If so, then the manipulation of ubiquitin might be a useful therapy in some catabolic states, such as multifactorial 'ITU' illnesses. This would presumably only be true if the U-P pathway was the principle proteolytic system in that state, which is unlikely.

Furthermore, in many catabolic states (such as fasting, without renal failure), where muscle breakdown may be a useful homeostatic response to free up amino acids for gluconeogenesis or energy, preventing ubiquitination may not be desirable.

4.11. Conclusions

The most important conclusion from this study is that better correction of metabolic acidosis improves the nutritional state, and possibly the morbidity, of PD patients in the first year of dialysis. The hypothesis is confirmed.

In this way, it demonstrates (for the first time) that the MA/protein metabolism effect has clinical relevance in humans with ESRF in the long-term. This is a remarkable discovery for six reasons:

(1) firstly, and most importantly, it is the first time *any* nutritional intervention has been shown to have anabolic effects in patients with ESRF in a properly controlled study;

(2) this nutritional gain occurred despite a tendency to less dialysis in the 'alkalotic' patients;

(3) if better correction of MA did 'cause' the improvement in hospitalisation, it is the first time for 15 years - since the National Cooperative Dialysis Study (NCDS) in 1983 (Gotch et al, **147**) - that *any* intervention has been shown to improve hospitalisation in a controlled study of ESRF patients;

(4) moreover, the *scale* of the improvement in morbidity (a nearly 75% improvement in hospitalisation), is greater than any previously reported;

(5) the effect was observed when neither group was truly acidotic or alkalotic; and,

(6) there were unexpected 'knock-on' effects in terms of cost and lifestyle: with a tendency to a lower dialysis dose, less peritonitis and less temporary technique failure in the HA group.

The individual conclusions of the study will now be summarised:

Matching and Acidosis

- Even though the two groups were reasonably well matched at baseline, there were some discrepancies, especially in terms of demographic data, and body weight at baseline. The haemoglobin reached the target level (>11g/dl) by six months in both groups, through the liberal use of EPO and blood transfusion.

- The two different dialysate/medication regimes *were* able to produce long-term differences in venous bicarbonate control, and probably acid/base balance. However, the combination of large doses of calcium carbonate and HA dialysate was not sufficient to maximally correct acidosis, to the target venous bicarbonate level of 30 mmol/L. For this reason, 48% of the HA patients also required sodium bicarbonate. Even then, the target venous bicarbonate level was not achieved in the HA group.
- The effects seen in this study were observed when neither group was alkalotic or acidotic. The possible role of bicarbonate loading in this study, and in the MA/protein metabolism effect in general, have been debated; as has the role of intracellular pH.

Nutrition

- At one year, the increase in body weight in the HA group was significantly greater than the LA group, as was the increase in midarm circumference. These are the most important findings of the study. The increases in triceps skinfold thickness were not significantly different, though may have been clinically relevant. It seems likely that a true metabolic change occurred, rather than that the HA patients were simply eating more. Evidence has been put forward that a decrease in protein degradation in the HA patients was largely responsible for the differences in differences in weight gain in the two groups.
- Fluid overload and changes in bone metabolism are the two most likely alternative explanations. Four arguments have been put forward suggesting that this greater weight gain was *not* due to fluid overload. Even if changes in bone metabolism are a factor in the extra weight gain in the HA patients, they are only likely to be a component.
- There was a (non-significant) tendency to greater muscle strength/endurance in the HA patients

- The serum cortisol was significantly *lower* in the 'acidotic' LA patients at one year. This is contrary to current ideas on the mechanism of the MA/protein metabolism effect. The ubiquitin-proteaosme pathway - which may be (at least in part) the mechanism behind the effect - has been shown to be activated by MA-associated *increased* glucocorticoid levels.

Dialysis dose and kinetic modelling

- There was a (non-significant) tendency to a lower dialysis dose in the HA patients. If confirmed, this finding could have cost and lifestyle benefits for PD patients. As importantly, it may help them to eat more.
- The three kinetic modelling parameters *decreased* during the study, in the HA group; whereas, in the LA group, two out of three parameters *increased.* For these reasons, it seems that there was a *decrease* in muscle breakdown in the HA patients over the year of study, and an *increase* in muscle breakdown in the LA group. This, in part, led to the hypothesis that a lesser degree of muscle breakdown occurred in the HA group - leading to less urea/creatinine etc that needed to be excreted. And so, the HA patients needed less dialysis.

Morbidity

- There were less hospital admissions in the HA group, and they spent less days in hospital per year than the LA patients. There was a tendency to fewer peritonitis episodes in the HA group.
- These improvements in morbidity in the HA group may be secondary to the improvement in nutritional state. They also could have cost and lifestyle benefits for PD patients.

Renal bone disease

- The target adjusted serum calcium of 2.5 mmol/L was achieved by six months in both groups. Serum phosphate was lower in the HA patients at one year, presumably due to the greater doses of calcium carbonate used (and possibly better control of acidosis). The control of serum alkaline phosphatase was also better in the HA group.
- Low doses of aluminium hydroxide drug did not lead to (biochemical) aluminium toxicity, in either group.
- Two parathyroidectomies were carried out in the HA group, and none in the LA group. Furthermore, the parathyroid hormone level was the same in the two groups at one year, as were the number and magnitude of hypercalcaemic episodes.
- These observations:

  (1) bring into question the claimed advantages of the combination of 'low calcium dialysate' and high dose calcium carbonate;

  (2) question the clinical significance of MA in the pathogenesis of secondary hyperparathyroidism; and,

  (3) demonstrate the importance of the serum calcium concentration in the aetiology of secondary hyperparathyroidism.

It would now seem that the association of metabolic acidosis and protein metabolism in renal failure, has clinical implications in patients receiving long-term dialysis.

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APPENDIX

Index of Tables and Figures