DIETARY PROTEIN AND THE PROGRESSION OF DIABETIC NEPHROPATHY

A Thesis submitted to the University of London for a Doctor of Medicine degree.

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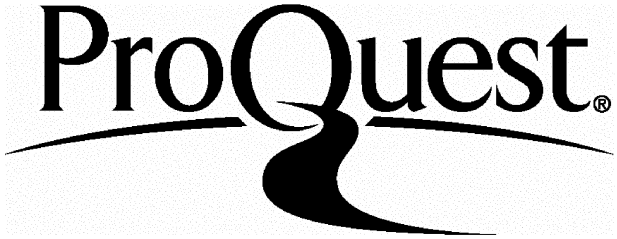

ABSTRACT

Nineteen insulin-dependent diabetic patients with diabetic nephropathy were studied prospectively for up to five years. Patients served as their own controls and were treated with a diet reduced in protein (low protein diet (LPD)) for a mean (range) of 33 (12-49) months following a run-in period of 29 (12-39) months on a normal protein diet (NPD).

The prescribed diet achieved a mean (SE) reduction of 41% in protein intake from 1.11 (0.06) on NPD to 0.66 (0.03) g/Kg/day on LPD ($p<0.0001$). On LPD carbohydrate intake rose by 15% and fat intake fell by 27% ($p<0.05$ and $p<0.006$ respectively). Dietary phosphate was lower (1484 (92) vs 1009 (33) mg/day, $p<0.0001$) and although total energy intake and body weight fell in the first six months of LPD they thereafter plateaued (1935 (116) to 1729 (106) kcal/day and 73 (3) to 70 (3) Kg respectively, $p<0.02$ for both). Plasma albumin increased (38 (0.8) to 40 (0.9) g/l, $p<0.002$) and mid-arm muscle circumference remained stable at 25 (1) cm.

Glycosylated haemoglobin level remained similar during the two diet periods (NPD 8.9 (0.3) vs LPD 9.0 (0.5) %, NS) while mean supine blood pressure fell by 4 mmHg ($p=0.001$) during the LPD period due to commencement or modification of antihypertensive therapy in nine patients.

During the period of NPD the mean glomerular filtration rate (GFR) fell at a rate of 0.61 (0.14) ml/min/mo. which was considerably higher than the rate of fall of 0.14 (0.08) ml/min/mo. on LPD ($p=0.001$). The effect of LPD on the rate of change of GFR remained highly significant ($p=0.002$) after adjustments were made for the potentially confounding variables of blood pressure and glycosylated haemoglobin. The effect of LPD on the rate of decline of GFR was heterogeneous with 10 of the 19 patients showing a significant ($p<0.05$) slowing in the rate of decline by

linear regression or a significant 'break' in the decline of GFR using a 'breakpoint' analysis. No factors could be identified to differentiate those who responded from those who failed to respond. The rise in the fractional clearance of albumin seen during NPD was halted on LPD.

A low protein diet effecting reductions in dietary protein and other dietary components such as phosphate and fat appears to retard the rate of decline of GFR in diabetic nephropathy, though in a heterogenous manner, and may be a useful therapy for certain patients.

During the two diet periods 164 simultaneous comparisons of the plasma clearance of ^{51}CrEDTA and creatinine clearance as markers of GFR were analysed in 19 patients. A high degree of correlation was seen between the two methods on both diets ($r=0.77$, $p<0.001$ and $r=0.85$, $p<0.0001$, for NPD and LPD respectively). However, during NPD creatinine clearance over-estimated the clearance of ^{51}CrEDTA by a mean of 5.8 ml/min/1.73 m^2 with wide scatter of differences (±2SD 28.2 to -39.8). During LPD the creatinine clearance again over-estimated the clearance of ^{51}CrEDTA on average by 5.3 ml/min/1.73 m^2 with a narrower scatter of differences (±2SD 19.0 to -29.6). Thus, on both the normal and low protein diets the creatinine clearance on average overestimated GFR (i.e. shows bias) and there were wide limits of agreements (standard deviations) between the two methods. The limits of agreement were 40% lower on LPD compared to NPD.

Creatinine clearance is not a reliable marker for the estimation of GFR compared to the clearance of ^{51}CrEDTA although it is more accurate on a low compared to a normal protein diet.

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STATEMENT

The work presented in this Thesis was carried out by myself during a period of research as a Research Assistant (Registrar grade) spent in the Unit for Metabolic Medicine at Guy's Hospital, London under the supervision of Professor GianCarlo Viberti, M.D., F.R.C.P. The study reported was clinically-based and prospective (spanning five years) and although I was not involved at commencement of the study I was responsible for the management of all the patients on the low protein diet (for nearly three years in all but one case) and the analyses of the findings.

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CHAPTER 1
INTRODUCTION

OVERVIEW AND HISTORICAL ASPECTS OF DIABETIC NEPHROPATHY

The association between proteinuria and diabetes was postulated over 200 years ago (Cotunnius 1764, Rollo 1798) and during the nineteenth century it was suggested that albuminuria may reflect an important renal disease specific to diabetes (Bright 1836, Rayer 1840). More contemporary interest and research was catalysed by the description by Kimmelstiel and Wilson of the glomerular nodular hyaline intercapillary lesions seen in some cases of diabetic renal disease (Kimmelstiel and Wilson 1936). Over the intervening 50 years significant advances in the understanding of the pathogenesis, pathophysiology, pathology, and treatment of this complication have been made.

MORPHOLOGICAL CHANGES

The description of glomerular lesions in seven elderly patients with non-insulin dependent diabetes mellitus in 1936 by Kimmelstiel and Wilson (Kimmelstiel and Wilson 1936) provided the impetus to the morphological and later morphometric study of diabetic renal disease. The nodular hyaline deposits that they described are rather unusual in biopsies from patients with diabetic nephropathy, however they were the first workers to recognise the increase in 'intercapillary connective tissue' (mesangial deposits) and suggested the term 'glomerulosclerosis'. The more characteristic diffuse form of glomerulosclerosis was described a decade later (Bell 1946). The developments of percutaneous renal biopsy and electron microscopy revealed that capillary basement membrane thickening was common soon after the onset of diabetes and subsequently the importance of mesangial expansion in the development of diabetic

nephropathy and functional decline was recognised (Østerby 1975, Mauer et al. 1984, Steffes et al. 1989, 1992).

STRUCTURE AND FUNCTION

The structure-and-function debate has evolved as quantitative methods for the measurement of low concentrations of urinary albumin, assessments of renal haemodynamics and the analysis of renal structure by light and electron microscopy have become available. The clinical abnormalities of persistent proteinuria, hypertension and declining glomerular filtration rate characterising clinical diabetic nephropathy are associated with advanced renal histological lesions (Østerby et al. 1983, Mauer et al. 1984). At early stages of nephropathy glomerular morphometric abnormalities already distinguish insulin-dependent diabetic patients with normal levels of urinary albumin excretion from those with slightly raised levels of urinary albumin excretion (microalbuminuria) (Chavers et al. 1989, Walker et al. 1992).

Recently Østerby has reported light and electron microscopic findings from 14 insulin-dependent diabetic patients with diabetic nephropathy from early to advanced (urinary albumin excretion 158-5494 μg/min, glomerular filtration rate (GFR) 30-128 ml/min/1.73m^2, mean arterial blood pressure 87-122 mmHg) (Østerby et al. 1990). An index of clinical nephropathy, calculated from urinary albumin excretion rate, glomerular filtration rate and mean arterial blood pressure correlated with an index of the structural lesions calculated from basement membrane thickness, mesangial expansion, and glomerular occlusion. A similar correlation has also been demonstrated in patients with normo- and microalbuminuria (Walker et al. 1992).

The exact morphological abnormalities that result in increased levels of albuminuria are not clear. Epithelial cell abnormalities causing changes in the ultrafiltration coefficient have been proposed (Myers

personal communication, 1993) but quantitative structural abnormalities have been difficult to demonstrate. However at the stage of microalbuminuria epithelial foot process width is increased (Østerby personal communication, 1992). What is clearer is that mesangial expansion and glomerular sclerosis are strongly correlated with a reduction in the glomerular filtration rate (Østerby et al. 1988, Harris et al. 1991).

MICROALBUMINURIA

In the early eighties an important finding was reported which enabled those diabetic patients destined to develop diabetic nephropathy to be identified up to 15 years before they presented with the full-blown clinical syndrome of persistent proteinuria, arterial hypertension, declining renal function, and accompanying retinopathy. This was the description that small sub-clinical elevations of urinary albumin excretion, a phenomenon termed microalbuminuria, predicted in almost 80% of cases the onset of overt nephropathy (Viberti et al. 1982, Parving et al. 1982, Mathiesen et al. 1984, Mogensen and Christensen 1984). The predictive levels of urinary albumin excretion varied between these studies (15, 30 and 70 μg/min.) as did the method of urine collection (4, 24 hour and overnight) and the length of follow-up (6, 10, and 14 years) but their overall findings and conclusions were similar. As microalbuminuria is not seen in the first 5 years of insulin-dependent diabetes it is likely to represent early disease rather than to be a marker of susceptibilty (Microalbuminuria Collaborative Study (MCS) 1992).

The significance of the precise level of microalbuminuria as a predictor has been investigated in a recent follow-up study of 8 years duration which suggests that in patients with higher levels of microalbuminuria (100-300 mg/24 hour) progression to overt nephropathy is more common than in patients with lower level

microalbuminuria (>30 and < 99 mg/24 hour) (Feldt-Rasmussen et al. 1991). In addition the prognostic significance of microalbuminuria in patients with a long duration of diabetes was recently examined in 18 insulin-dependent diabetic patients with microalbuminuria at screening performed after at least 15 years of diabetes. After a 10 year follow-up 5 (28%) developed macroalbuminuria. The authors suggested that the predictive value of microalbuminuria after a long duration of disease (25 years or more) is less than in patients with a shorter disease duration (12-14 years) (Forsblom et al. 1992).

Identifying patients 'at-risk' or with an early form of the complication has allowed the assessment of a number of interventions, including improved glycaemic control (Kroc Study 1984, Feldt-Rasmussen et al. 1986, 1991, Dahl-Jorgensen et al. 1988), blood pressure reduction using various agents (Christensen and Mogensen 1985, Marre et al. 1987, 1988, Mathiesen et al. 1991, Hallab et al. 1993) and dietary protein restriction (Cohen et al. 1987, Dullaart et al. 1993). All of these interventions have been associated with a reduction in urinary albumin excretion but the long-term implications of this are not yet known. However a recent update of the Steno studies of intensified glycaemic control on the course of microalbuminuria provides evidence that a period of improved glycaemic control is associated with less progression to overt nephropathy in patients with urinary albumin excretion rates in the 100-300 mg/24 hour range (Feldt-Rasmussen et al. 1991).

GLOMERULAR HYPERFILTRATION

The idea that diabetic patients may exhibit glomerular hyperfiltration is over 50 years old (Cambier 1934) and using accurate techniques for estimating glomerular filtration rate several more recent studies have found that up to 25% of patients have a GFR exceeding the upper limit of the normal range (Ditzel and Schwartz 1967, Mogensen

1971, Christiansen et al. 1981, Wiseman et al. 1985). The role of glomerular hyperfiltration in the susceptibility to diabetic nephropathy is not fully clarified at present. In one follow-up study hyperfiltration was suggested as a susceptibility factor but the association with microalbuminuria in these patients made it difficult to determine whether hyperfiltration was an independent risk factor (Mogensen and Christensen 1984). However data from a prospective study of 75 young diabetics carefully followed for 8 years revealed glomerular hyperfiltration (>125 ml/min/1.73 m^2) to be an independent risk factor for overt nephropathy (Rudberg et al. 1992). In contrast an 18 year follow-up of 29 insulin-dependent diabetic patients found no association between increased urinary albumin excretion rate with early glomerular hyperfiltration (Lervang et al. 1988). Finally, a five year prospective study of two matched cohorts of insulin-dependent diabetic patients with and without hyperfiltration, found no evidence of progression to microalbuminuria or a raised blood pressure in those with glomerular hyperfiltration, even though GFR fell more rapidly in this group (Jones et al. 1991).

Although in animal models of glomerular injury hyperfiltration has been suggested as a pathogenic mechanism (Hostetter et al. 1981) in man the evidence is less secure.

SUSCEPTIBILITY TO DIABETIC NEPHROPATHY

Epidemiological studies have revealed that diabetic nephropathy affects between 35-45% of insulin-dependent patients (Andersen et al. 1983, Krolewski et al. 1985, Kofoed-Envoldsen et al. 1987). What identifies this subset of patients has been a source of study for many workers. Recent data has suggested that diabetic nephropathy clusters in families (Seaquist et al. 1989, Pettitt et al. 1990, Borch-Johnsen et al. 1992). As cardiovascular disease is frequently associated with diabetic nephropathy known risk factors for cardiovascular disease were investigated in families of patients

with this complication in order to determine possible pathogenetic mechanisms for the familial association. The first clue to this association was the finding that parental blood pressure higher in probands with nephropathy (Viberti et al. 1987). At about the same time the activity of the red blood cell sodium-lithium countertransport (Na^+/Li^+ CTT), a marker of hypertension, was found to be higher in diabetics with nephropathy (Mangili et al. 1988, Krolewski et al. 1988). Further exploration of the familial association revealed that Na^+/Li^+ CTT activity was higher in parents of diabetic patients with nephropathy compared to parents whose diabetic offspring did not have nephropathy (Walker et al. 1990). The physiological role of the Na^+/Li^+ CTT is undetermined but it may reflect the activity of the cellular sodium-hydrogen antiporter (Na^+/H^+ AP). This ubiquitous cell cation transport system is involved in the regulation of intra-cellular pH, cell division and the absorption of sodium in the proximal renal tubule (Aronson 1983, Paris and Pouyssegur 1984, Owen 1985, Schwartz et al. 1989). Recently the activity of fibroblast Na^+/H^+ AP has been found to be elevated in diabetic patients with nephropathy (Trevisan et al. 1992, Davies et al. 1992, Lurbie et al. 1992). Abnormalities of the Na^+/H^+ AP could account for some of the pathogenic processes seen in diabetic nephropathy such as an increase in total body sodium, glomerular basement thickening and mesangial expansion.

Finally, cardiovascular disease is more frequent in the parents of diabetics with nephropathy and confers a 3-fold risk of renal disease to their diabetic offspring (Earle et al. 1992). These factors taken together suggest a familial, possibly genetic, contribution to this complication which is likely to be related to a susceptibility to cardiovascular events.

CLINICAL DIABETIC NEPHROPATHY

The diagnosis of overt diabetic nephropathy is usually based on clinical symptoms and signs. Rarely is renal biopsy considered necessary

to give pathological confirmation but this is useful if the clinical presentation is atypical, e.g., the absence of retinopathy, the presence of haematuria, or the finding of persistent proteinuria in an insulin-dependent diabetic patient of short disease duration. In non-insulin diabetics other renal diseases are common and renal biopsy may be more frequently indicated (Parving et al. 1992). The characteristic finding of diabetic nephropathy is persistent proteinuria usually detected by Albustix^{R} and corresponding to a urinary albumin concentration of around 250 mg/l. This usually reflects a total urinary protein loss of >0.5 g/24 hour. A recent report from Minneapolis described a subset of female patients with biopsy-proven diabetic glomerulopathy and a reduced glomerular filtration rate yet with low levels of urinary albumin excretion (Lane et al. 1992). This group of patients had a 'self-imposed' low dietary protein intake ranging from 44 to 51 g/day as calculated from urinary urea nitrogen.

Elevated blood pressure (BP), retinopathy (frequently proliferative) and lipid abnormalities are concomitants of clinical proteinuria (Winocour et al. 1987). Diabetic nephropathy characteristically leads to a progressive decline in renal function. The morphological correlates of the declining glomerular filtration rate are mesangial expansion and sclerosis of glomeruli. The rate of decline in GFR varies from 0.5 to 2.4 ml/min/month between individuals yet appears to be constant in a given individual (Mogensen 1976, Jones et al. 1979, Parving et al. 1981, 1987, Viberti et al. 1983, Jones et al. 1989).

TREATMENT

The methods available for the treatment of diabetic renal disease have improved considerably over the last 15 years. The identification of patients with early disease coupled with better insulin delivery systems which can produce improved metabolic control has enabled the effect of

'tight' metabolic control to be studied. The results of well-conducted trials involving small numbers of patients have suggested that at the stage of incipient nephropathy the progressive rise in urinary albumin loss is at least arrestable (Kroc Study 1984, Feldt-Rasmussen et al. 1986, 1991, Dahl-Jorgensen et al. 1988). The Diabetes Control and Complications Trial (DCCT 1987) will provide powerful evidence relating to the effect of glycaemic control on the development and progression of the disease and the main results will be available during 1993.

Reducing blood pressure levels and the intake of dietary protein also lowers urinary albumin excretion in microalbuminuric patients (Christensen and Mogensen 1985, Marre et al. 1987, 1988, Mathiesen et al. 1991, Hallab et al. 1993, Cohen et al. 1987, Dullaart et al. 1993). These interventions prevent progression to proteinuria (Marre et al. 1987, 1988, Mathiesen et al. 1991) yet to date no studies have demonstrated an effect on the rate of decline of GFR even after 4 years treatment with captopril (Mathiesen et al. 1991).

In patients with established diabetic nephropathy treating elevated levels of arterial blood pressure with various drugs has been shown to be of benefit in reducing the rate of decline of GFR in uncontrolled studies (Mogensen 1982, Parving et al. 1987, Björck et al. 1990, 1992).

Although data exists to link poor glycaemic control to the development of diabetic nephropathy (Krolewski et al. 1985, Nyberg et al. 1987) the rate of decline of glomerular filtration rate does not appear to be significantly affected by tightening blood glucose control in patients with established diabetic nephropathy (Viberti et al. 1983a, Bending et al. 1986).

Data reported in this thesis suggests that a reduction in dietary protein with other concomitant dietary changes has an overall benefit in reducing the rate of GFR loss in diabetic nephropathy although the effect is heterogeneous (Walker et al. 1989). Other studies with differing study

designs and methods have reported similar findings (Barsotti et al. 1988, Ciavarella et al. 1987, Evanoff 1989, Zeller et al. 1990).

Renal replacement therapy, including continuous ambulatory peritoneal dialysis, renal transplantation and haemodialysis, for diabetic patients who reach end-stage renal failure has been available for the last 20 years although even now in the United Kingdom there may be some deficiencies in the facilities provided (Renal Failure in Diabetics in the UK Working Party Reports, 1988, 1989). Generally the results of renal replacement therapy of whatever form are poorer in diabetic than in non-diabetic patients due to the excess morbidity and mortality that concomitant diabetic complications produce with cardiovascular causes predominating (Cameron and Challah 1986, Grenfell et al. 1988, Brunner and Selwood 1992, Basadonna et al. 1992, Manske et al. 1992, 1992a).

CONCLUSION

Knowledge of the important diabetic complication of nephropathy has increased greatly over the last two decades. It is clear that familial factors influence the risk of developing diabetic nephropathy in a given individual yet the exact pathophysiological mechanism is still undetermined and primary prevention is not possible. The identification of those patients with microalbuminuria provides an 'at-risk' population in whom secondary preventative manoeuvers can be applied and the long-term results of strict glycaemic control and reductions in blood pressure levels are awaited. The treatment of those with established renal disease has improved over the last 20 years but still nearly 600 diabetic patients entered end-stage renal failure in the United Kingdom during 1985.

THE EPIDEMIOLOGY AND NATURAL HISTORY OF DIABETIC NEPHROPATHY

THE EPIDEMIOLOGY OF DIABETIC NEPHROPATHY

The data that helps in the understanding of epidemiology of nephropathy in insulin-dependent diabetic patients stems chiefly from two sources - the Joslin Diabetes Center, Boston, Massachusetts, USA, and the Steno Memorial and Hvidøre Hospitals, Copenhagen, Denmark. These centres have been treating diabetic patients for over 70 years and have large patient bases and excellent record-keeping and follow-up facilities all of which are mandatory for accurate epidemiological studies. However, the nature of these specialized institutions will tend to attract a select group of patients and this needs to be kept in mind when assessing the data generated.

THE STENO STUDY

In this large study of 1475 insulin-dependent patients (onset before 31 years of age) diagnosed before 1953 at the Steno Memorial Hospital and followed for a period of 25 years or more all but 91 (6%) of eligible patients were traced (Andersen et al. 1983). Clinical diabetic nephropathy was defined as persistent proteinuria (urinary protein excretion >0.5 g/24 hours) in at least four consecutive 24-hour urine collection samples with an interval of at least a month between collections and in the absence of urinary infection. Forty-one percent of the patients developed diabetic nephropathy and 3% had persistent proteinuria from other causes than diabetes. A clear relation to the duration of the diabetes was seen with a peak prevalence after 20-25 years of the condition (Table 1.1, page 23). The peak incidence occurred after 16 years and fell away dramatically after 35 years of diabetes. The age of onset of diabetes did not influence the risk of developing nephropathy. The risk was higher in men (1.79 higher ratio)

and men appeared to develop nephropathy later than women. The year in which diabetes was diagnosed ('calendar year' effect) was associated with different incidence rates of nephropathy with those being diagnosed in the 1930's having higher rates than those diagnosed in later decades. This effect was also observed in the Joslin study (Krolewski 1985) (Table 1.1). Patients living in rural areas had a higher incidence of nephropathy compared to city dwellers. Glycaemic control was not specified in this study but patients with nephropathy had a higher insulin dose per kilogram body weight. Mortality was dramatically different between the two groups with 83% of the patients with nephropathy dying by the end of the follow-up compared to 25% of patients without proteinuria. Two-thirds of the nephropathic patient died from uraemia (these tended to be the patients who developed nephropathy within 20 years of diagnosis) and 20% from ischaemic heart disease (these tended to be the patients who developed nephropathy after 20 years of diagnosis). It was concluded from this large study was that diabetic nephropathy was 'the major life-threatening complication in Type 1 diabetes of juvenile onset'.

THE JOSLIN STUDY

Two years later a smaller but equally well-conducted study was published from the Joslin Center (Krolewski et al. 1985). Two-hundred and ninety-two patients with insulin-dependent diabetes were followed for between 20 and 40 years and the development of persistent proteinuria documented. The cumulative risk after 40 years of diabetes was 35% (Table 1.1). Again a marked effect of duration of diabetes was observed with a peak incidence after 10 to 14 years of the condition and a calendar year effect with those diagnosed in the 1930's having twice the risk of persistent proteinuria compared with those diagnosed in later decades. In contrast to the Steno study, an assessment of glycaemic control (quantified as 'an index of the frequency of hyperglycaemia' from clinic blood glucose

levels and expressed as quartiles) was made and hyperglycaemia was correlated with the risk of nephropathy for both men and women. An additional measure of diabetic nephropathy, the occurrence of end-stage renal failure, was also analysed in this study and this closely reflected the occurrence of persistent proteinuria. The median time between onset of persistent proteinuria and end-stage renal failure was 10 years. In patients whose diabetes was diagnosed before puberty, progression to end-stage renal failure took longer than in those with diabetes diagnosed after puberty.

This study, although smaller than the Steno study, has broadly the same findings and adds the important dimension of glycaemia to the equation. The calendar year effect is particularly intriguing. No definitive reason for this decline in incidence can be given, but the authors draw attention to the decrease in other glomerulonephritides during the second half of the twentieth century, changes in insulin formulations and the more widespread availability of antibiotics.

THE SECOND STENO STUDY

A more recent study from the Steno investigated whether the declining relative mortality in diabetics may be due to a decreasing rate of diabetic nephropathy (Kofoed-Envoldsen et al. 1987). Again this was a large study involving 2890 insulin-dependent patients diagnosed between 1933 and 1972 and before the age of 31 years. Interestingly, there was a 30% decrease in the incidence of nephropathy when comparing those patients diagnosed between 1953 and 1962 with those diagnosed between 1933 and 1942. The incidence was higher in males and peaked at 15-17 years of diabetes. In this Steno study insulin dose did not influence the incidence of proteinuria (Table 1.1).

OTHER STUDIES

Three recently reported prevalence studies involving insulin-dependent diabetic patients suggest some differences between the occurrence of diabetic nephropathy in England and Denmark and the U.S.A. (Gattling et al. 1988, Parving et al. 1988, Orchard et al. 1990).

Gattling obtained a 92% ascertainment rate in a population-based survey in Dorset, England and defined proteinuria as positivity to Albustix^R testing. This was found in only 6.4% of the insulin-dependent diabetic patients with a peak prevalence of 13.3% after 25-29 years of diabetes. However examining the hospital record of the patients with persistent proteinuria revealed that in 5 of 13 cases (38%) diabetic nephropathy was unlikely to be the cause of the proteinuria.

In contrast, Parving examining the insulin-dependent patients attending the outpatient department of the Hvidøre Hospital, Copenhagen in 1985 found a prevalence of macroalbuminuria (urinary albumin excretion rate >300 mg/24 hours and equivalent to Albustix^R positivity) of 19% with a peak prevalence of 25% after 15-29 years of diabetes (Parving et al. 1988). Recent data from the U.S.A. showed a peak plateau of macroalbuminuria (>200μg/min) or end-stage renal failure of 48% with a duration of 25 years (Orchard et al. 1990).

Different patient populations and methods of asssessment may acount for the marked discrepancies between studies. Patients are referred to a hospital clinic for many reasons and one reason may be concern about the development or presence of complications. The studies by Gatling and Parving used only one assessment of proteinuria. Urinary albumin excretion is very variable with a coefficient of variation up to 50% (Mathiesen et al. 1984, Feldt-Rasmussen and Mathiesen 1984, Cohen et al. 1987a) and a single reading may miss a number of patients with

intermittent proteinuria (Bending et al. 1986). A recent report demonstrated coefficients of variation for urinary albumin concentration or urinary albumin to creatinine ratios to be higher under routine clinical conditions than under study conditions (Johnson et al. 1993). This may influence the studies of Parving and Orchard in which the data was obtained from clinic records. Finally, the assessment of a slight positivity on an Albustix^R is difficult due to personal interpretation of subtle colour changes. The Hvidøre and Pittsburgh studies overcame this by quantifying the urinary albumin excretion rate.

TABLE 1.1. THE EPIDEMIOLOGY OF DIABETIC NEPHROPATHY. A COMPARISON OF THREE STUDIES.

STUDY	ANDERSEN 1983	KROLEWSKI 1985	KOFOED-ENVOLDSEN 1987
Location	Steno, Copenhagen	Joslin, Boston	Steno, Copenhagen
Number Of Patients	1384	292	2890
Peak Incidence	At 16 years	10-14 years	15-17 years
Cumulative Incidence	45% after 40 years	35% after 40 years	34% after 25 years
Sex Difference	M:F = 1.8:1	No difference	M:F = 1.5:1
Calendar Year Effect	Present	Present	Present
Associations	Domicile, Insulin-dose	Glycaemia	High BMI
Prognosis	50% dead after 7 years of proteinuria	ESRF in 75% within 15 years	Not stated

ESRF = End-stage Renal Failure

BMI = Body Mass Index

GEOGRAPHICAL AND ETHNIC FACTORS

Incidence and prevalence rates for diabetic nephropathy vary with location (WHO Multi-National Study 1985) and different ethnic groups in the United Kingdom (Asian and Afro-Caribbean) have higher rates than

indigenous Caucasians (Mather and Keen 1985, Samanta et al. 1986, Grenfell et al. 1988). Asian Indians living in West London also have a higher prevalence of microalbuminuria (Allawi et al. 1988). The reasons for these differences are likely to be multi-factorial and include genetic, environmental, dietary, and treatment influences.

THE NATURAL HISTORY OF DIABETIC NEPHROPATHY

The longitudinal studies which documented the progression from micro- to macroalbuminuria used baseline and a follow-up assessments separated by a 6 to 14 year interval (Viberti et al. 1982, Parving et al. 1982, Mathiesen et al. 1984, Mogensen and Christensen 1984). One study followed 64 young insulin-dependent patients prospectively for 8 years with assessments every 2 years (Rudberg et al. 1992). Fifteen of 53 (28%) initially normoalbuminuric patients developed microalbuminuria and 3 (6%) persistent proteinuria. In this study the initial GFR was the only independent predictor of microalbuminuria and proteinuria in patients with normoalbuminuria at baseline. In contrast a 4 year follow-up of 137 initially normoalbuminuric patients revealed a cummulative frequency of 8% for the development of persistent microalbuminuria: initial albumin excretion rate, blood pressure, glycosylated haemoglobin level and smoking were significant determinants of persistant microalbuminuria (MCS 1993). These finding are similar to those reported from a 6 year follow-up of 205 insulin-dependent diabetic patients with initial urinary albumin excretion in the normal range (Mathiesen et al. 1990). Microalbuminuria developed in 7% with initial albumin excretion rate and glycosylated haemoglobin levels being higher in those who progressed. Initial blood pressure levels were not higher in the progressors in this study however readings were taken infrequently. Finally a four year study of 15 insulin-dependent diabetic patients with levels of urinary albumin excretion above the normal range but below the level considered

by some to be in the microalbuminuric range (21±4 (SEM) mg/24 hours) revealed no change in the level of urinary albumin excretion on conventional insulin therapy (Dahl-Jorgensen et al. 1988).

A few studies have studied small numbers of microalbuminuric patients and have documented the development of macroalbuminuria (clinical diabetic nephropathy) in a proportion of cases. In a study reported by Feldt-Rasmussen, 5 of 18 (28%) microalbuminuric (urinary albumin excretion rate 30-300 mg/24 hours) insulin-dependent diabetic patients developed clinical nephropathy (urinary albumin excretion rate >300 mg/24 hours) during a two year observation period (Feldt-Rasmussen et al. 1986). An 8 year follow-up of this cohort suggests that only those patients with an initial albumin excretion rate above 100 mg/24 hours and who were randomised to conventional insulin treatment progress to proteinuria and declining GFR (Feldt-Rasmussen et al. 1991). In a study reported by Marre, 3 of 10 microalbuminuric (urinary albumin excretion rate 30-300 mg/24 hours) insulin-dependent diabetic patients developed clinical nephropathy (urinary albumin excretion rate >300 mg/24 hours) during a one year observation period (Marre et al. 1988). Finally, Mathiesen reported a 4 year follow-up of 23 normotensive microalbuminuric (geometric mean for urinary albumin excretion rate 105 mg/24 hour) insulin-dependent diabetic patients. Seven of these patients (30%) developed proteinuria by 4 years but this was not accompanied by a raised blood pressure or marked fall in GFR in every case (Mathisen et al. 1991). Six of the 7 progressors had a baseline urinary albumin excretion rate above 60 mg/24 hr.

The increase in urinary albumin excretion through the microalbuminuric range is thought to be expontential with an annual rate of increase between 7 and 19% (Christensen and Mogensen 1985, Feldt-Rasmussen et al. 1986). However not all patients show progression

(Christensen and Mogensen 1985, Messant et al. 1992) and it seems that those with lower levels of urinary albumin excretion and with a longer duration of disease are more likely to remain stable or progress at a slow rate (Feldt-Rasmussen et al. 1991, Forsblom et al. 1992).

Once persistent proteinuria is established, arterial blood pressure and urinary albumin excretion rise as the glomerular filtration rate falls with time and a number of studies have followed the rate of decline of the glomerular filtration rate prospectively (Mogensen 1976, Jones et al. 1982, Parving et al. 1981, 1987, Viberti et al. 1983, Jones et al. 1989). These studies which have used isotopic methods (except in the study by Jones et al. 1979) for the assessment of glomerular filtration rate have shown remarkably similar results. The rate of decline of glomerular filtration rate appears to be linear with time in a given individual although the inter-individual rates of decline of GFR may vary. In these studies the rate of decline in glomerular filtration rate was unrelated to age, sex, duration of diabetes or duration of proteinuria. Blood glucose control was associated with the rate of decline of GFR in a Scandinavian study (Nyberg et al. 1987). In some (Mogensen 1976) but not all studies (Jones et al. 1979, Parving et al. 1981, Viberti et al. 1983), a relationship between the rate of glomerular filtration rate decline and diastolic blood pressure was described. This lack of a strong relationship is surprising in view of the beneficial effects of treating blood pressure on the rate of decline of glomerular filtration rate (Mogensen 1982, Parving et al. 1987, Björck et al. 1990, 1992). The relationship may have been obscured by the introduction of antihypertensive therapy in some patients, but these patient were in the minority. Those patients with nephropathy who maintain a lying diastolic blood pressure below 90 mmHg have a significantly slower rate of loss of GFR compared to hypertensive patients (3.9 ml/min/yr vs. 9.6 ml/min/yr) and it has been suggested that the development of

hypertension is necessary for the rapid decline in GFR in diabetic nephropathy (Earle and Viberti 1991).

CONCLUSION

Diabetic nephropathy may eventually affect up to a third or even 40% of patients with insulin-dependent diabetes with a peak incidence after 10-20 years. Males are more likely to be affected and the incidence is decreasing over successive decades. The data for non-insulin dependent patients is not so readily available but they appear to develop nephropathy after a shorter duration of diabetes, and although in the United Kingdom incidence rates may be lower than for insulin-dependent diabetes patients, the larger number of patients with non-insulin dependent diabetes makes this complication a large problem in personal and health economic terms (Ballard et al. 1988, Parving et al. 1992).

Microalbuminuria signals early disease and recent data has suggested that low levels of microalbuminuria may be less predictive of later established nephropathy than higher levels (> 100 mg/24 hours) and that microalbuminuria in patients with more than 21 years of diabetes may be less predictive than in those with a shorter duration of disease (12-14 years) (Feldt-Rasmussen et al. 1991, Forsblom et al. 1992, Viberti and Friedman 1993).

In established diabetic nephropathy the loss of glomerular filtration rate is generally linear with time and in most cases is accompanied by a raised level of arterial blood pressure.

THE PATHOGENESIS AND PATHOLOGY OF DIABETIC NEPHROPATHY

The mesangial cell appears to be central in the pathology of diabetic nephropathy (Steffes et al. 1989, 1992, Lorenzi and Cagliero 1991). It is not known whether mesangial cell hyperplasia occurs however it is clear that

mesangial matrix accumulation is a critical lesion in diabetic glomerulopathy. In-vitro cultured mesangial cells will proliferate in response to growth factors, cytokines, matrix components and vasoactive substances (Mene et al. 1989) and recently levels of mRNA for a number of growth factors, including proliferating cell nuclear antigen (PCNA), TNF-α, PDGF-B chain, TGF-β and basic fibroblast growth factor have been shown to be elevated 4- to 5-fold in glomeruli from diabetic rats (Nakamura et al. 1993).

Widening of the glomerular basement membrane is seen uniformly in patients with diabetic glomerulopathy. Whether the increased width is due to increased synthesis of decreased degradation is unclear. In-vitro human endothelial cells over express basement membrane components in high glucose concentrations with control mediated at the level of transcription (Cagliero et al. 1988).

The role of glucose in the pathogenesis of diabetic nephropathy has obviously been the subject of considerable study. However as (a) diabetic nephropathy will only affect 35-40% of patients and thus the majority of patients escape serious renal disease, (b) the development of nephropathy is not linearly related to duration of disease and (c) the re-development of diabetic nephropathy in diabetics who have received a renal transplant is only weakly associated with an index of glycaemic control (Mauer et al. 1989) it is unlikely that hyperglycaemia alone is involved in the pathogenesis of diabetic nephropathy.

Glucose, or other metabolites, may exert their action at a number of different levels. A number of genes are regulated by glucose and translated proteins from mRNAs from human mononuclear cells differ between diabetics and non-diabetics (Mariash and Burmeister 1988). In-vitro studies demonstrate that glomerular cells cultured in 30mM glucose secrete 2- to 3-fold more type IV collagen than cells cultured in 5mM glucose (Danne et

al. 1993). Prolonged exposure to high concentrations of glucose is associated with abnormalities in cell replication, maturation and DNA damage in human cultured endothelial cells (Lorenzi et al. 1985, 1986).

Non-enzymatic glycosylation has been suggested as a mechanism for the increase in glomerular basement membrane and mesangial matrix (Brownlee et al. 1988) and additionally increases in renal cellular sobitol mediated through the polyol pathway have been proposed as a glucose-mediated pathogenic mechanism (Tilton et al. 1989). In humans no studies involving an inhibitor of non-enzymatic glycosylation such as aminoguanidine have been reported and one study involving non-insulin-dependent diabetic subjects with microalbuminuria demonstated no beneficial effects on renal function after treatment with Statil, an aldose reductase inhibitor (Cohen et al. 1989).

Data from the rat model suggests that elevations in glomerular pressures rather than hyperglycaemia are important in the development of glomerulopathy (Steffes et al. 1978, Mauer et al. 1978, Hostetter et al. 1981). Physical stress and shear forces which may damage the endothelial barrier and increase permeability of macromolecules in mesangial areas have been proposed as a possible pathogenic mechanism for mesangial expansion (Remuzzi and Bertani 1990). However, the rat, used as an animal model in many studies fails to develop the histological lesions characteristic of human diabetic nephropathy and there is considerable inter-species variation in renal haemodynamic responses to the diabetic state (Remuzzi and Bertani 1990). These factors make extrapolation to human diabetes difficult. The glomerular hyperfiltration seen in some diabetic patients may represent the human counterpart of the diabetic animal model but it is not clear whether glomerular hyperfiltration in man is an independent risk factor for the development of nephropathy.

FAMILIAL/GENETIC FACTORS AND THE SUSEPTIBILITY TO DIABETIC NEPHROPATHY

Diabetic nephropathy clusters in families, with diabetic siblings of diabetic probands with nephropathy having a frequency five times higher than diabetic siblings of diabetic probands without nephropathy (Seaquist et al. 1989). Among the Pima Indians with non-insulin dependent diabetes the prevalence of diabetic nephropathy in diabetic offspring of parents who both had diabetes and proteinuria is 46% (Pettitt et al. 1990). The importance of familial factors, and thus possibly heredity, has been further emphasized by the demonstration of a raised arterial pressure and an increased prevalence of cardiovascular death in the parents of proteinuric insulin-dependent diabetic patients (Krolewski et al. 1988, Viberti et al. 1987, Earle et al. 1992). These observations have been complemented by the finding of a raised sodium-lithium countertransport activity (Na^+/Li^+ CTT), a marker of risk for essential hypertension, in the red cells of insulin-dependent diabetic patients with either microalbuminuria or established nephropathy. While three independent studies confirmed the activity of this cation exchanger to be elevated in insulin-dependent diabetic patients with diabetic nephropathy (Krolewski et al. 1988, Mangili et al. 1988, Jones et al. 1990) a further study found that while the Na^+/Li^+ CTT activity was higher in diabetic patients there was no difference between those with and without nephropathy (Elving et al. 1992). A higher level of red cell sodium-lithium countertransport activity was reported in the parents of insulin-dependent diabetics with nephropathy (Walker et al. 1990) a finding not confirmed in a Danish study (Jensen et al. 1990) in which the method of measurement of Na^+/Li^+ CTT activity in red blood cells was different to that employed in other studies (Laffel et al. 1991, Canessa et al. 1992).

The pathogenic mechanism by which an elevation of red blood cell sodium-lithium countertransport activity contributes to renal disease in diabetes remains to be elucidated. It is possible that the red blood cell sodium-lithium countertransport activity is a mode of operation of the physiological sodium/hydrogen antiport (Na^+/H^+AP), a ubiquitous cell membrane cation transport system involved in the regulation of intracellular pH (pH_i), cell growth and renal proximal tubule sodium reabsorption (Aronson 1983, Paris and Pouyssegur 1984, Owen 1985, Schwartz et al. 1989). In skin fibroblasts the activity of the Na^+/H^+AP has been shown by three groups to be higher in diabetic patients with nephropathy compared to matched patients without nephropathy (Trevisan et al. 1992, Davies et al. 1992, Lurbe et al. 1992). In vitro an increase in the activity of the Na^+/H^+AP leads to a higher intracellular pH_i due to an apparent increase in the affinity of the internal hydrogen ion site, possibly due to increased phosphorylation of the antiport protein (Davies et al. 1992). In addition an increase in the incorporation of tritiated thymidine in cultures of skin fibroblasts from diabetic patients with nephropathy compared to matched patients without nephropathy suggests an intrinsic difference in cell cycle regulation (Trevisan et al. 1992). Cellular alkalinisation is necessary for cell growth in a number of in vitro systems (Schwartz et al. 1989). If the cells of certain diabetic patients have a genetically determined increase in the intrinsic activity of the Na^+/H^+AP or an increase response to growth factors or other stimuli increased intracellular alkalininity may stimulate cell growth and extracellular matrix production characteristic of diabetic nephropathy.

Changes in the Na^+/H^+ antiport activity in diabetes may be secondary to other factors such as extra-cellular matrix abnormalities (ECM) associated with the diabetic state. Adhesion of anchorage-dependent bovine capillary endothelial cells to fibronectin, mediated through

integrin $\alpha 5\beta 1$, leads to activation of the Na^+/H^+AP (Schwartz et al. 1991). Recently other ligands (including type III, IV and V collagens and laminin) acting through different integrins, have been shown to elevate pH_i by activation of the antiport (Schwartz et al. 1991a). Thus, increased Na^+/H^+AP activity in insulin-dependent diabetic patients with diabetic nephropathy could be reflecting altered ECM metabolism which is itself directly connected with nephropathy risk.

These recent findings strongly support the view that familial, possibly genetic, factors are important in the susceptibility to diabetic nephropathy.

TREATMENT MODALITIES

THE ROLE OF GLYCAEMIC CONTROL

The identification of microalbuminuria as the earliest stage of diabetic nephropathy and the introduction of novel methods for delivering insulin, such as continuous subcutaneous insulin infusion and insulin pens, enabled a number of groups to study the effect of strict glycaemic control on albumin excretion rates and glomerular filtration rate in prospective studies. Urinary albumin excretion is known to be associated with glycaemic control (Viberti et al. 1979, Wiseman et al. 1984) and thus it was important to investigate the effect of this intervention. Three important studies have been reported; the Kroc, the Steno, and the Oslo studies (Kroc Study 1984, Feldt-Rasmussen et al. 1986, 1991, Dahl-Jorgensen et al. 1988),.

These prospective controlled randomized studies were similar in their design yet varied in duration: the Kroc being eight months, the Steno two years, and Oslo four years (Table 1.2). The overall findings of these studies are relatively uniform suggesting that at the stage of microalbuminuria intensified glycaemic control can either lower urinary

albumin excretion rates or arrest the rise seen in the control group (Table 1.2).

There is cross-sectional evidence that proteinuria is associated with poor glycaemic control (Krolewski et al. 1985, Nyberg et al. 1987) yet few studies investigating the effects of improved glycaemic control have been carried out in these patients. Strict glyaemic control is associated with an increased frequency of hypoglycaemia (DCCT 1987) and ketoacidosis (Feldt-Rasmussen et al. 1991) and as many insulin-dependent diabetics with proteinuria have a duration of diabetes of 15-20 years the majority have defective secretion of counter-regulatory hormones, especially glucagon and adrenaline, and impaired awareness of hypoglycaemic symptoms (Gale 1990) making this intervention difficult and potentially dangerous. Indeed one studied found that strict glycaemic control, attempted by mean of continuous subcutaneous insulin infusion, was more difficult to achieve in diabetics with proteinuria than in patients with normal albumin excretion rates (Bending at al. 1984). In a small study of six proteinuric patients with a study design using each patient as his or her own control, improved glycaemic control had no influence on the rate of decline of GFR (Viberti et al. 1983a). In cases of intermittent proteinuria 12 months of improved blood glucose control has no significant effect on the rate of decline of the glomerular filtration rate (Bending et al. 1986).

TABLE 1.2. CONTROLLED STUDIES INVESTIGATING IMPROVED GLYCAEMIC CONTROL IN DIABETIC PATIENTS WITH MICROALBUMINURIA.

STUDY	TYPE/NUMBER OF PATIENTS		GLYCOSYLATED HAEMOGLOBIN	DURATION OF STUDY	OUTCOME
KROC	39 NA	20 CIT	9.9→9.9	8 MONTHS	No change in AER in either group
		19 CSII	10.2→8.0		
	20 MA	10 CIT	11.5→11.8		AER μg/min CIT 34→26
		10 CSII	11.3→8.2		CSII 30→10
STENO	36 MA	18 CIT	9.3→8.6	2 YEARS	θ ALB CIT 160→360 CSII 170→160
		18 CSII	9.5→7.2		
OSLO	30 'low' MA	15 CIT	9.5→10.5	4 YEARS	AER mg/24 h CIT 21→22
		15 CSII	10.1→9.0		CSII 26→16

KEY

NA = Normoalbuminuria
MA = Microalbuminuria
AER = Urinary albumin excretion rate
θ ALB = Fractional clearance of albumin
CIT = Conventional insulin treatment
CSII = Continuous subcutaneous insulin infusion

THE ROLE OF BLOOD PRESSURE CONTROL

Antihypertensive treatment using a variety of agents has been shown to be associated with a reduction in urinary albumin excretion in a number of studies involving microalbuminuric insulin-dependent diabetic patients studied for varying periods of time (Christensen and Mogensen 1985, Marre et al. 1987, 1988, Mathiesen et al. 1991). In all studies treatment was associated with a reduction in the level of urinary albumin excretion. In a 4 year study of normotensive microalbuminuric insulin-dependent diabetic patients reported by Mathiesen the reduction in the urinary albumin excretion rate was independent of blood pressure. Despite treatment with 100 mg of captopril a day and the addition of a thiazide diuretic blood pressure was similar between the treated and untreated groups (Mathiesen et al. 1991). Hallab et al recently reported that hydrochlorothiazide had no effect on reducing urinary albumin excretion in microalbuminuric insulin-dependent diabetic patients in contrast to enalapril which caused a mean reduction from 59 to 38 mg/24 hours over a one year period. Blood pressure reduction was similar although the average level of mean blood pressure was 4 mmHg lower in the enalapril group (Hallab et al. 1993). Most studies have been of insufficient duration to assess the effect of treatment on GFR. However in the study by Mathiesen there was no difference in the GFR between the treated and untreated groups after 4 years (Mathiesen et al. 1991). Treatment has been associated with a reduction in the progression to proteinuria (Marre et al. 1988, Mathiesen et al. 1991).

A number of studies have reported the beneficial effects of treating raised levels of blood pressure in established diabetic nephropathy (Mogensen 1982, Parving et al. 1987, Björck 1990, 1992). The study by Parving used a variety of agents excluding ACE inhibitors and using a self-

control design showed a slowing in the rate of decline of GFR although the responses of individual patients were heterogenous (Parving et al. 1987). Björck compared enalapril to metoprolol in a 2 year study found a slower rate of fall of GFR in the enalapril group compared to the metoprolol group (2.0 (SD 3.2) vs 5.6 (5.9) ml/min/year) although diastolic blood pressure levels were lower on enalapril therapy (Björck et al. 1992, Yeo et al. 1992, Sawicki and Berger 1992).

Whether angiotensin converting enzyme (ACE) inhibitors offer any particular advantage over other antihypertensive agents in diabetic nephropathy, either at the stage of micro- or macroalbuminuria, is not certain (Melbourne Diabetic Nephropathy Study Group 1991, Mogensen 1992). There is evidence in both the diabetic rat model and in patients with diabetic nephropathy that these drugs may reduce intraglomerular pressure and may enhance glomerular barrier size selectivity (Zatz et al. 1986, Marre et al. 1988, Pinto et al. 1990, Morelli et al. 1990, Björck 1990, 1992, Hallab et al. 1993). Additionally ACE inhibitors appear to have a side-effect profile which is less disadvantageous to the diabetic patient compared to other blood pressure lowering agents.

The level at which to treat blood pressure is debated. WHO cut-off levels for hypertension (160/95 mmHg) may be too high for these patients, many of whom are in their 20's. The use of centile charts (Acheson 1973, Walker et al. 1988, 1989a), correcting for the effects of age and sex on blood pressure, are more appropriate for assessing raised levels of arterial pressure although no studies have addressed the question of what level of blood pressure to treat in patients with microalbuminuria or proteinuria.

THE ROLE OF DIETARY PROTEIN

HISTORICAL ASPECTS

Nearly 90 years ago Folin showed that a diet of starch and cream caused a marked reduction in the urinary elimination of total nitrogen and urea in normal man (Folin 1905). By the 1940s, a number of workers were advocating low protein diets as a method for alleviating uraemic symptoms (Addis and Lew 1939, Addis et al. 1946). The exact amount of protein prescribed was variable, but when protein was severely restricted to prevent uraemic symptoms, it became apparent that patients were often in negative nitrogen balance (Bergstrom 1984). In 1963, Giordano reported that by supplementing a virtually nitrogen-free diet with essential L-amino acids, patients could be brought into a positive nitrogen balance (Giordano 1963). The proposals of Giordano were clinically applied by Giovannetti and Maggiore, who described a diet deficient in protein to which was added essential amino acids and small amounts of protein of high biological value (Giovannetti and Maggiore 1964). The diet they described was high in calories and all 8 patients treated for 3 to 10 months had an improvement in uraemic symptoms and 6 of the 8 were 'rehabilitated almost completely'. This diet, or variations on it, was adopted widely yet many patients were put into negative nitrogen balance, and it was monotonous and unpalatable (Bergstrom 1984). In order to maintain neutral nitrogen balance a diet providing 0.6 g/Kg/day of protein is necessary (Goodship and Mitch 1988).

In the 30 years since Giovannetti and Maggiore described their diet, low protein diets, either with or without essential amino acid supplementation or keto acid supplements (keto acid analogues of amino acids), have drifted in and out of favour and the role of dietary protein restriction in the management of chronic renal failure (CRF) from any

cause has been hotly debated (El Nahas and Coles 1986, Giovanetti 1986). Since 1975 there have been at least 46 trials of low protein diets in chronic renal failure published (Fouque et al. 1992). Many study designs have been employed with varying cohorts of patients. A recent meta-analysis of 5 trials selected from 46 suggested that low protein diet was associated with a reduced rate of 'renal death' (start of dialysis or death) compared to patients on a normal diet (odds ratio 0.54) (Foque et al. 1992). This meta-analysis has been criticized for a bias in selection (Walker 1992a, Fouque et al. 1992a).

CLINICAL STUDIES IN NON-DIABETIC PATIENTS

Using a prospective, controlled randomised study design Ihle studied 64 patients with severe renal failure (mean isotopic GFR 14.4 ml/min.) from glomerulonephritis, polycystic disease, reflux and analgesic nephropathy for 18 months on a LPD (Ihle et al. 1989). The prescribed diet provided 0.4 g/Kg/day of protein and 700 mg of phosphorous and was intended to be isocaloric with the normal protein diet (35 to 40 kcal/Kg/day) although body weight fell by about 5 Kg during the first 6 months of LPD and then plateaued. Serum albumin, mid-arm circumference and triceps skin fold thickness was unchanged on either diet. In the NPD group GFR fell from 15 to 6 ml/min. during the study as compared to 13.8 to 12 ml/min. in the LPD group. Blood pressure levels remained constant in each group. The urinary excretion of urea was lowered in the LPD group suggesting compliance with the diet although no specific data are given on the achieved protein intake as calculated from urinary urea nitrogen and it is not possible to calculate this figure from the data presented. Surprisingly plasma levels of phosphorous were no different between NPD and LPD groups and triglyceride levels were unchanged on LPD despite the increased carbohydrate intake. No data are given on the homogeneity of the response although in the last 3 months

of the study end-stage renal failure developed in 27% of patients on NPD and 6% on LPD. The conclusion from this study was that at an advanced stage of renal failure due to glomerular and tubulo-interstitial disease protein restriction, independent of changes in phosphorous of blood pressure, retards the progressive loss of GFR although maintaining energy intake initially is difficult.

Other studies employing non-controlled study designs and/or creatinine clearance or serum creatinine for the assessment of renal function have provided similar results but the intepretation of the results of these studies has to be balanced with the shortcomings of study design. In a study involving a large number of patients Rosman randomised 228 patients with creatinine clearance values from 10 to 60 ml/min. to 2 levels of protein restriction (0.4 and 0.6 g/Kg/day) depending on the degree of renal impairment (Rosman et al. 1984). Control patients continued their regular diet and the reciprocal of the serum creatinine was followed over an 18 month period. The rate of fall was less in the LPD groups independent of blood pressure levels. Studying the plots of the reciprocal of the serum creatinine against time on LPD it is clear that the first point on the graph, representing 3 months on LPD, is the highest of all the points (representing a fall in serum creatinine) due to the acute contraction of the creatine pool and a acute fall in the amount of ingested creatinine. This point has a marked influence on the slope of the line and largely contributes to the diffence in slope between LPD and NPD. A 4 year follow-up of this cohort concluded that only patients with primary glomerular disease responded to the LPD (Rosman et al. 1989). This contrasts with 2 smaller studies in which LPD was more efficacious in patients with CRF due to chronic pyelonephritis than in those with glomerular disease (El-Nahas et al. 1984, Oldrizzi et al. 1985). No explanations for these differences were suggested. In the 4 year follow-up

study by Rosman males had a faster rate of loss of creatinine clearance than females and it was only males who responded to LPD. The reason for the gender difference, previously observed in rats and man, remains to be elucidated but sex steriod responsive genes may play a role in the mechanism of this difference (Remuzzi et al. 1988, Hunt et al. 1988, Blantz et al. 1988).

Three studies from Italy using the reciprocal of the serum creatinine or creatinine clearance to assess GFR and employing a protein restriction of 0.6 g/Kg/day and a phosphorous restriction of 500 to 700 mg/day for periods of 11 to 44 months in patients with moderate degrees of renal impairment (mean serum creatinine 218-312 μmol/l) produced largely similar results demonstrating a beneficial effect of protein restriction (Maschio et al. 1982, Barsotti et al. 1983, Oldrizzi et al. 1985). The study designs included a control group yet blood pressure levels were not documented or corrected for in 2 of the studies (Barsotti et al. 1983, Oldrizzi et al. 1985). The study by Barsotti compared a low protein diet to a low protein and low phosphorous diet and demonstrated an additive effect of phosphorous restriction on the decline in creatinine clearance and increase in serum creatinine with the low protein and low phosphorous diet (Barsotti et al. 1983). A reduction in serum phospate levels, observed in this study in patients receiving the low protein and low phosphorous diet, was confirmed by some but not all studies demonstrating a beneficial response to LPD (Rosman et al. 1989, Ihle et al. 1989). Thus the role of a reduction in phosphorous in the effect of LPD on renal decline is still undefined.

Compliance with low protein diets has long been a problem due largely to their unpalatability and the marked change in dietary habits that low protein feeding entails. This has been highlighted recently by a large study of 380 patients who were prescribed a diet containg 0.6 g/Kg/day yet

achieved an intake of 0.8 g/Kg/day while the control patients achieved an intake of 1.0 g/Kg/day as calculated from urinary urea nitrogen excretion (Locatelli et al. 1991). Despite the poor compliance there were fewer 'renal end points' in the LPD group. Similarly in insulin-dependent diabetic patients with microalbuminuria only 7 of 14 patients randomised to a LPD of 0.6 g/Kg/day achieved an intake of less than 0.8 g/Kg/day (Dullaart et al. 1993).

Severely restricted protein diets (0.25 g/Kg/day) with amino- and keto-acid supplementation has been shown to be associated with weight loss and a reduction in muscle mass over a 6 month period despite a high energy intake of 3100 kcal/day (Lucas et al. 1986). The reduction in serum creatinine did not reflect any improvement in GFR in this study again highlighting the risks of using this parameters in these studies. In all dietary studies anthropometric measurements are essential to assess nutritional consequences of interventions.

CLINICAL STUDIES IN DIABETIC PATIENTS

Most of the studies designed to test the effects of a diet restricted in protein in insulin-dependent patients with diabetic nephropathy have used creatinine clearance or the reciprocal of the serum creatinine to assess renal function (Table 1.3, page 44). Data presented in Chapter 5 will demonstrate this is not an accurate or precise measure of GFR when compared to the plasma clearance of ^{51}Cr EDTA, an isotopic method which gives nearly identical values to inulin clearances. In addition changes in the creatine pool and creatinine intake seen in low protein diet studies further render this an unreliable measure of GFR.

Study designs have varied. Evanoff used patients as their own control. After a 12 month observation period a LPD of 0.6 g/Kg/day was instituted and, using creatinine clearance and the reciprocal of the serum creatinine to assess renal functional response, 11 patients were studied for

2 years (Evanoff et al. 1989). Nine of the 11 patients had antihypertensive agents initiated during the study and systolic blood pressure levels fell significantly. The LPD was associated with a slowing in the rate of decline of the reciprocal of the serum creatinine and no change in the creatinine clearance levels during the 2 years of study. It is difficult to separate the effects of the blood pressure reduction from those of LPD in this study. No attempts were made to correct for the substantial fall in systolic blood pressure (about 20 mmHg) and this makes interpretation of the findings of this study difficult. Using the same study design Barsotti followed 8 patients with more severe renal impairment for 16 months on NPD (1.2 to 1.4 g/Kg/day) and then instituted a more restrictive protein prescription of 0.25 to 0.35 g/Kg/day with essential amino acid supplementation for a mean duration of 17 months (Barsotti et al. 1988). Urinary urea and urinary protein levels fell during the diet, body weight, triceps skin thickness, midarm muscle circumference and plasma albumin levels were unchanged and insulin requirements fell despite an increase in the carbohydrate intake. The rate of decline of creatinine clearance was slowed on LPD in a heterogeneous manner.

A 6 month controlled study involving 7 patients in a LPD and 9 in a control group revealed no changes in creatinine clearance but a reduction in urinary albumin excretion on LPD (Ciavarella et al. 1987). The majority of patients in both groups had serum creatinine levels that were within the normal range indicating well preserved renal function and thus it was not surprising that no change in creatinine clearances were seen in this short-term study.

A larger controlled trial that employed an isotopic clearance method for measurement of GFR demonstrated a significant reduction in the rate of decline of GFR after 37 months on a low protein diet (0.72 g/Kg/day) compared to a control group on a normal protein diet (1.08 g/Kg/day) for

this period (Zeller et al. 1991). Blood pressure was lower in the group on the low protein diet but when included as an independent variable in a stepwise regression analysis with change in glomerular filtration rate as the dependent variable, it was found to exert no significant effect. Interestingly, the rate of decline of GFR was only significantly different between the patients on the two diets when those with initial glomerular filtration rates above 45 ml/min. were considered. This may be taken to suggest that dietary intervention should be introduced early in the course of diabetic nephropathy before significant reductions in glomerular filtration rate occur however this point was not raised in the discussion of the results. There was no indication that the LPD had any nutritional adverse effect. Serum cholesterol and triglyceride levels were increased during the LPD period but the changes failed to reach statistical significance.

Changes in glycaemic control were not associated with any renal functional effects in any of the above studies.

TABLE 1.3. CLINICAL STUDIES OF LOW PROTEIN DIET IN PATIENTS WITH DIABETIC NEPHROPATHY.

AUTHOR/ YEAR	STUDY DESIGN	DIETARY PRESCRIPTION	DIETARY ASSESSMENT	GFR ASSESS-MENT	DURA-TION OF LPD	OUTCOME
Zeller, 1991	Random-ised Con-trolled, 68 pts.	0.72g/Kg/d in pts. 1.08g/Kg/d in controls	Weighed food records Urinary Urea Nitrogen	Iothalamate clearances	37 months	Iothalamate clearances 0.26ml/ min/mo in pts. 1.01ml/min/mo. in controls MBP 102 in pts., 105 in controls
Evanoff, 1989	Self controls 11 pts.	1.2g/Kg/d on NPD→0.95g/ Kg/d on LPD	Dietary recall Urinary Urea Nitrogen	Creatinine clearance 1/serum creatinine	24 months	Δ 1/serum creatinine -0.18/yr on NPD -0.03/yr on LPD Systolic BP 147 LPD →126 NPD
Barsotti, 1988	Self controls 8 pts.	1.3g/Kg/d on NPD 0.3g/Kg/d on LPD (supplemented with essential amino and keto acids)	Urinary Urea Nitrogen	Creatinine clearan-ce	17 months	Decrease in TUP Increase in TPP Rate of decline in CrCl on NPD 1.38ml/min/mo on LPD 0.03 ml/min/mo
Ciavarel-la, 1987	Randomis-ed con-trolled 16 pts.	0.71g/Kg/d in pts. 1.44g/Kg/d in controls	Dietary Interview Blood Urea Nitro-gen Urinary Urea Nitrogen	Creatinine clearance	4.5 months	Decrease in AER in LPD group

AER = Urinary albumin excretion rate
LPD = Low protein diet
NPD = Normal protein diet
Δ = Delta (change in)
MBP = Mean blood pressure
TUP = Urinary total protein excretion rate
CrCl = Creatinine clearance
TPP = Total plasma protein level
All blood pressure values are in mmHg

REASONS FOR THE STUDY

The majority of previous studies designed to investigate the effect of LPD on the course of renal failure, in both diabetic nephropathy and renal failure from other causes, have used some measure of serum creatinine to assess GFR. This creates a particular problem due to the effects dietary modifications have on creatine and creatinine intakes and pool sizes (Crim et al. 1975, 1976, Perrone et al. 1992).

At the time of the conception of the study reported in the following chapters there were no published studies in patients with diabetic nephropathy that allowed (1) the effect of LPD on the rate of decline of GFR to be assessed using an isotopic clearance method thereby removing the confounding effects of changes in creatinine, (2) the effect of LPD to be separated from that of glycaemia and blood pressure changes, (3) the degree of homogeneity or heterogeneity of the response to LPD to be determined, (4) the medium-term effects of LPD to be assessed in terms of nutritional and anthropometric parameters, efficacy, tolerability and palatability and (5) the effect on serum lipids to be quantitated. The study described in the subsequent chapter was therefore designed and performed.

CHAPTER 2

A PROSPECTIVE STUDY OF A DIET REDUCED IN PROTEIN ON THE PROGRESSION OF DIABETIC NEPHROPATHY

AIMS OF THE STUDY

1. To examine, in a prospective study, the effects of a low-protein diet on the rate of progression of renal disease in a cohort of insulin-dependent diabetic patients with persistent proteinuria and glomerular filtration rates varying from normal to moderately impaired. A lower limit for GFR of 20 ml/min/1.73 m^2 was set as it has been previously shown that dietary protein restriction has no effect on the progression of renal failure in diabetic patients with mean serum creatinine values of 770 µmol/l (Attman et al. 1983) indicating severe renal impairment.

2. To examine the relationship between the clearance of ^{51}CrEDTA and creatinine in the assessment of GFR on normal and low protein diets.

STUDY DESIGN

Patients acted as their own controls with at least one year of follow-up, including 3 assessments of renal function, on a normal protein diet followed by a period of at least one year with 2 assessments of renal function on a low-protein diet (Figure 2.1).

This study design was selected for the following reasons:

1. It is known that in patients with diabetic nephropathy the rate of decline of GFR is linear with time in a given individual although inter-individual rates of decline may vary (Mogensen 1976, Jones et al. 1979, Parving et al. 1981, 1987, Viberti et al. 1983, Nyberg et al. 1987, Jones et al. 1989). Statistical methods are available to examine changes in the rate of decline associated with an intervention at a given time point using linear regression to compare NPD with LPD and a 'breakpoint' analysis to examine whether a single line fits all the data points for the whole study

better than a broken line (Draper and Smith 1981, Jones and Molitoris 1984, Zoccali et al. 1989). If a 'break' is detected the timing of this can be compared to the timing of the intervention.

2. The assessment of glomerular filtration rate using the clearance of ^{51}CrEDTA provides a reliable measure of renal function and is not influenced by changes in diet or renal failure (Chantler et al. 1969, Bröchner-Mortensen et al. 1969, Bröchner-Mortensen 1972, Bröchner-Mortensen and Rodbro 1976, 1976a, 1976b). Previous studies have used measurements of serum creatinine or creatinine clearance to estimate GFR which are influenced by changes in diet, muscle mass and the tubular secretion of creatinine (Perrone et al. 1992).

3. The length of the trial, a mean of 62 months, enabled 5 assessments of glomerular filtration rate on average on each diet. Previous studies of low protein diets have not exceeded 2 years in duration. A longer follow-up enables ascertainment of a more accurate rate of GFR decline, allows for delayed therapeutic and nutritional effects to be seen and provides data on the long-term acceptability of the diet.

4. A matched control group followed for an equal period of time on a normal protein diet would have been preferred but sufficient patient numbers were not available. It would be necessary to match patients on the diet and controls for a number of clinical characteristics including baseline rates of decline of GFR and large numbers of patients would be necessary for this to be done in a random fashion. A parallel group could not have been masked due to the nature of the intervention. However in order to compare the effects associated with the dietary intervention to a similar group of untreated patients, a cohort previously followed in the Unit was selected retrospectively in a non-random manner. This group will be termed the historical control group.

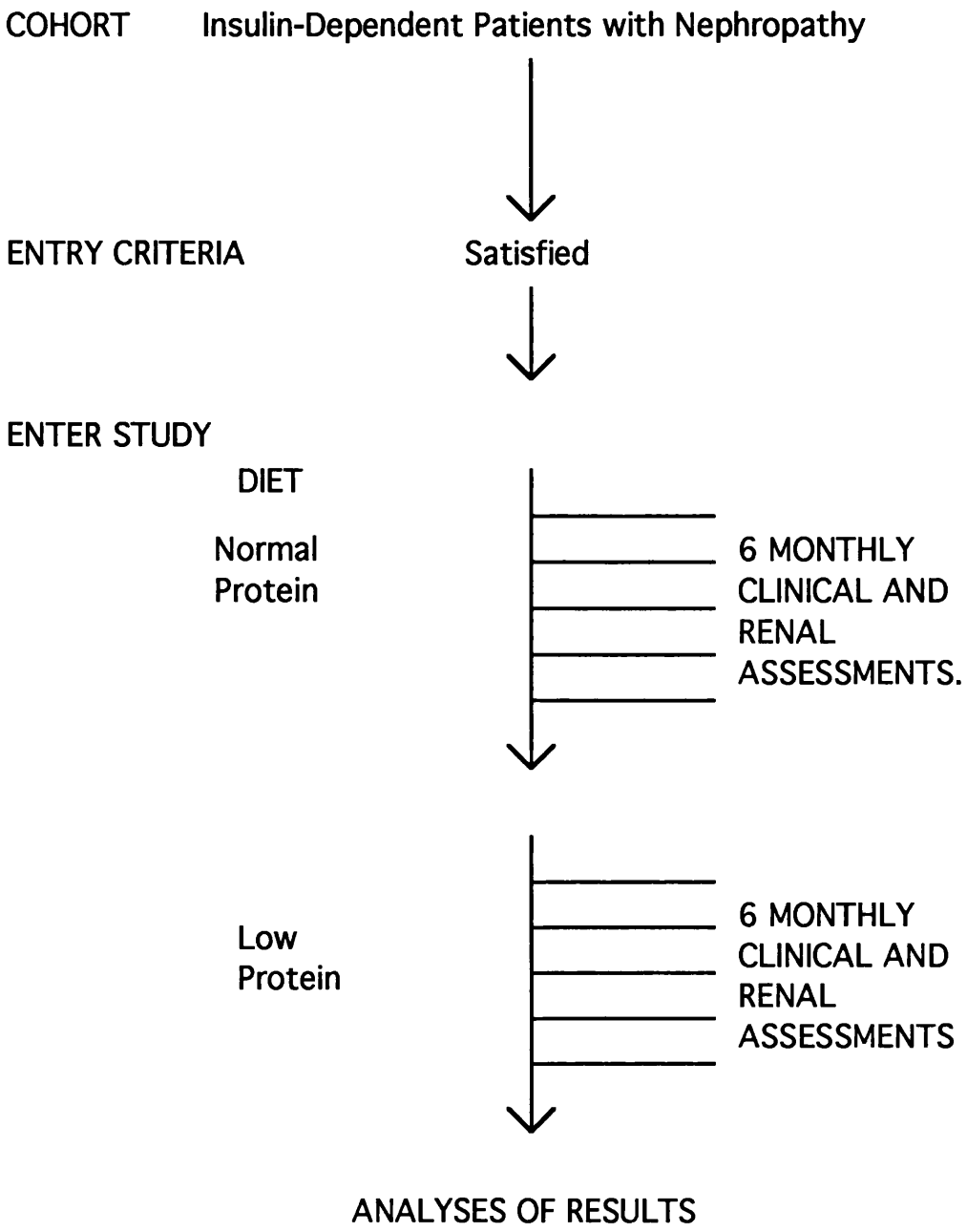


Figure 2.1 A Flow Sheet depicting the study protocol.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of Guy's Hospital.

All patients who were eligible to participate in the study were approached and the nature of the study explained to them by Professor GianCarlo Viberti, Dr. Jeremy Bending or myself and Mrs. Rosemary Dodds, a Nutritionist. All patients were aware that they had persistent proteinuria indicating diabetic nephropathy. They were told that the study was long-term and that six monthly assessments were involved: that the intervention would necessitate a large change in their dietary habits which may well have effects on other members of the family. Male patients were always interviewed with their partner if she performed most of the cooking. Patients were informed that they would be free to abandon the study at any time and that by participating in the study, not participating in the study, or withdrawing from the study their usual diabetic care at Guy's Hospital would in no away be affected. Verbal consent was obtained in those who were recruited into the study.

ENTRY CRITERIA

The entry criteria for the study were designed to enable as many patients as possible to enter. Patients had to be insulin-dependent as defined as ketosis-prone with an age at diagnosis usually less than 35 years. They had to have persistent proteinuria as defined by a total urinary protein excretion rate above 0.5 g/24 hours on two occasions separated by a six month period. The presence of persistent proteinuria was taken to indicate diabetic nephropathy only with co-existing diabetic retinopathy (background or proliferative), and in the absence of urinary sepsis or congestive cardiac failure. Glomerular filtration rate at entry to the study had to be above 20 ml/min/1.73m^2.

PATIENTS AVAILABLE FOR THE STUDY

All proteinuric insulin-dependent diabetic patients attending the Unit for Metabolic Medicine at Guy's Hospital are seen and monitored at least once every 6 months. Forty four patients with persistent proteinuria were available for the study yet 12 were ineligible for the reasons given in Figure 2.2.

PATIENTS ENTERED INTO THE STUDY

Of the 32 patients who were eligible to enter the study 13 did not complete the study protocol as shown in Figure 2.2.

PATIENTS AVAILABLE FOR ANALYSIS

All were of European origin and had a mean (range) age of 42 years (26-62) and a duration of diabetes of 24 (14-48) years (Table 2.1). Persistent proteinuria, as defined by consistently positive testing using Albustix^R and a total urinary protein excretion rate of greater than 500 mg/24 hours, had been present for a mean of 4 (1-10) years. The urinary albumin excretion rate had been above 300mg/day on 2 separate occasions in all patients. At enrollment it was below this level in 4 cases due to either antihypertensive therapy (subject numbers 3 and 8) or the variability seen in this parameter (subject number 5 and 7) (Tables 2.1 and 2.2). In no patients were there any clinical reasons to suspect other causes of persistent proteinuria in that no patients had clinical or laboratory evidence of other autoimmune diseases, and renal imaging in all patients revealed none with small kidneys. Renal biopsy was performed in 6 patients and histology revealed diabetic glomerulosclerosis only. One female patient (patient 2) was taking long-term low-dose amoxycillin (250 mg o.d.) for prophylaxis against urinary sepsis at enrollment into the study and remained on this medication throughout the normal- and low-protein phases of the investigation. Two patients (14 and 16) were on a phosphate binder (calcium carbonate) at enrollment.

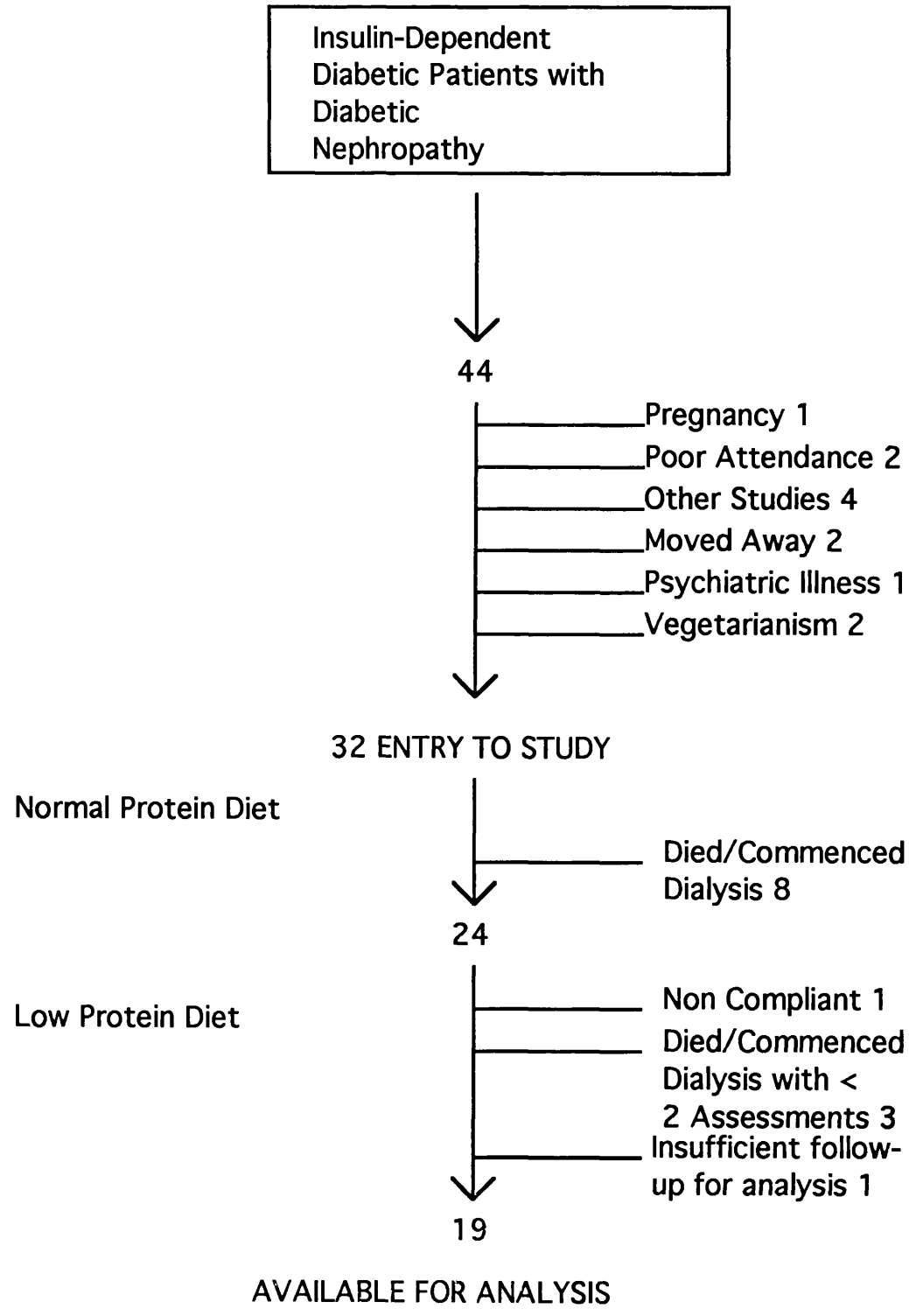


Figure 2.2 A Flow Sheet depicting the patients considered for the study.

TABLE 2.1. PATIENT DETAILS. ALL DATA ARE FOR RECRUITMENT INTO STUDY - (NORMAL PROTEIN DIET PERIOD).

Patient Initials	Sex	Subject Number	Age (yr)	Duration of Diabetes (yr)	Duration of Proteinuria (yr)	GFR ml/min/ 1.73m^2	AER mg/24 hr	HBA1 %	Blood Pressure (mmHg)	Blood Pressure Treatment
MB	F	1	58	48	2	43.0	996	8.5	170/86	No
LC	F	2	37	25	8	22.5	1071	9.3	129/85	No
AC	M	3	31	18	1	77.7	250	11.6	150/100*	Yes
ALC	M	4	51	22	6	61.9	589	11.7	156/80	No
PD	M	5	41	22	3	79.1	174	6.7	140/80	No
JD	M	6	29	14	1	36.9	4406	9.3	120/90	Yes
JH	M	7	45	39	3	124.8	187	11.1	122/77	No
KH	M	8	38	19	2	93.0	105	7.0	130/80	Yes
RH	M	9	30	14	2	95.1	338	11.5	136/80	No
WI	M	10	62	18	5	33.4	1160	7.5	152/78	No
ME	M	11	26	23	4	35.6	2008	7.1	190/80	Yes
MN	M	12	31	18	5	69.6	4177	8.7	111/72	No
AM	F	13	43	40	10	33.7	3312	8.4	178/100*	Yes
WP	M	14	58	16	3	48.5	458	7.8	130/80	No
PS	F	15	35	22	1	90.1	1303	9.8	162/82	No
RT	M	16	40	27	1	27.0	5064	12.5	154/96*	Yes
RW	M	17	34	19	1	65.4	537	7.3	162/110*	Yes
NW	M	18	51	31	5	69.0	1197	9.8	140/107*	Yes
BW	M	19	54	27	5	65.1	1095	10.5	140/80	Yes
		Mean	42	24	3.6	62	1496	9.3	146/86	Yes=9
		SD	11	9	2.5	27	1561	1.8	20/11	No=10
		Min	26	14	1	22.5	105	6.7	111/72	Total=19
		Max	62	48	10	124.8	5064	12.5	190/110	

TABLE 2.1 (Continued). PATIENT DETAILS. ALL DATA ARE FOR RECRUITMENT INTO STUDY - (NORMAL PROTEIN DIET PERIOD).

Patient Initials	Sex	Subject Number	Weight (kg)	BSA (m^2)	Plasma Creatinine (µmol/l)	Plasma Urea (mmol/L)	Insulin daily dose (u)
MB	F	1	95.4	2.06	120	9.2	60
LC	F	2	49.7	1.53	200	11.0	15
AC	M	3	92.0	2.11	124	11.0	66
ALC	M	4	69.5	1.93	114	6.3	44
PD	M	5	69.2	1.98	110	9.7	38
JD	M	6	69.6	1.81	170	14.0	40
JH	M	7	67.3	1.84	102	9.0	36
KH	M	8	70.8	1.97	89	5.5	58
RH	M	9	80.8	2.06	110	4.5	48
WI	M	10	82.0	2.02	178	12.0	40
ME	M	11	66.8	1.85	184	5.4	35
MN	M	12	63.1	1.75	118	6.8	44
AM	F	13	57.7	1.63	160	11.0	24
WP	M	14	82.0	2.09	155	11.0	74
PS	F	15	64.5	1.71	81	7.1	38
RT	M	16	56.6	1.74	165	20.0	36
RW	M	17	81.2	1.98	123	9.8	78
NW	M	18	80.5	2.07	122	4.7	27
BW	M	19	83.7	2.02	112	8.5	60
		Mean	72.7	1.85	133	9.3	45
		SD	11.8	0.15	33	3.6	16
		Min	49.7	1.5	81	4.5	15
		Max	95.4	2.11	200	20.0	78

BLOOD PRESSURE TREATMENT

Nine patients were receiving antihypertensive medication at commencement of the study (Table 2.2). During NPD antihypertensive therapy was commenced in 5 further patients so at the start of LPD 14 patients were receiving antihypertensive therapy (Table 2.2). During LPD antihypertensive medication was commenced in 4 patients and in 6 antihypertensive medication was modified; a drug was added in 5 cases and withdrawn in 1 case (metoprolol was withdrawn as the patient developed left ventricular failure secondary to an ischaemic cardiomyopathy). By the end of the study, 18 of the 19 patients were receiving antihypertensive medication. In 14 of the 18 (78%) antihypertensive therapy was on-going at the time of commencement of LPD. Ten (55%) patients had modification of their antihypertensive regimens during LPD.

SPECIFIC ANTIHYPERTENSIVE DRUGS USED DURING THE STUDY.

Captopril was the only drug which was used during LPD but not during NPD. Nifedipine was used in 3 patients during the low protein diet and only 1 during the normal protein diet. These differences reflect changes in prescribing habits that occurred during the course of the study. The protocol did not specify drugs to be used or avoided. Frusemide was used in more patients during the low protein diet (9 vs 5) due, in part, to its diuretic as well as hypotensive action as a number of patients had symptoms of volume overload in addition to hypertension.

TABLE 2.2. ANTI-HYPERTENSIVE AND DIURETIC THERAPY OF PATIENTS DURING THE STUDY. NUMBERS IN PARENTHESIS REPRESENT DATE OF COMMENCEMENT OR DISCONTINUATION OF THERAPY IN MONTHS ON RESPECTIVE DIET.

Patient Initials	Sex	Subject Number	Normal Protein Diet Period	Low Protein Diet Period
MB	F	1	--	Metoprolol(21) Nifedipine(27)
LC	F	2	--	Frusemide(12) Captopril(18)
AC	M	3	*Prazosin Bendroflurazide	Frusemide Captopril(3) Nifedipine(7)
ALC	M	4	--	Captopril Frusemide(24)
PD	M	5	--	Metoprolol(24)
JD	M	6	*Metoprolol Nifedipine	Frusemide(15) Captopril(20)
JH	M	7	--	--
KH	M	8	*Bendroflurazide Metoprolol	
RH	M	9	Metoprolol(22)	
WI	M	10	Hydrallazine(14) Frusemide(20)	
ME	M	11	*Metoprolol	
MN	M	12	Metoprolol(35)	
AM	F	13	*Frusemide Metoprolol	Metoprolol d/ced(12)†
WP	M	14	NavidrexK®(12) Metoprolol(15)	Hydrallazine(3)
PS	F	15	Frusemide(13)	
RT	M	16	*Frusemide	
RW	M	17	*Metoprolol Bendroflurazide	Prazosin(11)
NW	M	18	*Metoprolol Hydrallazine	Nifedipine(5)
BW	M	19	*Metoprolol Frusemide	

* On this therapy at recruitment.
† Discontinuation (d/ced) due to left ventricular failure secondary to ischaemic cardiomyopathy.

GLYCAEMIC REGIMEN

At enrollment 15 patients used a regimen of twice daily injections of a mixture of soluble and an intermediate-acting insulin and 4 used soluble insulin delivered by a continuous subcutaneous insulin infusion device (Nordisk infusors in 3 and a Grasby pump in 1). Only 1 patient substantially changed insulin regimen during the study by changing from twice daily injections of a soluble and an intermediate-acting insulin to 3 injections of a soluble insulin pre-prandially with a lente insulin at night. This occurred during the low protein diet period.

OTHER DIABETIC COMPLICATIONS

These are summarised in Table 2.3.

TABLE 2.3. EXTRA-RENAL COMPLICATIONS OF PATIENTS AT RECRUITMENT.

Patient Initials	Sex	Subject Number	Retino-pathy	Peripheral Neuropathy	Autonomic Neuropathy	PVD/Foot Problems	Known IHD †
MB	F	1	PR	Yes	Yes	Foot ulcers	No
LC	F	2	BR	Yes	Yes	No	No
AC	M	3	BR	Yes	Yes	(R) BKA	No
ALC	M	4	PR	Yes	Yes	Foot ulcers	Yes
PD	M	5	BR	No	No	No	No
JD	M	6	BR	No	No	No	No
JH	M	7	PR*	Yes	Yes	No	No
KH	M	8	PR	Yes	Yes	Charcot feet	No
RH	M	9	BR	No	No	No	No
WI	M	10	PR	Yes	Yes	No	No
ME	M	11	PR	No	No	No	No
MN	M	12	PR Blind	Yes	Yes	No	No
AM	F	13	BR	Yes	No	No	Yes
WP	M	14	PR	Yes	Yes	No	No
PS	F	15	PR Blind	Yes	Yes	No	No
RJ	M	16	PR*	Yes	No	No	No
RW	M	17	PR	No	No	No	No
NW	M	18	BR	No	No	No	No
BW	M	19	PR	Yes	No	No	No

* Registered partially-sighted
† As defined from symptoms and resting electrocardiogram
BR Background Retinopathy
PR Proliferative Retinopathy
BKA Below Knee Amputation

CLINICAL ASSESSMENTS

Once recruited into the study the patients were seen on a metabolic ward every six months for formal assessment. At each assessment the patient would arrive fasting on the Metabolic Ward at approximately 0800 with 24 hour urine collection from the previous 24 hours. The patient was weighed and 30 ml. of venous blood drawn from a cannula in an antecubital vein. 3 MBq of ^{51}CrEDTA (81 μCi) in a volume of 10ml was then injected into an antecubital vein in the opposite arm to that in which the cannula was sited and the exact time of injection noted. Supine blood pressure was taken in the right arm using a standard mercury sphygmomanometer after the patient had rested supine for 10 minutes. Two readings were taken and the mean recorded. Systolic levels consistently above 160 or diastolic levels above 95 mmHg were treated. After these reading the patient then injected the morning doses of insulin and had breakfast.

Height was measured at the beginning of the study using a stadiometer for calculation of body mass index and body surface area. The body surface area at commencement of the study was used for the correction of the glomerular filtration rate for body surface area throughout the study.

After breakfast the patient any problems with glycaemic control, complications, the diet, or any other medical complaint were discussed and a physical examination performed. The current medication including insulin dosage was recorded.

At 2, 3, 4, 6, and 8 hours after the injection of ^{51}CrEDTA 10 ml of venous plasma was drawn via cannula for measurement of ^{51}CrEDTA level for assessment of glomerular filtration rate.

Mid-arm muscle circumference was used as an anthropometric measure. For this measurement the non-dominant arm was used. The

arm was held out at 90 degrees to the body and the mid-point between the tip of the olecranon and the acromium process of the scapula ascertained using a flexible tape-measure and marked. The mid-arm circumference was measured at this point to the nearest 1mm without allowing the tape to compress the skin and with the arm now hanging loosely at the patients' side. Triceps skinfold thickness was measured at the same point three times using Harpenden calipers marked in divisions of 1mm. A fold of skin and subcutaneous tissue was held between thumb and forefinger and drawn away from the underlying muscle. The width of the skinfold was then measured by applying the caliper to an area under the pinch (Tanner and Whitehouse 1975). The mean of 3 measurements was used for calculations.

Mid-arm muscle circumference (MAMC) was calculated as

MAMC = MUAC - (0.3412 x TST)

where MUAC is mid-upper arm circumference and TST is triceps skinfold thickness in mm (Thomas 1988).

During the day spent on the metabolic ward the patient was seen by a nutritionist (Mrs. Rosemary Dodds) to discuss the dietary weighed food records and any problems with the diet. Supplies and recipes were given as necessary.

BLOOD TESTS

Venous blood was taken for the measurement of full blood count, plasma sodium, potassium, calcium, creatinine, urea, albumin and glycosylated haemoglobin. Serum lipoproteins including total cholesterol, LDL-cholesterol, VLDL-cholesterol, HDL-cholesterol, total triglyceride, LDL-triglyceride, VLDL-triglyceride, HDL- triglyceride were measured in 15 of the patients during the last six months of the normal protein diet and within one year of commencing the low protein diet. Venous blood was taken into a plain glass tube from an uncuffed antecubital vein after a 12-

hour fast. The blood was allowed to clot and the serum then collected after centrifugation.

MEASUREMENT OF GLOMERULAR FILTRATION RATE

An isotopic clearance method was employed using the clearance of ^{51}CrEDTA after an intravenous injection of 3MBq (Chantler et al. 1969). Venous plasma was sampled via an in-dwelling cannula at 2, 3, 4, 6 and 8 hours post injection. Using an in-house computer programme devised by the Department of Nuclear Medicine at Guy's Hospital and employing a single compartment model the glomerular filtration rate was derived from the decay of radioactivity.

URINE TESTS

At each visit the patients brought up to the ward a timed 24-hour urine collection for the measurement of urinary albumin, total protein, sodium, urea and creatinine. A mid-stream specimen (MSU) was collected at each ward visit for microscopy, culture, and if any organisms were identified, sensitivity. If the MSU suggested a urinary tract infection appropriate treatment was initiated and the collection repeated. Each urine collection for each patient was recorded on an index card which contained information regarding the patient's name and hospital number, the date of the urine collection, the volume of the urine collection, and the exact time over which the collection was made.

A 5 ml aliquot of each urine specimen was stored in duplicate at -20^{o} centigrade until assay.

ANALYTICAL METHODS

Full blood count was measured in the routine hospital laboratories using a Coulter counter analyser as were plasma electrolytes, albumin and urinary urea and creatinine using a multichannel analyser (Vickers Medical, UK). Plasma creatinine was assayed using a reaction rate method

(LKB Pharmacia, Milton Keynes, UK) and glycosylated haemoglobin by the Corning method (Ciba-Corning, Halstead, UK). Urinary albumin was assayed in duplicate in batches of approximaely 50 samples in the Unit for Metabolic Medicine using a radioimmunoassay (Keen and Chlouverakis 1963). Prior to assay each sample was tested with a dipstix (Multistix, Boehringer-Mannheim) to semi-quantitatively assess the albumin concentration and if high appropriate dilutions were made to ensure the albumin concentration fell within the range of the assay. Samples that fell above the standard curve were diluted and re-assayed. The intra-assay coefficient of variation (CV) was 5% and the inter-assay CV 9.5%. Results were rejected if duplicate samples were greater than 10% different from each other and the sample was re-assayed.

Major serum lipoprotein fractions were separated using a MSE 50 preparative ultracentrifuge at 12^{o} centigrade (M.S.E. West Sussex, UK) following the method of Carlson (Carlson 1973, Mattock et al. 1982). Serum and lipoprotein fraction triglyceride and cholesterol were assayed by enzymatic methods (Boehringer-Mannheim, FRG) using a Cobas Bio analyser (Roche Diagnostica, Welwyn Garden City, UK). Results were acceptable if subfraction recovery rates fell between 89 and 115% of the total triglyceride or total cholesterol.

DIETARY ASSESSMENTS

All dietary assessments were performed by a nutritionist, Mrs. Rosemary Dodds. All patients were provided with self-zeroing dietary scales (Miniscale PC International, Cambridge; 2000x1g) at enrollment into the study.

Prior to the start of the low protein diet a comprehensive diet history was taken and a weighed food record was obtained. These weighed food records were performed on one typical weekday and one day during a

weekend. Once the patient had started the low protein diet a dietary history was taken every three months and the patients were instructed to perform a 24-hour weighed food record on one day per month and sent this to the nutritionist. Patients were instructed to weigh all foods and liquids consumed at home and record this information in a log book. Brand names of foods and methods of preparation and cooking were recorded. For foods and fluids eaten away from the home, patients were instructed to provide a full description of the food, the location, and the price. On NPD the mean (range) number of weighed food records provided was 4 (3-8) and on LPD 21 (6-42). Records were checked with each patient and then coded and analysed using the DIET programme on the University of London computer. From this data, the nutritionist was able to estimate the composition of the food and fluid consumed (Paul and Southgate 1978).

Compliance with the diet was assessed by the measurement of urinary urea excretion calculated from the timed 24-hour urine collections (Steffe et al. 1976, Issaksson 1980). From the urinary urea excretion urinary urea nitrogen was calculated as follows. The fraction of urea that is nitrogen is 28/60. As urea is given in mmol/l, this fraction is multiplied by the molecular mass of urea (60) to convert it to mg. This figure is the urinary urea nitrogen (UUN) which is divided by 1000 to convert to grams. This figure was added to a standard figure for non-urea nitrogen loss given as 31 mg N/Kg/day. This figure was multiplied by 6.25 (the reciprocal of the fraction of a protein that is nitrogen - 16%) to produce the protein intake, in grams, per day.

Thus, urinary urea (mmol/l) x 28 = urinary urea nitrogen in mg/1000 = urinary urea nitrogen in grams. Urinary nitrogen in grams + (non urea nitrogen = 0.031 x weight in kg) = Nitrogen intake. Nitrogen intake x 6.25 = protein intake in grams/day. This is simplified to:

$$UUN + NUN = I_N$$

$$I_N \times 6.25 = \text{protein intake g/day}$$

where NUN indicates non-urea nitrogen and was taken to be 31 mg/N/Kg per day on the assumption of nitrogen balance and I_N indicates nitrogen intake.

A mean of 4 (range 2-8) collections were available for each patient on the normal protein diet and 7 (1-14) on the low protein diet (Table 3.3, page 91).

ACCEPTABILITY

A questionnaire assessing dietary acceptability was completed by the patients after at least 6 months on LPD (Appendix). Questions were designed to cover satiety, taste, cost, complexity of the diet, effect on social life, ability to eat out, shopping and preparation of food and overall satisfaction with the diet. Patients were instructed to score each question on a 5 point Lickert scale with 5 indicating the highest acceptability. The mean and mode values were calculated for the group as a whole.

DIETARY PRESCRIPTION

The prescription of a low protein diet in Western countries has been hindered by the traditional central role of animal protein, usually meat, as the main component of a substantive meal with vegetables having a low priority. When patients and their partners were counselled concerning the low protein diet they were advised to consider animal protein as a garnish rather than a main feature. The central component of the meal was to be the low protein products such as pasta, rice, and bread with vegetables playing a more important role than is traditionally considered.

The prescribed diet contained 40 g of protein daily with an additional 1.6 g of dietary protein for every gram of urinary protein

excreted if the patient had a total urinary protein level above 3 g per day indicating nephrotic proteinuria in order to prevent negative nitrogen balance. Approximately half of the protein came from animal and half from vegetable sources. This resulted in a lower energy intake for the diet compared to traditional low protein diets (Renal Dialysis Group 1984) and so this was balanced by the allowance of a variety of high carbohydrate foods such as bread and potatoes. The use of sugar, double cream and glucose polymers, prescribed in low protein diets in non-diabetic patients, were not allowed as it was felt that these foods may worsen glyaemic control and be difficult for patients to become accustomed to. Rice and potatoes were encouraged since they have a low protein to carbohydrate ratio.

ANIMAL PROTEIN CONTENT OF THE DIET

This was calculated using the concept of three 7 g 'exchanges', for example:

- 200 ml of full milk
- 1 egg (55 g)
- 1 oz (28 g) average lean meat

Food containing a high animal protein content were listed in List A (Table 2.4).

CARBOHYDRATE CONTENT OF THE DIET

The traditional system involving the 10 g carbohydrate exchanges with emphasis on high fibre and low glycaemic index foods was maintained in the study. All the patients were familiar with this system. Carbohydrate exchanges were subdivided into two lists; those containing an appreciable quantity of protein, List B (Table 2.5), and those containing little protein, List C (Table 2.6). List B foods averaged less than 2 grams of protein per 10 grams of carbohydrate, and the patients were recommended to take nine exchanges from this list daily. The remaining carbohydrate

intake, depending upon calorific requirement, was derived from the low protein List C foods. List C low protein carbohydrate exchanges consisted almost entirely of fruits and specially manufactured low-protein, high-carbohydrate bread, bread mix, biscuits, pasta and crackers supplied free of charge and subsequently on prescription (FP 10) from Nutricia, Procea and Ultrapharm in the United Kingdom.

FAT CONTENT OF THE DIET

The dietary prescription was to restrict fat to less than 35% of energy intake with an increase in the ratio of polyunsaturated to saturated fatty acids in line with the dietary recommendations of the British Diabetic Association (British Diabetic Association Nutrition Sub-Committee Medical Advisory Committee 1982, 1992) and initial targets suggested by the Committee on Medical Aspects of Food Policy (COMA) (Committee on Medical Aspects of Food Policy 1984, 1989). The reduction in animal protein foods (List A) such as meat, cheese, eggs, and milk was envisaged to cause a concomitant reduction in saturated fat intake.

OTHER NUTRIENTS

Dietary sodium was not specifically restricted but patients were instructed not to add salt to their food. This is standard advice as the majority of patients with diabetic nephropathy have elevated levels of blood pressure and a no-added salt diet is a part of their management. Dietary phosphorus was not specifically restricted. The importance of dietary fibre (British Diabetic Association Nutrition Sub-Committee Medical Advisory Committee 1982) was emphasized when the patients were instructed on the prescribed low protein diet and patients were encouraged to select high fibre food options whenever possible and to add bran to home-made low protein foods.

A demonstration of some low protein recipes was provided by the nutritionist at various times during the study, at which times patients and

their partners were able to taste the recipes and ask questions about their preparation. These meetings also provided a forum for patients to exchange ideas and experiences. In addition patients were provided with a number of sample recipes (Table 2.7, page 71) and the nutritionist spent time with each patient and their partner, if appropriate, demonstrating some of the suggested recipes.

TABLE 2.4. LIST A (PROTEIN EXCHANGES).

The following foods are rich in protein. Any one item can be "swopped" for another item in the amounts given according to your daily allowance.

The amounts quoted are portions of cooked foods (i.e., as eaten) and exclude bones, gristle and fat, so it is essential that you pick lean cuts of meat and remove any bones and visible fat.

Each food contains 7 g of protein.

1	egg
$1\frac{1}{2}$	egg yolks
3	egg whites
1 oz (28 g)	chicken, beef, veal, lamb/mutton, pork, liver, kidney, turkey, etc.
1 oz (28 g)	lean bacon, ham corned beef
$1\frac{1}{2}$ oz (42 g)	kipper (weighed with bones)
$1\frac{1}{2}$ oz (42 g)	white fish (e.g., cod, haddock)
1 oz (28 g)	oily fish (e.g., herring, mackerel, prawns)
1 oz (28 g)	hard cheese (e.g., cheddar, cheshire, stilton, wensleydale, edam, camembert)
2 oz (55 g)	cottage cheese, curd cheese, cheese spread

The following foods also contain carbohydrate:

1/3 pint (200 ml)	milk (whole or skimmed)
3/4 oz (21 g)	dried skimmed milk powder
2	small sausages or
1	large sausage or
4	chipolatas
2	fishfingers
1 (150 g)	carton natural yogurt
1 (150 g)	small carton flavoured/fruit yogurt

TABLE 2.5. LIST B (CARBOHYDRATE EXCHANGES).

The following items contain approximately 10 g carbohydrates (1 carbohydrate exchange) in the amounts quoted. The foods in this list contain small amounts of protein which can add up to an appreciable quantity over the course of the day. It is therefore necessary to restrict the total number of exchanges from this list to a total of ____ exchanges each day. The rest of your total daily carbohydrate allowance should therefore be made up from List C which are protein free carbohydrate exchanges.

The foods which are underlined are **high fibre foods**, within your total allowance you should try to have as many of these as possible.

The foods on this list contain 2 g of protein or less.

	wgt oz (grams)	Handy Measure
Cereals:		
Flour wholemeal	1/2 oz (15 g)	1 heaped tablespoon
Macaroni (raw, brown)	1/2 oz (15 g)	4 level tablespoons
Macaroni, boiled, brown	$1\frac{1}{2}$ oz (42 g)	3 tablespoons
Oatmeal (raw)	1/2 oz (15 g)	1 heaped tablespoon
Porridge (cooked with water)	4 oz (225 g)	8 level tablespoons
Rice, raw, brown	1/2 oz (15 g)	1 level tablespoon
Rice, cooked, brown	1 oz (30 g)	1 heaped tablespoon
Semolina (raw)	1/2 oz (15 g)	3 level tablespoons
Spaghetti, raw, brown	1/2 oz (15 g)	10 long strands (10")
Spaghetti, boiled, brown	$1\frac{1}{2}$ oz (42 g)	7 heaped tablespoons
Breads:		
Bread, wholemeal	3/4 oz (25 g)	2/3 slices - long medium cut loaf 2/3 slices - large thin cut loaf 1/2 slice from tall medium cut loaf 1 slice from small thin cut loaf
Wholemeal pitta bread		1/4 an average
Rolls, white, crispy	3/4 oz (20 g)	1/2 roll
Rolls, soft, white	3/4 oz (20 g)	1/3 roll
Breakfast Cereals:		
All Bran	3/4 oz (20 g)	10 level tablespoons
Cornflakes	1/2 oz (15 g)	4 level tablespoons

Muesli (unsweetened)	1/2 oz (15 g)	4 level tablespoons
Puffed Wheat	1/2 oz (15 g)	9 level tablespoons
Readybrek (raw)	1/2 oz (15 g)	1 heaped tablespoon
Rice Krispies	1/3 oz (10 g)	6 level tablespoons
Shredded Wheat	1/2 oz (15 g)	2/3 biscuit
Weetabix	1/2 oz (15 g)	1 'biscuit'
Bran Flakes	1/2 oz (15 g)	1/2 cup
Biscuits:		
Crispbreads (Rye)	1/2 oz (15 g)	2 biscuits
Cream Crackers	1/2 oz (15 g)	2 biscuits
Digestive biscuit	1/2 oz (15 g)	1 biscuit (large)
Oatcakes	1/2 oz (15 g)	1 biscuit
Plain biscuits e.g., Rich T, Marie, Nice Osbourne	1/2 oz (15 g)	2 biscuits
Puddings:		
Ice cream	1.75 oz (50 g)	$1^1/_2$ brickettes/1 scoop
Custard	2 oz (60 g)	1/2 small ladle
Trifle	2 oz (60 g)	1/2 small portion
Vegetables:		
Potatoes, boiled/ mashed	2 oz (50 g)	1 egg-sized
Jacket Potato	2 oz (50 g)	1 small-sized
Roast Potato	1.5 oz (42 g)	1 egg sized
Chips	1 oz (30 g)	4 large chips
Crisps	1 oz (25 g)	1 small packet
Soup - tomato & other creamed soups	5 oz (140 ml)	8 tablespoons (1/4 pint)
Soup - vegetable (thick)	4 oz (115 ml)	6 tablespoons (1/5 pint)

Beverages:

Horlicks	1/2 oz (15 g)	2 heaped teaspoons
Ovaltine	1/2 oz (15 g)	2 heaped teaspoons
Bournvita	1/3 oz (10 g)	2 heaped teaspoons

TABLE 2.6. LIST C (CARBOHYDRATE EXCHANGES CONTAINING VERY LITTLE PROTEIN)

The following foods contain carbohydrate but only small protein, and will therefore be used to make up your total carbohydrate allowance each day together with those from List B. Each item is equal to one 'carbohydrate exchange' (10 g) in the amounts quoted unless indicated otherwise, so that any item can be 'swopped' for another item according to your allowance. You will be allowed a total of ____ exchanges from this list.

Again the higher fibre options are underlined. Please eat the skin of fruit such as apples and pears to increase the fibre content of the diet.

	wgt (oz)	Handy Measure
Cornflour/Custard powder	1/3 oz (10 g)	1 level tablespoon
Sugar	1/3 oz (10 g)	2 teaspoons
Jam/Marmalade	1/2 oz (15 g)	2 teaspoons
Fruit:		
Apple (eating)	4 oz (110 g)	1 medium
Apple (stewed)	5 oz (140 g)	6 level tablespoons
Apple (baked plus skin)	5 oz (140 g)	1 medium
Apricots (fresh, raw)	6 oz (170 g)	3 large
Bananas	2 oz (55 g)	1 small peeled
Cherries raw	3.5 oz (100 g)	12
Dried currants, sultanas, raisins	0.5 oz (15 g)	2 level tablespoons
Dates without stones	0.5 oz (15 g)	3
Figs dried, raw	0.7 oz (20 g)	1
Gooseberries, raw	8 oz (225 g)	24
Grapes	2.5 oz (70 g)	10 large
Oranges without peel	4 oz (115 g)	1 medium
Orange & Grapefruit Juice (unsweetened)	4 oz (110 ml)	1 small glass

Peaches fresh	5 oz (140 g)	1 large
Pears raw	5 oz (130 g)	1 large
Pineapple, fresh	3 oz (170 g)	2 heaped tablespoons
Pineapple in natural juice	3 oz (85 g)	1 small glass

TABLE 2.7. AN EXAMPLE OF FOODS EATEN DURING ONE DAY ON NPD AND LPD FROM A SUBJECT WITH A RELATIVELY HIGH ENERGY INTAKE.

	Normal Diabetic Diet:	Low Protein Diet:
	2,800 kcal	2,833 kcal
	119 g protein	45.6 g protein
	286 g carbohydrate	417 g carbohydrate
	131 g fat, 38 g fibre	109 g fat, 45 g fibre
Milk for the day:	630 ml	200 ml
Breakfast:		
	2 Shredded Wheat	30 g Oat Bran
	1 rasher bacon	28 g sultanas
	1 slice wheatgerm bread	1 slice low protein bread
	1 egg	with soya margarine and
	all fried in lard	low sugar marmalade
	2 cups tea	tea with lemon
Mid-morning:		
	1 slice wheatgerm bread	Lp currant bread with bran*
	soya margarine	soya margarine
	20 g hard cheese	coffee with creamer
	milky coffee	banana
Lunch:		
	3 slices brown bread	2 slices low protein bread*
	soya margarine and cheese	1 slice Hi Bran bread
	potato crisps	soya margarine
	peanuts	vegetable pate and salad
	apple	2 apples
	tea with milk	coffee with creamer
Mid-afternoon:		
	coffee with milk	LP currant bread with bran*
	biscuits	apple
		low protein date cake*
		coffee with creamer
Supper:		
	grapefruit juice	grapefruit juice
	1 pork chop	small portion cod steak
	mashed potatoes	mashed potatoes, tomatoes
	frozen peas	carrots, runner beans
	carrots, cabbage	cauliflower
	orange, banana	orange, banana
	tea with milk	apple slices with sultanas
		tea with lemon
During Evening:		
	milky coffee	coffee with creamer
	digestive biscuit	Lp, high fibre cake*
Bedtime:		
	milky coffee	coffee with creamer
	biscuits, soya margarine	LP cookies and crackers
	cheese	

Note: * refers to home-made foods baked with low protein flour.
Lp refers to special low protein products: Aglutella, Juvela, Nutricia, Rite Diet; Stanmore, UK.

DURATION OF THE TWO DIET PERIODS

To establish a rate of decline of glomerular filtration rate at least three assessments were necessary on the normal protein diet spanning 12 months of follow-up (Table 2.8). This represents the shortest duration on the normal protein diet. On the low protein diet all patients had at least four assessments with the exception of patient 16 who commenced renal replacement therapy after two assessments. The mean number of assessments and duration on each diet were similar (Table 2.8).

TABLE 2.8. DURATION AND NUMBER OF ASSESSMENTS ON NORMAL PROTEIN DIET (NPD) AND LOW PROTEIN DIET (LPD)

Patient Initials	Sex	Subject Number	Normal Protein Diet		Low Protein Diet	
			Duration (mo)	Assess-ments	Duration (mo)	Assess-ments
MB	F	1	29	5	33	5
LC	F	2	39	5	36	5
AC	M	3	13	3	45	8
ALC	M	4	35	4	30	5
PD	M	5	20	5	30	5
JD	M	6	17	3	26	4
JH	M	7	38	4	36	5
KH	M	8	32	6	31	5
RH	M	9	29	8	31	5
WI	M	10	35	5	35	6
ME	M	11	32	4	25	4
MN	M	12	39	4	33	6
AM	F	13	12	3	29	5
WP	M	14	36	8	39	7
PS	F	15	21	4	31	5
RT	M	16	30	4	12	2
RW	M	17	36	3	23	4
NW	M	18	25	4	49	7
BW	M	19	36	7	45	8
		Mean	29	5	33	5
		SD	8.5	1.5	8.3	1.4
		Min	12	3	12	2
		Max	39	8	49	8

HISTORIC CONTROL COHORT

ENTRY CRITERIA

Patients had to be insulin-dependent with diabetic nephropathy (clinically or histologically determined) and have had at least one year follow-up including three assessments of renal function.

PATIENTS AVAILABLE FOR STUDY

Fifteen patients fulfilled the entry criteria and had undergone routine assessments of renal function approximately every six months from 1979 until 1984. One patient was excluded as she was of Afro-Carribean descent and all patients in the low protein diet study were Europids; three were excluded as they had completed a number of intervention studies which may have influenced the natural history of their diabetic nephropathy such as intensified glycaemic control.

PATIENTS ENTERED INTO THE STUDY

The details of 11 patients are given in Table 2.9.

TABLE 2.9. HISTORICAL CONTROL GROUP PATIENT DETAILS. ALL DATA ARE FOR RECRUITMENT INTO STUDY.

Patient Initials	Sex	Subject Number	Age (yr)	Duration of Diabetes (yr)	Duration of Proteinuria (yr)	GFR ml/min/ $1.73m^2$	AER mg/24 hr	HBA_1 %	Blood Pressure (mmHg)	Blood Pressure Treatment
WB	M	1	59	26	1	122.0	920	13.0	140/70	No
EB	F	2	49	22	2	45.1	4653	14.7	160/100	Yes
AC	M	3	26	24	2	132.0	406	11.5	130/80	No
JC	M	4	33	19	2	68.0	2156	11.5	120/75	No
AF	M	5	28	15	7	98.5	5083	10.7	130/83	No
MG	M	6	32	18	4	70.0	1324	13.6	171/109	Yes
RJ	M	7	44	28	2	54.9	1065	14.1	151/94	No
SK	F	8	33	26	9	40.5	2512	13.5	150/100	Yes
DO	M	9	38	6	1	89.0	1270	9.5	103/83	No
BW	M	10	15	12	1	207.0	1620	9.3	129/96	No
CW	F	11	29	19	5	11.5	1240	15.1	110/70	Yes
		Mean	35	20	3.3	85	2023	12.4	136/87	Yes=4
		SD	11.5	6	2.5	51	1450	2.0	21/13	No=7
		Min	15	6	1	11.5	406	9.3	103/70	Total=11
		Max	59	28	9	207	5083	15.1	171/109	

TABLE 2.9 (continued). HISTORICAL CONTROL GROUP PATIENT DETAILS. ALL DATA ARE FOR RECRUITMENT INTO STUDY.

Patient Initials	Sex	Subject Number	Weight (kg)	BSA (m^2)	Plasma Creatinine (µmol/l)	Plasma Urea (mmol/L)	Insulin daily dose (u)
W B	M	1	67.7	1.78	89	5.9	68
EB	F	2	61.0	1.65	160	12.5	40
AC	M	3	65.9	1.74	102	7.2	70
JC	M	4	74.0	1.84	113	5.6	52
AF	M	5	71.0	1.84	110	3.4	96
MG	M	6	67.0	1.85	103	6.3	100
RJ	M	7	65.2	1.86	173	15.0	60
SK	F	8	72.0	1.89	113	9.3	34
DO	M	9	67.5	1.79	113	4.7	60
B W	M	10	40.8	1.27	86	3.8	50
C W	F	11	68.5	1.81	298	26.3	34
		Mean	65.5	1.76	133	9.1	60
		SD	8.5	0.16	58	6.4	21
		Min	40.8	1.27	86	3.4	34
		Max	74.0	1.89	298	26.3	100

BLOOD PRESSURE TREATMENT AND DRUGS USED

Four patients (36%) were receiving antihypertensive agents at the start of the follow-up and a further 3 had therapy added during the period of study (Table 2.10). Four patients remained off antihypertensive agents throughout the period of follow-up.

TABLE 2.10. ANTIHYPERTENSIVE AND DIURETIC THERAPY OF HISTORICAL CONTROL GROUP PATIENTS. NUMBERS IN PARENTHESIS REPRESENT DATE OF COMMENCEMENT OR DISCONTINUATION OF THERAPY IN MONTHS.

Patient Initials	Sex	Subject Number	Therapy
WB	M	1	--
EB	F	2	*Navidrex K® Frusemide Spironolactone (7) Prazocin (4)
AC	M	3	--
JC	M	4	--
AF	M	5	--
MG	M	6	*Tenoretic® Tenoretic® discontinued (6)† Metoprolol Hydralazine Bendroflurazide (6) Frusemide (24)
RJ	M	7	Navidrex K® (43) Tenoretic® (47)
SK	F	8	*Frusemide Hygroton®
DO	M	9	Navridex K® Atenolol (65)
BW	M	10	Metoprolol (6)
CW	F	11	*Frusemide Hygroton®

*On this therapy at recruitment.
†Discontinuation of fixed combination drug.

GLYCAEMIC REGIMEN

Ten patients used a twice daily subcutaneous injection of a soluble an intermediate-acting insulin. One patient used soluble insulin twice a day.

OTHER DIABETIC COMPLICATIONS

The pattern of extra-renal complications is summarized in Table 2.11.

DURATION OF FOLLOW-UP

The mean (range) follow-up time was 57 (16-114) months enabling 9 (6-14) assessments of renal function to be performed (Table 2.12).

TABLE 2.11. EXTRA-RENAL COMPLICATIONS OF HISTORICAL CONTROL GROUP PATIENTS AT RECRUITMENT

Patient Initials	Sex	Subject Number	Retino-pathy	Peripheral Neuropathy	Autonomic Neuropathy	PVD/Foot Problems	IHD
WB	M	1	BR	Yes	Yes	No	No
EB	F	2	PR	Yes	Yes	No	No
AC	M	3	PR	No	No	No	No
JC	M	4	PR	Yes	Yes	No	No
AF	M	5	PR	No	No	No	No
MG	M	6	PR	Yes	No	No	No
RJ	M	7	PR	Yes	Yes	No	No
SK	F	8	Blind	Yes	Yes	No	No
DO	M	9	PR	No	No	No	No
BW	M	10	PR	No	No	Cheiroar-thropathy	No
CW	F	11	Blind	Yes	Yes	Foot Ulcers	No

TABLE 2.12. DURATION OF FOLLOW-UP AND NUMBER OF ASSESSMENTS OF PATIENTS IN THE HISTORICAL CONTROL GROUP

Patients' Initials	Sex	Subject Number	Duration (months)	Number of Assessments
WB	M	1	33	9
EB	F	2	16	6
AC	M	3	49	11
JC	M	4	39	8
AF	M	5	66	9
MG	M	6	43	9
RJ	M	7	92	14
SK	M	8	54	11
DO	M	9	114	7
BW	M	10	77	10
CW	F	11	42	8
		Mean	57	9.3
		SD	27	2.1
		Min	16	6
		Max	114	14

CLINICAL AND LABORATORY ASSESSMENTS

The clinical examinations and laboratory assessments, including the method for measurement of glomerular filtration rate, were identical to those for the patients in the low protein diet study. The only exception was the method used for measuring glycosylated haemoglobin which was changed from a microcolumn method employing ion exchange chromatograph (normal range 5.8 - 8.2%) to an Agar Gel electrophoretic method (normal range 4.9 - 7.5%) in 1981.

MATCHING DETAILS

The patients in the low protein diet study and those in the historical control cohort were adequately matched for age, duration of diabetes, duration of proteinuria, glomerular filtration rate, urinary albumin excretion rate, and blood pressure at recruitment (Table 2.13). Slightly more patients recruited for the low protein diet study were receiving antihypertensive medication (9 vs 4, X^2 = 0.34, NS). Glycosylated haemoglobin levels were higher in the historical control cohort which were likely due to methodological changes.

TABLE 2.13. COMPARISON OF PATIENT DETAILS IN HISTORICAL CONTROL GROUP AND DIETARY STUDY GROUP. (Data are mean (SD) at recruitment).

Parameter	Historical Control Group N=11 8M 3F		Diet Study Group N=19 15M 4F		t value (df)*		P value
Age (yrs)	35	(11.5)	42	(11)	1.65	(28)	>0.1
Duration of Diabetes (yrs)	20	(6)	24	(9)	1.31	(28)	0.2
Duration of Proteinuria (yrs)	3.3	(2.5)	3.6	(2.5)	0.32	(28)	>0.5
GFR (ml/min/1.73m^2)	85	(51)	62	(27)	1.62	(28)	>0.1
AER (mg/24 hrs)	2023	(1450)	1359	(1597)	1.13	(28)	>0.2
Log AER	3.3	(3.16)	3.13	(3.2)	0.14	(28)	>0.5
Blood Pressure							
Systolic	138	(21)	146	(20)	1.06	(28)	>0.2
Diastolic	87	(13)	86	(11)	0.67	(28)	0.5
Number on % on BP Therapy (%)	4	*(36)*	9	*(47)*	X^2 0.344		>0.5
HbA1	12.4	(2.0)	9.3	(1.8)	4.37	(28)	<0.001
Duration of Study Period (mo)	57	(27)	62	(12)	0.70	(28)	>0.4
Number of Assessments	9	(2.1)	10	(2.4)	1.15	(28)	>0.2

*(df) = degrees of freedom

DATA COLLECTION, ANALYSES AND STATISTICS

All clinical and laboratory data was recorded in the patients' clinical notes and subsequently transcribed onto data sheets. Each patient had a separate sheet. The data from these sheets was collated onto a spreadsheet programme (Reflex^{R}) and checked for inaccuracies. Data was then transferred to an SPSS (Statistical Programme for Social Sciences) spreadsheet.

Descriptive statistics were used for comparisons of groups. For normally distributed data differences were tested using Student's t-test either paired or unpaired as appropriate and Chi squared tests. For non-normally distributed data log transformation of the data and subsequent t-tests were used. These analyses were performed using an Apple MacIntosh LC personal computer using the statistical programmes StatWorks (version 1.1, Cricket Software Inc, 1985), StatView 512 (Abacus Concepts Inc, 1988) and Systat Software (version 5.2, Systat Inc, 1992). Data are expressed as means or median with standard deviations, standard errors of the mean or 95% confidence intervals as indicated.

The rate of change in glomerular filtration rate during the normal protein diet and the low protein diet was estimated using an ordinary least squares regression (Draper and Smith 1981). An F-test was then performed for each individual to test the hypothesis that the slopes on the normal and low protein diets were equal. The intercepts of the regression equations for the normal and low protein diet were tested for statistical differences, i.e., the predicted level of glomerular filtration rate at zero time (when the low protein diet commenced) calculated from the regression equation of the normal and low protein diets. Changes in blood pressure and/or glycaemic control were added to the regression equation and the slopes and intercepts were re-calculated. In the model used, glomerular filtration rate was the dependent variable with months as the

independent variable and blood pressure or glycosylated haemoglobin added as co-variables.

A further analysis of the rate of change of the individual glomerular filtration rates was made using a 'Breakpoint' test (Jones and Molitoris 1984, Zoccali et al. 1989). This method establishes whether the best fit of a series of points (in this case GFR over time) is linear or whether a broken line provides a better fit. It does not take into account the timing of an intervention (in this case the date at which the low protein diet was instigated). Briefly, the analysis is based on a four-parameter linear regression and one non-linear parameter. Different values of this non-linear parameter are selected as the breakpoint (x_0). A linear regression problem is solved for this point and the residual sum of squares is calculated. Difference values of x_0 are tried until the value that minimises the residual sum of squares is found. A straight line fits the data better if the residual sum of squares is less than that calculated for the point at which the line 'breaks'.

Most parameters were statistically compared for each patient during NPD and LPD in addition to group mean comparisons and where appropriate results are expressed for both individual and mean group differences.

Bland Altman plots were constructed to test the agreement of 2 methods of measurement. Firstly, urinary urea and weighed food records for the assessment of dietary protein intake and secondly creatinine clearance and the plasma clearance of ^{51}CrEDTA for the assessment of GFR (Bland and Altman 1986). In the construction of a Bland Altman plot the difference between the methods is plotted on the Y axis and the mean for each measure plotted on the X axis. This plot then allows the determination of the mean difference (bias) and the variance (±2SD) around this mean (limits of agreement).

CHAPTER 3
RESULTS OF THE STUDY

COMPARISON OF DIETARY PROTEIN INTAKE USING URINARY UREA EXCRETION AND WEIGHED FOOD RECORDS

Table 3.1 gives the values for protein intake on each diet period calculated from urinary urea excretion and weighed food records. The two methods were in good agreement for the normal diet periods and showed a highly significant degree of correlation ($r=0.81$, $p<0.001$). Figure 3.1 shows a Bland Altman plot in which the mean of the 2 methods is plotted against the difference between the 2 methods. Each data point represents one patient and the values are calculated from the means of all weighed food records and urinary urea excretion values during NPD and LPD. Although the bias is virtually zero, the limits of agreement are wide, suggesting that the two methods may vary by as much as 26 grammes of protein in the assessment of protein intake.

For the low protein diet the mean values calculated from urinary urea excretion were on average 6 grammes higher than the values calculated from the weighed food records. The correlation coefficient between the two methods gaves an r value of 0.32 (NS) and a Bland Altman plot (Figure 3.2) displays the bias of 6 grammes and shows the limits of agreement which are similar to those seen for the normal diet (Figure 3.1).

TABLE 3.1. DIETARY PROTEIN INTAKE CALCULATED FROM URINARY UREA EXCRETION AND WEIGHED FOOD RECORDS.

	NORMAL PROTEIN DIET		LOW PROTEIN DIET	
Patient Number	Calculated from urinary urea (g/day)	Calculated from weighed food records (g/day)	Calculated from urinary urea (g/day)	Calculated from weighed food records (g/day)
1	77	52	59	44
2	49	67	36	41
3	130	114	74	52
4	62	72	45	60
5	122	124	46	48
6	101	87	48	46
7	89	93	61	44
8	84	103	55	50
9	95	93	55	43
10	73	94	37	52
11	45	55	32	41
12	73	64	38	38
13	64	59	37	34
14	87	77	58	45
15	59	54	55	42
16	77	72	67	54
17	88	74	52	42
18	79	94	64	38
19	98	99	66	51
MEAN	82	81	52	46
SD	21	20	12	6
		NS		NS

Figure 3.1.
Bland Altman plot of 2 Methods for assessing dietary Protein intake on NPD

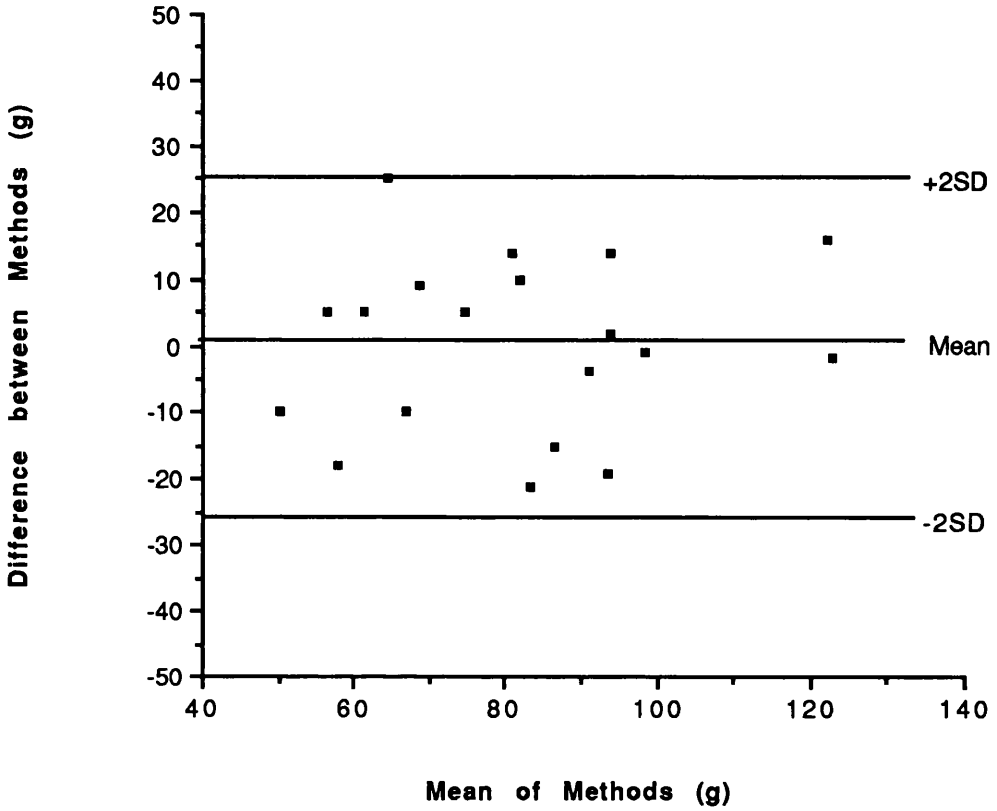
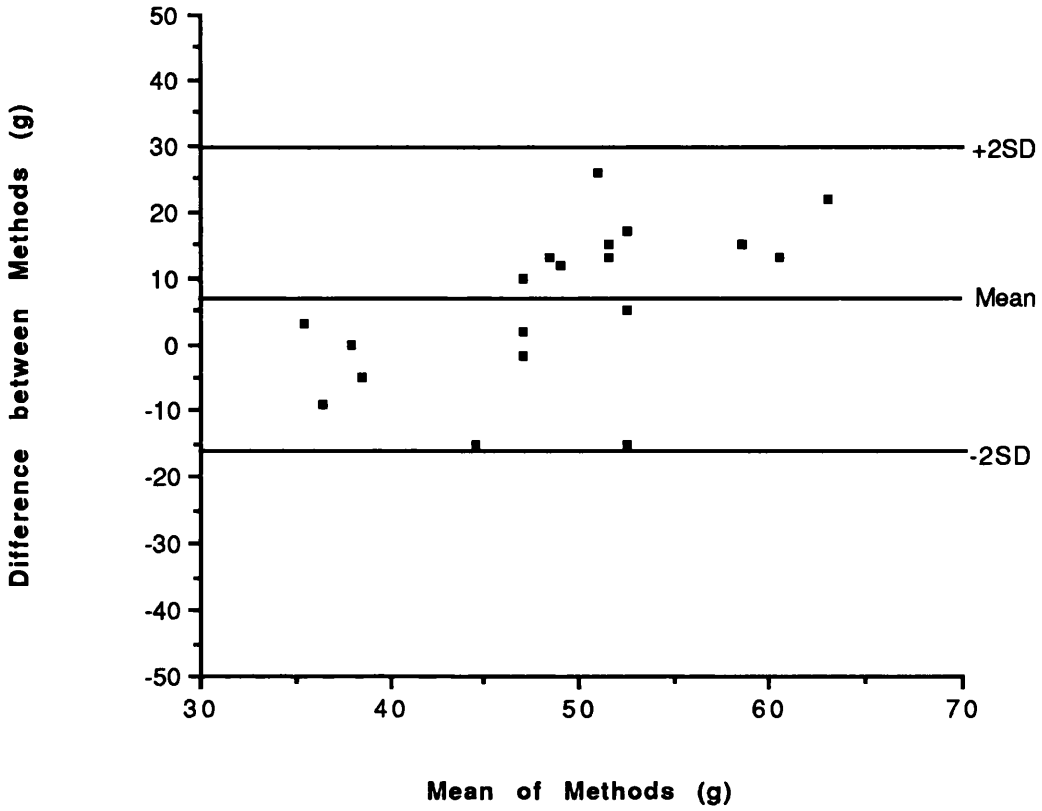


Figure 3.2.
Bland Altman plot of 2 Methods for assessing dietary Protein intake on LPD



These data indicate that on NPD weighed food records were on average a precise and accurate method for assessing dietary protein intake when compared to urinary urea excretion. However in certain individuals these 2 values could vary by as much as 26 grammes. During LPD patients tended to under-estimated their dietary protein intake using weighed food records. It is unlikely that methodological factors influenced this difference as patients were thoroughly instructed in the techniques of both weighed food records and 24 hour urine collections. The most likely explanation is that in an attempt to be compliant patients recorded less protein on the weighed food records than they consumed. However the discrepancy was, on average, of small magnitude.

DIETARY CHANGES

From the data taken from the dietary records, the ingested protein fell by 43% on the low protein diet from a level of 1.11 (0.06) g/Kg/day on NPD to 0.66 (0.03) g/Kg/day on LPD (Table 3.2). This reduction was mainly due to a reduction of 58% in animal protein with a smaller reduction of 17% in vegetable protein on LPD. The fat content of the diet fell by 27% due mainly to the reduction in ingested meat. The polyunsaturated/unsaturated fat ratio (P/S ratio) increased during LPD again due to reduction in saturated fat in meat. Commensurately phosphate intake fell by 32%. In order to maintain energy intake the carbohydrate intake increased by 14.5% but despite this total energy intake was lower by 10%. When corrected for changes in body weight, however, energy intake was similar during the two diet periods at 26.6 (1.5) kcal/Kg/day on NPD and 25.0 (1.5) kcal/Kg/day on LPD.

Sodium intake fell on LPD as judged by a reduction in urinary sodium excretion which fell to 143 (12) mmol/day from 178 (14) mmol/day on NPD ($p<0.02$).

TABLE 3.2. DIETARY DATA AND BODY WEIGHT ON NPD and LPD. DATA ARE MEAN ± SEM PER DAY.

	NPD	LPD	Percent Change	Significance (p value)
Energy (kcal)	1935 ± 116	1739 ± 104	-10	0.02
Protein (g)	81 ± 5	46 ± 1	-43	0.0001
Animal protein (g)	52 ± 4	22 ± 1	-58	0.0001
Vegetable protein (g)	29 ± 2	24 ± 1	-17	0.01
Fat (g)	85 ± 7	62 ± 4	-27	0.006
P/S ratio	0.33 ± 0.04	0.57 ± 0.06	+73	0.0004
Carbohydrate (g)	200 ± 11	229 ±17	+14.5	0.05
Phosphate (mg)	1484 ± 92	1009 ± 33	-32	0.0001
Body Weight (kg)	73 ± 3	70 ± 3	-4	0.02

URINARY UREA AND URINARY CREATININE EXCRETION

When all 19 patients were considered together, mean urinary urea and urinary creatinine levels were lower on LPD (p=0.001 for both) (Figure 3.3, Table 3.3). On an individual basis urinary urea was significantly lower in 10 and urinary creatinine lower in 6 patients on the low protein diet (Table 3.3).

Figure 3.3. Urinary urea (UU) and creatinine (UCr) on NPD and LPD. Error bars are SE.

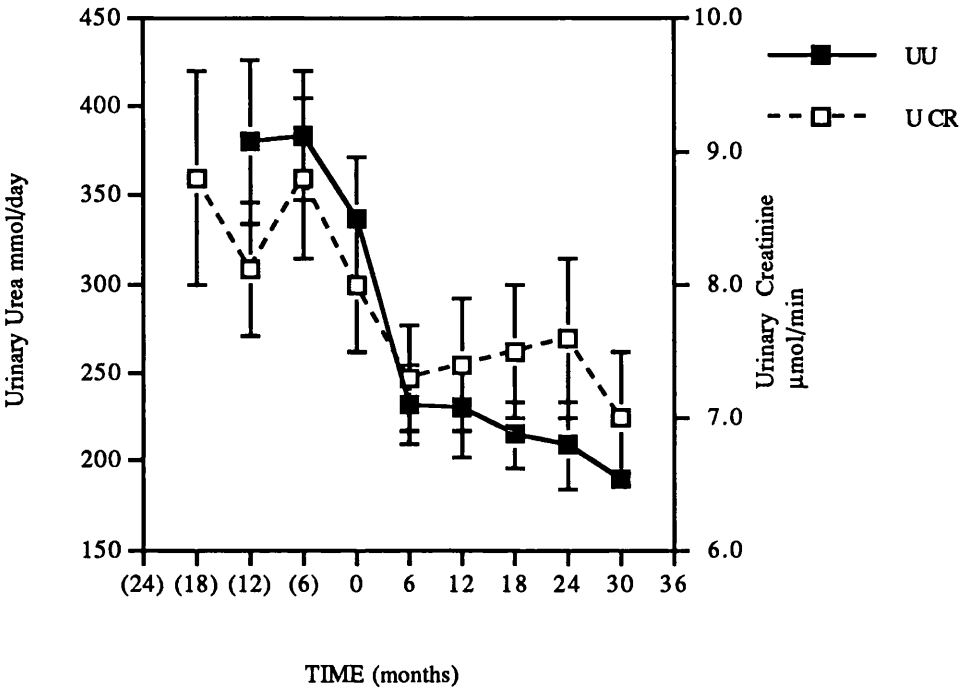


Figure 3.3. Values in parentheses and the 0 time point on the horizontal-axis indicate the NPD period. From 6-30 months represents the LPD period.
Data for all 19 patients are given at time points (6), 0 and 6 months, for (12) months 17 patients, for (18) months 8 patients, for 12 and 18 months 18 patients, for 24 months 15 patients and for 30 months 13 patients.

TABLE 3.3. URINARY UREA AND CREATININE EXCRETION RATES ON NDP and LPD. Data are mean (SD) and the number of urine collections is stated for each patient on NPD and LPD.

	NORMAL PROTEIN DIET			LOW PROTEIN DIET		
PT No.	No. of collections	Urinary Urea mmol/24hr	Urinary Creatinine µmol/min	No. of collections	Urinary Urea mmol/24hr	Urinary Creatinine µmol/min
1	5	322 (54)	7.12 (0.45)*	6	245 (89)	5.22 (0.38)
2	3	207 (62)	5.07 (0.38)	8	141 (40)	5.17 (0.69)
3	2	599 (270)	12.35 (1.2)	10	353 (190)	9.40 (2.7)
4	3	268 (50)*	5.60 (0.8)	8	173 (32)	4.64 (0.8)
5	8	614 (159)*	9.32 (0.43)	9	193 (57)	8.94 (1.95)
6	4	472 (62)*	10.5 0(1.06)*	4	182 (66)	8.16 (0.88)
7	4	435 (52)*	9.80 (0.72)	6	274 (64)	8.55 (0.75)
8	7	402 (58)*	10.10 (2.5)	9	240 (84)	7.90 (0.90)
9	3	446 (111)*	11.20 (2.3)	5	223 (32)	10.70 (1.6)
10	3	317 (14)*	7.90 (0.9)*	8	120 (43)	5.78 (1.03)
11	3	162 (19)	6.90 (0.24)	5	111 (47)	6.63 (1.5)
12	4	306 (39)*	9.10 (1.67)*	10	139 (26)	7.15 (0.7)
13	4	287 (72)*	5.07 (0.4)	4	152 (30)	4.49 (1.3)
14	2	406 (171)	7.70 (1.46)	14	239 (59)	8.00 (1.15)
15	3	250 (85)	5.90 (0.7)	6	240 (34)	6.60 (0.6)
16	3	317 (48)	7.20 (0.98)	1	294 (-)	5.60 (0.4)
17	3	403 (77)	10.90 (0.3)*	4	204 (87)	8.48 (0.23)
18	2	357 (16)	8.16 (0.64)	3	260 (41)	8.56 (0.71)
19	6	451 (55)*	10.3 (0.75)*	9	281 (86)	8.50 (1.1)
Mean	4	369	8.43	7	214	7.3
SD	1.6	116	2.2	3	63	1.7

p<0.001

p<0.001

* Individual significant differences NPD vs LPD

WEIGHT AND MID-ARM MUSCLE CIRCUMFERENCE CHANGES

Fourteen patients lost weight on the low protein diet and in 10 this was significant at the 5% level (Table 3.2, Figure 3.4). In cases where weight loss occurred on the low protein diet this tended to occur within the first 6 to 12 months of the diet and thereafter weight plateaued and showed a trend to increase (Figure 3.4). Acceptance of an increase in carbohydrate intake to maintain energy levels was initially difficult due to concerns that this would worsen glycaemic control. Patients who exceeded 110% of their ideal body weight (3 patients), who retired from work (3 patients) or whose failing vision necessitated a more sedentary lifestyle (1 patient) reduced their total energy intake on the low protein diet and consequently lost weight. The mean reduction in weight between the two diet periods was 3.1 g (4.25% of the original weight). This reduction includes below-knee amputations performed on two patients on low protein diet.

Further evidence that the weight reduction did not signify an important change in body composition was provided by the finding of unchanged mid-arm muscle circumference measurements, at 25 (1) cm, during each of the diet periods.

Figure 3.4. Weight during NPD and LPD.
Error bars are SE.

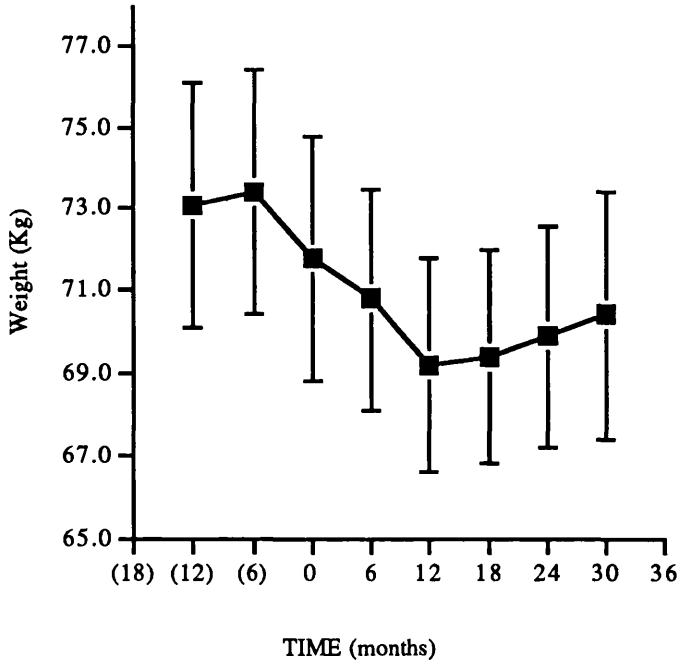


Figure 3.4. Values in parentheses and the 0 time point on the horizontal axis indicate the NPD period. From 6-36 months represents the LPD period.
Data for all 19 patients are given at time points (18), (12), (6), 0, 6 and 12 months, for 18 and 24 months 18 patients and for 24 months 15 patients.

BLOOD PRESSURE CHANGES

Changes in this variable are important as they may independently affect the rate of decline in glomerular filtration rate (Mogensen 1982, Parving et al. 1987).

BLOOD PRESSURE LEVELS DURING THE TWO DIET PERIODS

Because standing blood pressure levels can be significantly affected by differences in postural response to vasoactive drugs and autonomic neuropathy lying blood pressure was chosen for the analysis. Systolic blood pressure for the whole group was 5 mmHg lower during the low protein diet (p=0.06), diastolic presssure 6 mmHg lower and mean (1/3 pulse pressure and diastolic pressure) pressure 4 mmHg lower (p=0.001 for both) (Table 3.4).

When blood pressure levels were examined on an individual patient basis, diastolic blood pressure was significantly lower during the low protein diet in 1 patient and systolic blood pressure was lower in 2 patients. Mean blood pressure was not significantly different in any patient.

TABLE 3.4. LYING BLOOD PRESSURE LEVELS ON NPD AND LPD. Data are Mean (SD).

	Normal Protein Diet	Low Protein Diet
Systolic mmHg	149 (15)	144* (21)
Diastolic mmHg	86 (9)	80** (8)
Mean mmHg	106 (9)	102** (10)

*p=0.06
**p=0.001

While 14 patients were receiving antihypertensive therapy at commencement of the low protein diet 4 patients had such therapy instituted during LPD and in 6 it was modified (Table 2.2, page XX) due to protocol requirements for control of blood pressure. Exact titration of blood pressure levels is difficult with antihypertensive drug therapy and it was not surprising that mean blood pressure levels were slightly lower on LPD. It should be emphasised that in only 3 individuals were blood pressure levels significantly lowered on LPD.

GLYCAEMIC CHANGES

Mean levels of glycosylated haemoglobin were not different between each diet period when all the patients were considered together (Table 3.5). On an individual basis glycosylated haemoglobin was significantly lower during the low protein diet in 2 patients and higher in 2 patients.

Insulin daily dose corrected for body weight was on average non-significantly lower on low protein diet (Table 3.5). In 3 patients values were significantly lower on LPD and in 1 patient significantly higher.

TABLE 3.5. GLYCOSYLATED HAEMOGLOBIN LEVELS AND INSULIN DOSE ON NPD AND LPD. Data are Mean (SD).

	Normal Protein Diet	Low Protein Diet
Glycosylated Haemoglobin %	8.9 (1.1)	9.0 (2.0)
Insulin Dose U/Kg/day	0.56 (0.13)	0.52 (0.16)

RENAL FUNCTION CHANGES

GLOMERULAR FILTRATION RATE

When all 19 patients were considered together, the mean (SD) rate of decline of glomerular filtration rate fell by 77% from 0.61 (0.61) ml/min/month on the normal protein diet to 0.14 (0.37) ml/min/month on the low protein diet ($p<0.001$) (Table 3.6). The linearity of the rate of decline was higher on NPD compared to LPD (r values = 0.84 (0.15) vs. 0.69 (0.23), $p=0.008$). The mean glomerular filtration rate at the start of the normal protein diet was 62 (27.6) ml/min and by the end of this diet period (a mean period of 29 months) it had fallen to 47 (25) ml/min. There was a non-significant mean step-down in the glomerular filtration rate of the commencement of the low protein diet (46.9 (24.7) to 45.8 (23.8). This 2.3% reduction is within the error of the GFR measurement of 4%. During the low protein diet period (a mean period of 33 months), the mean glomerular filtration rate declined from 46 (24) to 42 (25) ml/min.

When the data is considered on an individual patient basis a more complex picture emerges (Table 3.6, Figure 3.5). In eight patients (numbers 1, 3, 5, 6, 7 12, 15 and 18) the slopes of the rate of decline of glomerular filtration rate were significantly different at the 5% level when compared by simple regression analysis (Table 3.6, Figure 3.5). When the 'Breakpoint' analysis was employed to test whether one line fitted all the data points (that is all points on both diets) or whether a line with a 'break' gave a better fit, seven patients (numbers 1, 2, 3, 5, 6, 7 and 10) had a significant break at a mean (SD) of 13 (9) months into the low protein diet (Table 3.6). Thus 10 patients showed a significant slowing in their rate of decline of glomerular filtration rate on simple regression analysis and/or a 'break' in their rate of decline on the low protein diet (Table 3.7, page 101).

In a further five patients (numbers 4, 9, 11, 13 and 14) the rate of decline of the glomerular filtration rate was slower on the low protein diet

but the slowing failed to reach significance. In three patients (numbers 8, 17 and 19) the glomerular filtration rate declined at a non-significantly faster rate on the low protein diet and in patient 16 the low protein diet was associated with a significantly faster rate of decline.

TABLE 3.6. RATE OF DECLINE OF GLOMERULAR FILTRATION RATE ON NORMAL (NPD) AND LOW (LPD) PROTEIN DIETS EXPRESSED AS ML/MIN/MO. THE P VALUE REPRESENTS THE DIFFERENCE BETWEEN SLOPES. THE BREAKPOINT P VALUE SIGNIFIES A SIGNIFICANT BREAK IN A LINEAR DECLINE AND THE MONTHS INDICATE WHEN THIS OCCURS DURING THE LPD.

Patient Number	NPD	LPD	P	Breakpoint p value	Months*
1	-0.72	-0.07	0.01	0.05	4
2	-0.10	+0.16	NS	0.01	23
3	-2.24	-0.66	0.05	0.01	16
4	-0.06	+0.13	NS	NS	--
5	-0.64	+0.26	0.001	0.01	2
6	-1.01	-0.24	0.001	0.01	2
7	-0.20	+0.39	0.02	0.05	23
8	-0.50	-0.60	NS	NS	--
9	-0.92	-0.43	NS	NS	--
10	-0.11	+0.18	NS	0.01	22
11	-0.10	+0.01	NS	NS	--
12	-0.13	+0.34	0.03	NS	--
13	-0.49	+0.30	NS	NS	--
14	-0.62	-0.21	NS	NS	--
15	-1.95	-0.54	0.04	NS	--
16	-0.33	-0.78	0.03	NS	--
17	-0.23	-0.39	NS	NS	--
18	-0.94	-0.06	0.03	NS	--
19	-0.25	-0.37	NS	NS	--
Mean	-0.61	-0.14	0.001	--	13
SD	0.61	0.37			9.3
SE	0.14	0.08			3.5
95%CI	-0.33 - -0.88	-0.3 - 0.03			

*months into the low protein diet

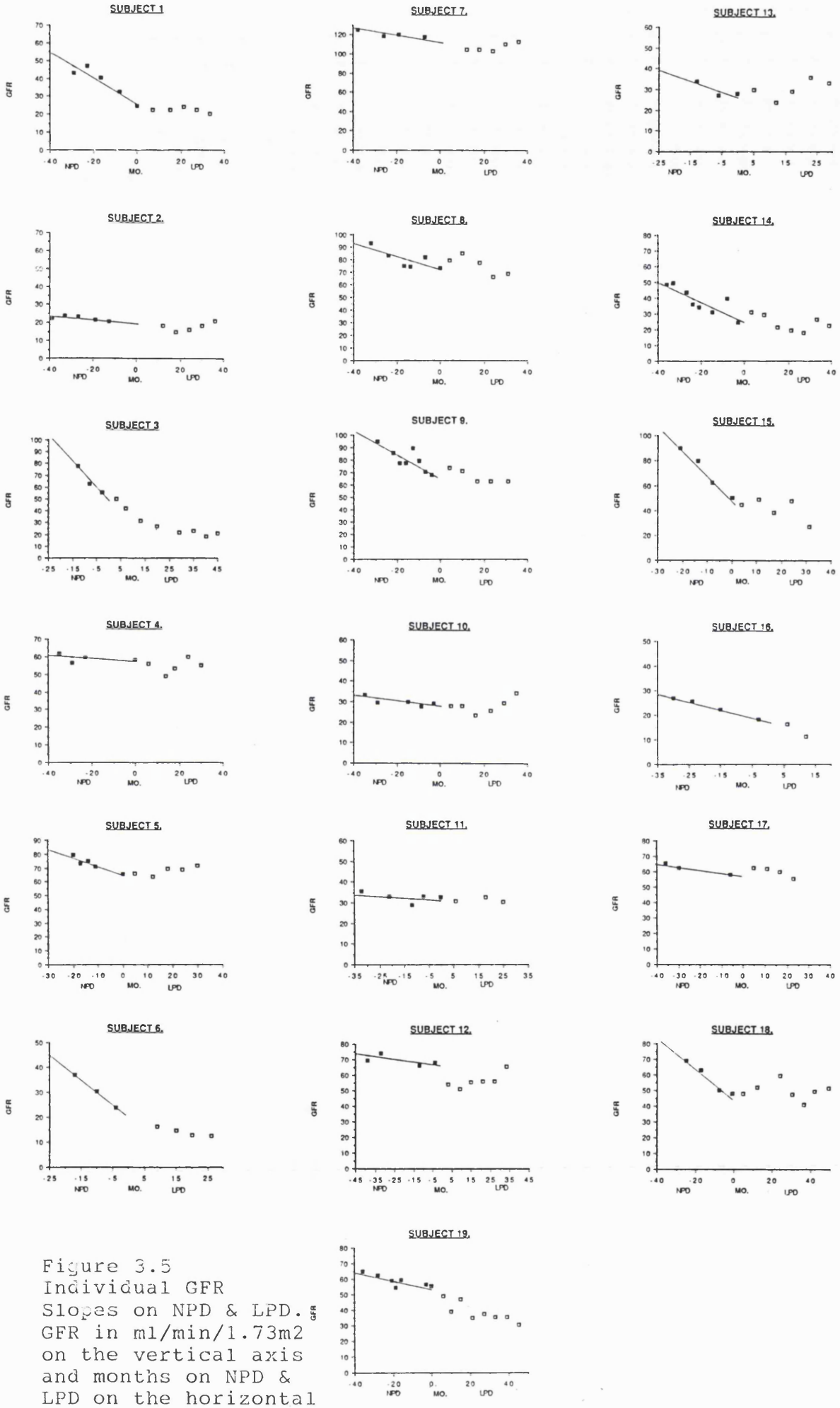


Figure 3.5
Individual GFR Slopes on NPD & LPD. GFR in ml/min/1.73m2 on the vertical axis and months on NPD & LPD on the horizontal axis.

TABLE 3.7 GLOMERULAR FILTRATION RATE (GFR) RESPONSE TO LOW PROTEIN DIET - BY PATIENT IDENTIFICATION NUMBER.

RESPONDERS		PARTIAL RESPONDERS	NON-RESPONDERS	
Signficant Difference in Δ GFR Using Simple Regression Analyses	Significant 'Breakpoint'	Non-significantly Slower using Simple Regression Analyses	Non-significantly Faster Using Simple	Significantly Faster Regression Analyses
1	1			
	2			
3	3	4		
5	5			
6	6			
7	7	9	8	
	10			
		11		
12		13		16
15		14	17	
18			19	
Totals 8	7	5	3	1
and/or 10				

GLOMERULAR FILTRATION RATES CORRECTED BLOOD PRESSURE AND GLYCAEMIA

The rates of decline of glomerular filtration rate were adjusted for the effects of blood pressure and glycaemia by adding these co-variates to the regression equation of GFR against time (Table 3.8). Only a small contribution to the rate of decline of GFR was made by these two variables on both diets.

TABLE 3.8. RATE OF DECLINE OF GLOMERULAR FILTRATION RATE (ΔGFR) IN PATIENTS ON NPD AND LPD CORRECTED FOR INFLUENCES OF BLOOD PRESSURE (BP) AND GLYCAEMIC CONTROL (HbA1).

	Normal Protein Diet			Low Protein Diet		
Subject Number	ΔGFR	ΔGFR +BP	ΔGFR +HbA1	ΔGFR	ΔGFR +BP	ΔGFR +HbA1
1	-0.72	-0.72	-0.94	-0.07	-0.08	-0.08
2	-0.10	-0.10	-0.06	+0.16	+0.38	+0.15
3	-2.24	-2.36	-1.81	-0.66	-0.71	-0.46
4	-0.06	-0.10	-0.03	+0.13	-0.05	+0.19
5	-0.64	-0.64	-0.75	+0.26	+0.25	+0.20
6	-1.01	-1.01	-0.84	-0.24	-0.32	-0.29
7	-0.20	-0.25	-1.48	+0.39	+0.38	-0.59
8	-0.50	-0.60	-0.63	-0.60	-0.46	-0.79
9	-0.92	-0.87	-1.03	-0.43	-0.69	-0.49
10	-0.11	-0.11	-0.23	+0.18	+0.09	+0.28
11	-0.10	+0.07	-0.16	+0.01	+0.002	+0.09
12	-0.13	-0.15	-0.13	+0.34	+0.39	+0.34
13	-0.49	-0.11	0.00	+0.30	+0.31	+0.45
14	-0.62	-0.59	-0.54	-0.21	-0.21	-0.15
15	-1.95	-1.66	-1.89	-0.54	-0.63	-0.58
16	-0.33	-0.33	-0.36	-0.78	-0.80	-0.85
17	-0.23	-0.23	-0.23	-0.39	-0.37	-0.39
18	-0.94	-0.88	-1.12	-0.06	-0.04	-0.03
19	-0.25	-0.23	-0.05	-0.37	-0.42	-0.41
Mean	-0.61	-0.58	-0.64	-0.14	-0.16	-0.18
SD	0.61	0.61	0.60	0.37	0.39	0.39
95%CI	-0.33	-0.30	-0.38	-0.3	-0.33	-0.35
	- -0.88	- -0.85	- -0.92	- 0.03	- 0.19	- 0.04

PLASMA CREATININE AND UREA

When all measurement from all 19 patients were considered together the mean serum creatinine increased by 20% from 141 (40) μmol/l on NPD to 169 (82) μmol/l on LPD ($p=0.031$) (Figure 3.6). In 7 patients there was a significant increase in plasma creatinine on the low protein diet. These 7 patients had a non-significantly higher mean level of plasma creatinine on the normal protein diet compared to the 12 patients who showed no significant increase during the low protein diet (153 (46) vs 132 (33)). However, when the last creatinine values on NPD were compared between these 7 patients versus the other 12 patients the differences were more marked (188 (73) vs 127 (35) μmol/l, $p<0.005$). This suggests that in those patients who had a plasma creatinine levels significantly above the normal range (>120 μmol/l) the creatinine level rose more than in those whose creatinine was within or just above the normal range. This is in keeping with the observation that plasma creatinine levels are only useful indirect markers of the glomerular filtration rate when they are above the normal range (Shemesh et al. 1985).

Plasma urea was no different between the two diet periods when all 19 patients were considered together. However, there was an initial non-signficant reduction of plasma urea to a mean level of 8.8 (5.6) mmol/l six months after the start of LPD from a mean level of 10.9 (4.8) mmol/l at the end of the normal protein diet (Figure 3.6) and the rising trend seen on NPD was attenuated on LPD. On an individual basis plasma urea was significantly lower during the low protein diet in 6 patients and higher in 2 patients. These latter 2 patients also had a significant elevation in their serum creatinine levels during the low protein diet.

Figure 3.6. Plasma Urea (pu) and Creatinine (p CR) Levels during the Study

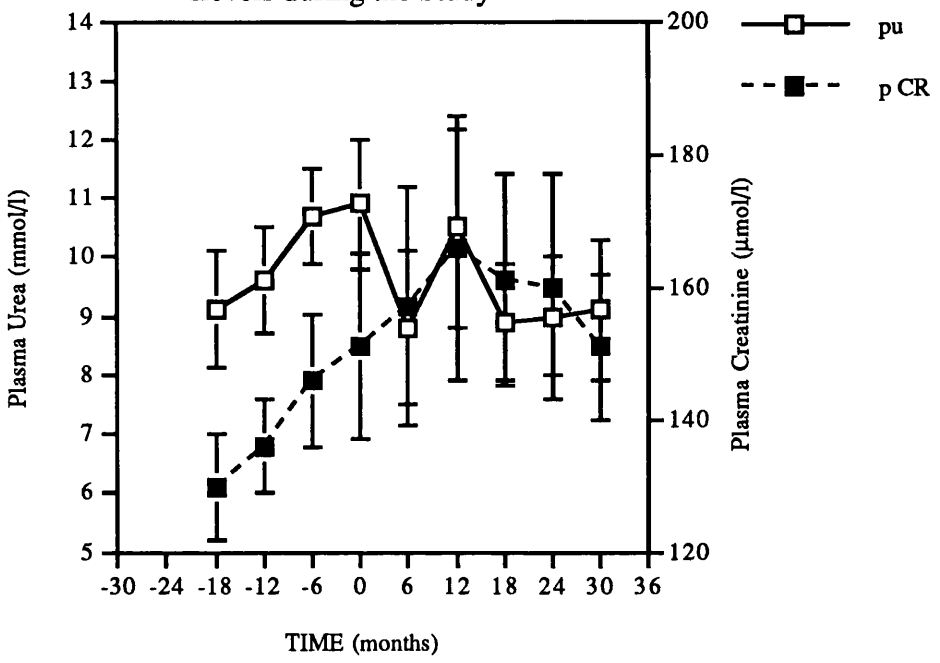


Figure 3.6. Negative values and the 0 time point on the horizontal axis indicate the NPD period. From 6-36 months represents the LPD period. Data for all 19 patients are given at time points -6, 0, 6 and 12 months, for -12, 18 and 24 months 18 patients and for -18 and 30 months 15 patients. Data are mean and error bars SE.

URINARY ALBUMIN EXCRETION

In considering group means, both the mean and geometric mean for urinary albumin excretion rate were lower on the low protein diet (p=0.036 and 0.001 respectively) (Table 3.9, Figures 3.7, 3.8). When the urinary albumin excretion was corrected for the glomerular filtration rate (thereby expressing the fractional albumin excretion = UA/PA/GFR x 1440 where UA is the urinary albumin excretion rate per day, PA the plasma albumin, GFR the glomerular filtration rate which is multiplied by 1440 to convert from minutes to 24 hours) there was no significant difference between the two diet periods. The mean (SE) rate of change of fractional clearance of albumin was 0.006 (0.03) x 10^{-4} per month on NPD and -0.18 (0.12) x 10^{-4} per month on LPD. Although the increase in urinary albumin excretion on NPD was halted on LPD the magnitude of this difference failed to achieve statistical significance (p=0.15).

On an individual patient basis 5 patients showed a significant drop in urinary albumin excretion rate on LPD and the fractional clearance of albumin was lower in 2 patients and higher in 1 patient.

TABLE 3.9. URINARY EXCRETION RATES AND FRACTIONAL CLEARANCES (θ) DURING THE TWO DIET PERIODS. DATA ARE MEAN (SD).

	Normal Protein Diet	Low Protein Diet	p
Mean Urinary Albumin mg/24 hr	1067 (1341)	694 (849)	0.036
Geometric Mean urinary albumin* mg/24hr	490 (179-4749)	288 (41-3480)	0.001
θ Alb $x10^{-4}$	4.21 (7.3)	3.86 (5.9)	NS

* geometric mean (range).

Figure 3.7 - Urinary Albumin Excretion Rate during the Study

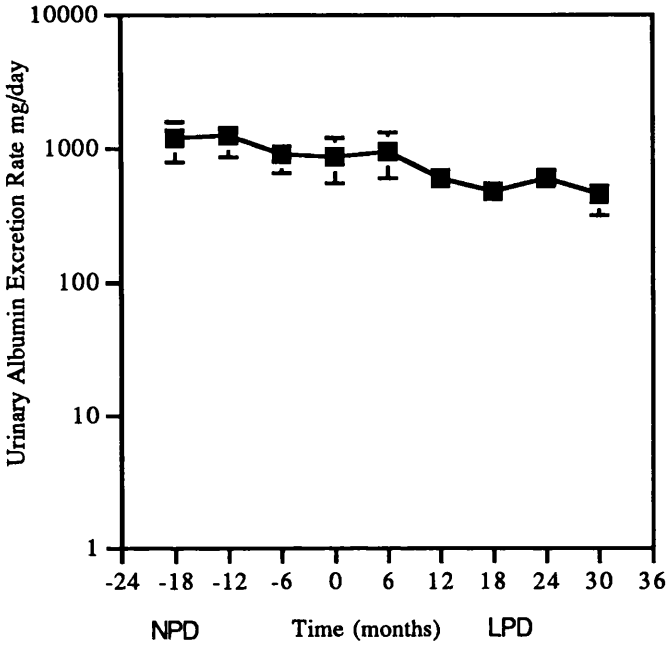
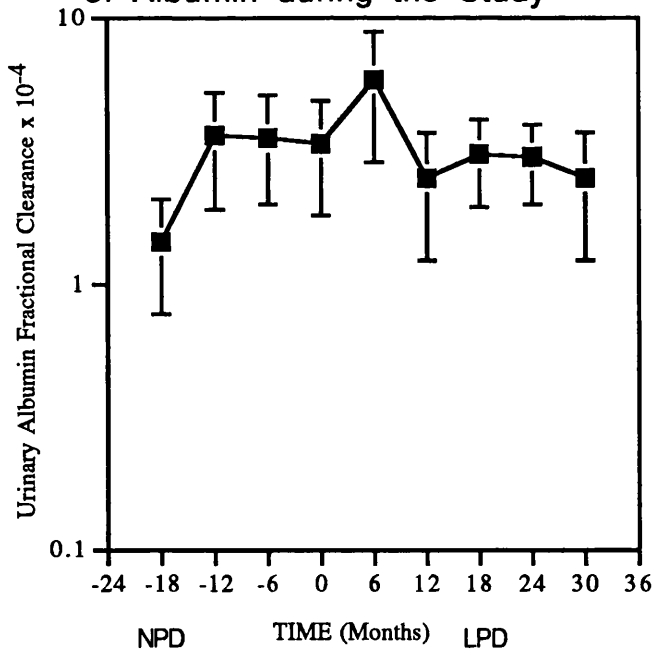


Figure 3.8 - Fractional Clearance of Albumin during the Study



Figures 3.7 and 3.8. Negative values and the 0 time point on the horizontal axis indicate the NPD period. From 6-36 months represents the LPD period.
Data for all 19 patients are given at time points -12, -6, 0, 6 and 12 months, for 18 months 18 patients, 24 months 17 patients, for -18 months 15 patients and for 30 months 12 patients.
Data are mean and error bars SE. Log. scale used.

HAEMOGLOBIN AND ELECTROLYTE CHANGES

Mean haemoglobin levels were remarkably similar during the two diet periods (Table 3.10). However in two patients mean levels were lower during LPD and in two levels were higher. There were no clinical or biochemical features in these 4 patients to explain these variations.

Mean levels of plasma calcium levels were very similar on the two diets and only dropped significantly in two patients for no diagnosed reason during the LPD. Mean levels of sodium, potassium, and phosphate were all lower on LPD (Table 3.10). On an individual basis plasma sodium was significant reduced in 1 patient and increased in 1 patient on LPD. For potassium the picture was slightly different with 5 patients having a significant reduction on LPD. In none of these 5 patients was this reduction associated with the introduction of Frusemide or any other diuretic during LPD. Plasma phosphate level was significantly lower in 1 patient.

TABLE 3.10. HAEMOGLOBIN AND ELECTROLYTE LEVELS DURING THE TWO DIET PERIODS (MEAN (SD)).

	Normal Protein Diet	Low Protein Diet
Haemoglobin mg/dl	13.0 (2.02)	13.12 (2.07)
Sodium mmol/l	139 (3.2)	137* (1.9)
Potassium mmol/l	4.2 (0.37)	4.0** (0.35)
Calcium mmol/l	2.29 (0.09)	2.27 (0.09)
Phosphate mmol/l	1.14 (0.18)	1.09*** (0.22)

*p=0.014
**p=0.018
***p=0.033

CHANGES IN PLASMA ALBUMIN AND TOTAL PLASMA PROTEINS

Mean plasma albumin levels increased on the low protein diet and a significant increase was seen in six individual patients (Table 3.11). In one patient plasma albumin levels fell on the low protein diet. The increase in plasma albumin was not related to the reduction in the urinary loss of albumin. A linear regression demonstrated a non-significant association between the change in urinary albumin excretion and the change in plasma albumin (r=0.32, NS).

Mean total plasma protein levels were not different between the two diet periods (Table 3.11). In one patient the value was lower on the low protein diet and in one it was higher.

TABLE 3.11. PLASMA ALBUMIN AND TOTAL PLASMA PROTEIN LEVELS DURING THE TWO DIET PERIODS (MEAN (SD)).

	Normal Protein Diet	Low Protein Diet
Plasma Albumin g/l	37.8 (3.5)	40.2* (3.8)
Total Plasma Proteins g/l	68.1 (4.5)	68.9 (5.4)

*$p < 0.05$

SERUM LIPOPROTEIN LEVELS

In 15 patients serum lipoprotein levels were compared on each diet. No values were available in 4 patients due to the fact that the recovery of the fractions in the sample taken on the normal protein diet were unsatisfactory or the patients commenced the low protein diet before baseline lipoprotein levels were assayed using the method employed for this study.

The reduction in the level of total cholesterol on LPD failed to reach significance (p=0.063) however LDL-cholesterol was significantly lower (p=0.009) (Table 3.12). Additionally, HDL-cholesterol was lower in the low protein diet (p=0.009). Total triglyceride was slightly but significantly higher on the low protein diet (p=0.018) probably due to the increased carbohydrate intake.

TABLE 3.12. SERUM LIPOPROTEIN LEVELS IN 15 PATIENTS ON A NORMAL PROTEIN DIET AND LOW PROTEIN DIET. Data are mean (SD).

Diet	Total Cholesterol	LDL Cholesterol	VLDL Cholesterol	HDL Cholesterol	Total Triglyc-eride	LDL Triglyc-eride	VLDL Triglyc-eride	HDL Triglyc-eride
Normal Protein	5.17 (1.32)	3.37 (1.18)	0.50 (0.435)	1.22 (0.28)	1.10 (0.65)	0.28 (0.09)	0.65 (0.52)	0.14 (0.03)
Low Protein	4.68 (1.18)	2.73 (0.93)	0.52 (0.36)	1.10 (0.3)	1.26 (0.60)	0.32 (0.09)	0.67 (0.47)	0.27 (0.08)
Difference	0.49	0.64	0.02	0.12	0.16	0.04	0.02	0.13
P Value	0.063	0.009	0.35	0.009	0.018	0.07	0.64	0.0001

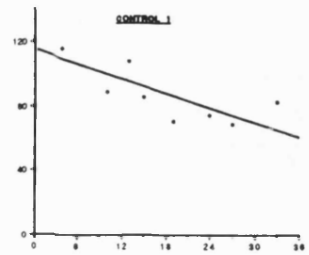
COMPARISON WITH HISTORICAL CONTROL GROUP

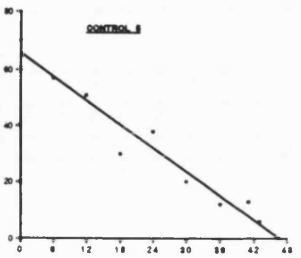
RATE OF DECLINE OF GLOMERULAR FILTRATION RATE

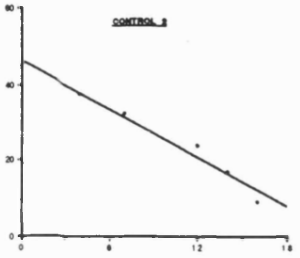
The rate of decline of glomerular filtration rate in the historical control group was 0.96 (0.59) ml/min/mo. and adjustment for the effects of blood pressure and glycaemia made very little difference to this value (Table 3.13, Figure 3.9). Analyzing the data using the 'Breakpoint' test revealed that 2 patients had a rate of decline which was better fitted with 2 regression lines rather than a single, unbroken line. One patient had a breakpoint at 12.5 months at which time the rate of decline of glomerular filtration rate accelerated and in another patient the rate of decline slowed at 52.7 months (Table 3.13, Figure 3.9). The linearity of the rate of decline was high with a mean (SD) r value of 0.90 (0.1) ($p<0.001$).

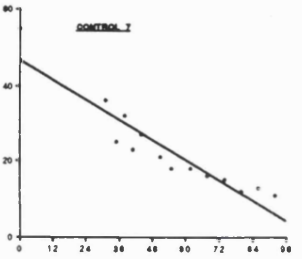
TABLE 3.13. RATES OF DECLINE OF GLOMERULAR FILTRATION RATE IN THE HISTORICAL CONTROL GROUP CORRECTED FOR INFLUENCES OF BLOOD PRESSURE (BP) AND GLYCAEMIC CONTROL (HbA1). THE BREAKPOINT P VALUE SIGNIFIES A SIGNIFICANT BREAK IN A LINEAR DECLINE AND THE MONTHS INDICATE WHEN THIS OCCURED DURING THE STUDY PERIOD.

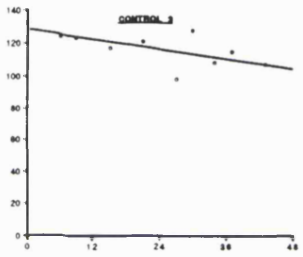
Patient Number	Δ GFR	Δ GFR + BP	Δ GFR +HbA1	Breakpoint p value	Months
1	-1.5	-1.54	-1.50	NS	-
2	-2.13	-2.02	-2.26	0.01	12.5
3	-0.51	-0.52	-0.33	NS	-
4	-1.29	-1.20	-0.82	NS	-
5	-1.06	-1.02	-1.06	NS	-
6	-1.41	-1.36	-1.31	NS	-
7	-0.44	-0.46	-0.40	0.01	53
8	-0.55	-0.56	-0.58	NS	-
9	-0.56	-0.45	-0.45	NS	-
10	-1.02	-0.91	-1.40	NS	-
11	-0.10	-0.14	-0.10	NS	-
Mean	-0.96	-0.92	-0.93		33
SD	0.59	0.56	0.64		29
SE	0.18	0.17	0.19		20
95%CI	-0.61 - -1.32	-0.60 - -1.25	-0.55 - -1.30		

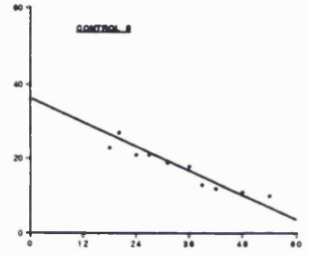


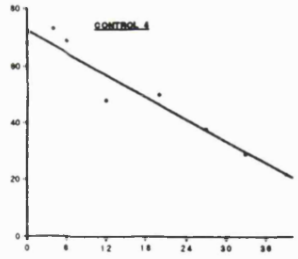


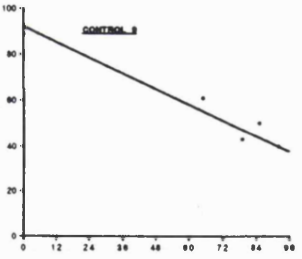


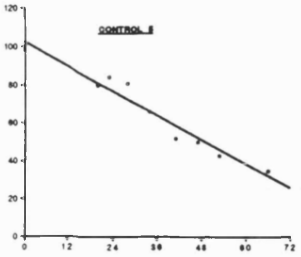


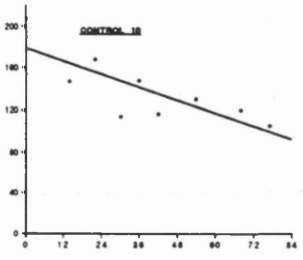












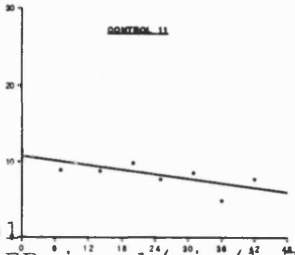
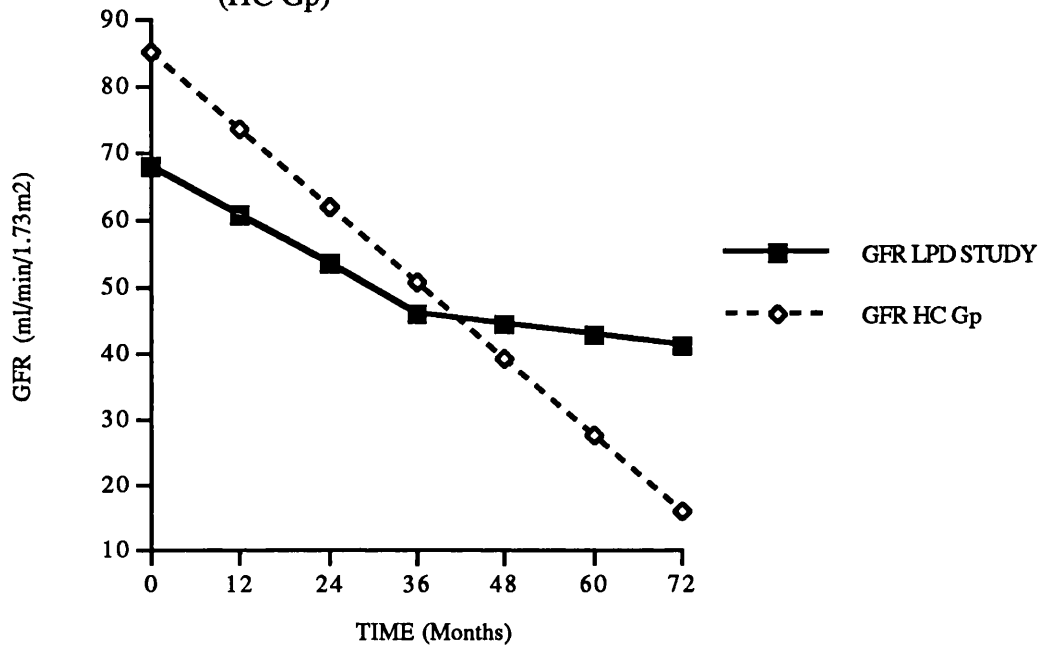


Figure 3.9
Individual GFR
Slopes of
Historical Control
Group Patients. GFR in ml/min/1.73m2 on the vertical axis and months on the horizontal axis.

The rate of fall was significantly less on LPD compared to the rate of fall in the Historical Control Group as depicted in Figure 3.10 ($p < 0.001$).

Figure 3.10. Mean GFR Slopes of Patients in LPD study and Historical Control Group (HC Gp)



ACCEPTABILITY

The results of the questionnaire completed by the patients after at least 6 months on LPD are summarised in Table 3.14. Whilst patients found the diet palatable they found that choosing food was difficult and cooking with low protein foods was unpopular. The diet did influence their social life with eating out being inconvenient. Overall the diet was well accepted and only 1 patient (a male in his late-teenage years) was unable to comply and was excluded from the analysis (Figure 2.2, page 51). Patients with a regular lifestyle and regular, similar meals had less difficulty in adhering to the diet compared to those patients with a more varied lifestyle who tended to eat out and travel more.

TABLE 3.14. MEAN AND MODE ACCEPTABILITY SCORES OF THE LOW PROTEIN DIET WHERE 1 IS THE LOWEST SCORE AND 5 IS THE HIGHEST.

ACCEPTABILITY

	Mean	Mode
Palatability/Satiety	4.29	5
Affect on Social Life	4.15	5
Eating Out	3.32	5
Choosing Food, Using Exchanges	3.25	3
Comparison With Previous Diet	2.25	2.5
Cooking With Low Protein Foods	1.71	1

MORBIDITY AND MORTALITY

During the course of the study 8 of the 19 (42%) patients had significant clinical events (Table 3.15). These included two deaths both from complications of coronary artery disease. Two patients developed foot ulcers that became infected leading to septicaemia and neccesitating below-knee amputations. One patient commenced CAPD after 14 months on LPD.

TABLE 3.15. SIGNIFICANT CLINICAL EVENTS DURING THE COURSE OF THE STUDY.

Patient Initials	Sex	Subject Number	Event
MB	F	1	septicaemia from foot ulcer necessitating right below-knee amputation (41)
AC	M	3	septicaemia from foot ulcer leading to right below-knee amputation (10)
ALC	M	4	left ventricular failure secondary to ischaemic heart disease (59)
KM	M	8	foot ulcers (56)
AMF	F	13	death from left ventricular failure secondary to ischaemic heart disease (45)
WP	M	14	death from acute myocardial infarction (78)
PS	F	15	fracture of right humerus due to trauma
RT	M	16	renal replacement therapy (CAPD) (44)

Values in parenthesis are the time in months from enrollment in the study at which the event occurred.

CAPD - Chronic ambulatory peritoneal dialysis

CHAPTER 4

A COMPARISON OF THE 'RESPONDERS' AND THE 'NON-RESPONDERS'

INTRODUCTION

Ten of the 19 patients involved in the low protein diet study had a significant reduction in their rate of decline of glomerular filtration rate associated with LPD (Table 3.7, page 101). This reduction was defined using simple regression analysis in eight patients and breakpoint analysis in a further two patients. Patients 2 and 10 had a significant breakpoint and significance values for simple regression of 0.08 and 0.09 respectively. Had the level of significance been 10% for the regression these patients would have been considered to have had a significant slowing in the decline of glomerular filtration rate on low protein diet. Similarly, patients 12, 15, and 18 had a significant change in the simple regression analysis but the F values of the breakpoint analysis lay betweeen the 2.5 and 10% of the F distribution. Thus the use of the traditional level of significance of 5% caused some patients to demonstrate a significant response in only one of the two analyses. Six patients had a statistical response in both statistical tests.

As the response to the low protein diet was heterogeneous, an attempt was made to identify factors that differentiated the 10 patients who responded from the 9 who did not.

PARAMETERS INVESTIGATED

In order to systematically study any factors that may be associated with a response or lack of response to the low protein diet the following parameters were investigated:

At the start of the low protein diet: Age, duration of diabetes and proteinuria, glomerular filtration rate, rate of fall of GFR on NPD, urinary albumin excretion rate, glycosylated haemoglobin, blood pressure levels, and anti hypertensive treatment treatment.

Duration the low protein diet: Compliance with the diet, changes in diet, weight, blood pressure, blood pressure therapy, glycosylated haemoglobin and albumin excretion rate.

STATISTICAL METHODS

For each parameter assessed patients were divided into Responders and Non-Responders and differences between these groups compared using t-tests on normally distributed data.

RESULTS

BASELINE ASSESSMENTS

There were no significant differences in age, duration of diabetes and proteinuria, glomerular filtration rate, albumin excretion rate, glycosylated haemoglobin and blood pressure at the beginning of the low protein diet period between the 10 patients who showed a significant response to the diet and the 9 who did not (Table 4.1). Both systolic and diasolic blood pressure levels tended to be higher in the non-responder group despite a higher proportion of these patients receiving antihypertensive therapy.

TABLE 4.1. CLINICAL PARAMETERS OF PATIENTS WHO HAD A SIGNIFICANT (RESPONDERS) AND NON-SIGNIFICANT (NON-RESPONDERS) RESPONSE TO THE LOW PROTEIN DIET.

	RESPONDERS N=10	NON-RESPONDERS N=9
Age (yr)	44 (12)	44 (11)
Duration of Diabetes (yr)	28 (11)	26 (7)
Duration of Proteinuria (yr)	5.6 (3.3)	6.5 (2.6)
Glomerular Filtration Rate ($ml/min/1.73m^2$)	50 (29)	46 (20)
Albumin Excretion Rate (mg/24hr)	389 (120-3467)	380 (132-5754)
Glycosylated Haemoglobin (%)	8.9 (1.6)	8.6 (1.4)
Systolic Blood Pressure (mmHg)	137 (20)	144 (13)
Diastolic Blood Pressure (mmHg)	77 (12)	84 (11)
Blood Pressure Therapy	6 (60%)	8 (89%)
Duration of NPD (mo.)	28 (10)	31 (8)
Duration of LPD (mo.)	35 (7)	30 (9)

Data are mean (SD) except for Albumin Excretion Rate which is Geometeric mean and range. All data relate to the start of the low protein diet period.

CHANGES ON LOW PROTEIN DIET

DIETARY CHANGES

An obvious reason for certain patients to show a response to the low protein diet while others did not would be lack of, or reduced, compliance with the diet. Table 4.2 gives data comparing the achieved protein intake on LPD using the 2 methods of assessment (weighed food records and urinary urea excretion). There were no differences between the groups whether the total achieved protein intake was considered or the intake corrected for body weight (Table 4.2). Table 4.3 details the

amount of protein reduction in g/day and g/Kg for each patient on NPD and LPD. These values are calculated from subtracting the protein intake on LPD from that on NPD and data are given for both methods of estimation of protein intake. Although the responders tended to reduce their protein intake more on the low protein diet when expressed as a gram reduction per kg body weight using either method of estimation, these differences failed to reach statistical significance (from weighed food records responders 0.55 (0.27) vs. non-responders 0.43 (0.18) g/Kg from urinary urea 0.46 (0.30) vs. 0.35 (0.11) g/Kg).

Dietary phosphate intake was not different when responders were compared to non-responders. The change in serum phosphate during LPD was on average -0.03 (0.06) mmol/l in the responders and -0.09 (0.12) mmol/l in the non-responders (NS).

Energy intake was not different between responders and non-responders (data not shown).

TABLE 4.2. ACHIEVED REDUCTION IN THE AMOUNT OF PROTEIN INGESTED ON LPD. Data are expressed for urinary urea estimations and weighed food records in grams per day and grams per kg per day (parentheses). Patients are divided into responders and non-responders.

Patient No.	RESPONDERS		NON-RESPONDERS	
	Protein intake from		Protein intake from	
	Urinary Urea	Weighed Food Records	Urinary Urea	Weighed Food Records
1	59 (0.77)	44 (0.57)		
2	36 (0.78)	41 (0.90)		
3	74 (0.88)	52 (0.62)		
4			45 (0.61)	60 (0.82)
5	46 (0.68)	48 (0.71)		
6	48 (0.73)	46 (0.70)		
7	61 (0.90)	44 (0.65)		
8			55 (0.79)	50 (0.72)
9			55 (0.69)	43 (0.54)
10	37 (0.46)	52 (0.65)		
11			32 (0.50)	41 (0.63)
12	38 (0.66)	38 (0.66)		
13			37 (0.68)	34 (0.63)
14			58 (0.74)	45 (0.57)
15	55 (0.94)	42 (0.72)		
16			67 (1.15)	54 (0.92)
17			52 (0.65)	42 (0.52)
18	64 (0.79)	38 (0.46)		
19			66 (0.82)	42 (0.52)
Mean	52 (0.76)	44 (0.66)	52 (0.74)	47 (0.66)
SD	13 (0.14)	5 (0.11)	12 (0.18)	8 (0.13)

TABLE 4.3. REDUCTION IN THE AMOUNT OF PROTEIN INGESTED ON THE LOW PROTEIN DIET COMPARED TO THAT INGESTED ON THE NORMAL PROTEIN DIET. Data are expressed for urinary urea estimations and weighed food records in grams per day and grams per kg per day (parentheses). Patients are divided into responders and non-responders.

Patient No.	RESPONDERS		NON-RESPONDERS	
	Protein intake from		Protein intake from	
	Urinary Urea	Weighed Food Records	Urinary Urea	Weighed Food Records
1	18 (0.18)	8 (0.08)		
2	13 (0.27)	26 (0.54)		
3	56 (0.60)	62 (0.66)		
4			17 (0.23)	12 (0.16)
5	76 (1.1)	76 (1.10)		
6	53 (0.77)	41 (0.59)		
7	28 (0.42)	49 (0.73)		
8			29 (0.41)	53 (0.75)
9			40 (0.49)	50 (0.62)
10	36 (0.43)	42 (0.50)		
11			13 (0.20)	14 (0.22)
12	35 (0.54)	26 (0.41)		
13			27 (0.46)	25 (0.43)
14			29 (0.36)	32 (0.40)
15	4 (0.06)	12 (0.18)		
16			10 (0.17)	18 (0.31)
17			36 (0.45)	32 (0.40)
18	15 (0.19)	56 (0.71)		
19			32 (0.38)	48 (0.57)
Mean	33 (0.46)	40 (0.55)	26 (0.35)	32 (0.43)
SD	21 (0.30)	21 (0.27)	10 (0.11)	15 (0.18)

WEIGHT CHANGES

Patients tended to lose weight on the low protein diet and this was more marked in the responders (-5.05 (7.04) Kg) than the non-responders (-1.01 (2.25) Kg, p=0.079). The 2 patients who who underwent lower limb amputations on LPD were both in the responder group and as these 2 patients lost weight due to the operation and the period of illness associated with these events (9 and 5 Kg) a large proportion of the difference was accounted for by these patients.

BLOOD PRESSURE CHANGES

Mean decreases in blood pressure on LPD tended to be greater in responders for diastolic and mean blood pressure. In contrast systolic blood pressure tended to fall more in non-responders but none of these differences achieved statistical significance (Table 4.4).

TABLE 4.4. CHANGES IN MEAN SYSTOLIC, DIASTOLIC AND MEAN BLOOD PRESSURE VALUES ON THE LOW PROTEIN DIET IN PATIENTS WHO RESPONDED TO THE DIET (RESPONDERS) AND NON-RESPONDERS.
THE DELTA (Δ) VALUES ARE FOR ALL VALUES ON NORMAL DIET MINUS ALL VALUES ON LOW PROTEIN DIET. VALUES ARE MEAN (SD).

	RESPONDERS	NON-RESPONDERS	P
ΔSystolic BP mmHg	3.6 (12)	9.5 (7)	NS
ΔDiastolic BP mmHg	7.4 (7.8)	4.6 (3.3)	NS
ΔMean BP mmHg	5.9 (6.0)	3.8 (4.4)	NS

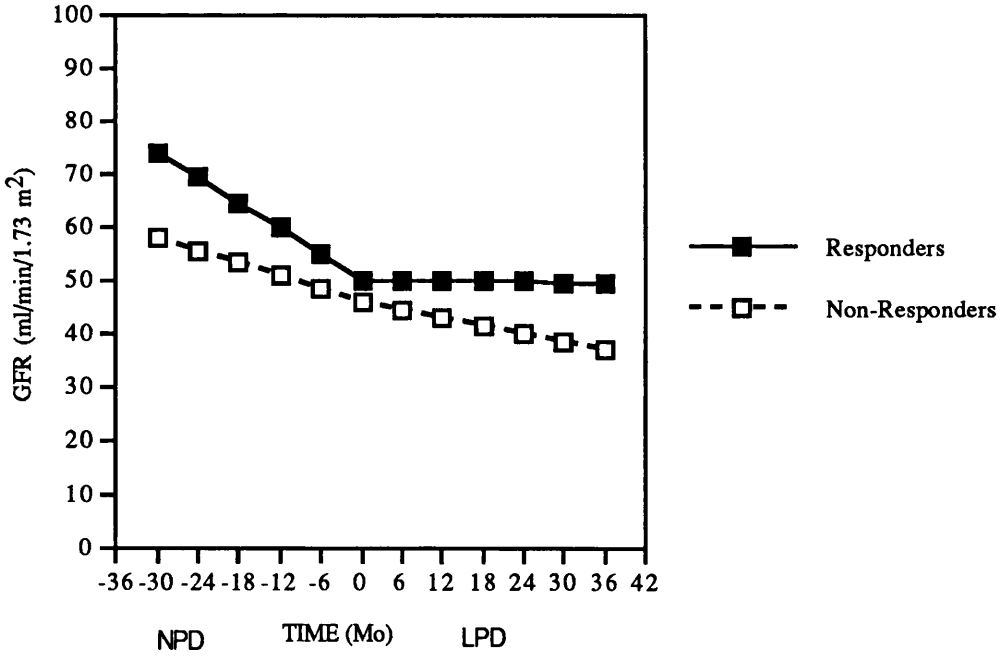
RENAL CHANGES

Responders would be expected to have had a more pronounced change in their rate of change of GFR compared to non-responders and the magnitude of this difference is seen in Table 4.5. Responders tended to have a non-significantly faster rate of decline of GFR on NPD compared to non-responders (Figure 4.1).

TABLE 4.5. CHANGES IN GLOMERULAR FILTRATION RATE ON NORMAL AND LOW PROTEIN DIETS IN RESPONDERS (R) AND NON-RESPONDER (NR) CORRECTED FOR EFFECTS OF BLOOD PRESSURE AND GLYCAEMIA. DATA ARE MEAN (SD).

	GFR		GFR + BP		GFR + HbA1	
	R	NR	R	NR	R	NR
NPD	-0.80	-0.39	-0.80	-0.33	-0.92	-0.34
	(0.76)	(0.27)	(0.74)	(0.30)	(0.66)	(0.34)
LPD	-0.02	-0.26	-0.03	-0.30	-0.11	-0.26
	(0.36)	(0.35)	(0.41)	(0.35)	(0.35)	(0.44)
p	0.0003	0.34	0.0001	0.73	0.0001	0.56

Figure 4.1 - GFR decline in Responders and Non-Responders on NPD and LPD.



Urinary albumin excretion and the fractional clearance of albumin were no different between responders and non responders throughout LPD and the change in these parameters in the first 6 and 12 months on LPD were also similar in responders and non responders (data not shown).

LIPOPROTEIN CHANGES

No differences were seen between responders and non responders for any of the lipoprotein sub-fractions measured (data not shown).

CHAPTER 5

A COMPARISON OF THE PLASMA CLEARANCE OF ^{51}CrEDTA AND CREATININE CLEARANCE FOR THE ESTIMATION OF GLOMERULAR FILTRATION RATE

INTRODUCTION

Creatinine clearance has been used as an estimate of GFR for many decades and although recognized to be a less-than-ideal measure of glomerular filtration it has been used in a number of studies which have tested the effect of various therapeutic manoeuvres on the rate of decline of renal function over time (Shemesh et al. 1985, Payne 1986, Levey 1990, Klahr 1991, Levey et al. 1991, Fouque et al. 1992). Creatinine is not a perfect filtration marker for a number of reasons (Perrone et al. 1992). Firstly since creatinine is formed as a result of the dehydration of creatine production depends on the creatine pool which in turn is largely determined by muscle mass and the dietary intake of creatine ingested in meat. Dietary creatine intake has been shown to affect the creatine pool and urinary creatinine excretion independent of nitrogen balance and muscle mass (Crim et al. 1975, 1976). Meat is also the major dietary source of creatinine thereby providing a further mechanism whereby meat feeding leads to an increase in urinary creatinine excretion (Perrone et al. 1992) (Figure 3.3, page 91, Figure 3.6, page 105). Secondly renal tubular secretion of creatinine has long been recognized (Rehberg 1926, Shemesh et al. 1985) and blocking this secretion by the administration of oral cimetidine at appropriate doses lowers the ratio of creatinine to inulin clearances to approach unity (Van Acker et al. 1992). Finally for the calculation of a clearance an accurate, timed urine collection is necessary. These are often inconvenient for the

patient and prone to error in terms of completeness and accuracy in recording start and finish times.

Measurement of GFR using exogenous markers such as inulin, ^{125}I-iothalamate, ^{99}Tc-DTPA or ^{51}CrEDTA gives a truer estimate (Klahr 1991) and by measuring the plasma disappearance of an isotope abolishes the need for a 24 hour urine collection.

AIM OF THE STUDY

To examine the relationship between creatinine clearance and the clearance of ^{51}CrEDTA from plasma as estimates of the GFR in patients with diabetic nephropathy while on a normal and subsequently a low protein diet in an attempt to quantitate the accuracy and precision of these measurements.

METHODS

All 19 patients involved in the low protein diet study were used. The 24 hour urine collections used for measurement of urinary creatinine were normally commenced on the morning prior to the patient's admission for measurement of GFR by the clearance of ^{51}CrEDTA. Patients finished the collection at about the time the ^{51}CrEDTA was injected and the creatinine clearance measurement was in all cases within eight hours of the ^{51}CrEDTA clearance. All patients had collected repeated 24 hour collections of urine and were fully versed in the method of collection. Patients wrote the times of the start and finish of the collection on the urine bottle provided. In all cases creatinine and ^{51}CrEDTA clearances were corrected for 1.73m^2 body surface area. The mean (SD) number of simultaneous comparisons per patient were 3.5 (1.6) on NPD and 5.1 (1.6) on LPD.

STATISTICAL METHODS

Bland-Altman plots were constructed in which the difference between the 2 methods was plotted against the mean value of the 2 methods (Bland and Altman 1986). Pearson correlation coefficients were calculated for the two methods on different diets and the Durbin-Watson statistic was calculated to determine whether the level of clearances affected the correlation between them (Durbin and Watson 1971). Data are expressed as mean and standard deviations unless otherwise stated.

RESULTS

There was a good correlation between the two methods using Pearson correlation with an r value of 0.77 ($p<0.001$) for NPD and 0.85 for LPD ($p<0.0001$).

During NPD the mean glomerular filtration rate estimated from the clearance of ^{51}CrEDTA was 56.4 (24.1) ml/min/1.73 m^2 and from the creatinine clearance 62.2 (25.8) ml/min/1.73m^2 giving a mean difference between the methods of 5.8 ml/min/1.73m^2 and a standard deviation of the mean differences 17.0 ml/min/1.73m^2 as shown in Figure 5.1. During LPD the mean ^{51}CrEDTA clearance was 42.9 (22.7) ml/min/1.73m^2 and the mean creatinine clearance 48.2 (20.8) ml/min/1.73m^2 giving a mean difference of 5.3 ml/min/1.73m^2 with a standard deviation 12.1 (Figure 5.2).

On both the normal and low protein diets the creatinine clearance on average overestimated the glomerular filtration rate (showed bias) and there were wide limits of agreements (standard deviations) between the two methods. The limits of agreement were 40% better on the low protein diet compared to the normal protein diet.

The amount of difference between the two methods varied in relation to the clearance levels (Figures 5.1 and 5.2) with a greater disparity

between the two methods at higher clearance levels (Durbin-Watson statistic = 1.42 for NPD and 1.20 for LPD; $p<0.01$ for both).

Fig 5.1 Bland-Altman Plot of Creatinine Clearance and Plasma Clearance of 51 Cr EDTA

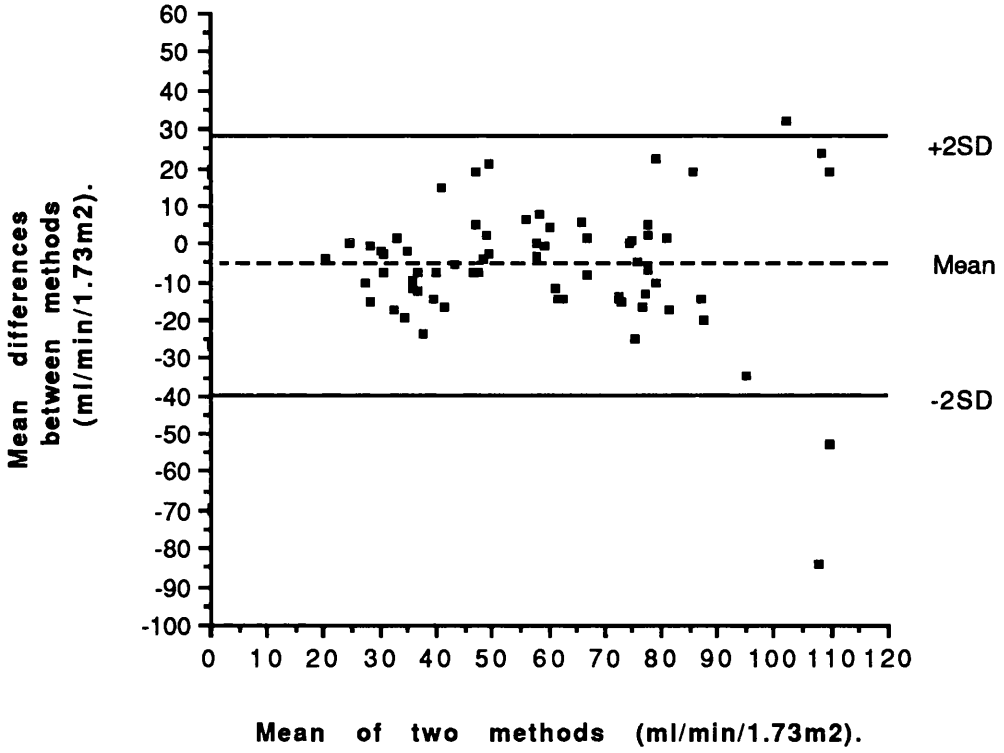


Fig 5.2. Bland-Altman Plot of Creatinine Clearance and Plasma Clearance of 51Cr EDTA

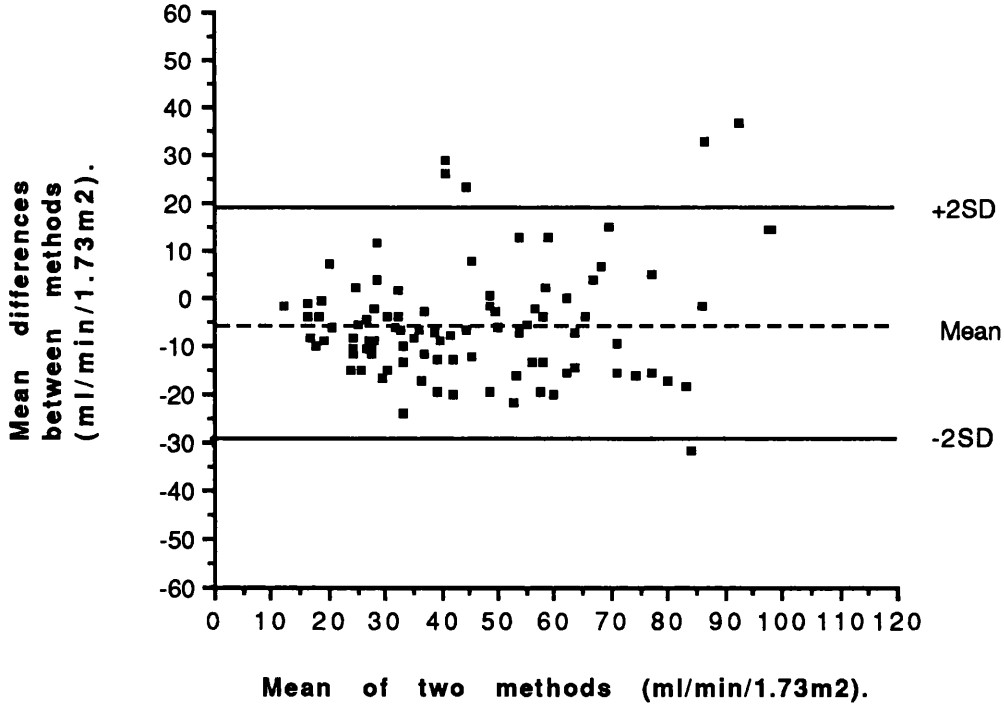


Figure 5.1, 5.2. Bland-Altman plots for NPD (5.1) and LPD (5.2).

CHAPTER 6
DISCUSSION OF THE FINDINGS

SUMMARY OF RESULTS

The main findings of the study may be summarised as follows:

1. A period of up to 49 months on LPD is feasible in a cohort of insulin-dependent diabetic patients with diabetic nephropathy.

2. A substantial reduction in protein intake (on average 43%) is achievable.

3. No untoward nutritional effects were detectable after a mean follow-up of 33 months despite an initial reduction in energy intake and weight.

4. The rate of decline of GFR was slowed by an average of 0.47 ml/min/mo. on LPD. At commencement of LPD the mean GFR was 46 ml/min and had the decline seen on NPD continued (0.61 ml/min/mo.) it would have taken a mean 75 months for patients to have needed renal replacement therapy. On LPD the GFR decline was 0.14 ml/min/mo. and if this slowing remained constant 328 months would elapse until patients required renal replacement therapy.

5. The GFR response to LPD was heterogenous with just over half the patients showing a statistically significant response. No factors differentiated the responders from the non-responders.

6. The rise in albuminuria was halted on LPD.

7. Serum lipoproteins were modified on LPD in a manner likely to confer benefit.

8. Creatinine clearance cannot be recommended as a measure for GFR on either NPD or LPD as it is imprecise, with wide limits of agreement, and inaccurate, having bias, compared to the plasma clearance of ^{51}CrEDTA.

CRITICAL DISCUSSION OF THE STUDY

STUDY DESIGN

Using patients as their own controls is justifiable as the decline in GFR in the range studied is believed to be linear over time (Mogensen 1976, Jones et al. 1982, Parving et al. 1981, 1987, Viberti et al. 1983, Jones et al. 1989). The high degree of linearity in the rate of decline of GFR on both diets supports this as does data from the historical control group. The historical control group was retrospectively selected in a non-random fashion. Given the disadvantages of this selection a comparison of the rate of decline of GFR in this cohort with that on LPD serves to further illustrate the deviation from the expected rate of decline associated with the dietary intervention (Figure 3.10, page 114).

A strength of the study design was the method used for the measurement of GFR. Data from the study comparing the creatinine clearance with the clearance of ^{51}CrEDTA (Chapter 5) reveals that creatinine clearance overestimates the clearance of ^{51}CrEDTA. On average, this overestimate was similar on both normal and low protein diets, however, the precision of creatinine clearance compared to the clearance of ^{51}CrEDTA was higher on LPD. This is likely to be due in part to the more uniform food intake, especially protein, whilst on LPD. The amount of difference between the two methods has a relation to the actual clearance levels with the differences being greater with higher clearances.

CREATININE AND 51CrEDTA CLEARANCES

Creatinine clearance has traditionally been the method used to estimate GFR in clinical practice, however, it is generally accepted that measurement of this parameter using exogenous markers such as inulin, ^{125}I-iothalamate, ^{99}Tc-DTPA or ^{51}CrEDTA gives a truer estimate (Klahr 1991). Creatinine is a less than ideal filtration marker, since it is secreted by the tubules in addition to being filtered by the glomerulus (Levey 1990).

Creatinine clearance tends to over-estimate glomerular filtration rate in normal individuals for this reason, and as renal function deteriorates and serum creatinine rises there is a greater tubular secretion of creatinine, making this measure of glomerular filtration rate less precise (Payne 1986, Shemesh et al. 1985). Additionally, serum creatinine is affected by muscle mass and the intake of meat, which has important consequences when using this measure of renal function in studies involving changes in diet (Crim et al. 1975, 1976, Payne 1986, Levey 1990). This is reinforced by data in the present study (Figure 3.6, page 105).

The exogenous compound used for the estimation of glomerular filtration salt in the study was ^{51}Cr-ethylene diamine tetraacetic acid (^{51}CrEDTA). This substance is not metabolised and is excreted only by the kidneys. In the sheep the finding that the renal clearance of ^{51}CrEDTA was similar to the clearance of inulin (Stacy and Thorburn 1966) prompted its use in man by Chantler (Chantler et al. 1969). A high degree of correlation was found between the clearance of ^{51}CrEDTA and that of inulin. In addition, the clearances calculated using the classical method (clearance = UV/P) and clearances calculated from the volume of distribution and the decay of the plasma activity of ^{51}CrEDTA were described and a good correlation was found between these two methods. An overall correction factor was derived, assuming a single compartment model, the use of venous blood and a consistent slight difference between the standard clearances of inulin and of ^{51}CrEDTA, enabling the plasma clearance of ^{51}CrEDTA to be used as precise and accurate measure of GFR.

The work of Chantler was confirmed by Bröchner-Mortensen (Bröchner-Mortensen et al. 1969, Bröchner-Mortensen 1972, Bröchner-Mortensen and Rodbro 1976, 1976a), who employed a different method to estimate clearance of ^{51}CrEDTA from the plasma disappearance after a single injection and a measure of plasma volume. Chantler calculated the

$T_{1/2}$ of the isotope disappearance from the plasma from a graph of the plasma activity versus time using the linear part of the graph. The volume of distribution of the isotope was calculated by plotting the logarithm of the plasma activity as a function of time and extrapolating the linear part of the curve back to zero in order to calculate the effective initial activity of the plasma at zero time (P_0). The apparent volume of distribution was given by dividing the activity of a given standard at a given dilution by P_0. The plasma clearance was then calculated from plasma clearance constant multiplied by the volume of distribution. Bröchner-Mortensen used the area under the clearance curve and T-1824 (Evans blue dye) as a measure of the volume of distribution to calculate clearance. His studies in 17 normal subjects also revealed a high degree of correlation when compared to inulin clearances. In both studies the curve of plasma activity versus time approximates to a near exponential slope after 100-120 minutes post injection. Samples were collected up to 5 hours.

^{51}CrEDTA has a number of favourable features which make it most reliable as a tracer for the assessment of glomerular filtration rate. It agrees well with inulin clearance. It is relatively inexpensive (£50 per 10mls = 3mBq). The EDTA chelate is easily prepared and the γ-radiation emitted form the source is measurable on standard equipment. Due to the low radiation dose (3mBq = 81μCi) it may be safely used in children and repeated estimates can be made in a single patient. The extra renal clearance of ^{51}CrEDTA is approximately 4ml/min (Bröchner-Mortensen and Rodbro, 1976b). The day-to-day variation (coefficient of variation) of a single determination of glomerular filtration rate by the clearance of ^{51}CrEDTA is in the order of 2-10% when the glomerular filtration rate is above 30ml/min and 2-15% when less than 30ml/min. The values for creatinine clearance are 4-60% and 4-36% respectively (Bröchner-Mortensen and Rodbro 1976). In the protocol used plasma samples were

drawn at 2, 3, 4, 6, and 8 hours after the injection of the isotope. The optimum mean time for sampling has been quoted as 2 hours (Bröchner-Mortensen and Rodbro 1976a). The later samples were collected to ensure a true estimate of the monoexponential part of the decay curve.

Given that the plasma clearance of ^{51}CrEDTA provides a reliable assessment of GFR the data presented in Chapter 5 adds to the considerable literature that support the view that creatinine clearance is a poor measure of the glomerular filtration rate, especially in the context of dietary changes and in both the clinical and experimental settings isotopic clearance methods should be employed in the measurement of this important renal parameter.

DIETARY PRESCRIPTION, ASSESSMENT AND COMPLIANCE

A value of 40 g of protein a day was selected for the level of protein restriction. Protein intakes below 0.6 g/Kg/day have been associated with protein malnutrition (Lucas et al. 1986, Goodship and Mitch 1988) and the aim of the dietary prescription was to reduce protein intake to a non-harmful level, to a level which was likely to be achievable by the majority of patients and was likely to have a therapeutic effect. In so doing the proposed hypothesis could be tested. There were no specific restrictions on the dietary intake of phosphate, sodium and fat although these all fell pari-passu with the protein reduction, which was primarily a reduction in animal protein. Despite the increase in carbohydrate energy levels were not equal on NPD and LPD and patients initially lost weight. After 12 months on LPD weight plateaued and the stable mid-arm muscle circumference and elevation in plasma albumin are reassuring objective measurement that LPD caused no significant harmful nutritional effects. Mean energy intake expressed as kcal per Kg was similar on NPD and LPD. An initial reduction in energy intake and fitness was seen in 25 patients with moderate CRF on a 0.6 g protein/Kg diet, however after 3 months

aerobic fitness improved and perceived energy and emotional reaction improved significantly (Hart et al. 1992). In the present study no patients complained of decreased energy levels and the diet was considered acceptable in a number of respects with the exception of cooking with low protein foods (Table 3.14, page 115).

Two methods were used to estimate dietary protein intake - weighed food records, which also provided an estimate of other nutrient intakes, and urinary urea excretion. Recently the accuracy of weighed dietary food records has been questioned (Livingstone et al. 1990). In a study of 31 free living adults, energy intake was measured by seven day weighed records and concurrently using the double labelled water technique (Livingstone et al. 1990). The method employed for the weighed food record was very similar to that employed in the present study. The double labelled water method ($^2H_2{}^{18}O$) assesses the rate of production of carbon dioxide by measuring the differential disappearance of the stable isotopes deuterium and oxygen-18 after subjects have been given an oral loading dose of labelled water. Total energy expenditure is calculated from carbon dioxide production using classic respirometry quotient formulae. This carefully conducted study revealed serious discrepancies between estimates of energy intake (weighed food records) and expenditure (doubly labelled water technique) in the order of 19% for males and 18% for females with energy intake being lower than expenditure. As the measure of energy expenditure is thought to have only a 5% degree of bias, it seems likely that weighed food records under-reported habitual food and fluid intake either for conscious or sub-conscious reasons. These differences were most marked in those subjects in the lower percentiles of energy intake. In the present study any bias in weighed food records would be expected to be similar on both NPD and LPD and if values were not accurate they would be precise.

A discrepancy was found between the estimation of dietary protein intake from weighed food records and the estimate derived from urinary urea nitrogen in the present study (Table 3.1, page 86, Figures 3.1, 3.2, pages 87, 88). This was most marked during the low protein part of the study when protein intake from weighed food records were lower than levels calculated from urinary urea nitrogen. Compliance has been reported as a problem in 2 recently reported studies of LPD, one involving diabetic patients (Locatelli et al. 1991, Dullaart et al. 1993). In the present study all patients reduced their protein intake and although only 4 patients achieved intakes of less than 40 g of protein a day on LPD (Table 4.2, page 121) the mean reduction to 0.66 g/Kg/day suggests good compliance.

HETEROGENEITY OF RENAL RESPONSE

A new finding in this study was the clearly quantified heterogeneity of GFR response to LPD (Chapter 4). Surprisingly no factors could be identified to distinguish the responders from the non-responders either at commencement or during LPD. Small differences between these 2 groups were present, such as a slightly greater dietary protein reduction in responders, but these were non-statistically significant differences and unlikely to explain the marked difference in the GFR response to LPD. An interesting observation was the non-significantly faster fall in the decline of GFR on NPD in the responders (Table 4.5, page 124, Figure 4.1, page 124). This may have made a response to LPD easier to detect however the slowing of GFR decline to -0.02 ml/min/mo. on LPD in responders was so marked that had the non-responders slowed to this level a statistically significant response would have been observed in this group (-0.39 (0.27) NPD vs. -0.02 (0.36) LPD, $p<0.005$). Reductions in urinary albumin excretion often accompany improvements in GFR, or at least a reduced rate of loss (Mogensen 1982, Parving et al. 1987, Zeller et al. 1991), yet in the present study levels of albuminuria were similar during LPD in

responders and non-responders and the change in the fractional clearance of albumin during the first year of LPD did not predict response.

CONFOUNDING EFFECTS OF GLYCAEMIC AND BLOOD PRESSURE CHANGES

Both blood glucose and blood pressure changes on LPD could have been associated with the change in the rate of GFR decline. Previous data would suggest that changes in glycaemia would exert a small effect on this change (Viberti et al. 1983a, Bending et al. 1986) whereas an effect of blood pressure is more likely (Mogensen 1982, Parving et al. 1981, 1987, Björck et al. 1990, 1992). Changes in glycaemia and blood pressure were adjusted for in the linear regression by adding these parameters as covariables and recalculating the rates of decline on the 2 diets (Table 3.8, page 103). The uncorrected difference between the 2 diets was 0.47 ml/min/mo., the difference after adjustment for glycaemic changes 0.46 ml/min/mo. and after adjustment for blood pressure changes 0.42 ml/min/mo. Thus as a percentage of the uncorrected difference, blood pressure reduction accounted for 11% of the effect on GFR during LPD. The difference in glycosylated haemoglobin levels between the 2 diets was very small (0.1%) (Table 3.5, page 96) and mean blood pressure was only 4 mmHg lower on LPD (Table 3.4, page 95). Levels of blood pressure in a previous study of proteinuric patients, using the same design as that employed in the present study and using antihypertensive therapy as an intervention, were markedly higher during a run-in period than those observed on NPD (mean blood pressure, 111 vs. 106 mmHg on NPD in present study) (Parving et al. 1987). After the initiation of antihypertensive therapy mean blood presure fell by 12 mmHg to 99 mmHg in the study reported by Parving compared to the 4 mmHg reduction during LPD in the present study. The reduction in the rate of GFR decline associated with this large fall in blood pressure was similar to that effected by LPD. The assumption

has been made that the effects of blood pressure reduction and dietary protein reduction are additive. Evidence for an additive effect on reduction in proteinuria is provided by a short-term study of 17 patients with proteinuria due to a variety of non-diabetic renal diseases (Ruilope et al. 1992). Addition of enalapril to LPD resulted in a further decrease in proteinuria and a reversal of some acute renal haemodynamic changes associated with LPD which include a reduction in GFR and renal plasma flow (RPF) (Klahr and Alleyne 1973). Similar findings have been reported in normal rats and lead to the suggestion that intrarenal angiotensin II (AII) may mediate some of the intrarenal haemodynamic changes associated with LPD (Fernandez-Repollet et al. 1987). Whether the long-term the effects of blood pressure and dietary protein intake reduction on GFR decline are additive is not determined but assumed.

LIPID CHANGES

Insulin-dependent diabetic patients with proteinuria have a markedly increased cardiovascular mortality compared to similar non-proteinuric patients and an atherogenic lipoprotein profile associated with the proteinuric state has been proposed as a possible mediator (Borch-Johnsen and Kreiner 1987, Winocour et al. 1987, Brunner and Selwood 1992). In addition lipid nephrotoxicity has been hypothesised as a mechanism for chronic progressive glomerular and tubulo-interstitial disease (Moorhead et al. 1982). The clinical and experimental evidence for this hypothesis is scant and inconclusive. In unilateral nephrectomised rats 19 weeks of a high cholesterol diet was associated with more glomerulosclerosis and tubulo-interstitial damage than in rats fed chow (Kasiske et al. 1990) and micropuncture studies demonstrated increased intra-glomerular capillary pressure in the high cholesterol group. Rats fed a high linoleic acid diet for 5 weeks after a $1^3/4$ nephrectomy had lower blood pressure levels, higher GFRs and lower levels of proteinuria than

rats fed a low linoleic acid diet (Heifets et al. 1987). Glomerular lesions were more severe in the latter group. In contrast, insulin-dependent diabetic patients with microalbuminuria had a higher rate of rise of urinary albumin excretion (58%) after 2 years of a linoleic-acid-enriched diet compared to a group fed a usual diet (16%) (P/S ratio 0.96 vs. 0.56) (Dullaart et al. 1992). This well-conducted prospective controlled clinical trial argues against alterations in saturated fatty acids, cholesterol and the P/S ratio being associated with changes in glomerular permselectivity. Linoleic acid has been a subject of interest as a high linoleic acid diet in normal subjects elevated creatinine clearance possibly through enhanced synthesis of vasodilatory prostaglandins (Adam and Wolfram 1984).

In the present study mean levels of total cholesterol and triglyceride during NPD were similar to value from a sample of meat-eating UK subjects and lower than levels reported by Winocour who studied a similar number of proteinuric insulin-dependent patients with better preserved renal function (Thorogood et al. 1987, Winocour et al. 1987). No obvious explanation for this discrepancy can be given. Even with relatively normal lipoprotein levels on NPD the significant reduction in LDL-cholesterol on LPD and the fall in total cholestrol are encouraging and welcome (Table 3.12, page 111). Not so encouraging is the observation of a small fall in HDL-cholesterol and rise in total triglyceride. Overall the influence of LPD on serum lipoproteins is likely to be modest and the clinical implications unclear. A large prospective controlled trial would be necessary to answer such questions.

DIETARY PROTEIN AND RENAL FUNCTION

IN NORMAL HUMANS

Vegans who eat less total protein than omnivores (0.95 vs. 1.29 g/kg/day) and 100% of their protein intake is in the form of vegetable

protein, have glomerular filtration rates that are 11% lower than matched omnivores (Wiseman et al. 1987). In addition, urinary albumin excretion and blood pressure levels are lower (Wiseman et al. 1987). A similar effect of diet on blood pressure was observed during a 6 week period of a lacto-ovo-vegetarian diet in 59 healthy habitually omnivorous subjects (Rouse et al. 1983). Mean systolic blood pressure fell by 6 mmHg and diastolic by 3 mmHg independent of changes in weight and sodium or potassium intake. The lower blood pressure seen on LPD in the present study may, in part, been mediated by undefined effects of protein reduction on blood pressure. In normal humans consuming a usual diet GFR increases by 7-18% and urinary albumin excretion by 100-300% in response to a meat meal of 80g of protein as lean cooked beef (Viberti et al. 1987a, Fioretto et al. 1990). In contrast a 3 week period of low protein diet (43 g/day) causes a 14% reduction in the baseline GFR, a 9% reduction in renal plasma flow (RPF) and a 50% reduction in the urinary albumin excretion rate (Viberti et al. 1987a).

IN DIABETIC PATIENTS

Normoalbuminuric insulin-dependent patients completing 3 weeks of LPD (45 g/day) had a reduction in GFR with no difference in RPF and thus a reduction in filtration fraction (FF) (FF=GFR/RPF) (Wiseman et al. 1987a). Glycaemic control and blood pressure levels were unchanged while the fractional clearance of albumin was lower on LPD. A larger study involving 35 normoalbuminuric insulin-dependent diabetic patients investigated the response to a $100g/1.73m^2$ protein load in the form of a meat meal (Fioretto et al. 1990). The area under the glomerular filtration rate curve rose more in normals than in the diabetic patients by a factor of 3.8. The impaired response of glomerular filtration rate to the meat meal in the diabetic patients was not due to differences in absorption of the meal since plasma levels of branched-chain amino acids were not

different between normals and diabetics. Possible mechanisms of the differing responses were investigated and glucagon-mediated increases in the vasodilatory prostaglandins, prostaglandin E_2 and 6-keto prostaglandin $F_{1\alpha}$ were found to be impaired in diabetics.

In diabetic patients with increased urinary albumin excretion short-term studies of reductions in dietary protein have revealed similar results. At the stage of microalbuminuria a reduction in GFR, urinary albumin excretion rate and fractional clearance of albumin was seen after 3 weeks of a low protein diet (47 g/day) (Cohen et al. 1987). These changes were independent of changes in glycaemia or blood pressure. In insulin-dependent diabetic patients with diabetic nephropathy 3 weeks of LPD was associated with an improvement in glomerular permselectivity while no differences were seen in renal haemodynamics (GFR, RPF, and FF) between the two diet periods (Rosenberg et al. 1987, Bending et al. 1988). The reabsorption rate of B_2 microglobulin was similar in both diet periods, suggesting that tubular function was not influenced by the different diets.

MEDIATORS OF RENAL EFFECTS OF DIETARY PROTEIN

In order to define some of the determinants of the change in glomerular filtration rate in response to different dietary protein intakes, Krishna and colleagues administered a 1g of protein/kg body weight, as beef steak, to 9 healthy males (Krishna et al. 1988). The renal haemodynamic studies were repeated on three separate occasions after pre-treatment with either placebo, indomethacin (to inhibit renal prostaglandin synthesis) or enalapril (to inhibit angiotensin II synthesis). Following placebo, GFR increased by 29% with an accompanying increase in RPF and a fall in renal vascular resistance (RVR). Pretreatment with indomethacin attenuated the rise in the GFR (12% rise) whereas treatment with enalapril was not different to placebo. Urinary excretion rates of

prostaglandin E_2 fell significantly in the indomethacin group, levels of plasma renin activity were increased in the enalapril group while plasma noradrenaline and adrenaline were unchanged in all groups. From these data it appears that the protein-mediated elevation in glomerular filtration is in part associated with prostaglandins. In diabetics the attenuated glucagon response to a protein challenge may mediate the reduced prostaglandin effect (Fioretto et al. 1990).

The effects of similar amounts of animal and vegetable protein ingestion on renal haemodynamics were investigated in a short-term study of 10 normal males (Kontessis et al. 1990). GFR, RPF and the fractional clearance of albumin were all lower on the vegetable diet while RVR was higher compared to the animal protein diet. In a separate experiment, seven normal subjects were given an 80g protein load of animal protein (lean cooked beef) and subsequently 80g of diluted soya powder (vegetable protein). While an elevation in GFR and RPF and a reduction in RVR was seen after animal protein no changes occurred after soya. The incremental glucagon area was greater for meat than soya and whereas the vasodilatory prostaglandin 6-keto $PGF_{1\alpha}$ rose significantly after the animal protein, it did not change after soya challenge. From these data, it appears that the same quantity of vegetable protein causes different renal effects as compared to animal protein, and that this difference is associated with a smaller glucagon and vasodilatory prostaglandin response. These hormonal mediators have been also implicated from a study in which infusion of somatostatin dimished the renal response to amino-acid infusion (Castellino et al. 1988).

Elevated levels of plasma renin activity on high protein diets has also been observed in man and experimental animals (Rosenberg et al. 1987, Paller and Hostetter 1986). This difference could not be explained in terms of differences in sodium or potassium intakes, which were identical

between the two diets. As prostaglandins are known to mediate renin release (Oates et al. 1979, Paller and Hostetter 1986), the elevated urinary prostaglandins may have caused the elevated renin levels. Although there were no changes in mean arterial pressure between the two diet periods the elevated renin levels may have resulted in increased levels of angiotensin II to cause constriction of both the afferent and efferent glomerular arterioles. The role of angiotensin II in the physiological and pathophysiological response to low protein feeding is likely to be important since in the rat captopril reverses the reduced GFR and RPF and increased renal vascular resistance seen with LPD (Fernandez-Repollet et al. 1987).

Other mediators in addition to glucagon, prostaglandin and the renin/angiotensin/aldosterone system may act in the renal response to dietary protein. Recently increased levels of mRNA for PDGF-A and -B chain and TGF-β have been shown to correlate with glomerulosclerosis in a rat model (Fukui et al. 1993). LPD reduced the prevalence of glomerulosclerosis and attenuated the abnormally high expression of the PDGF-A and -B chain and TGF-β genes. This data suggest that growth factors may play a role in the development of glomerulosclerosis and can be modulated by LPD.

CONCLUSIONS AND IMPLICATIONS

There is now enough evidence to strongly support the hypothesis that a reduction in the dietary intake of protein retards the rate of decline of renal functional loss in cases of established diabetic nephropathy in patients with insulin-dependent diabetes mellitus. Subjecting a patient to this dietary regime should not be undertaken lightly and needs full co-operation of the patient and full involvement of a nutritionist who is experienced in this field. The data presented demonstrates that the GFR

response to LPD is heterogeneous thus making it is vital to assess whether an individual is benefiting from the intervention. Patients should have a run-in period on their usual diet and therapy with 3 isotopic measurement of GFR to establish a baseline rate of decline against which the response to LPD can be compared. Protein restriction of 0.66g/Kg/day had no untoward effects in this study.

Addendum

Limitations of Study Design

This was not a controlled study and the effect of the intervention has to be viewed with this limitation in mind. As detailed on pages 46 and 47 the study design employed is of value in patients with diabetic nephropathy. If a control group had been studied, and patient numbers precluded this, it would have been necessary to match patients and controls for the rate of decline of GFR before the intervention of LPD. This crucial matching parameter was not performed in the only long-term controlled trial in patients with diabetic nephropathy (Zeller et al. 1991)

Compliance with LPD

The level of compliance with the LPD was high (Table 3.1, page 86). In contrast two recent studies have shown poor levels of compliance with a LPD (Locatelli et al. 1991, Dullaart et al. 1993) and due, in part, to patient selection a particularly compliant population of patients may have been recruited in the present study. This does not detract from the conclusions of the study but in routine clinical practice it may be more difficult to achieve the reduction in dietary protein than experienced in the reported study. The role of an experienced nutritionist in any dietary manipulation is crucial.

Historical Control Group

The 11 patients included in this group were not studied contemporaneously with those in the LPD study. However, the management of patients with diabetic nephropathy was relatively uniform during the late 1970s and 1980 and all parameters, including blood pressure levels were similar between the two cohorts (Table 2.13, page 82). The historical control group should not be viewed as a 'control' group for the LPD cohort but it serves to highlight further the effect of LPD on the rate of GFR decline.

'Responders' and 'Non-Responders'

It would have been satisfying to have identified a factor or factors that associated with a response or lack of response to LPD, and Chapter 4, page 117, addresses this issue.

No such factor was identified. Of the 4 patients who commenced captopril during the LPD period, 3 were in the responder group and of the 4 patients who started any hypotensive treatment during the LPD periods, 3 were in the responder group (Table 2.2, page 55 and Table 4.2, page 121). Mean blood pressure levels were non-significantly lower in the responder group during LPD (Table 4.4. page 123). Due to small numbers interpreting the clinical relevance of these non-significant differences is difficult. At present we are not in a position to know whether a few millimeters of mercury difference in blood pressure levels exerts a clinically useful renal effect. We are now at a point where studies of defined levels of blood pressure reduction should be instigated to determine whether there is a threshold below which further lowering a blood pressure exerts no additional reno-protective effect.

APPENDIX I

Name:.....

FEEDBACK FORM CONFIDENTIAL

se questions are designed to give us a clearer idea of your experience of the low protein diet and how it fects your daily life. Please circle the number on the scale of 1 to 5 which most accurately indicates how feel. There are no right or wrong answers, we are interested in your opinion.

at is your overall satisfaction with the way you are eating?	Dislike extremely	1	2	3	4	5	Like very much
ow often are you hungry?	Hungry often	1	2	3	4	5	Almost never
ow would you describe your appetite?	Poor	1	2	3	4	5	Excellent
n general, are you satisfied with the taste of the food you are eating?	Not satisfied	1	2	3	4	5	Very satisfied
n general, are you satisfied with the amount of food you are eating?	Not satisfied	1	2	3	4	5	Very satisfied
f you are not satisfied, is the amount?	Too much	1	2	3	4	5	Not enough
s the diet made your blood glucose control?	Much better	1	2	3	4	5	Much worse
ow different do you feel your eating pattern is compared to other people?	Not different at all	1	2	3	4	5	Very different
w do feel about other people knowing you are on a diet?	It bothers me quite a lot	1	2	3	4	5	I don't mind at all
other people seem bothered if you eat differently than they do?	They seem to be bothered quite a lot	1	2	3	4	5	They don't mind at all
es eating out in restaurants cause you difficulty?	It causes me a lot of difficulty	1	2	3	4	5	It's not difficult
es eating out at someone else's home cause you difficulty?	It causes me a lot of difficulty	1	2	3	4	5	It's not difficult
w much does now and what you eat interfere with other activities in your life?	It doesn't interfere at all	1	2	3	4	5	It interferes a lot
w much do you think what you eat affects your health?	It has no affect	1	2	3	4	5	It affects it a lot
you think the diet has made you feel?	Much better	1	2	3	4	5	Much worse
es your eating pattern affect your family?	Not at all	1	2	3	4	5	A great deal
difficult do you (or whoever does the shopping) nd choosing food when you are shoppping?	Very difficult	1	2	3	4	5	Very easy
difficult is it to plan and prepare your meals?	Very difficult	1	2	3	4	5	Very easy

9. Compared to your previous diet, how much time is spent in planning, shopping and preparing your meals?

Much less 1 2 3 4 5 Much more

10. Do you find choosing what to eat?

Very difficult 1 2 3 4 5 Very easy

11. Do you find the diet?

Easy to follow 1 2 3 4 5 Complicated

12. Do you find using the exchanges?

Very easy 1 2 3 4 5 Very difficult

13. Do other members of your household eat the same diet as you?

None of the time 1 2 3 4 5 All of the time

14. Do you find your current eating pattern?

Much more expensive 1 2 3 4 5 Much cheaper

15. What percentage of the time do you 'cheat' on the diet?

Less than 10% 11-20% 21-30% 31-40% More than 50% of the time

16. How do you enjoy eating now as compared to how you ate in the past

I liked my previous diet much better 1 2 3 4 5 I like my present diet much better

17. Are there any special food products which you use and enjoy?

If no, please go on to question 25. If yes, please list them here:

....

....

....

....

....

18. Are there any special food products you have tried but do not enjoy?

If not, please go on to question 26. If yes, please list them here:

....

....

....

....

....

19. Do you (or whoever does most of the cooking) find cooking with low protein foods?

Very easy 1 2 3 4 5 Very difficult

20. What do you find most difficult about following the diet?

.....

.....

21. Which foods do you miss normal amounts of most?

.....

.....

22. Are there any specific problems, additional comments or suggestions you would like to make about your current eating pattern or special foods?

.....

.....

.....

.....

THANKS VERY MUCH FOR YOUR HELP!

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