

THE KIDNEY IN DIABETES MELLITUS
URINARY TRANSFERRIN EXCRETION,
HYPERTENSION,
THE **RENIN-ANGIOTENSIN-ALDOSTERONE** SYSTEM,
AND THE ROLE OF
ANGIOTENSIN CONVERTING ENZYME INHIBITORS
IN THERAPY

by

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SYNOPSIS

Patients with diabetic renal disease develop elevated urinary albumin excretion rates [AER] and hypertension. Preliminary data from several groups suggest that diabetic patients handle transferrin, a protein similar in size and weight, in a different fashion from albumin. In the first part of this thesis I report the results of clinical studies of urinary transferrin excretion [TER] in diabetes mellitus.

More than **80%** type 1 [insulin dependent] diabetic patients have increased TER but less than 40% have increased AER. TER may be provoked by exercise in uncomplicated type 1 diabetes and the rise is proportionally far greater than that for AER. Newly diagnosed type 2 [non-insulin dependent] diabetic subjects have increased TER which falls with improved glycaemic control. Interventional studies with lisinopril, an angiotensin converting enzyme [ACE] inhibitor, in microalbuminuric and macroalbuminuric diabetic subjects show a reduction in TER independent of reduction in blood pressure. Data are presented suggesting a role for altered renal tubular function in TER in diabetes.

The second part of the thesis examines the role of the **Renin-Angiotensin-Aldosterone** system in the hypertension of diabetic renal disease. Patients with elevated AER have increased resting plasma renin activity. Those with uncomplicated diabetes show an exaggerated blood pressure response to exercise. ACE inhibition reduces blood pressure in hypertensive patients and AER in both hypertensive and normotensive patients.

DEDICATION

To my parents with love and thanks

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AIMS OF THE THESIS

part1.

1. To investigate the utility of measurement of urinary **transferrin** excretion as a marker for renal disease in diabetes mellitus. This aim to be achieved by a series of clinical studies:

i. To establish the prevalence of diabetic renal disease [defined by **the** presence of elevated urinary albumin excretion rate] in a defined clinic population.

ii. To establish the prevalence of elevated urinary **transferrin** excretion in the same population.

ii. To examine the influence of glycaemic control on transferrin excretion.

iv. To examine the influence of exercise on transferrin excretion.

v. To examine the influence of angiotensin converting enzyme [ACE] inhibition on transferrin excretion.

2. By means of these studies to offer an explanation for differences in behaviour between transferrin and albumin in diabetes.

part2.

1. To study the role of the **Renin-Angiotensin-Aldosterone** System in the hypertension of diabetic renal disease; at rest and in exercise.

2. To study the influence of angiotensin converting enzyme **[ACE]** inhibition on hypertension and the progression of renal disease in diabetes mellitus.

PERSONAL CONTRIBUTION TO THE THESIS

I personally conceived the idea for each of the **studies** described in this thesis.

Over the course of 12 months I examined every type 1 diabetic patient **from** the clinic at East Birmingham Hospital and organised urine collections from them to screen **for** diabetic nephropathy. I performed autonomic function testing on them all and analysed the results. This population was subsequently screened for transferrinuria.

I recruited and examined normal subjects from among the hospital staff who acted as controls for estimation of transferrin excretion.

I devised the study protocol and, with the help of Dr John Watson [Registrar], recruited and studied newly diagnosed type 2 diabetic patients referred to clinic. Each patient was examined and urine collections were made on three occasions.

I recruited and, with the help of Dr David Cavan [Registrar], exercise tested normal control and uncomplicated type 1 diabetic subjects.

I devised two major studies involving the use of angiotensin converting enzyme [ACE] inhibitors in diabetic nephropathy. These were both double blind and single centre. To date these remain the largest studies of their kind in the world. I performed all the measurements of renal blood flow and glomerular filtration myself - giving **the** radioisotopes and taking blood samples as necessary. This

also involved the organisation of two timetables to **coordinate** trial visits for both studies, the collection of data, and maintenance of the trial record books.

With the exception of data presented in chapters 11 and 12 (performed by ICI Pharamceuticals) I have analysed **all** data myself. Much of the data presented in this thesis **has** been published in medical and scientific journals and reprints of these papers are appended.

CHAPTER 1

DIABETIC NEPHROPATHY - LITERATURE REVIEW

DIABETIC NEPHROPATHY

Diabetes mellitus is a common condition which affects **approximately 1%** of the population in the United Kingdom. The hyperglycaemia of diabetes is thought to be responsible for **the** recognized complications of retinopathy, neuropathy and nephropathy.

1.1. Definition of Diabetic Nephropathy

The traditional clinical definition of diabetic nephropathy rests upon the observation of a mean urinary protein excretion rate greater than **0.5 g 24h⁻¹** in at least 3 collections in a patient who has been diabetic for more than 10 years [Mogensen 1988a]. Diabetic nephropathy is characterised by morphological and functional alterations in the kidney. These changes, however, do not necessarily occur in parallel: histological changes may be present in the absence of proteinuria [Deckert et al 1985]. Mauer et al [1984] studied **45** type 1 diabetic patients and found no correlation between histological abnormality and degree of proteinuria.

1.1.1. Renal Morphology

The classical histological lesion of diabetic nephropathy is glomerulosclerosis as described by Kimmelsteil and Wilson [1936]. Within this description there are five lesions which may occur in variable combinations; a diffuse lesion with uniform mesangial expansion; a nodular lesion

where the mesangial matrix takes a globular shape; a fibrinoid cap which has a crescentic shape and is situated in the peripheral capillary wall; a capsular drop situated on the inner side of the capsule of Bowman; arteriolar hyalinosis in which eosinophilic material collects in the walls of the afferent and efferent arterioles. The thrust of this thesis, however, deals with the functional abnormalities found in diabetic nephropathy.

1.1.2. Renal Function

Renal function in diabetic nephropathy passes through five stages as defined by Mogensen [Table 1.1]. Briefly, the stages may be described as follows:

Stage 1.

Glomerular hyperfunction and hypertrophy. This is present at diagnosis.

Stage 2.

Silent stage with normal albumin excretion but structural lesions present. This may persist for many years and be revealed only as increased AER in times of stress e.g. under exercise provocation.

Stage 3.

Incipient nephropathy with persistent microalbuminuria and systemic blood pressure levels which although not frankly hypertensive are higher than those of matched subjects without proteinuria.

Stage 4.

Overt, or frank, nephropathy - characterised by macroalbuminuria, systemic hypertension and falling GFR.

Stage 5.

End stage renal failure.

[Mogensen and Schmitz 1988b]

Table 1.1

The Stages of Diabetic Nephropathy

Stage	Designation	Characteristics	Main Structural Changes	GFR [ml/min]	AER [µg/min]	BP
Stage 1	Hyperfunction Hypertrophy	Large kidneys & hyperfiltration	Glomerular hypertrophy	= 150	May be increased	Normal
Stage 2	'Silent stage'	Normal AER	Increased membrane thickness & mesangial expansion	± Hyperfiltration	Normal	Normal or slightly high
Stage 3	Incipient Nephropathy	AER persistently between 20-200 µg/min		= 160	20-70	High compared with normals
Early						
Late				= 130	70-200	Rises 3.5% per year
Stage 4	Overt Nephropathy	Clinical Proteinuria	Further membrane thickening and mesangial expansion. Glomerular closure	= 130-70	> 200	Frank hypertension.
Stage 5	Uraemia	End stage renal failure	Generalized glomerular closure	0-10	May fall	Hypertension.

GFR - glomerular filtration rate, AER - albumin excretion rate, BP - blood pressure.

1.2.1. Prevalence of Overt Diabetic Nephropathy

The true prevalence of diabetic nephropathy is unknown since population based studies have not been performed. In selected patient groups, however, nephropathy has been reported to affect as many as 40% of type 1 [insulin dependent] diabetic patients [Andersen et al 1983, Krolewski et al 1985, Borch-Johnsen et al 1986] although this figure may be declining [Kofoed-Enevoldsen et al 1987]. A similar proportion of type 2 [non-insulin dependent] patients may suffer from nephropathy [Kunzelman et al 1985, Sasaki et al 1986] although many workers have reported lower prevalence figures for this group of patients. Two studies from this country reported prevalences of 2.7% [Daggett 1986] and 6.8% [Gatling et al 1987]. A population based study from Rochester, Minnesota found a cumulative incidence of persistent proteinuria of 24.6% after twenty years' disease [Ballard et al 1988].

1.2.2. Prevalence of Incipient Diabetic Nephropathy

Microalbuminuria, now conventionally defined as AER between 20 $\mu\text{g min}^{-1}$ and 200 $\mu\text{g min}^{-1}$, predicts the development of diabetic nephropathy [Viberti et al 1982, Mathiesen et al 1984] and has been used as a measure of prevalence for nephropathy. The reports quote prevalence figures of 20% [Mathiesen et al 1986], 10.7% [Mills and Hocken 1986], 23% [Niazy et al 1987], 16% [Berglund et al 1987] and 22% [Parving et al 1988a]. These results, however, are confounded by the fact that not only did the patients have different disease

durations but also the studies were performed before there was a standard definition of microalbuminuria. Hence, each group adopted its own definition - AER greater than 15 $\mu\text{g min}^{-1}$ [Mogensen and Christensen 1984a], between 30 $\mu\text{g min}^{-1}$ and 140 $\mu\text{g min}^{-1}$ [Viberti et al 1982], greater than 70 $\mu\text{g min}^{-1}$ [Mathiesen et al 1984], and between 30 mg 24h^{-1} and 300 mg 24h^{-1} [Niazy et al 1987, Parving et al 1988a].

There appears to be a bimodal prevalence of nephropathy with peaks at 20-40 years and 60 years of age [Bjerkelund 1951, MacNeal and Rogers 1955, El Mahallawy and Sabour 1960]. The earlier peak probably represents those patients with type 1 and the latter those with type 2 disease.

1.3. Morbidity and Mortality

Patients with type 1 [insulin dependent] diabetes mellitus have a mortality rate up to twenty times higher than the non diabetic population [Borch-Johnsen et al 1986]. This excess has been ascribed predominantly to cardiovascular disease which may account for 50% of deaths [Kessler 1971], and renal disease which accounts for a similar proportion [Moloney et al 1983, Andersen et al 1983]. Patients who do not develop persistent proteinuria have a relative mortality about twice that of the general population whereas those with proteinuria have an absolute mortality rate of 17% per year [Borch-Johnsen et al 1985]. Mortality from renal disease shows a positive correlation with the duration of persistent proteinuria. In an early study five year survival after

onset of persistent proteinuria was 65% but the corresponding figure at 10 years was only 28% [Caird 1961]. A more recent study, however, suggests that mortality may be falling and that this may be because of a fall in the prevalence of nephropathy [Borch-Johnsen et al 1986].

It is estimated that nearly 600 patients per year in the United Kingdom will develop end stage renal failure as a consequence of diabetes mellitus [Joint Working Party on Diabetic Renal Failure 1985]. In the United States diabetes accounted for 24.5% of new cases of renal failure due to end stage renal disease in 1984 [Sepe and Teutsch 1988]. A study of Scandinavian diabetic patients reported 51% survival seven years after the development of overt nephropathy [Andersen et al 1983] while in the United Kingdom there was only a 49% survival at three years for patients with end-stage renal disease as a consequence of diabetes [Cameron and Challoh 1986].

Microalbuminuria, an early marker for diabetic renal disease, is a predictor of increased mortality especially in type 2 [non-insulin dependent] diabetes [Mogensen 1984b, Jarrett et al 1984]. In a 10 year follow up study of more than 400 type 2 diabetic patients with microalbuminuria 56% died from cardiovascular causes but less than 3% from renal failure [Mogensen et al 1985]. This may be a manifestation of the increased large vessel disease to which [elderly] type

2 diabetic patients are prone.

Hypertension has been recognized to be a complication of diabetes for many years [Hintzenberger 1921]. **An** increase in the body's exchangeable sodium is thought to be responsible for the increase in blood pressure. Normotensive diabetic patients without nephropathy have increased exchangeable body sodium compared with non-diabetic control subjects and hypertensive diabetic patients with nephropathy have even higher levels [O'Hare et al 1985].

1.4. Factors involved in the development of diabetic nephropathy.

Factors which may be involved in the development and progression of diabetic nephropathy are race, sex, metabolic state, dietary protein and systemic blood pressure.

1.4.1. Race and Sex.

Race and gender may be risk factors since Native and Afro-Americans are at increased risk of nephropathy [Waring 1970, West 1974, Friedman 1987] and some workers have reported an increased prevalence in males [Andersen et al 1978, West et al 1980]. It has been reported recently that Afro-Americans have an increased incidence of end-stage diabetic renal disease compared with white Americans [Cowie et al 1989]. The Afro-Americans, however, had higher systemic blood pressures than the white hence it is unclear whether the increased incidence of renal disease was a consequence of this or race.

1.4.2. Metabolic State.

It should be self-evident that diabetic nephropathy does **not** occur in the absence of diabetes. However, a direct causative link between hyperglycaemia and the development of diabetic nephropathy in humans remains to be established [Larkins and Dunlop 1992]. In a study of 292 type 1 diabetic patients followed between 20 and 40 years from diagnosis, those with the worst glycaemic control [highest blood sugars] during the first 15 years of their disease had more than four times the risk of developing nephropathy as those with good glycaemic control [Krolewski et al 1985]. Chase and **co** workers [1989] reported 3.6 times the prevalence of microalbuminuria in patients with poor glycaemic control compared with those with good control in a study of 230 type 1 diabetic subjects. A population based study of the Nauru islanders, who have a high prevalence of impaired glucose tolerance and type 2 diabetes, identified blood glucose and blood pressure as risk factors for the development of micro- **and** macroalbuminuria [Collins et al 1989].

1.4.2.1. Improved Glycaemic Control

Improved glycaemic control has been demonstrated to reduce proteinuria in animal models of diabetes [Pennell et al 1981] and in a series of papers Rasch reported prevention of diabetic glomerulopathy in streptozocin diabetic rats treated with insulin [Rasch 1979a, 1979b, 1980]. Petersen et al [1988] reported that sub-cutaneous insulin therapy could

arrest the progress of the disease even after the development of glomerular pathology.

Many studies have addressed the question of whether improved metabolic control can prevent or retard the progression of nephropathy in human diabetes [Viberti et al 1983, Kroc Collaborative Study Group 1984, Wiseman et al 1985, Feldt-Rasmussen et al 1986, Dahl-Jorgensen et al 1988, Bending et al 1986]. The results of these studies, however, have been variable. Mauer et al [1989a] studied normal kidneys transplanted into patients with type 1 diabetes. They found only an imprecise relationship between histological changes of diabetic nephropathy in the transplanted kidney and the degree of glycaemic control and suggest that in addition to glycaemic control there are risk factors intrinsic to the kidney itself. There is one report in the literature of kidneys taken from a patient with type 1 diabetes and transplanted into nondiabetic recipients [Abouna et al 1983]. Renal biopsies seven months post transplant showed almost complete resolution of diabetic nephropathy indicating that even severe lesions can be reversed. Factors other than normoglycaemia, however, may have been important. A recent re-examination of patients entered into two independent studies at the Steno hospital looked at the outcome of patients treated with either conventional insulin regimes or insulin infusion [Feldt-Rasmussen et al 1991]. This study showed that insulin infusion treated patients had better glycaemic control; fewer developed frank nephropathy; they had a slower decline in GFR. These

results suggest that nearly normalising blood glucose in diabetic patients for prolonged periods of time does protect against deterioration in renal function.

There is, as yet, no convincing evidence that tight glycaemic control is of major benefit in the long term prevention of diabetic nephropathy.

1.4.3. Dietary Protein.

Brenner et al [1982] have proposed a mechanism whereby high protein intake may stimulate glomerulosclerosis; and Mauer et al [1989b] reported increased fractional mesangial volume, an abnormality seen in diabetic nephropathy, in streptozocin diabetic rats fed high protein diets. Nyberg et al [1987] assessed protein intake by dietary history in three groups of diabetic patients; those with long standing diabetes but no evidence of nephropathy; those with nephropathy and reduced but stable GFR; those with progressive renal impairment. There was no discernible difference between the groups' protein intake and no correlation between protein intake and rate of decline in renal function.

1.4.3.1. Reduction in Dietary Protein

Despite the fact that reductions in dietary protein have been used for many years to treat renal failure arising from a variety of causes there have been only two prospective randomised trials of this treatment, neither of them in diabetes [Rosman et al 1984, Ihle et al 1989]. There have been no long term studies of dietary protein restriction in the treatment of diabetic nephropathy. Medium to short term studies in patients with established nephropathy [Evanoff et al 1987, Walker et al 1989] and incipient nephropathy [Wiseman et al 1987, Cohen et al 1987] report reductions in albuminuria and decline of renal function [GFR]. Bending et al [1988], however, in a cross-over study comparing usual-protein with restricted-protein diets in patients with nephropathy found reductions in urinary protein excretion but no change in glomerular filtration rate or renal plasma flow on the restricted diet. They concluded that the diet had somehow altered glomerular permselectivity. Limitation of dietary protein in normoalbuminuric diabetic patients appears to reduce glomerular hyperfiltration and so offer some protection from subsequent nephropathy [Kupin et al 1987, Pedersen et al 1989]. There is not, however, enough evidence at the moment to implicate dietary protein in the pathogenesis of diabetic nephropathy nor to recommend protein restriction to patients with this complication.

1.4.4. Systemic Hypertension

There is now a considerable body of evidence that raised

blood pressure plays a major role in the development and progression of diabetic nephropathy and, moreover, that lowering blood pressure may affect the rate of disease progression.

1.4.4.1. Aetiology of hypertension in diabetic nephropathy.

A proportion of diabetic patients may have essential [idiopathic] hypertension in association with nephropathy. In a study of non-diabetics with essential hypertension and diabetics with incipient or overt nephropathy there was a correlation between mean arterial pressure and rate of urinary albumin excretion but with considerable overlap between the two groups. When the two variables were evaluated for each group individually, however, the diabetic patients were found to have a much higher albumin excretion rate at any given blood pressure [Christensen et al 1987]. On this basis it may be possible to separate patients with essential hypertension from those with diabetic nephropathy. The majority of patients with diabetic nephropathy will have hypertension as consequence of this complication. The aetiology of this type of hypertension has not yet been fully elucidated but is almost certainly multifactorial and dependent upon genetic and hormonal factors.

1.4.4.2. Genetic influences

Several recent reports suggest that there is a genetically determined tendency to hypertension which may be important in the development of diabetic nephropathy. First,

hypertension is more common in the hypertensive siblings of patients with type 1 or type 2 diabetes than in the siblings of those who are normotensive [Kelleher et al 1988]. Second, the parents of patients with type 1 diabetes who have proteinuria tend to have higher blood pressures than the parents of patients who do not develop proteinuria [Viberti et al 1987]. The third piece of evidence comes from a study of **89** patients with type 1 diabetes [Krolewski et al 1988]. At recall, approximately 18 years after diagnosis, 33 patients had developed diabetic nephropathy. The risk of developing nephropathy was tripled if they had a parent with hypertension. The patients with nephropathy had increased erythrocyte sodium-lithium cotransport. Sodium lithium cotransport has been reported to be normal or elevated in patients with variable stages of diabetic nephropathy [Mangili et al 1988, Jones et al 1990, Carr et al 1990, Elving et al 1991]. The abnormality has been shown to be associated with hypertension [Canessa et al 1980] and may be a marker for its heritability [Canali et al 1981, Walker et al 1990]. A study of two groups of diabetic patients, with and without nephropathy, and their siblings showed that the siblings of nephropaths had increased albumin excretion rates compared with siblings of non-nephropaths [Seaquist et al 1989]. In this study, however, there was no significant difference in blood pressure between the two groups of siblings. Jensen et al [1990], however, measured arterial blood pressure and maximal sodium-lithium cotransport in the parents of patients

with type 1 diabetes with nephropathy, normoalbuminuric diabetes and age matched non diabetic controls. They found **no** difference between the blood pressures in the two groups of patients. Patients with diabetes had higher sodium-lithium cotransport than non-diabetic control subjects independent of the presence of nephropathy. Those diabetic patients with nephropathy had non-significantly higher [$P = 0.06$] sodium-lithium cotransport than those without. These workers conclude that genetic predispositions to hypertension and increased nephropathy sodium-lithium cotransport activity are not risk markers for the development of diabetic nephropathy.

1.4.4.3. Volume expansion

Hypertensive diabetic patients have increased plasma volume. The volume increase is mediated by increased exchangeable body sodium and these may precede the onset of hypertension [De Chatel et al 1977]. Nephropathic diabetics have a further increase in their exchangeable sodium compared with those who do not have nephropathy [O'Hara et al 1985, Feldt-Rasmussen et al 1987]. The reason(s) for the high levels of exchangeable sodium are not known, although insulin has been shown to promote renal tubular absorption of sodium [De Fronzo 1981]. The increased body sodium leads to fluid retention and expanded circulating volume hence favouring the development of hypertension.

1.4.4.4. Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system [**RAAS**] plays a

role in the modification of vascular tone and sodium homeostasis. There have been contradictory reports of the activity of the **RAAS** in diabetes mellitus. Some workers have found activity to be normal [Feldt-Rasmussen et al 1987, O'Hare et al 1988], while others have found it to be increased [De Chatel et al 1977] or decreased [Christlieb et al 1976, Tuck et al 1979]. Leutscher and Kraemer [1988] reported recently that patients with microproteinuria have increased levels of circulating prorenin. In a prospective study of teenage diabetics **34%** of those who had elevated rates of albumin excretion at re-examination after five years tended to have significantly increased plasma renin activity [Paulsen et al 1989]. These workers hypothesise that elevated angiotensin II levels associated with high plasma renin activity may increase glomerular capillary pressure and provoke proteinuria and glomerular damage.

1.4.5. Glomerular Hypertension

Glomerular hyperfiltration appears to be a risk factor for the development of diabetic nephropathy [Hostetter et al 1982, Zatz et al 1986]. Diabetic patients with persistent hyperfiltration are more likely to progress to persistent Proteinuria than patients with lower initial glomerular filtration rates [Mogensen and Christensen 1984a]. The amount of any substance filtered from the plasma at the glomerulus depends not only in the intrinsic permeability of the glomerular basement membrane which, in turn, is determined by the pore size and the electrical charge, but also on the

amount of blood passing through the capillaries in a unit time [perfusion] and the intracapillary pressure [Figure 1-1]. Animal data have suggested that single-nephron hyperfiltration **is** dependent on both glomerular capillary hyperperfusion and hypertension [Hostetter et al 1981]. Measures which increase glomerular capillary perfusion in the remaining nephrons, such as partial or unilateral nephrectomy, accelerate the development of diabetic nephropathy [Steffes et al 1978, O'Donnell et al 1986]. Zatz and co-workers [1986] treated diabetic rats with enalapril, an angiotensin converting enzyme [ACE] inhibitor. A dose which lowered the systemic blood pressure in these normotensive animals also reduced their glomerular capillary pressure and transglomerular capillary pressure gradient. Treated animals did not develop proteinuria or histological evidence of diabetic nephropathy whereas untreated control animals did. Both groups had comparable elevations of blood glucose, thus supporting the hypothesis that glomerular hypertension, rather than metabolic abnormalities, is of major importance in the evolution of diabetic nephropathy [Zatz et al 1986,1987].

A recent study of renal function in diabetic children, however, does not support the animal data [Drummond **et al** 1989]. This was a double-blind randomized crossover trial comparing enalapril with placebo in diabetic children with raised glomerular filtration rates, but normal blood Pressure and albumin excretion rates. During enalapril treatment there was a significant reduction in systemic blood

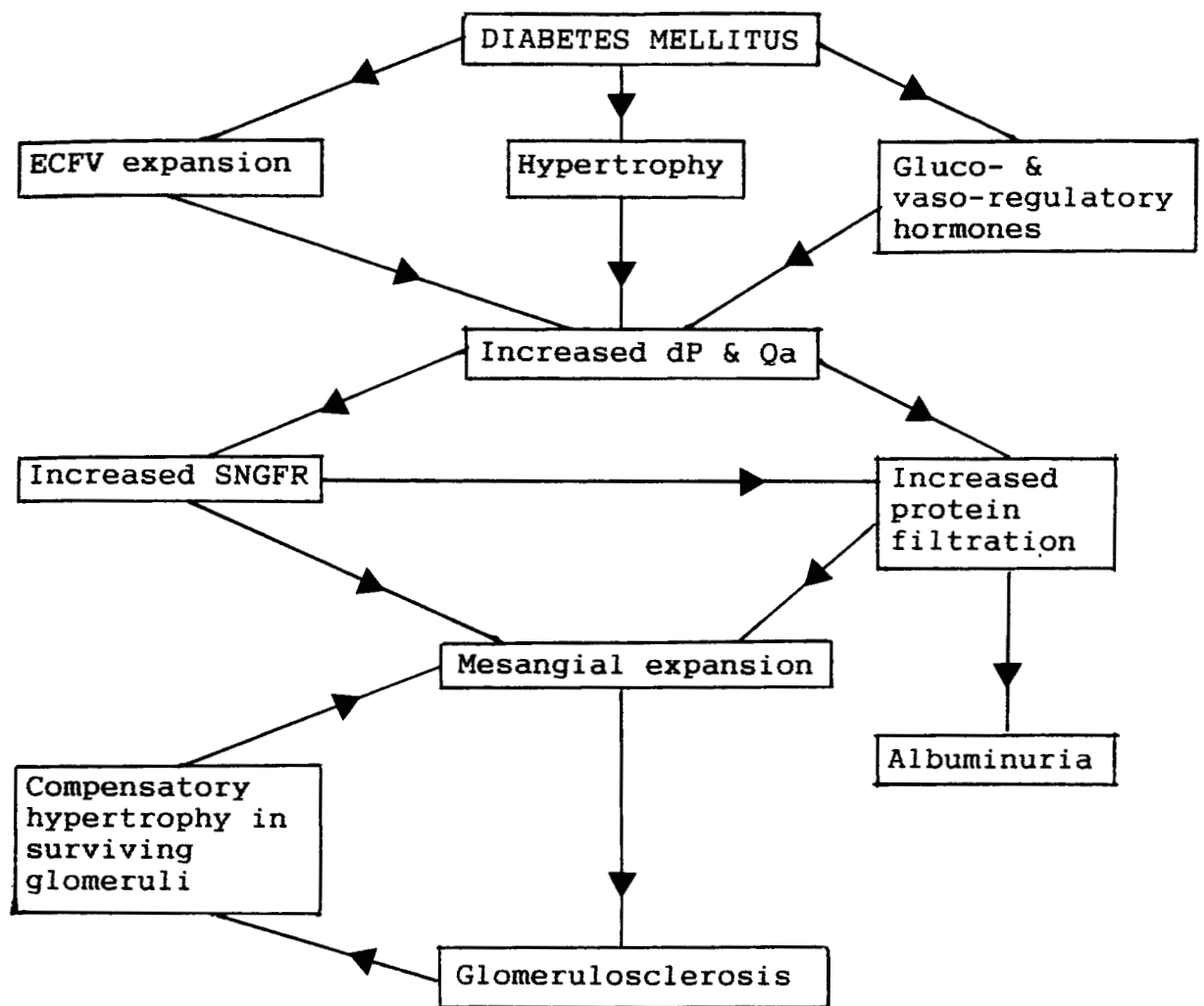


FIGURE 1.1.

Hypothetical role of glomerular hyperfiltration in the initiation and progression of diabetic nephropathy.

Hyperfiltration is stimulated by extracellular fluid volume [ECFV] expansion, renal hypertrophy, and vasoactive hormones. increased glomerular transcapillary pressure [dP] and glomerular plasma flow rate [Qa] are responsible for hyperfiltration leading to albuminuria, mesangial deposition of circulating proteins and glomerulosclerosis. [SNGFR = single nephron glomerular filtration rate].

pressure but no change in glomerular filtration rate, renal plasma flow or filtration fraction. The authors concluded that the renin-angiotensin-aldosterone system plays no role in the hyperfiltration of diabetes, and inferred that there is no evidence of glomerular hypertension in this group of patients. In the absence of glomerular micropuncture studies it is not possible to be dogmatic on this point. Elevation of glomerular filtration rate is common early in diabetes but not all patients progress to nephropathy. Glomerular hypertension may be an important factor in the initiation of nephropathy in susceptible patients later in the course of their disease.

1.4.6. Renal Prostaglandins

The kidney is one of the most active prostaglandin producing tissues. The glomeruli synthesise mainly PGI_2 , a vasodilator, [determined as its metabolite 6-keto- $\text{PGF}_{1\alpha}$] but also some thromboxane [TXB_2], PGE_2 and $\text{PGF}_{2\alpha}$. [Schlondorff and Ardaillou 1986]. Prostaglandins play a contributory role in renal autoregulation [Schnerman et al 1984]. They may influence glomerular filtration rate by altering efferent and afferent arteriolar tone [Schor et al 1981, Schnerman et al 1984], by influencing the renin-angiotensin-aldosterone system [Schor et al 1981], and by effecting mesangial Contraction [Scharschmidt et al 1983].

Esmatjes et al [1985] studied 21 type 1 diabetic Patients and 15 non-diabetic control subjects. Diabetic Patients had increased glomerular filtration rate and renal

plasma flow but urinary excretion of **PGE₂** and **6-keto-PGF_{1α}** was no different from control subjects'. Administration of lysine acetylsalicylate, a cyclo-oxygenase inhibitor, significantly reduced glomerular filtration rate and renal plasma flow in the diabetic group only suggesting that renal prostaglandins are involved in the hyperfiltration of type 1 diabetes. In a similar study using a different cyclo-oxygenase inhibitor, piroxicam, there were similar reductions in glomerular filtration rate and urinary prostaglandin excretion during treatment [Gambardella et al 1988]. Barnett et al [1987] compared glomerular and mesangial prostaglandin synthesis and glomerular contraction in two rat models of diabetes. They found that even though streptozocin rats had enhanced **PGE₂** production in vivo the contractile response of isolated glomeruli to different pressors was the same in each group. This argues against prostaglandins being important for hyperfiltration in diabetes. Jenkins et al [1989] found that indomethacin reduced urinary excretion of **PGE₂** and **6-keto-PGF_{1α}** without altering renal blood flow or glomerular filtration rate. Studies of the effects of prostaglandin inhibition in diabetic nephropathy suggest that prostaglandins are important influences upon renal haemodynamics and proteinuria. Hommel et al [1987] treated nephropathic type 1 diabetic patients with indomethacin and found reductions in urinary excretion of **PGE₂** and albumin and of glomerular filtration rate. In a similar study of type 1 diabetic

patients with incipient nephropathy there were reductions in **albuminuria** but no change in glomerular filtration **rate** [Mathiesen et al 1988]. Barnett et al [1984] have reported reduction in diabetic albuminuria in patients treated with a specific thromboxane synthetase inhibitor.

1.5. Modifying the Course of Diabetic Nephropathy

From the above it may be seen that the most useful was in which to affect the course of diabetic nephropathy is by adjustment of blood pressure.

1.5.1. Hypertension in Overt Diabetic Nephropathy.

Systemic hypertension is associated with diabetic nephropathy and plays a role in its development [Parving et al 1983a]. Those patients with persistent hypertension have a faster decline in renal function than those without [Hasslacher et al 1985].

Several studies have shown that treatment of hypertension in overt diabetic nephropathy may retard, but not halt, the inevitable decline into end-stage renal failure. The first of these was reported by Mogensen [1976]. This study involved 8 patients with persistent proteinuria in the range 0.5 to 8.8 g 24 h^{-1} and mean systemic blood Pressure 161/101 mm Hg. These patients were examined between two and four times over a six to sixty month period. Treatment commenced with propranolol or combined with hydralazine and frusemide. During the 47 day treatment

period mean blood pressure fell to **143/93 mm Hg** and albumin excretion rate was significantly reduced from a mean of **3547 $\mu\text{g min}^{-1}$** to **2412 $\mu\text{g min}^{-1}$** . It is unclear in this report whether all patients had type 1 diabetes mellitus.

In an extended follow-up study by the same worker six men with type 1 diabetes were followed for a mean of **73 months** [Mogensen 1982]. During the **28 month** control period before active treatment the mean glomerular filtration rate was **86.1 ml min^{-1}** and the mean monthly decline was **1.23 ml min^{-1}** . During treatment of hypertension the decline in glomerular filtration rate was **0.49 ml min^{-1}** and the mean blood pressure fell from **162/103 mm Hg** to **144/95 mm Hg**. There was a corresponding fall in the mean yearly increase of albumin excretion [expressed as a percentage of glomerular filtration rate] from **107%** during the control period to only **5%** during active treatment.

A similar study following ten diabetic patients with persistent proteinuria for **29 months** before and **39 months** after treatment with metoprolol, hydralazine and frusemide or thiazide reported a fall in mean blood pressure from **144/97 mm Hg** to **128/84 mm Hg**, and a fall of albumin excretion from **977 $\mu\text{g min}^{-1}$** to **433 $\mu\text{g min}^{-1}$** [Parving et al 1983b]. The rate of decline of glomerular filtration rate fell from **0.91 $\text{ml min}^{-1} \text{ month}^{-1}$** before treatment to **0.39 $\text{ml min}^{-1} \text{ month}^{-1}$** during treatment.

The results of a long term follow-up study of the treatment of hypertension in patients with persistent proteinuria has been published recently [Parving and Hommel 1989a]. Forty-five patients were followed until death or for **at** least ten years following the onset of persistent proteinuria. Forty-one patients received treatment for hypertension, 19 of whom were receiving triple therapy. The mean blood pressure at the onset of treatment was 148/95 mm Hg. Systolic blood pressure was virtually unchanged whereas diastolic blood pressure fell by 0.87 mm Hg per year during treatment. The cumulative death rate was 18% at ten years after the onset of nephropathy and the overall mortality was 31%. These results are in marked contrast to previous reports of death rates [between 50 and 77%] at ten years after the onset of nephropathy [Andersen et al 1983, Krolewski et al 1985]. The authors attribute this improvement in mortality to closer attention to blood pressure control in the study group over the previous decade.

1.5.2. Hypertension in Incipient Diabetic Nephropathy.

Interest has focussed recently on the possibility of treating patients at an earlier stage of the nephropathic Process in an effort to arrest its progress. Incipient nephropathy [the phase of microproteinuria] may last 15 years **or** longer before overt nephropathy develops. Although not hypertensive by conventional criteria these patients have significantly elevated blood pressure compared with non-

diabetic controls [Mathiesen et al 1984, Wiseman et al 1984, Christensen et al 1985]. There is also a correlation between diastolic blood pressure and albumin excretion rate [Wiseman **et** al 1984]. Most workers have regarded hypertension as an important causative factor in the genesis of diabetic nephropathy but Mathiesen et al [1990] have published data showing a rise in albumin excretion rate preceding sustained rises in blood pressure in their patients.

Because incipient nephropathy may have such a protracted course long term studies of blood pressure control have not yet been performed. Short term studies, however, have yielded promising results. A study of the effect of acute reduction in blood pressure, using a stat dose of clonidine in patients with incipient nephropathy, produced a reduction in systemic blood pressure accompanied by a fall in albumin excretion rate [Hommel et al 1986a]. Christensen and colleagues [1985] followed six male patients with incipient nephropathy for a mean of 5.4 ± 3.0 years before and 4.7 ± 1.0 years after treatment with a combination of β -blocker and diuretic. During treatment there was a significant reduction **in** mean arterial pressure [diastolic + one third of pulse Pressure] and an annual decrease of $19 \pm 10\%$ in mean albumin excretion rate. Prior to the commencement of antihypertensive treatment there had been an increase in mean albumin excretion rate of $18 \pm 17\%$ per year. In this study there **was** a small, but significant, fall in glomerular filtration rate. Although the results of these studies suggest that

treatment of blood pressure in incipient nephropathy is beneficial they are based on small numbers of patients and of relatively short duration. Longer duration studies of patients with incipient nephropathy are required to establish whether treatment of supranormal or hypertensive blood pressure has a place in their management.

1.5.3. The Place of Angiotensin Convertins Enzyme [ACE] Inhibitors in the Treatment of Diabetic Nephropathy

There is at present great interest in the role of ACE inhibitors in the treatment of hypertension in patients with diabetic nephropathy. ACE inhibitors work by preventing the conversion of angiotensin I to angiotensin II. Angiotensin II stimulates aldosterone secretion and causes vasoconstriction, both of which tend to raise blood pressure. ACE inhibitors exert their intra-renal effects predominantly at the efferent glomerular arteriole, causing vasorelaxation, and thus lowering the intraglomerular capillary pressure [Zatz et al 1986, Anderson et al 1986]. As mentioned earlier there is evidence that glomerular hypertension is important in the pathogenesis of diabetic nephropathy and that its reduction Prevents structural injury and proteinuria [Steffes et al 1978]. Reduction of intraglomerular pressure with ACE inhibitors exerts a protective effect in both normotensive-and hypertensive diabetic animals [Cooper et al 1989]. In this Paper normotensive and spontaneously hypertensive rats were rendered diabetic by injection of streptozocin and then treated with enalapril or placebo. Both groups of enalapril

treated rats showed a reduction in glomerular basement membrane thickening, albuminuria and systemic hypertension.

1.5.3.1. ACE Inhibitors and Overt Diabetic Nephropathy

ACE inhibitors are efficacious in the treatment of systemic hypertension and, along with other antihypertensive drugs, reduce protein excretion in diabetic nephropathy. Taguma and co-workers [1985] described a reduction of proteinuria in patients with overt diabetic nephropathy who were treated with captopril. These patients were taking other antihypertensive medications which were not altered during the study. The mean urinary protein excretion fell from $10.6 \pm 2.2 \text{ g } 24\text{h}^{-1}$ to $6.1 \pm 1.4 \text{ g } 24\text{h}^{-1}$ after two weeks treatment and with no significant fall in systemic blood pressure. A succession of papers from workers in Scandinavia has shown that ACE inhibitors reduce blood pressure and proteinuria in hypertensive diabetic patients with nephropathy.

In his first paper on this subject Björck et al [1986] followed 15 patients with diabetic nephropathy treated with captopril and found a reduction in mean blood pressure of 5 mm Hg and a reduction in the rate of fall of glomerular filtration rate from $10.3 \text{ ml min}^{-1} \text{ year}^{-1}$ to $2.4 \text{ ml min}^{-1} \text{ year}^{-1}$. There was no correlation between the reduction in blood pressure and the reduction in the rate of deterioration of renal function. Neither was there any change in mean urinary protein excretion ($2.9 \pm 2.0 \text{ g } 24\text{h}^{-1}$ before and $2.8 \pm$

1.9 g 24h^{-1} after treatment]. Hommel and co-workers [1986b] studied the effect of 12 weeks captopril treatment on 16 hypertensive patients with diabetic nephropathy. They found **that** blood pressure fell from 147/94 mm Hg to 135/88 mm Hg, **and** albuminuria from a median of 1549 [range 168-2198] $\mu\text{g min}^{-1}$ to 1170 [range 352-2238] $\mu\text{g min}^{-1}$. Parving et al [1988b] treated 18 hypertensive nephropaths for about two and a half years with captopril and frusemide or bendrofluazide. Mean arterial blood pressure fell by 8.7 ± 1.3 mm Hg in the treated group and rose by 6.6 ± 1.5 mm Hg in a placebo group. Albumin excretion fell from $982 \mu\text{g min}^{-1}$ to $390 \mu\text{g min}^{-1}$ in the captopril group and rose from $936 \mu\text{g min}^{-1}$ to $1367 \mu\text{g min}^{-1}$ in the placebo group. The rate of decrease of glomerular filtration rate was lower in the captopril treated group $5.8 \pm 0.7 \text{ ml year}^{-1}$ vs. $10.0 \pm 1.3 \text{ ml year}^{-1}$. This study, however, is open to criticism in that it was non-randomised and used historical controls. Björck et al [1990] in a prospective, open randomised study comparing enalapril with metoprolol in diabetic nephropathy found a greater reduction in albuminuria in the enalapril treated group after eight weeks. After 2.2 years treatment the rate of reduction in glomerular filtration rate was $2.0 \pm 3.2 \text{ ml}^{-1} \text{ min}^{-1} \text{ year}^{-1}$ and in the metoprolol group was $5.6 \pm 5.9 \text{ ml}^{-1} \text{ min}^{-1} \text{ year}^{-1}$ [Björck et al 1992].

It has been suggested that ACE inhibitors offer better renal protection than other classes of antihypertensive drug. This is born out by Björck's data [1990,1992] and by Holdaas's

[1991]. Holdaas and colleagues compared the effects of lisinopril with those of nifedipine on twelve diabetics in an open randomized cross over study. There were four-week wash out periods and active treatment periods lasting three weeks. Lisinopril reduced albumin excretion from $1343 \pm 337 \mu\text{g min}^{-1}$ to $879 \pm 299 \mu\text{g min}^{-1}$ but nifedipine had no effect $1436 \pm 336 \mu\text{g min}^{-1}$ to $1319 \pm 342 \mu\text{g min}^{-1}$. Neither drug had any effect on glomerular filtration rate.

1.5.3.2. ACE Inhibitors and Incipient Diabetic Nephropathy.

If observed reductions in rates of decline of renal function, and changes in albumin excretion rates, in patients with nephropathy are independent of changes in blood pressure it supports the view that ACE inhibitors have intrinsic renal protective properties. Several recent papers are of interest in this respect because they have reported the effects of ACE inhibition on renal function in normotensive diabetic patients. The earliest of these came from Marre et al [1988]. They studied 20 normotensive diabetic patients with persistent microalbuminuria for 12 months. Patients were randomised to receive either placebo or enalapril and were followed double blind for six months and then single blind for the remainder of the study. Three placebo treated patients developed frank nephropathy whereas none of the enalapril treated patients did and five had become normoalbuminuric. Mean arterial pressure fell with enalapril but rose with placebo. Similarly renal resistance and filtration fraction

fell with enalapril suggesting a specific intra-renal effect. Parving et al [1989b] reported on normotensive patients with $AER > 200 \mu g \text{ min}^{-1}$ who were treated with placebo or captopril in an open randomised study. At one year there were reductions in mean arterial blood pressure, albuminuria and fractional albumin clearance in the treated group and rises in the placebo group and the rate of decline of glomerular filtration rate was less than in the placebo group. The importance of this study is that it has demonstrated that, in normotensive patients with overt nephropathy, the increase in albumin excretion rate may be not only slowed, but arrested completely. Mathiesen et al [1991] reported the four year results for normotensive microalbuminuric patients given captopril or placebo in an open randomised study. The trends were similar to Parving's study; no captopril treated patient had developed nephropathy but seven from the placebo group had. Interestingly there was no difference between the groups for glomerular filtration rate.

CHAPTER 2.

DIABETIC PROTEINURIA - LITERATURE REVIEW

DIABETIC PROTEINURIA

Hippocrates observed more than 2,000 years ago that bubbles on the surface of the urine are a sign of diseases of the kidney: we know now that this is a clinical sign of proteinuria. It was recognized as early as 1770 that the urine of some diabetic patients may contain protein [Cotunnus]. Rayer [1840] postulated that diabetes may cause a form of Bright's disease. It is now recognized that proteinuria is an important marker for the presence of diabetic nephropathy. While several proteins may be lost in the urine in diabetic nephropathy most workers have concentrated their attention upon the behaviour of albumin. In this chapter I will review briefly the literature regarding the mechanisms of proteinuria in diabetes mellitus and then go on to consider other proteins and enzymes which have been investigated and the most suitable means of collecting urine specimens for study.

2.1. Mechanisms of Proteinuria in Diabetes Mellitus.

The non-diabetic glomerular basement membrane acts as a size and charge selective barrier [Dennis and Robinson 1986]. Evidence for this comes from dextran filtration studies in which dextrans of different molecular size have been infused and their appearance in the urine studied [Brenner et al 1978]. An ideal test macromolecule is neither secreted nor reabsorbed and if excretion of the reference solute is not altered by tubular function then the ratio of urinary clearance of test to reference solute is

equal to the ratio of the concentration of the test solute in Bowman's space to its concentration in plasma. This ratio of clearances is known as the permselectivity and varies from 0 where no test molecule appears in the urine, to 1 when there is perfect clearance. It has been shown that neutral dextrans with molecular radius $< 14\text{\AA}$ have permselectivity of 1. As the radius of the dextran increases towards that of albumin $[36\text{\AA}]$ the permselectivity falls [Arturson et al 1971]. Hence it has been inferred that the glomerular basement membrane is size selective.

Chang et al [1975] studied the effect of charge on the sieving of molecules at the glomerular basement membrane by comparing permselectivities of neutral dextran and [anionic] sulphated dextran. He demonstrated that neutral dextrans were size-limited beyond 20\AA whereas anionic dextrans were restricted over the entire range of molecular sizes studied. Since there is no evidence for tubular secretion or reabsorption of dextrans the observed differences must be a consequence of another process - basement membrane charge. The fact that this is likely to be a negative charge is further suggested by enhanced excretion of cationic proteins [Rennke et al 1978]. The glomerular basement membrane is composed largely of glycoproteins with many carboxyl residues and sulphate rich proteoglycans which are both negatively charged [Cotran and Rennke 1983]. Glomerular polyanion can be demonstrated histochemically by staining with colloidal iron and Alcian Blue [Michael et al

1970]. Simplification of the foot processes of glomerular epithelial cells occurs in nephrotic man and animals, possibly as a consequence of alteration in glomerular basement membrane charge. This may lead to alterations in the size and shape of slit pores and facilitate the passage of proteins [Luke 1984]. Similar mechanisms may operate in diabetes.

2.1.1. Glomerular Basement Membrane in Diabetes Mellitus

Ala-Houhala and Pasternak [1987] studied fractional dextran clearances in diabetic nephropathy and concluded that altered glomerular permeability was a consequence of reductions in charge rather than altered size of the membrane pores. Nakamura et al [1988] infused dextrans into patients with diabetic nephropathy and found similar results. The mechanism of this proteinuria probably relates to alterations of glomerular basement charge. Decreased de-novo synthesis of glomerular proteoglycans has been demonstrated in diabetic rats [Kanwar et al 1983]. A reduction of glycosaminoglycan component of the glomerular basement membrane has been demonstrated in humans [Parthasarathy et al 1982] and altered charge has been reported by Cohen and Surma [1984].

2.1.2. Alterations in Serum Proteins

A further factor of importance in the glomerular excretion of protein is the charge of the filtered molecule

itself. It is well recognized that glycosylated proteins traverse glomerular membrane more easily than native unglycosylated proteins [Ghiggeri et al 1984a, Williams and Siegal 1985, Bertolatus and Hunsicker 1985, Layton and Jerums 1988].

The three dimensional shape of a protein may affect its renal handling. Enhanced glomerular excretion of dextrans may be a consequence of their unfolding from a globular structure into a string-like shape [reptation] [Ryan 1981]. Ghiggeri et al [1984b] demonstrated urinary excretion of albumin with altered three dimensional conformation in patients with diabetic nephropathy. Molecular conformation may also be important for tubular reabsorption since filtered proteins with similar iso-electric points and sizes may be taken up at different rates by the tubules [Purtell et al 1979].

2.1.3. Altered Renal Tubular Function

It is thought that nearly all of the albumin filtered at the glomerulus is reabsorbed in the healthy renal tubule [Galaske et al 1979] by energy dependent endocytosis [Straus 1964]. The concentration of albumin in glomerular filtrate has not been measured directly in the human but Mogensen [1977] inhibited tubular reabsorption by infusing lysine and reported urinary albumin excretion in the order of 500 mg $24h^{-1}$. Impaired renal tubular reabsorption could account for some part of diabetic proteinuria. Results from different studies, however, have been conflicting with some workers

finding altered, [Abrass 1984, Fletcher et al 1986, Gibb et al 1989] and others normal tubular function [Mathiesen et al 1984].

2.2. Proteins and Enzymes other than Albumin

A range of urinary proteins and enzymes have been studied as potential markers for diabetic nephropathy. These include those thought to originate or be handled primarily by the renal tubules, β_2 -microglobulin and N-acetyl- β -D-glucosaminidase [NAG], and the glomerulus, transferrin.

2.2.1. β_2 -Microglobulin.

β_2 -microglobulin is a low molecular weight protein present as part of the histocompatibility antigen on cell membranes and is turned over at a constant rate. It is readily filtered at the glomerulus and nearly 100% reabsorbed in the proximal renal tubule where it is catabolised [Bernier 1969]. Hence, the protein should be useful as a measure of renal function. Walton et al [1988], however, found β_2 -microglobulin excretion to be a poor marker for tubular function in diabetic children with microalbuminuria. Viberti et al [1981] compared plasma β_2 -microglobulin and creatinine levels in proteinuric diabetic patients whose renal function was assessed by clearance of $^{51}\text{Cr-EDTA}$. They found that β_2 -microglobulin levels were elevated in all patients with glomerular filtration rate less than $80 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ whereas some patients had normal serum creatinine. This work

has been criticised, however, since their **reference** range for normal **β_2 -microglobulin** levels was based on data from only 12 patients. **β_2 -microglobulin** has been studied in patients with diabetic nephropathy [Jones et al 1980]. It appears that in these patients when GFR is normal so too is urinary **β_2 -microglobulin** excretion. Fletcher et al [1986] studied low molecular weight proteinuria by polyacrylamide gel electrophoresis [PAGE] in diabetic patients with increased albuminuria. They found that no patient had increased **β_2 -microglobulin** levels. In a study of Albustix negative patients glycaemic control appeared to be an important modifying influence on **β_2 -microglobulin** excretion [Watanabe et al 1987]. **β_2 -microglobulin** excretion may also be influenced by exercise [vide inf].

2.2.2. N-acetyl- β -D-glucosaminidase [NAG]

Urinary N-acetyl- β -D-glucosaminidase [NAG] is a lysosomal enzyme in the proximal renal tubule [Pugh & Walker 1960]. It may be elevated in diabetics with and without nephropathy [Whiting et al 1979a, 1979b, Cohen et al 1981]. Watanabe et al [1987] measured serum and urinary NAG levels in 61 diabetic patients who did not have macroalbuminuria and 19 age and sex matched control subjects. They found that all the diabetic subjects, even those with disease duration less than 2.5 years, had increased NAG levels. Levels were strongly correlated with glycaemic control suggesting that they are not particularly useful as a marker of renal function in this group of patients. Other workers studying

NAG excretion in diabetic patients and non-diabetic control subjects found increased levels in all diabetic subjects and higher levels in those with proteinuria [Jung et al, 1988]. They concluded that NAG may be a useful marker for diabetic nephropathy. Type 1 diabetic children without nephropathy have increased urinary NAG levels [Gibb et al 1989] and these correlate with urinary transferrin but not albumin excretion [Martin et al 1990a].

2.2.3. Transferrin

Transferrin is similar in size and molecular weight to albumin [38A, 77,000]. Elevated urinary transferrin excretion rates have been reported recently in patients with complications from type 2 diabetes [Cheung et al 1989]. These workers studied 157 type 2 diabetic patients and 53 non diabetic control subjects and found significantly higher transferrin excretion in the diabetic group. When the diabetic patients were split into three sub groups with progressively higher urinary albumin excretion there was also progressively higher transferrin excretion which correlated with arterial pressure.

Two groups have suggested that urinary transferrin may be an early marker for renal damage in diabetes mellitus. Bernard et al [1988] studied transferrin and albumin excretion in type 1 (n=84), type 2 (n=23), and type 3 (n=69) diabetic subjects. They found increased excretion of both proteins in

78 subjects, with increased transferrin alone in 23 and increased albumin alone in only 7. There was a better correlation between the two proteins for those subjects with the highest albumin excretion rates. They were able to reproduce these observations in streptozocin diabetic rats. The second group of workers [Martin et al, 1988] studied patients with either Albustix negative type 1 diabetes mellitus [n=54], systemic lupus erythematosus [n=13], rheumatoid arthritis [n=17], lymphoma [n=16] or childhood nephropathy [n=15]. In all groups there was a relatively greater proportional rise in transferrin than albumin excretion compared with a healthy control group [n=31]. The same group have reported increased urinary transferrin excretion in children with type 1 diabetes [Martin et al 1989]. In a comparison of 74 diabetic children with 40 non diabetic control subjects the urinary transferrin excretion was significantly elevated and 17 diabetic children had excretion rates above the 95th centile. In contrast there was no significant increase in albumin excretion in the diabetic children. Ellis et al [1983] studied 67 type 1 diabetic children and found increased transferrin, albumin, IgG, and β_2 -microglobulin excretion compared with controls. These children had normal or increased glomerular filtration rates. The observed patterns of proteinuria in this study suggest both glomerular and tubular dysfunction. Disproportionate increases of transferrin over albumin excretion have been reported previously in a wide range of glomerular and tubular diseases [Coimbra et al 1984].

2.3. Which Urine Sample?

The traditional clinical definition of diabetic nephropathy rests upon the observation of a mean urinary protein excretion rate greater than **0.5 g 24h⁻¹** in at least 3 collections in a patient who has been diabetic for more than 10 years [Mogensen 1988a]. Patients with this degree of proteinuria are Albustix positive i.e. their proteinuria may be detected on simple stick testing of random urine samples. This level represents an albumin excretion rate of **200 µg min⁻¹**. Problems arise in the detection of patients with lesser degrees of proteinuria. As mentioned above [1.2.2. Prevalence of Microproteinuria] different workers have used not only different reference values but also different time periods for urine collection. Early morning urine samples with albumin:creatinine ratio greater than 3.0 reliably predict an albumin excretion rate greater than **30 µg min⁻¹** in overnight urine collections [Marshall 1991]. It is generally agreed, however, that early morning specimens are useful only for screening purposes [Hutchison and Paterson 1988, Winocour 1992]. Albumin excretion rates demonstrate a day to day variability of as much as 37% [Howey et al 1987, Cohen et al 1987] and may be affected by many factors, especially exercise [Mogensen 1975, 1979; Viberti et al 1978].

Twenty-four hour urine collections are notoriously difficult to make accurately. Overnight collections have the

advantages that they do not interfere with the patients daily routine; they do not necessitate carrying a large collection bottle during the day; they are less prone to variability, perhaps because they are not subject to the influences of exercise which pertain during the day [Chachati et al 1986, Cohen et al 1987].

2.4. The Influence of Exercise on Diabetic Proteinuria

As mentioned above albumin excretion may be influenced by exercise; indeed, it has been suggested that exercise be used as a provocative test to identify patients at risk of diabetic renal disease [Feldt-Rasmussen et al 1985]. The mechanisms underlying exercise induced proteinuria are unknown. Huttunen et al [1981] found increased albumin but decreased β_2 -microglobulin excretion in exercising non-diabetic children and adolescents. Poortmans et al [1988] exercised healthy non-diabetic men with and without lysine infusion on bicycles. [Lysine is thought to interfere with tubular reabsorption of filtered protein]. They found increased albumin and β_2 -microglobulin excretion after short term exhaustive exercise suggesting both increased glomerular permeability and reduced tubular reabsorption. Viberti et al [1978] used **albumin: β_2 -microglobulin** ratios as a measure of proteinuria in exercising diabetic patients and found evidence for a glomerular but not tubular leak. Ala Houhala [1990] studied fractional protein and dextran clearances in 12 proteinuric diabetic and 12 non-diabetic subjects at rest

and after exercise. Exercise reduced glomerular filtration rate and renal blood flow and increased the filtration fraction in both groups. In addition there was increased clearance of large molecular radius dextran in the diabetic group. He concludes that exercise induced proteinuria is a consequence of altered renal haemodynamics and increased size of the glomerular basement membrane pores.

THE KIDNEY IN DIABETES MELLITUS:

PART I

URINARY TRANSFERRIN EXCRETION

IN DIABETIC RENAL DISEASE

CHAPTER 3.

METHODS

3.1. URINE COLLECTION

For screening of the type 1 diabetic patients to establish prevalence of proteinuria and during interventional studies with angiotensin converting enzyme [ACE] inhibitors overnight collections of urine were made.

Patients were provided with plastic collection bottles without preservative. They were instructed to empty their bladder prior to retiring for the night and to record the time on the bottle's label. They were then instructed to collect any urine passed during the night and the first urine passed the following morning and to record the time of the morning specimen. Samples were returned to the hospital on the morning that the collection was completed. The total volume and the time of the collection were noted. A fresh aliquot was sent to exclude urinary tract infection and other aliquots were frozen at -70 °C for later assay for protein content.

For study of the prevalence of transferrinuria in newly diagnosed type 2 diabetic patients and of the effect of exercise on transferrinuria in type 1 diabetic patients timed urine samples were collected as described later.

3.2. BLOOD PRESSURE

Blood pressures were measured at heart level in the right arm after at least ten minutes rest using a Hawksley random zero sphygmomanometer. Diastolic blood pressure was recorded as Korotkow phase V. Mean arterial

blood pressure [MAP] was calculated as Diastolic pressure + **[0.33 x Pulse pressure]**.

3.3. VENESECTION

All blood specimens were drawn from an antecubital vein without venous stasis after blood pressure had been measured.

3.4. SERUM ELECTROLYTES AND ALBUMIN

Serum sodium, potassium, urea, creatinine, and albumin were measured using a Vickers SB-120 multi-channel autoanalyser.

3.5. GLUCOSE and GLYCATED HAEMOGLOBIN

Glucose was measured using a glucose oxidase method [Trinder 1969], glycated haemoglobin [**HbA₁**] by affinity chromatography using Glycogel-B [Mallia et al 1981].

3.6. PLASMA RENIN ACTIVITY

This was measured using an endogenous Substrate assay. Four plasma sample **[0.2ml]** from each patients were incubated at 37 °C with 0.2 ml of 0.2 mol l⁻¹ sodium phosphate buffer [pH 6.0] containing the following angiotensin converting enzyme and angiotensinase inhibitors; 3.0 mmol l⁻¹ EDTA [Ethylene diamine tetra-acetic acid], 8.5 mmol l⁻¹ **8-hydroxyquinoline**, and 5.0 mmol l⁻¹ dimercaprol. Incubations were stopped at 0, 15, 45, and 180 min by plunging the incubation tubes into liquid nitrogen. The amount of angiotensin I produced from endogenous angiotensin was

determined by an in-house **radioimmunoassay** technique using radiolabelled ^{125}I -angiotensin I prepared by the chloramine T method. Samples [0.1 ml] of the enzyme incubations were added to tubes containing 0.1 ml 50 mmol l^{-1} Tris-HCl buffer [pH 7.5] containing 3 g l^{-1} human serum albumin and 2 g l^{-1} neomycin sulphate; 0.2 ml of angiotensin I antiserum [CIS, High Wycombe, UK] diluted 1:8000 in Tris-HCl buffer before use; 0.05 ml of ^{125}I -angiotensin I [diluted 1:1000 in Tris-HCl buffer before use]. At the same time angiotensin I standards between 156-5000 pmol l^{-1} were set up. The **radioimmunoassay** was performed by incubating for 72 h at 4 °C and stopped by adding 1 ml 0.05 mol l^{-1} Tris-HCl buffer [pH 7.5] containing 10% [v/v] human plasma and 10 g l^{-1} activated charcoal [Norit SX1, Hopkins and Williams, Chadwell Heath, UK] at 4 °C. After centrifugation at 3000g for 10 min the radioactivity of the pellet was counted and the angiotensin I in each assay tube calculated. The plasma **renin** activities were then determined employing linear regression on 0, 15, 45, and 180 min angiotensin I concentrations. Enzyme activities were given in nmol angiotensin I produced litre plasma $^{-1}$ hour $^{-1}$ at 37 °C. The reference range was determined from 10 healthy recumbent adults and was 0.3-2.7 nmol l^{-1} h $^{-1}$. Three controls [low, medium, and high] were included with each batch, and **inter-batch CV** were 8.3, 7.1, and 7.3 % for plasma renin activities of 1.2, 4.2, and 15.0 nmol l^{-1} h $^{-1}$, respectively.

3.7. PLASMA ALDOSTERONE

Plasma aldosterone concentrations were determined in duplicate by a **radioimmunoassay** kit 'Coat-a Count' [Diagnostics Products **Corp**', Los Angeles, USA]. Quoted reference values for healthy recumbent adults were **28-444 pmol l⁻¹**. Three control samples were assayed with each batch of samples, and the calculated inter batch CV were 10.8, 13.1, and 8.1 % for plasma aldosterone concentrations of 193, 490, and 1357 pmol l⁻¹ respectively.

3.8. ATRIAL NATRIURETIC FACTOR

This was measured using a two-site **immunoradiometric** assay according to the method of Lewis et al [1989].

3.9. ADRENALINE, NOR-ADRENALINE, AND DOPAMINE

These were measured by high performance liquid chromatography with **electrochemical** detection using the method described by Hugh et al [1987].

3.10. URINARY TRANSFERRIN

Antiserum to human serum **transferrin** [Sigma, Poole, **UK**] was produced in rabbits following subcutaneous multisite injection of 1 mg kg⁻¹. ¹²⁵I-**transferrin** label was prepared by the liquid phase **iodogen** method [Fraker 1978] and purified on a **Sephadex G-75**, 25 ml column [Pharmacia, Uppsala, Sweden]. Rabbit antiserum to human serum **transferrin** was diluted 25,000 fold in assay buffer [phosphate buffer 50 mmol l⁻¹, pH 7.5, with 1g l⁻¹ bovine serum albumin] Containing rabbit carrier serum [**RCS**] **optimised** against

precipitating antibody [2.5 ml l^{-1}]. The diluted antiserum [$400 \text{ } \mu\text{l}$] was incubated at 25°C with $50 \text{ } \mu\text{l}$ of human serum transferrin standard or unknown and $100 \text{ } \mu\text{l}$ transferrin tracer solution for 18h. Sheep antiserum [$100 \text{ } \mu\text{l}$ of 60 ml l^{-1}] to rabbit IgG [Sigma, Poole, UK] [raised by subcutaneous multisite injection of 0.3 mg kg^{-1}] in 500 ml l^{-1} horse serum/phosphate buffer was then added to each sample immediately followed by $500 \text{ } \mu\text{l}$ of 100 g l^{-1} polyethyleneglycol, incubated at 25°C for 40 min, centrifuged and the precipitate counted. Urine specimens were diluted 5 fold in assay buffer initially and further diluted where appropriate. Recovery was 98.2% [range 96.1-101.2 %]. Cross reactivity of the antibody with albumin was $< 0.1\%$ at 2 g l^{-1} of albumin. The range of the assay was $70\text{--}2200 \text{ } \mu\text{g l}^{-1}$. Interassay coefficients of variation were: at $100 \text{ } \mu\text{g l}^{-1}$ 11.8%, at $290 \text{ } \mu\text{g l}^{-1}$ 5.6%, at $710 \text{ } \mu\text{g l}^{-1}$ 4.3%.

3.11. URINARY ALBUMIN

Albumin content of urine was measured using a double antibody radioimmunoassay employing radiolabelled albumin prepared by a chloramine T method. Assays were conducted in duplicate after pre-diluting albumin standards [$3\text{--}200 \text{ mg l}^{-1}$ stored at -20°C] and specimens 1 in 20 with 0.2 mol l^{-1} potassium phosphate buffer [pH 7.2] containing 70 mmol l^{-1} sodium chloride, 3 mmol l^{-1} sodium azide, and 1.5% by volume inactivated rabbit serum. This buffer was also used to make any other dilutions. To each tube was added 0.075 ml of diluted standard or specimen, 0.1 ml of a 1:1000

dilution of ^{125}I -albumin and 0.2 ml of a 1:20000 dilution of albumin antisera [Atlantic Antibodies, Scarborough, ME 04074, USA]. Tubes were vortex mixed and incubated at 37 °C for 150 min after which time 0.1 ml of **antisheep/goat** Sac-cel [Immunodiagnosics Systems, Washington, UK] was added. After a further 30 min at room temperature 1 ml of water was added to each tube and the tubes were centrifuged at 3000g for 15 min. After aspiration the radioactivity was counted in the cellulose pellets and the concentration of albumin was calculated from the generated standard curve. Interbatch CV was 7.0, 4.3, and 4.2 % for urine concentrations of 10, 44, and 161 mg l⁻¹ respectively.

3.12. MARKERS OF RENAL TUBULAR FUNCTION

N-acetyl-β-D-glucosaminidase [NAG] was measured by spectrofluorimetry as described by Leback and Walker [1961]. **Alpha-1-microglobulin** was measured by radial immunodiffusion using the method of Mancini et al [1965].

3.13. URINARY PROSTAGLANDINS

Thromboxane-B₂ and **6-keto-PGF_{1α}** were measured in urine using commercially available kits [Amersham, UK] employing a double antibody radioimmunoassay. all specimens were assayed in duplicate.

3.14. GLOMERULAR FILTRATION RATE

This was measured as follows:

3.14.1. Preparation of Dose and Standard

1. 10 ml 10% dextrose was aseptically dispensed into a sterile 20 ml vial.
2. The required volume {3 MBq} of ^{51}Cr EDTA was dispensed into a 1 ml syringe and diluted to 1 ml with sterile water.
3. The ^{51}Cr EDTA/water was added to the dextrose and volume made up to 12 ml using sterile water.
4. 10 ml of this solution was withdrawn and used for the patient dose. This syringe was weighed and before and after filling with the patient dose and the weights noted.
5. 1 ml of the remaining solution was dispensed into a 500 ml volumetric flask and made up to 250 ml using sterile water.
6. Dose and standard were calibrated by weighing accurately two syringes each fitted with needle and guard before and after drawing up required volumes of radio pharmaceutical. The net weights of dose and standard were calculated and the ratio of dose:standard was derived.

3.14.2. Technique

1. A 10 ml heparinized blood sample was taken from the patient for count of background activity.
2. The patient was injected via an antecubital vein and the time noted.
3. Four 10 ml heparinized blood samples were taken at 90, 150, 210 and 270 min post injection from a site different from that used for injection.
4. The blood samples were centrifuged and, from each, 2 ml plasma was dispensed into sample tubes. 2ml was dispensed from the standard flask into a further sample tube.
5. The samples were counted along with the standard and room background for the same time. All plasma counts and standard counts were corrected for background activity. All samples were counted in duplicate.
6. Using log-linear graph paper the plasma count was plotted [log scale] against time. Using the best straight line fit the intercept at time zero [time of injection] and half time of clearance [$t_{1/2}$] in minutes were determined.
7. The volume of dilution [V] and glomerular filtration rate [GFR] were calculated from the following formulae;

$$V \text{ [litres]} = \frac{[\text{standard counts}] \times [\text{dose/standard ratio}]}{\text{Intercept}}$$

$$\text{GFR [ml min}^{-1}\text{]} = \frac{V \times 0.693}{t_{1/2}} \times 1000$$

3.15. EFFECTIVE RENAL PLASMA FLOW

3.15.1. Preparation of Dose and Standards

These were prepared in the same manner as those for glomerular filtration rate but using a dose of 2 MBq ^{125}I -hippuran.

3.15.2. Technique

The technique was the same as for measurement of glomerular filtration rate except that a single blood sample was taken 44 min after injection. samples were counted as above and the volume of distribution [V'] and effective renal plasma flow [ERPF] calculated as follows:

$$V' [\text{litres}] = \frac{[\text{standard counts}] \times [\text{dose/standard ratio}]}{[44 \text{ min plasma counts}]}$$

$$\text{ERPF [ml min}^{-1}\text{]} = 5.5 \times V'$$

3.16. CORRECTION FOR SURFACE AREA

To correct for differences in body size glomerular filtration rate and effective renal plasma flow were corrected for 'a **body** surface area of 1.73 m^2 with a nomogram derived from the formula of Dubois and Dubois [1916] using weight and height.

3.17. DERIVED MEASURES

$$\text{Filtration Fraction [FF]} = \text{GFR} / \text{ERPF}$$

$$\text{Renal resistance [RR]} = \text{MAP} / \text{ERPF}$$

$$\text{Fractional albumin clearance [F}_{\text{ALB}}] = \text{Albumin clearance} / \text{GFR}$$

3.18. STATISTICAL METHODS

For all studies data were found to be non-parametrically distributed. For multiple group comparisons the Kruskal Wallis test was used and if this demonstrated a difference groups were compared using the Mann Whitney U test. Correlations were established with the Spearman Rank Order test. These calculations were performed on a PC using the Microstat statistical package. For the two studies involving ACE inhibitors analyses were performed on an IBM mainframe computer using the **SAS** statistical package.

3.18.1. ACE inhibitor in Overt Nephropathy

For blood pressures, GFR, RBF, ERPF, FF, and RR changes from baseline [visit 7 minus visit 2] were analysed using the analysis of covariance [ANCOVA] technique: the baseline [visit 2] values were further included in the model as the covariate as this was found to substantially contribute to the models and hence improve the precision of the estimates of treatment difference. The assumptions underlying the ANCOVA technique were checked against these data and were not found to be violated. For AER and Quantitative protein, however, the changes from baseline were found to violate the normality assumption underlying the ANCOVA technique as they were heavily skewed with extreme outliers even after logarithmic transformation. Therefore

the non parametric Mann Whitney U test was applied to the untransformed changes from baseline for these endpoints.

3.18.2. ACE inhibitor in Incipient Nephropathy

Primary end points for the study were changes in AER, urinary prostaglandins, GFR and RBF after 48 weeks treatment. Secondary end points were changes in heart rate, blood pressure and metabolic control. Changes from baseline [visit 2] to the end of randomised treatment [visit 7] were analysed using the analysis of covariance [ANCOVA] technique. The baseline values were further included in the model since this was found to contribute substantially to the model and hence improve the precision of the estimate of treatment difference. The assumptions underlying the ANCOVA technique [normality and homoscedasticity] were checked against the data and found not to be violated. Patients whose compliance was less than 80% were excluded from the final analyses.



CHAPTER 4.

SCREENING OF CLINIC POPULATION AND IDENTIFICATION OF PATIENTS WITH PROTEINURIA

4.1. PATIENT SCREENING

All patients involved in the studies which comprise this thesis were recruited from the diabetic clinic at East Birmingham Hospital. The clinic has approximately 3,000 patients with type 1 [insulin dependent] or type 2 [non-insulin dependent] diabetes mellitus and those with type 1 diabetes are reviewed at four month intervals. Hence, all patients with type 1 diabetes should pass through the clinic in a twelve month period.

For the twelve month period beginning 31st May 1987 all type 1 diabetics attending the clinic were invited to participate in a study to ascertain the prevalence of albuminuria. Type 1 diabetic patients were defined as those who were diagnosed prior to the age of 35 years, who had at least one episode of ketosis, and showed an absolute requirement for insulin therapy. Since this is an NHS clinic with large numbers of patients it was felt justified to make the diagnosis on these clinical grounds. C-Peptide levels were not measured as a routine.

The aims of the study were explained to the patients. A full medical history was taken and all patients were examined to establish the presence or absence of any complications of diabetes or the presence of any other disease. Retinopathy was assessed by direct ophthalmoscopy; neuropathy by clinical assessment of vibration sense, light touch and reflexes; peripheral vascular disease from history

and the presence or absence of peripheral pulses [brachial:ankle pressures were not measured]: coronary artery disease from history and resting electrocardiogram [ECG]. Blood pressure was measured twice in the right arm after at least ten minutes rest using a Hawksley random zero sphygmomanometer. The mean of these readings was recorded. Autonomic nervous function was assessed using the battery of tests as described by Ewing and Clark [1982]. Briefly, this involves recording the ECG during slow maximal respiration, during the Valsalva manoeuvre while the patient maintains a pressure of 40 mm Hg for 30 seconds, and during the change from supine to upright posture. In addition the blood pressure response to sustained hand grip is measured and any postural drop in blood pressure is recorded. By measuring alterations in blood pressure and the maximal and minimal RR intervals on the ECG during the different phases of respiration and comparing these with standard ratios it is possible to establish whether autonomic neuropathy is present.

In addition to routine bloods taken in clinic for the management of diabetes a sample was taken from all patients for measurement of serum creatinine.

Patients were instructed how to perform an overnight collection of urine and provided with a collection bottle and printed instructions to reinforce what had been said in clinic. They were asked to return the urine

collection to the hospital the morning of its completion. Two collections were made within a six month period. On return to the hospital the urines were aliquotted; a fresh aliquot **was** sent to exclude urinary tract infection; the remainder were frozen at -70 °C pending assay, by radio-immunoassay [RIA], for albumin and transferrin. the volume and times of collection were recorded for later calculation of protein excretion rates. These were calculated from the formula

$$\text{PER} = \frac{\text{Pc} \times \text{V}}{\text{T}}$$

where **PER** is protein excretion rate, **Pc** is protein concentration in urine, **V** is volume of urine, and **T** is time **of** collection of urine. Conventionally the excretion rates are expressed as $\mu\text{g min}^{-1}$.

4.2. RESULTS

One hundred and eight patients fulfilling the criteria for diagnosis of type 1 diabetes participated in the study. Patient characteristics are shown in table 4.1. The median [range] age was 27 [12-68] years and disease duration 12 [1-49] years. **The** patients were sub-divided on the basis of albumin excretion rate [AER] into normoalbuminuric [AER < 20 $\mu\text{g min}^{-1}$, n = 79], microalbuminuric [20 $\mu\text{g min}^{-1}$ < AER < 200 $\mu\text{g min}^{-1}$, n = 19], and macroalbuminuric [AER > 200 $\mu\text{g min}^{-1}$, n = 10]. Those with macroalbuminuria had longer median[range] disease duration than the other groups [34[20-68] years **vs** 26[15-65] years normo- and 29[12-60] years microalbuminuric, **P** < 0.05]. Forty patients had retinopathy

of some degree and this was significantly more common in those with elevated AER [19 normo-, 12 micro- and 9 macroalbuminuric, $P < 0.01$]. Autonomic and peripheral neuropathy, as well as systolic hypertension were more common in patients with macroalbuminuria than either of the other groups. More patients in the macroalbuminuric group were treated for hypertension [9 compared with 6 normo-, and 3 microalbuminuric, $P < 0.01$]. Macroalbuminuric patients had higher serum creatinine than the other groups [median[range] 111[86-215] $\mu\text{mol l}^{-1}$ vs 89[52-126] $\mu\text{mol l}^{-1}$ normo- and 84[66-133] $\mu\text{mol l}^{-1}$ microalbuminuric, $P < 0.01$]. One patient in the macroalbuminuric group with peripheral vascular disease had had a lower limb amputation. There was no significant difference between groups for any other variable, including the number who were smokers.

Table 4.1

Patient characteristics on screening for normo-, micro-, and macroalbuminuria.

	All Patients [n = 108]	Normo- [n = 79]	Micro- [n = 19]	Macro- [n = 10]
AER [$\mu\text{g min}^{-1}$]***	8.4[0.3-2810]	4.8[0.3-19.7]	44.8[26.5-134]	1555.0 [203-2810]
Age [yrs]	27 [12-68]	26 [15-65]	29 [12-60]	34 [20-68]
Disease Dur'n [yrs]**	12 [1-49]	11 [1-49]	10 [1-28]	21 [10-41]
Smoker [n=]	28	18	07	03
Retinopathy [n=]*	40	19	12	09
IHD [n=]	12	05	02	05
CVD [n=]	01	01	0	0
PVD [n=]	08	02	03	03
Auto' Neuropathy [n=]*	13	04	02	07
Periph' Neuropathy[n=]*	10	03	01	06
SBP [mm Hg]*	120 [80-190]	110 [80-190]	114 [100-150]	140 [120-184]
DBP [mm Hg]	70 [60-100]	70 [60-96]	70 [60-90]	90 [60-100]
BP Rx*	18	06	03	09
Creatinine [$\mu\text{mol l}^{-1}$]*	91 [52-215]	89 [52-126]	84 [66-133]	111 [86-215]

IHD - ischaemic heart disease, CVD - cerebrovascular disease, PVD - peripheral vascular disease, SBP - systolic blood pressure, DBP -diastolic blood pressure ,BP Rx - receiving antihypertensive medication. Comparisons between normo-, micro-, and macroalbuminuric groups was by Kruskal Wallis test; *** P <0.0001, ** P <0.01, * P <0.05.

4.3. DISCUSSION

4.3.1. Prevalence of proteinuria

Diabetes mellitus is a common disease affecting approximately 1.0 % of the population in the United Kingdom. It has been reported that as many as **40%** of type 1 [insulin dependent] diabetic patients may develop nephropathy as a disease complication [Andersen 1983]. Other authors, however, report a reduction in the incidence of nephropathy [Krolewski 1985, Kofoed-Enevoldsen 1987]. These workers examined cohorts of type 1 diabetic patients diagnosed in different decades and found that those diagnosed later had a lower incidence of nephropathy independent of duration of diabetes. The reasons for this are unclear; male sex, and high body mass index, in addition to year of diagnosis were found to be risk factors; social class, insulin dose, place of abode, and readmission to a teaching centre did not influence the incidence of nephropathy. Smoking habit does not appear to have had a major part to play in the development of nephropathy since there was an increased number of women smokers in later years but still a male preponderance of patients with nephropathy. There was, however, no account of total amount of tobacco smoked. A possible factor in the decreased incidence of nephropathy is the treatment of hypertension.

The high relative mortality of patients with type 1 diabetes is restricted mainly to those with proteinuria [Andersen 1983, Borch-Johnsen 1985]. A decrease in the

relative mortality of patients with type 1 diabetes has been reported from Denmark although the reason for this was unrecognized [Green 1985]. Parving and Hommel [1989a] have recently published data showing improved prognosis in diabetic nephropathy which they attribute to better treatment of hypertension in this group of patients. This study, however, may be criticised because of the relatively small number of patients [45], the fact that it used historical controls, an absence of data regarding duration of diabetes in the study group compared with controls, and the effects of insulin dosage and glycaemic control. Allowing for these criticisms, however, there was a greatly decreased cumulative mortality ten years after the onset of nephropathy.

4.3.2. Prevalence of Microproteinuria

In the population studied for this thesis nineteen [17.6%] patients had microalbuminuria. This figure is in broad agreement with the prevalence quoted by Berglund [1987] but lower than the figures from other workers [Mathiesen 1986, Niazy 1987, Parving 1988a]. As mentioned earlier the difference in observed prevalences may relate to different definitions of microalbuminuria chosen by different groups of researchers. I chose the range 20-200 $\mu\text{g min}^{-1}$ since this is now commonly accepted as a working definition.

4.3.3. Prevalence of Macroproteinuria

Ten [9.3%] patients had macroalbuminuria. All of these had disease duration of at least 10 years and 9 were

treated for hypertension. Hence, it appears that all had developed frank nephropathy. A prevalence of nearly 10% is higher than those reported by Daggett [1986], 2.7%, and Gatling [1988], 6.8%. Both of their studies, however, were of larger groups of unselected [type 1 and type 2] diabetic patients. It is possible that my study population has an artificially high prevalence because of a sampling error. Conversely, it is possible that Daggett's and Gatling's figures would be higher if they had examined only type 1 diabetic patients.

4.3.4. Prevalence of Retinopathy

Retinopathy became progressively more prevalent with increasing proteinuria in the present study [24% normo-, 63% micro-, and 90% macroalbuminuric patients]. These figures are similar to those reported in previous studies by other workers. An association between diabetic retinopathy and nephropathy is widely accepted; the interaction between nephropathy and retinopathy has been termed the diabetic renal-retinal syndrome [Friedman 1980]. Severe retinopathy is more likely to be found in patients with renal disease [Klein 1988] and diabetic retinopathy is more advanced in patients with microalbuminuria than those with normal albumin excretion rates [Barnett 1985]. Proliferative retinopathy also becomes more frequent with increasing albuminuria, being found in 28% of patients with microalbuminuria and 58% of those with macroalbuminuria in one study [Parving 1988a].

When end-stage diabetic nephropathy is present 70% of patients will have proliferative retinopathy and the remaining 30% have non-proliferative disease [Ramsay 1979]. None of the patients in the present study had such advanced disease.

4.3.5. Nephropathy and Cigarette Smoking

An association between cigarette smoking and nephropathy has been claimed by some authors [Telmer 1984, Norden 1984]. I found no difference between groups for the number of patients who were current cigarette smokers. In a case control study of 192 cigarette smoking patients with type 1 diabetes and 192 non smokers there was significantly more macroproteinuria [19.3% vs 8.3%, $P < 0.001$] and proliferative retinopathy [12.5% vs 6.8%, $P < 0.025$] in the smoking group [Mühlhauser et al 1986]. My study and that of Mühlhauser looked at crude indices of smoking habit: Stegmayr [1987] has suggested that 'pack years' i.e. total amount of tobacco smoked is a more appropriate measure of risk. [A person smoking ten cigarettes per day for 4 years would accumulate two pack years, etc.] This difference in method of calculation of tobacco habit may explain the lack of an observed association between smoking and nephropathy in the present study.

In summary, it appears that the population of type 1 diabetics in the present study are typical in terms of the numbers with micro- and macroalbuminuria and the numbers with retinopathy. There does appear to be an increased

prevalence of retinopathy with increasing albuminuria and, similarly, an increased prevalence of hypertension. I could not confirm previous workers' reports of an association between cigarette smoking and diabetic nephropathy; this may relate to the crude method chosen to measure smoking habit.

CHAPTER 5

URINARY TRANSFERRIN EXCRETION IN TYPE 1 [INSULIN DEPENDENT] DIABETES MELLITUS.

5.1. INTRODUCTION.

As mentioned above some workers have reported a prevalence of 40% for nephropathy in type 1 [insulin dependent] diabetic patients [Andersen et al 1983, Krolewski et al 1985. Prevalence figures from my study were slightly lower [Chapter 4]. Frank nephropathy is preceded by consistently elevated urinary albumin excretion rates [Viberti et al 1981, Wartha et al 1984, Fletcher et al 1986, Jung et al 1988] which do not occur until diabetes has been present for several years [Mogensen 1984, Viberti et al 1982]. Elevated urinary transferrin and N-acetyl- β -D-glucosaminidase excretion rates may precede elevation of albumin excretion rate in diabetic children [Ellis et al 1983, Martin et al 1990] and may be earlier predictors of renal disease. I studied urinary transferrin and albumin excretion in adult [type 1] diabetic patients to investigate this possibility.

5.2. SUBJECTS, MATERIALS AND METHODS.

Subjects were 47 type 1 [insulin dependent] diabetic patients who had participated in the initial screening of the clinic. Since proteinuria in type 1 diabetes is uncommon until diabetes has been present for several years only patients with disease duration of at least 5 years were studied. Twenty eight healthy medical and laboratory personnel with no family history of diabetes were studied as sex-matched controls.

All subjects were examined as described previously

for evidence of large vessel disease or retinopathy. Patients were grouped according to albumin excretion rate [AER] derived from overnight urine collections [normo- $<20 \mu\text{g min}^{-1}$, micro- $20\text{--}200 \mu\text{g min}^{-1}$, and macroalbuminuric $> 200 \mu\text{g min}^{-1}$]. Subjects' characteristics and biochemistry results are shown in Table 5.1.

Although macroalbuminuric patients tended to be older and to have had diabetes for longer the difference between these and other groups was not significant. All macroalbuminuric patients were treated for hypertension but no other patient or control subject was. There was no significant difference in plasma biochemistry or glycaemic control between patients with and without elevated transferrin excretion rate. There was no significant difference in age or sex ratio between diabetic and control subjects.

On the morning of the test, having eaten their usual breakfast, all subjects voided urine and were given 200ml water to drink per hour. After a 1h rest period a 2h timed recumbent urine collection was made. Aliquots of urine were stored at -20°C for quantification of albumin, transferrin, **N-acetyl- β -D-glucosaminidase** [NAG], creatinine, and electrolytes. A sample of fresh urine was sent for bacteriological examination. Blood was drawn from an antecubital vein after the rest period for estimation of plasma glucose, glycated haemoglobin [HbA_{1c}], creatinine, and

albumin. Serum was frozen and stored at -70 °C for measurement of transferrin.

5.3. RESULTS

One patient had an asymptomatic urinary tract infection and was excluded from further analyses.

Control urinary transferrin excretion rates [TER] ranged from 0.01 to 0.2 $\mu\text{g min}^{-1}$ (median 0.04 $\mu\text{g min}^{-1}$) but were skewed by the highest value. The median transferrin excretion rate was significantly higher in the diabetic than the control group [0.58 vs. 0.04 $\mu\text{g min}^{-1}$, $P < 0.001$]. Forty [85%] diabetic patients had transferrin excretion rates above the 95th centile [0.11 $\mu\text{g min}^{-1}$] for the range derived from the control group. There was no significant difference in age, duration of diabetes, or glycaemic control between these patients and those with transferrin excretion rates below the 95th centile value. There were significant differences between median transferrin excretion rates of the normo-, micro-, and macroalbuminuric groups [0.2, 3.38, and 34.7 $\mu\text{g min}^{-1}$ respectively, $P < 0.001$, Table 5.2].

Transferrin excretion rates and urinary transferrin/creatinine ratios were significantly correlated in the diabetic patients [$r=0.95$, $p < 0.02$]. There were significant differences in urinary transferrin/creatinine ratios between the diabetic groups and between diabetic and control groups [Table 5.2]. Transferrin excretion rate did not correlate with age, disease duration or metabolic

Table 5.1.

Characteristics, plasma glucose, glycated haemoglobin, serum albumin, transferrin and creatinine for diabetic and control groups.

	Control	Albumin excretion rate [$\mu\text{g min}^{-1}$]		
		<20	20-200	>200
Number	28	29	11	7
Age [years]	36+11	37+5	43+18	42+17
Diabetes' duration [years]	-	14+12	14+11	18+6
IHD	-	2	1	-
PVD	-	2	1	-
<u>Retinopathy</u>				
Simple	-	7	3	3
Complex	-	1	3	4
Plasma glucose [mmol l^{-1}]	5.4+1.3	11.7+6.4	10.4+4.4	8.7+4.3
HbA _{1c} [%]	6.3+1.4	9.8+2.7	10.0+3.0	8.8+3.3
Albumin [g l^{-1}]	35+4	38+4	35+3	37+2
Transferrin [g l^{-1}]	1.7+0.5	1.7+0.4	1.8+0.4	1.8+0.5
Creatinine [$\mu\text{mol l}^{-1}$]	86+22	87+23	105+40	86+19

Glycated haemoglobin [HbA_{1c}], reference range 5.0-9.0%. IHD - Ischaemic heart disease, PVD - Peripheral vascular disease. Values shown are Mean + SD.

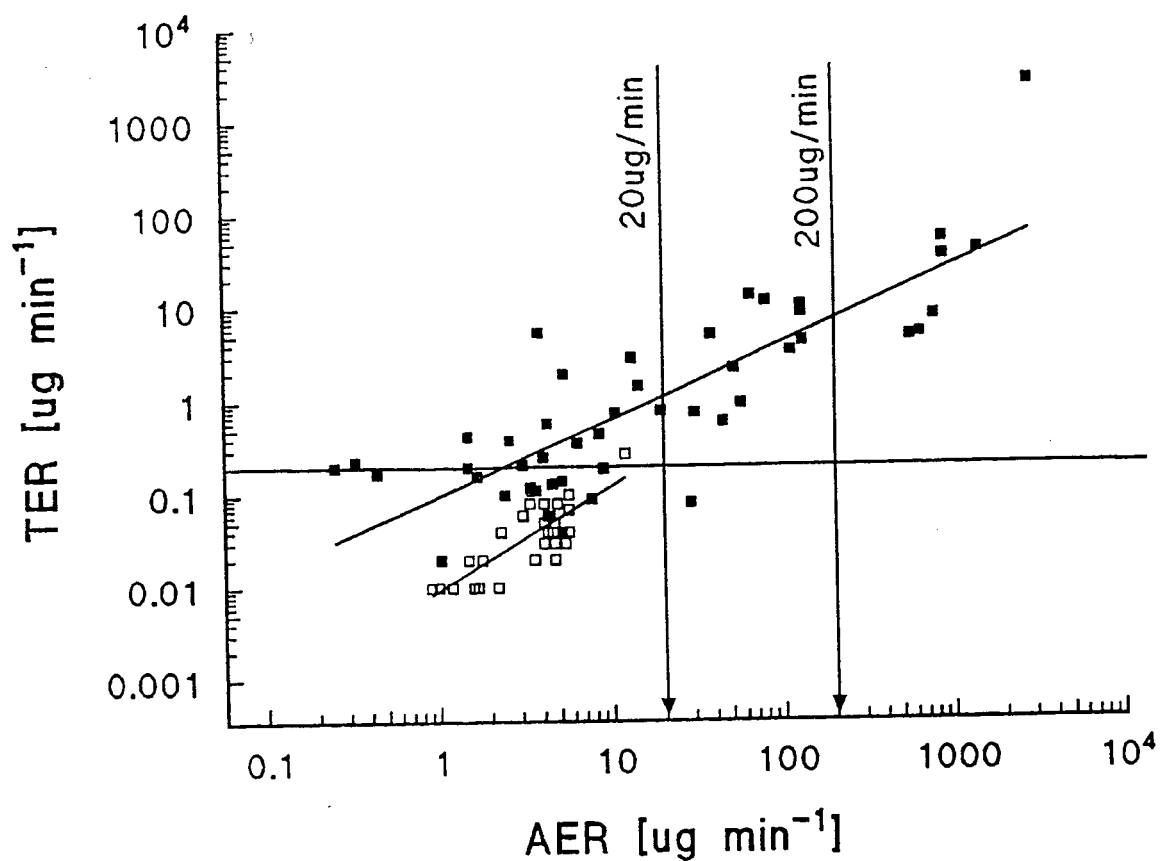


FIGURE 5.1.

Transferrin [TER] and albumin [AER] excretion rates for diabetic [closed square] and non-diabetic [open square] subjects on screening. Horizontal line represents 95th centile for TER defined from non-diabetic subjects. Vertical lines at 20 and 200 represent limits of microalbuminuria.

Table 5.2.

Urinary transferrin [TER] and albumin excretion rates [AER], creatinine concentration, transferrin: creatinine ratios [T:Cr], and N-acetyl- β -D-glucosaminidase [NAG] excretion for all groups.

	Control	Diabetic		
	[n = 28]	[n = 29]	[n = 11]	[n = 7]
AER [$\mu\text{g min}^{-1}$]	3.6 [0.9-12.0]	4.1 [0.3-9.5]	56.8 [20.0-153.3]	868.0 [562.0-2810.0]*
TER [$\mu\text{g min}^{-1}$]	0.04 [0.01-0.28]	0.2 [0.02-5.4]	3.38 [0.08-13.25]	34.7 [4.8-2663.3]*
Creatinine [mmol l^{-1}]	4.6 [0.7-20.5]	3.2 [1.0-17.3]	8.6 [3.5-18.5]	5.3 [3.6-18.1]
T:Cr [$\mu\text{g mmol}^{-1} \cdot 10^2$]	0.7 [0.06-2.3]	3.1 [0.6-51.1]	16.0 [4.0-126.0]	576.0 [80.0-958.0]
Albumin:Transferrin	90.0 [24.3-235]	14.7 [0.7-130.0]	26.7 [4.8-360.0]	34.5 [1.1-125.4] *
NAG [$\mu\text{mol h}^{-1} \text{ l}^{-1}$]	58 [0-218]	76 [0-531]	153 [8-743]	269 [157-1300]
Results expressed as median [range]. * p < 0.001.				

Table 5.3.

Correlations between N-acetyl- β -D-glucosaminidase [NAG],
transferrin excretion rate [TER] and albumin excretion rate [AER],
for diabetic group [n=47].

	NAG	TER	AER
NAG	1.0	-	-
[TER]	0.67*	1.0	-
[AER]	0.63*	0.65*	1.0

* P < 0.01

control [**HbA_{1c}** or plasma glucose during testing] in any group.

There was a correlation between albumin and transferrin excretion rates in the control [**r=0.81**, $P < 0.05$] and diabetic groups [**r=0.78**, $P < 0.001$, Fig 5.1].

All patients with albuminuria $> 20 \mu\text{g min}^{-1}$ had transferrin excretion rates greater than the 95th centile value [Fig 5.1]. Twenty-one patients had elevated transferrin excretion rates but normal albumin excretion rates. These did not differ from other patients with respect to age, disease duration or glycaemic control.

All patients with clinical retinopathy had elevated transferrin excretion rates but not all patients with elevated transferrin excretion rates had retinopathy [Table 5.1].

N-acetyl- β -D-glucosaminidase excretion was significantly higher in the diabetic than control groups [Table 5.3]. There was a correlation between albumin, transferrin and **N-acetyl- β -D-glucosaminidase** excretion rates in diabetic patients [Table 5.3]. There was no significant correlation between **N-acetyl- β -D-glucosaminidase** and glycaemic control [random plasma glucose or glycated haemoglobin].

5.4.DISCUSSION

Elevated urinary transferrin excretion rates have been described recently in adults with complications of

type 2 diabetes [Cheung et al 1989] and children with type 1 diabetes [Ellis et al 1983, Martin et al 1990]. A higher prevalence of elevated urinary transferrin than albumin excretion rates has been reported in a mixed group of type 1 and type 2 diabetic patients [Bernard et al 1988] and these authors suggested that microtransferrinuria may be a more sensitive marker of glomerular damage in diabetes than microalbuminuria.

5.4.1. Reference Range for Urinary Transferrin Excretion

There are few data regarding urinary transferrin excretion in health and in particular no data regarding normal urinary excretion rates. I studied a group of 28 non-diabetic subjects to establish a reference range for urinary transferrin excretion. All of the control subjects were normotensive. They were aged between 21 and 60 years [median 31] which is comparable with the age range of patients in the present studies. The urinary transferrin excretion rates ranged from $0.01 \mu\text{g min}^{-1}$ to $0.28 \mu\text{g min}^{-1}$ with a median value $0.04 \mu\text{g min}^{-1}$. The values, however, were skewed to the lower end of the range and even after transformation did not conform to a normal distribution hence the 95th centile value [$0.11 \mu\text{g min}^{-1}$] was taken as the upper limit of normal. Urinary transferrin excretion increases with age in the rat and is also higher in males than females [McCormick et al 1989]. I found no correlation between urinary transferrin excretion and age or sex in this group of control subjects.

5.4.2. Prevalence of transferrinuria

Most of the [type 1] diabetic subjects [85%] had elevated urinary transferrin excretion rates. This prevalence is similar to that found in type 2 diabetic subjects [Bernard et al 1988]. All of the diabetic subjects with albumin excretion rate $> 20 \mu\text{g min}^{-1}$ had increased transferrin excretion rate [$> 0.11 \mu\text{g min}^{-1}$] as did all patients with retinopathy. However, not all patients with increased transferrin excretion rates had evidence of complications. This observation offers some support to the suggestion that increased urinary transferrin excretion may be a marker for the complications of diabetes.

5.4.3. Mechanism of Transferrinuria

Possible explanations for elevated transferrin excretion rate include:

5.4.3.1. Altered glomerular capillary basement membrane function.

The normal non-diabetic glomerular capillary membrane is both size and charge selective [Dennis and Robinson 1986]. Since transferrin is larger than albumin [Tietz 1987] difference in size is unlikely to explain increased transferrin excretion. In diabetes there may be altered glomerular basement membrane charge [Parthasarathy and Spiro 1982, Kanwar et al 1983, Cohen and Surma 1984] which may facilitate the passage of charged macromolecules.

Glycated serum proteins pass more easily across glomerular basement membrane [Layton and Jerums 1988, Bertolatus and Hunsicker 1985, Williams and Segal 1985, Ghiggeri et al 1984]. Transferrin, however, is less glycated than albumin in diabetes [Austin et al 1987] and so this is an unlikely explanation.

5.4.3.2. Normal glomerular handling of altered serum proteins.

Enhanced glomerular excretion of anionic dextrans may be a consequence of unfolding of the normally globular structure into a more string-like shape [reptation] [Ryan 1981]. There are reports of altered three-dimensional conformation of albumin in diabetic microproteinuria [Ghiggeri 1984]. A similar change in transferrin might favour **its** excretion. There are, however, no data from the present studies to confirm or refute this.

5.4.3.3. Decreased tubular reabsorption of transferrin.

Tubular function in diabetes has been reported as altered [Fletcher et al 1986, Abrass 1984, Gibb et al 1989] or normal [Mathiesen et al 1984]. Transferrin [**pI** 5.8] should be preferentially reabsorbed over albumin [**pI** 4.9] [Mathiesen et al 1984, Christensen et al 1983]. Molecular conformation is also important, however, since proteins with similar sizes and isoelectric points are taken up at different rates by renal tubules [Purtell et al 1979]. Glycation induced changes in transferrin's structure and/or

charge may affect its tubular handling.

N-acetyl- β -D-glucosaminidase is a lysosomal enzyme originating in the renal tubule [Pugh and Walker 1960] and urinary excretion is elevated in various renal disorders [Dance and Price 1970, Kunin et al 1978, Whiting et al 1979]. Glycaemic control may make a significant contribution to the levels of urinary **N-acetyl- β -D-glucosaminidase** in diabetes mellitus [Watanabe et al 1987]. I found no correlation between plasma glucose or glycated haemoglobin and N-acetyl- β -D-glucosaminidase in the patients in this study. There was, however, increased urinary excretion of N-acetyl- β -D-glucosaminidase in diabetic subjects compared with non-diabetic control subjects. There was higher N-acetyl- β -D-glucosaminidase excretion in patients with greater degrees of proteinuria. The correlation between transferrin excretion rate and **N-acetyl- β -D-glucosaminidase** suggests that there may be altered renal tubular handling of this protein. Bernard et al [1990] studied urinary transferrin excretion in workers exposed to cadmium, a metal known to cause renal tubular damage. They found that urinary transferrin excretion rose to a greater extent, and at lower blood levels of cadmium, than **N-acetyl- β -D-glucosaminidase** and other markers of tubular function. From these data they suggest that there are glomerular basement membrane rather than tubular changes.

5.4.3.4. Increased transferrin Presentins to the glomerulus.

This is an unlikely explanation since serum levels of transferrin were the same in all study groups.

It has been suggested that elevated transferrin excretion rate may be an early marker of glomerular dysfunction [Bernard et al 1988, Martin et al 1988]. The data from this cross-sectional study suggest that albumin and transferrin are handled differently by the diabetic kidney. The majority of patients had elevated transferrin excretion rates compared with non-diabetic control subjects. Only **40%**, however, of type 1 diabetic patients will progress to nephropathy. Hence elevated transferrin excretion rate is a **poor** predictor for this complication but may be a marker for renal dysfunction.

CHAPTER 6

TRANSFERRINURIA IN TYPE 2 DIABETES: EFFECTS OF GLYCAEMIC CONTROL

6.1. INTRODUCTION

Increased levels of albuminuria have been reported in newly diagnosed type 1 diabetic patients [Mogensen and Schmitz 1988] and these return to normal once diabetes has been controlled. Similar findings have been reported in type 2 diabetes [Martin et al 1990b]. Having observed elevated levels of urinary transferrin excretion in adults with type 1 diabetes and different degrees of albuminuria, and paying regard to recent reports of transferrinuria in type 2 diabetic patients with complications [Cheung et al 1989, Bernard et al 1988] it seemed logical to try to answer three questions: First, is transferrinuria present at diagnosis in type 2 diabetes mellitus? Secondly, how does urinary transferrin excretion alter with improved metabolic control? Thirdly, does transferrinuria truly relate to the presence of diabetic microangiopathy?

6.2. PATIENTS AND METHODS

All newly diagnosed type 2 diabetic patients without a history of renal disease who attended clinic during a six month period were invited to participate. Patients who had been prescribed oral hypoglycaemic agents or insulin by their general practitioner prior to their first clinic attendance were excluded. Patients were studied at their initial visit and again after 6 and 12 weeks treatment with diet alone or diet and oral hypoglycaemic drugs as appropriate.

Alterations in blood pressure may alter urinary

protein excretion. Anti-hypertensive therapy, therefore, was **not** commenced or altered for the duration of the study in any patient.

At the initial consultation the study aims were explained, patients were given a general medical examination and evidence of diabetic complications was sought. Macrovascular disease was assessed on the basis of history, physical examination and resting ECG. Retinopathy was assessed by direct ophthalmoscopy and was graded as absent, simple [background or pre-proliferative changes] or complex [proliferative changes, macular or peri-macular exudates]; neuropathy was assessed by clinical tests and was graded as present or absent.

At each visit patients were asked to empty their bladders and rest recumbent for 2h having 200 ml water per hour to drink. The volume of urine passed during this time was recorded and aliquots were frozen at -20 °C for later batch analysis of transferrin, albumin, α -1-microglobulin [AlM], **N-acetyl- β -D-glucosaminidase** [NAG] and creatinine. A fresh mid-stream specimen of urine was sent **for** bacteriological examination.

Blood was drawn from an ante cubital vein for estimation of glucose, glycated haemoglobin [HbA1], urea, creatinine, albumin and full blood count. Blood pressure was measured in the right arm after at least 10 min recumbent rest. Diastolic pressure was recorded as the disappearance of

sounds [Korotkow phase V].

6.3. RESULTS

Forty patients participated in the study. Ages ranged from 31 to 80 years [median 61]. There were 26 men and 14 women, 30 were Caucasian, 7 Asian, 2 Afro-Caribbean and 1 Oriental. Most subjects were normotensive: 6 patients, however, had diastolic blood pressure greater than 95 mm Hg. Three of these and 13 others were on anti-hypertensive medication. Fifteen patients had complications at presentation. Five had peripheral vascular disease, 8 ischaemic heart disease [all 8 had symptomatic angina pectoris and 3 had suffered myocardial infarction], 4 had clinical neuropathy and 6 had retinopathy.

There was a decrease in plasma glucose at each visit and glycaemic control, judged by HbA_{1c}, was significantly improved by treatment [Table 6.1].

To correct for variability of urinary volume the albumin:creatinine and transferrin:creatinine ratios were calculated. These are shown with urinary transferrin and albumin excretion rates in Table 6.2. There were reductions in the urinary transferrin excretion rates over the period of the study. Similar results were seen in the urinary transferrin:creatinine ratios. There was a trend towards reduction of albumin excretion rate during the study but this was significant only in patients with abnormally high albumin excretion rate at entry. There was no significant change in

urinary albumin:creatinine ratios.

Eleven [28%] patients initially had albumin excretion rate greater than 20 $\mu\text{g min}^{-1}$ [60.8, 20.4-315.3 $\mu\text{g min}^{-1}$ median, range]. Ten of these patients had elevated transferrin excretion rate. All had significant falls in albumin excretion rate with treatment [$P < 0.001$]. After 6 weeks treatment there were 6 [15%] patients with elevated albumin excretion rate [44.8, 28.2-76.1 $\mu\text{g min}^{-1}$ median, range]. All of these had elevated transferrin excretion rates. At 12 weeks only 4 [10%] had elevated albumin excretion rate [60.0, 22.1-152.8 $\mu\text{g min}^{-1}$ median, range] three had elevated transferrin excretion rates. Only one patient whose initial rate was less than 20 $\mu\text{g min}^{-1}$ subsequently had an elevated excretion rate.

AlM and NAG levels both fell significantly after 6 and 12 weeks treatment of diabetes [Table 6.2].

Initially there were 21 [53%] patients with elevated transferrin excretion rate [0.65, 0.12-10.9 $\mu\text{g min}^{-1}$ median, range]. All of these had highly significant falls in transferrin excretion rate with treatment [0.34, 0.15-2.24 $\mu\text{g min}^{-1}$ at 6 weeks and 0.27, 0.12-1.11 $\mu\text{g min}^{-1}$ at 12 weeks, $P < 0.0001$]. At 6 weeks 12 [30%] patients had elevated transferrin excretion rate and at 12 weeks there were 8 [20%]. Four patients who started with normal transferrin excretion rate had elevated rates at subsequent visits.

Median urinary transferrin excretion rates were similar in patients presenting with or without complications [0.09, 0.01-3.12 $\mu\text{g min}^{-1}$ and 0.12, 0.01-10.94 $\mu\text{g min}^{-1}$ respectively, median and range, $P = \text{NS}$]. There were similar findings for albumin excretion rates [9.9, 2.4-143.5 $\mu\text{g min}^{-1}$ and 8.75, 1.3-315.3 $\mu\text{g min}^{-1}$ respectively, $P = \text{NS}$].

Transferrin excretion rate did not correlate with age or disease duration in these patients. There was a strong correlation between transferrin excretion rate and albumin excretion rate [$r=0.86$, $P < 0.0001$] and between A1M and NAG [$r=0.70$, $P < 0.0001$], transferrin excretion rate and A1M [$r=0.55$, $P < 0.0001$] and transferrin excretion rate and NAG [$r=0.46$, $P < 0.0001$] at each visit. There were weaker correlations between albumin excretion rate and NAG [$r=0.46$, $P < 0.01$] and A1M [$r=0.44$, $P < 0.01$] at each visit. There were correlations between HbA1 levels and each urinary protein: transferrin excretion rate [$r=0.43$, $P < 0.0001$], albumin excretion rate [$r=0.33$, $P < 0.001$], A1M [$r=0.45$, $P < 0.0001$] and NAG [$r=0.47$, $P < 0.0001$]. There was no correlation between transferrin excretion rate and systolic or diastolic blood pressure.

Sixteen patients with hypertension [13 treated] were considered as a sub-group. Their ages were not different from the other patients but they included 8 of the 15 patients with complications at presentation. Ten Patients had elevated transferrin excretion rate initially

Table 6.1.

Plasma glucose, glycated haemoglobin [HbA_{1c}], urea and creatinine in diabetic patients [n=40] at diagnosis and after 6 and 12 weeks treatment.

	0 Weeks	6 Weeks	12 Weeks
Glucose [mmol l ⁻¹]	12.5 [3.4-29.1]	8.3 [3.5-20.9]	6.6 [2.5-19.4]*
HbA _{1c} [%] [§]	10.8 [6.3-24.1]	8.6 [5.2-15.9]	6.9 [2.6-13.1]*
Urea [mmol l ⁻¹]	5.7 [3.4-10.7]	6.0 [3.3-11.7]	5.7 [3.4-11.8]
Creatinine [μmol l ⁻¹]	94 [71-146]	95 [62-158]	92 [55-158]

§ Reference range 5-9%

* P < 0.001

Table 6.2.

Urinary excretion of transferrin [TER], albumin [AER], α -1-microglobulin [A:M], N-acetyl- β -D-glucosaminidase [NAG] and urinary transferrin:creatinine [T:Cr] and albumin:creatinine [A:Cr] ratios in diabetic patients [n=40] at diagnosis and after 6 and 12 weeks treatment.

	0 Weeks	6 Weeks	12 Weeks
TER [$\mu\text{g min}^{-1}$]	0.12 [0.01-10.95]	0.04 [0.01-2.24]	0.04 [0.01-1.12] *
AER [$\mu\text{g min}^{-1}$]	7.84 [1.3-315.3]	4.2 [1.6-76.1]	4.3 [1.3-152.8]
T:Cr [$\mu\text{g mmol}^{-1}$]	20.8 [1.1-910.4]	5.0 [1.0-840.6]	7.5 [0.9-120.6] *
A:Cr [$\mu\text{g mmol}^{-1}$]	0.8 [0.1-33.3]	0.5 [0.2-20.3]	0.6 [0.2-26.0]
A:M [$\mu\text{g min}^{-1}$]	14.5 [1.7-102.2]	5.8 [2.0-75.4]	3.0 [2.0-33.3] *
NAG [u min^{-1}]	232 [0 - 1856]	97 [0 - 1109]	59 [0 - 625] *

=====

All variables shown as median [range].

* P < 0.001

and **8** had elevated albumin excretion rate. The numbers fell to **4** and **3** patients after 12 weeks treatment. These patients do not appear to have behaved differently from those without hypertension.

6.4.DISCUSSION

6.4.1. Transferrinuria and glycaemic control

Elevated albumin excretion rates have been reported in patients with newly diagnosed, uncontrolled type 1 or type 2 diabetes mellitus and these return to normal when diabetes is controlled [Mogensen and Schmitz 1988b, Martin et al 1990b]. I studied urinary transferrin excretion in newly diagnosed type 2 diabetic patients and examined the effects of glycaemic control.

Twenty-seven per cent of patients had microalbuminuria and 53% had elevated urinary transferrin excretion rates at presentation. Prevalence data for microalbuminuria in type 2 diabetes are scarce. Mogensen [1984b] reported that 12.5% of outpatient diabetics had albumin excretion rate between 30 and **140 $\mu\text{g min}^{-1}$** . The patients in his study, however, were not newly diagnosed. The Bedford study [Keen et al 1969], which was community based, found increased prevalence of elevated albumin excretion rates at diagnosis in diabetic patients compared with borderline diabetic patients or control subjects. A study of 510 patients with type 2 diabetes found that 48% had urinary protein excretion $> 150 \text{ mg } 24\text{h}^{-1}$ [approximately 105

$\mu\text{g min}^{-1}$] [Fabre et al 1982].

In my study the number of patients with elevated transferrin or albumin excretion rates fell after treatment of diabetes. One possible explanation for this observation is that hyperglycaemia may cause an osmotic diuresis with consequent proteinuria. Control of diabetes by reducing the level of glycosuria would subsequently decrease proteinuria. I did not measure urinary glucose but the findings may be explained in part by the [presumed] high urinary levels of glucose in newly diagnosed patients.

6.4.2. Altered Renal Function

An alternative explanation for the decreased proteinuria after treatment of diabetes would be altered glomerular filtration rate secondary to control of blood sugar. Mogensen [1971a] demonstrated elevated glomerular filtration rates in type 1 diabetes but found no correlation with serum glucose concentration. The same worker also demonstrated increased maximum tubular reabsorption capacity for glucose [T_{mG}] in type 1 diabetic patients rendered hyperglycaemic by glucose infusion [Mogensen 1971b]. The rise in [T_{mG}] was paralleled by a rise in glomerular filtration rate indicating that glomerulo-tubular balance is maintained. Similarly, Sandahl-Christiansen et al [1981] demonstrated that hyperglycaemia contributes to disturbed renal haemodynamics in type 1 diabetes mellitus. These studies indicate that glycaemic control can affect renal function. Two groups of workers have published data regarding

renal function during and after 12 months improved glycaemic control. Wiseman et al [1985] reported normalisation of glomerular filtration rate but no change in renal size. Feldt-Rasmussen et al [1986] reported reduction of kidney size but no change in glomerular filtration rate with no change in proteinuria. I do not have adequate data to examine the possibility that changes in renal haemodynamics were responsible for the reductions in transferrinuria and albuminuria seen in the present study.

In the non diabetic kidney the majority of albumin filtered at the glomerulus is reabsorbed in the proximal tubule [Dennis and Robinson, 1986]. Increased amounts of urinary albumin in diabetes are thought to be a consequence of glomerular leak [Abrass 1984]. Transferrin and albumin have similar molecular sizes [38A and 36A respectively] and weights [77,000 and 66,000 respectively] [Tietz 1987]. Increased urinary transferrin loss, therefore, may reflect glomerular leak.

As noted above glycation of serum proteins facilitates their transport across the glomerular basement membrane. Transferrin, however, is relatively less glycated than albumin in diabetes and so its transport should not be facilitated at the expense of albumin. If glycated albumin and transferrin compete for the same tubular reabsorption pathway it is possible that because of differences in isoelectric point and molecular conformation albumin is preferentially reabsorbed. Increased transferrin excretion

rate, therefore, would be most likely the result of decreased tubular reabsorption.

Other workers have demonstrated that there is altered tubular function in diabetes [Mathiesen et al 1984, Gibb et al 1989]. The suggestion of altered tubular function is supported by data from diabetic children [Martin et al 1990a]. Yaqoob et al [1992] have reported recently that tubular damage and transferrinuria precede microalbuminuria in type 1 diabetes. I found correlations between transferrin and tubular proteins in this study. Although there were correlations between the tubular markers and albumin excretion rates these were weaker than with transferrin excretion rates suggesting that tubular handling is more important for transferrin than albumin.

6.4.3. Transferrinuria and Complications in type 2 Diabetes

Elevated urinary transferrin excretion rates have been reported in patients with type 2 diabetes and complications [Cheung et al 1989]. In 15 [**37.5%**] of my patients there was evidence of one or more complications [ischaemic heart disease, peripheral vascular disease, retinopathy, neuropathy] at diagnosis. There was no difference in transferrin or albumin excretion rate between Patients with and without these complications. There was no difference in transferrin excretion rate or albumin excretion rate between patients with and without these complications. These patients, however, unlike those of Cheung et al were

newly diagnosed and may not have been diabetic for long enough to develop complications by the time of the study.

In conclusion, elevated urinary transferrin excretion rate is common in newly diagnosed type 2 diabetic patients and levels fall with glycaemic control. The mechanism for this proteinuria is unknown but may relate to altered tubular function. Elevated urinary transferrin excretion rate does not appear to be a marker for complications in newly diagnosed type 2 diabetic patients.

CHAPTER 7

INCREASED URINARY TRANSFERRIN EXCRETION IN EXERCISING NORMOALBUMINURIC INSULIN DEPENDENT DIABETIC PATIENTS.

7.1. INTRODUCTION

Presently the most reliable predictor for diabetic nephropathy is the presence of microalbuminuria, an albumin excretion rate between 20 and 200 $\mu\text{g min}^{-1}$ in two from three timed urine samples in a six month period. Elevated urinary transferrin excretion rates, however, may precede elevated albumin excretion rates in diabetic children [Martin et al 1990] and it has been suggested that elevated transferrin excretion rates may predict patients at risk of diabetic nephropathy [Martin et al 1988, Bernard et al 1988]. No more than 40% of type 1 diabetic patients are likely to develop nephropathy but I have shown that more than 80% of patients have elevated urinary transferrin excretion rates. Hence, it is apparent that spontaneous urinary transferrin excretion is of low predictive value for this nephropathy. Stimulated transferrin excretion, however, may be useful. Vigorous exercise is known to provoke elevated albumin excretion rates in type 1 diabetic patients who have normal albumin excretion rates at rest [Viberti et al 1978, Brun et al 1987, Feldt-Rasmussen et al 1985]. To investigate whether transferrin may behave in a similar fashion I studied the urinary excretion of transferrin in response to exercise in patients with uncomplicated type 1 diabetes mellitus.

7.2. MATERIALS AND METHODS

Eight men with Type 1 diabetes mellitus for at least five years and eight sex and age matched healthy control subjects were studied. The diabetic subjects were

normoalbuminuric [urinary albumin excretion rate < 20 $\mu\text{g min}^{-1}$, confirmed by at least two timed urine collections], normotensive [blood pressure < 160/95 mm Hg], and had no retinopathy or clinical evidence of peripheral or autonomic neuropathy. The control subjects had no family history of diabetes mellitus, hypertension or renal disease. Diabetic and control subjects were matched as far as possible for their habitual level of exercise.

7.2.1. Exercise Protocol

All subjects underwent a preliminary progressive exercise test to determine maximal workload capacity. The test commenced at a workload of 50w using an electromagnetically braked cycle ergometer [Rodley Elektronik 820, Sweden]. The workload was then increased by 20w min^{-1} until exhaustion. To standardize the physical stress for individuals, since not all had the same maximal exercise capacity, a steady state sub-maximal test at 50% maximal workload was performed not less than 1 week and no more than 2 months after the maximal test. Data from the steady state tests are presented here.

On the study day subjects attended between 1300-1600 hours having eaten as normal at midday. Diabetic subjects took their usual insulin. At the start of the study period subjects emptied their bladders and drank 200 mL water. They then rested recumbent for 1h after which urine was collected for estimation of albumin, transferrin, α -i-microglobulin, and **N-acetyl- β -D-glucosaminidase** content.

Blood was drawn with minimal venous stasis for measurement of glucose, glycated haemoglobin, plasma electrolytes, urea, creatinine, and albumin. Subjects then exercised for 20 min at 50% maximal workload. Further blood and urine samples were collected immediately after exercise and following one hour's rest. To maintain fluid balance during the study subjects were given 200ml water plus a volume equivalent to their previous hour's urine output to drink per hour. A fresh mid-stream specimen of urine was sent for bacteriological examination.

7.2.2. Measurements

All blood was centrifuged immediately [2000g, 4 °C] for 10 min. Plasma and urine were frozen and stored at -20 °C for later analysis.

7.3. RESULTS

Although diabetic and control subjects were well matched for age and level of habitual exercise, workloads median [range] for steady state exercise were slightly higher in the control 260 [150-370w] than diabetic group 210 [150-290w], $P < 0.05$.

Table 7.1 shows subject characteristics and plasma biochemistry at the start of the study.

Table 7.2 shows urinary flow rates and protein excretion rates for diabetic and control subjects at rest, immediately after exercise and following one hour's recovery.

Urine flows were no different between diabetic and control groups and did not alter with exercise.

In the diabetic group the median transferrin and albumin excretion rates after exercise were significantly higher than at rest [1.5 vs $0.2 \mu\text{g min}^{-1}$, $P < 0.001$, and 29.8 vs $7.8 \mu\text{g min}^{-1}$, $P < 0.001$ respectively]. The proportional rise in transferrin excretion rate [$>600\%$] was more than twice that in albumin excretion rate [$<300\%$]. Median N-acetyl- β -glucosaminidase excretion levels were higher in the diabetic than control subjects at rest and after exercise [Table 7.2] and did not alter significantly. There was no change in α - 1 -microglobulin excretion.

In the non-diabetic control group albumin excretion rose from 6.3 to $11.4 \mu\text{g min}^{-1}$ [$P < 0.05$]. There was no alteration in transferrin, N-acetyl- β -glucosaminidase, or α - 1 -microglobulin excretion.

After exercise there was a correlation between transferrin and albumin excretion rates in the diabetic group [$r = 0.79$, $P < 0.02$]. No correlation was observed between any other proteins. There was no correlation between N-acetyl- β -D-glucosaminidase excretion and glycated haemoglobin.

7.4. DISCUSSION

This study confirmed the observation of

Table 7.1.

Subject characteristics and biochemistry at the start of the study.

	AGE [yrs]	DURATION [yrs]	GLUCOSE [mmol L ⁻¹]	HbA1 [%]*	ALBUMIN [g L ⁻¹]	CREATININE [μmol L ⁻¹]
PATIENTS	20	11	3.9	8.8	37	85
	20	10	11.0	11.8	32	82
	26	13	6.7	9.2	34	86
	26	18	14.1	6.5	34	108
	31	07	24.0	12.8	37	105
	32	19	13.6	9.8	34	97
	34	18	10.3	8.8	40	97
	35	17	3.9	9.4	41	104
CONTROLS	20	-	3.7	5.5	42	92
	23	-	5.4	3.8	37	95
	24	-	6.1	4.9	43	96
	27	-	3.5	4.5	43	111
	30	-	7.0	6.1	38	102
	31	-	7.5	3.3	39	93
	32	-	2.0	5.2	37	126
	35	-	5.6	4.3	43	87

* Reference range 5.0-9.0%.

Table 7.2.

Urinary flow rates and albumin [AER], transferrin [TER], α -1-microglobulin [AlM], and N-acetyl- β -D-glucosaminidase [NAG] excretion [median and range] in diabetic and control subjects at rest, immediately after exercise and following recovery.

	REST	RECOVERY	EXERCISE
<u>URINE [mL min⁻¹]</u>			
DIABETIC	1.8 [0.9-4.3]	1.6 [0.6-2.3]	1.4 [0.9-8.6]
CONTROL	1.2 [0.7-10.0]	2.2 [0.9-8.0]	1.7 [0.8-8.3]
<u>AER [μg min⁻¹]</u>			
DIABETIC	7.8 [3.1-8.4]	29.8 [16.6-343.8] *	13.7 [3.2-52.2]
CONTROL	6.3 [1.5-9.0]	11.4 [2.7-114.9] **	4.3 [1.9-12.1]
<u>TER [μg min⁻¹]</u>			
DIABETIC	0.2 [0.05-0.4]	1.5 [1.3-26.3] *	0.6 [0.1-2.5]
CONTROL	0.1 [0.01-7.9]	0.2 [0.02-8.9]	0.09 [0.01-12.6]
<u>AlM [μg min⁻¹]</u>			
DIABETIC	5.9 [3.4-17.3]	13.8 [3.6-33.8]	11.4 [1.9-26.9]
CONTROL	2.9 [1.4-20.0]	9.8 [1.8-26.7]	4.8 [1.6-16.7]
<u>NAG [μmol min⁻¹]</u>			
DIABETIC	0.25 [0.12-0.85] ‡	0.17 [0.08-0.54] ‡‡	0.17 [0.04-0.26]
CONTROL	0.14 [0.04-0.18]	0.11 [0.02-0.2]	0.05 [0.01-0.23]

* P < 0.001 compared with resting values. ** P < 0.05 compared with resting values.
 ‡ P < 0.001 compared with control values. ‡‡ P < 0.05 compared with control values.

increased urinary albumin excretion rate following exercise in type 1 diabetic patients [Viberti et al 1978, Brun et al 1978, Feldt-Rasmussen et al 1985]. The finding of increased transferrin excretion in diabetic subjects following exercise is novel. Although quantitatively much less transferrin than albumin is excreted there is a proportionally far greater increase. Although there was a slight rise in albumin excretion following exercise there was no increase in urinary transferrin excretion in the control group. This suggests altered handling of transferrin in the diabetic state. Albumin excretion rate has been shown to increase on exercise in poorly controlled diabetes. The same may be true for other proteins, including transferrin, but there are no data regarding this. The increase in protein excretion could not have been a consequence of altered urine flow rates since there was no difference between diabetic and control groups nor within the groups during the study.

7.4.1. Evidence for Renal Tubular Dysfunction

Viberti et al [1978] interpreted their findings of increased urinary albumin excretion but normal β_2 -microglobulin excretion [a marker of renal tubular function] following exercise as evidence for a glomerular leak of albumin. A study of non-diabetic children and adolescents reported similar results [Huttunen et al 1981]. Poortmans and co-workers [1988], however, found that there was a pattern of proteinuria suggestive of mixed glomerulo-tubular loss during an exercise test in diabetic subjects

given lysine, an amino acid which blocks tubular reabsorption of filtered protein. The finding of increased albumin excretion following exercise in the diabetic group in the present study is evidence for a glomerular leak. Data from the cross sectional study showed a correlation between urinary excretions of transferrin and N-acetyl- β -D-glucosaminidase [a marker of renal tubular function] suggesting a tubular component to transferrin loss.

7.4.2. N-acetyl- β -D-glucosaminidase excretion

In the present study N-acetyl- β -D-glucosaminidase excretion was higher in the diabetic than the control group at rest and after exercise. The finding of increased **N-acetyl- β -D-glucosaminidase** excretion at rest in diabetic subjects with proteinuria has been taken as evidence of tubular dysfunction [Ratzmann et al 1988]. Increased **N-acetyl- β -D-glucosaminidase** excretion following treadmill exercise and a correlation between N-acetyl- β -D-glucosaminidase and glycated haemoglobin levels has been reported in diabetic children [Brouhard et al 1985]. Neither **N-acetyl- β -D-glucosaminidase** nor α -1-microglobulin excretion altered with exercise in my study; there was no correlation with serum levels of glycated haemoglobin. These observations suggest that any effect of exercise on tubular protein leak is small, if present at all.

The mechanism[s] underlying the increased loss of protein following exercise is unknown. Ala-Houhala and Pasternak [1987], and Ala-Houhala [1990] demonstrated

decreased glomerular filtration rate and renal blood flow with net increased filtration fraction and fractional clearance of dextrans of radius greater than 4.8nm after exercise in subjects with diabetic nephropathy. These results indicate that there is increased glomerular permeability as a consequence of altered renal haemodynamics. Loss of the negative charge on glomerular capillary walls is important in the development of diabetic proteinuria [Parthasarathy and Spiro 1982]. Results from animal work reveal a reduction in this charge following exercise [Zambraski et al 1981]. This may explain partially the development of post exercise proteinuria in the human. The anionic glomerular basement membrane represents less of a barrier to the passage of cationic than anionic molecules. Since native albumin, is more anionic than transferrin [pI albumin 4.9, pI transferrin 5.8] it should be preferentially retained by the glomerular basement membrane. My observation of increased transferrin excretion after exercise is not in keeping with this mechanism and suggests that haemodynamic factors may be more important in this setting.

In summary, I have demonstrated increased transferrin excretion in exercising diabetic subjects without evidence of complications. There was no rise in urinary transferrin excretion following exercise in a well matched control group. In the diabetic group the proportional increase in transferrin excretion was greater than for

albumin and there is evidence for altered glomerular leak in these patients. The data suggest that even patients with apparently uncomplicated diabetes mellitus may have abnormal renal function. Elevated urinary transferrin excretion rates after exercise may be better discriminators than elevated urinary albumin excretion rates. The mechanism underlying the exercise induced increase in transferrin excretion remains to be elucidated.

CHAPTER 8

THE EFFECTS OF AN ANGIOTENSIN CONVERTING ENZYME INHIBITOR AND CALCIUM CHANNEL BLOCKER ON URINARY TRANSFERRIN EXCRETION IN DIABETIC PATIENTS WITH ELEVATED ALBUMIN EXCRETION RATES.

8.1. INTRODUCTION

I have demonstrated elevated transferrin excretion rates in patients with type 1 diabetes mellitus and this may indicate impaired renal function. My second study showed that newly diagnosed type 2 diabetic patients have increased transferrin excretion rates and the third that urinary transferrin excretion can be provoked by exercise in patients with uncomplicated type 1 diabetes. Despite these apparent similarities with the behaviour of albumin, transferrin's relationship with markers of renal tubular function suggests that the diabetic kidney handles it in a different fashion from albumin.

Several groups have reported decreases in urinary albumin excretion in hypertensive and normotensive diabetic patients treated with angiotensin converting enzyme [ACE] inhibitors [Taguma et al 1985, Marre et al 1988]. I have studied the effects of an ACE inhibitor on urinary transferrin excretion in normotensive diabetic patients with microalbuminuria and hypertensive diabetic patients with macroalbuminuria.

8.2. PATIENTS AND METHODS

Subjects had micro- or macroalbuminuria and had been identified from an extension of the screening programme described earlier. There were two study protocols which are described more fully in Chapters 11 and 12.

The first was a double blind placebo

controlled study of the effects of an ACE inhibitor, lisinopril, in normotensive [BP < 160/90 mm Hg], microalbuminuric [AER, > 20 $\mu\text{g min}^{-1}$ but < 200 $\mu\text{g min}^{-1}$] patients. Patients gave informed consent and followed the protocol as detailed in Figure 8.1. Briefly, after a two week placebo run in period those whose compliance was worse than 80% were excluded and the remainder were randomised double blind to receive lisinopril or placebo. Patients randomised to lisinopril received a test dose of 2.5 mg and then lisinopril 10 mg daily for the duration of the study [48 weeks]. Those randomised to placebo continued placebo matching lisinopril 10 mg daily. Patients were reviewed on four further occasions at 12 week intervals.

The second [Fig 8.2] was a double blind, double dummy comparison of lisinopril with nifedipine, in hypertensive [blood pressure \geq 160/90 mm Hg off treatment] patients with persistent macroproteinuria [AER \geq 200 $\mu\text{g min}^{-1}$]. After a two week placebo run in period to exclude those with compliance less than 80% patients were randomised double blind to receive lisinopril or nifedipine. Patients randomised to lisinopril received a test dose of 2.5 mg with placebo matching slow release nifedipine 10mg twice daily followed by lisinopril 5 mg daily with placebo matching slow release nifedipine 10 mg twice daily [Visit 3]. Patients randomised to nifedipine were given a test dose of slow release nifedipine 10 mg twice daily and placebo matching 2.5 mg lisinopril followed by slow release nifedipine 10 mg twice

FIGURE 8.1.

Study Protocol for Normotensive Patients Treated with Lisinopril or Placebo

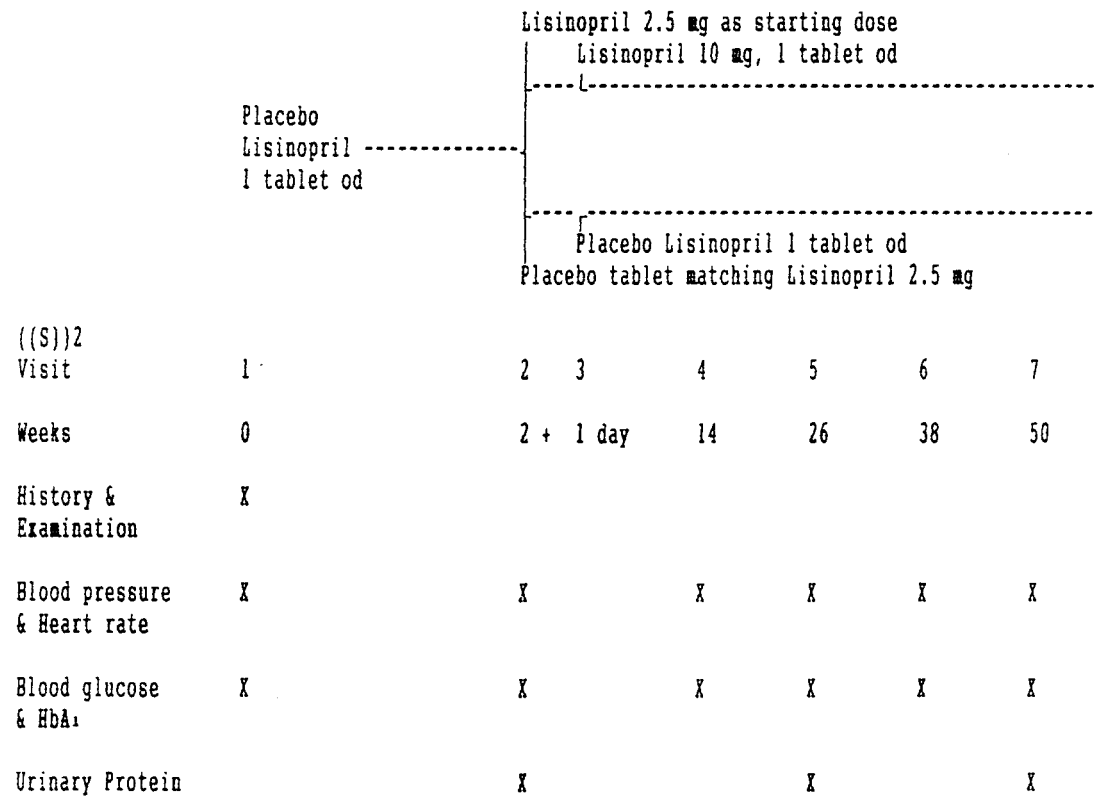


FIGURE 8.2.

Study Protocol for Hypertensive Patients Treated with Lisinopril or Nifedipine

		Lisinopril [L] 2.5mg as starting dose					
		L 5 mg od		L 5/10 mg od		L 5/10/20 mg od	
Placebo Lisinopril ----- and Nifedipine		-----					
		N 10 mg bd		N 10/20 mg bd		N 10/20/40 mg bd	
		Nifedipine SR [N] 10 mg bd					
((S))2							
Visit	1	2	3	4	5	6	7
Weeks	0	2 + 1 day		5	8	11	19
History & Examination	X						
Blood pressure & Heart rate	X	X		X	X	X	X
Blood glucose & HbA _{1c}	X	X		X	X	X	X
Urinary Protein	X	X				X	X

daily and placebo matching lisinopril 5 mg one tablet daily. All were reviewed three times at three week intervals. The dose of lisinopril was increased stepwise to 10 mg [Visit 4] or 20 mg daily [Visit 5] and that of nifedipine to 20 mg twice daily [Visit 4] or 40mg twice daily [Visit 5] as necessary with the aim of achieving diastolic blood pressure ≤ 90 mm Hg. Antihypertensive medication was continued for a further eight weeks at which time the study was terminated [Visit 7].

In both studies blood pressure was measured at each visit after at least 10 minutes rest. Two readings were taken from the right arm, at heart level, using a Hawksley random zero sphygmomanometer and the mean was recorded. Diastolic blood pressure was taken as Korotkoff phase 5. Mean arterial blood pressure [MAP] was calculated as diastolic blood pressure + pulse pressure/3.

Timed overnight urine collections were made immediately prior to clinic visits.

8.3.RESULTS

8.3.1. Normotensive Patients

Thirty-two patients entered the first study. Three lisinopril treated and 2 placebo treated patients were withdrawn because of adverse effects or failure to attend for follow up. Hence, 27 patients [12 lisinopril, 15 placebo] completed the study. The groups were well matched for age,

Table 8.1.

Characteristics of normotensive patients at entry to the study.

	Lisinopril n=15	Placebo n=17
M:F	11:4	12:5
Age [years]	48.3±13.0	49.1±16.0
Weight [kg]	80.8±16.1	76.6±17.4
Age & weight presented as mean±SD.		

Table 8.2.
Transferrin excretion rates [median, range] for
lisinopril and placebo treated patients during study of
normotensive diabetics.

VISIT	LISINOPRIL	PLACEBO
2	0.76, 0.04-11.9 [n=12]	1.89, 0.07-30.52 [n=17]
4	0.23, 0.04-13.49 [n=13]	3.4, 0.10-18.39* [n=16]
5	0.32, 0.04-12.44 [n=13]	1.6, 0.04-10.46* [n=17]
7	0.39, 0.03-4.28 [n=10]	2.84, 0.09-46.2* [n=15]

* P > 0.01 between lisinopril and placebo groups.

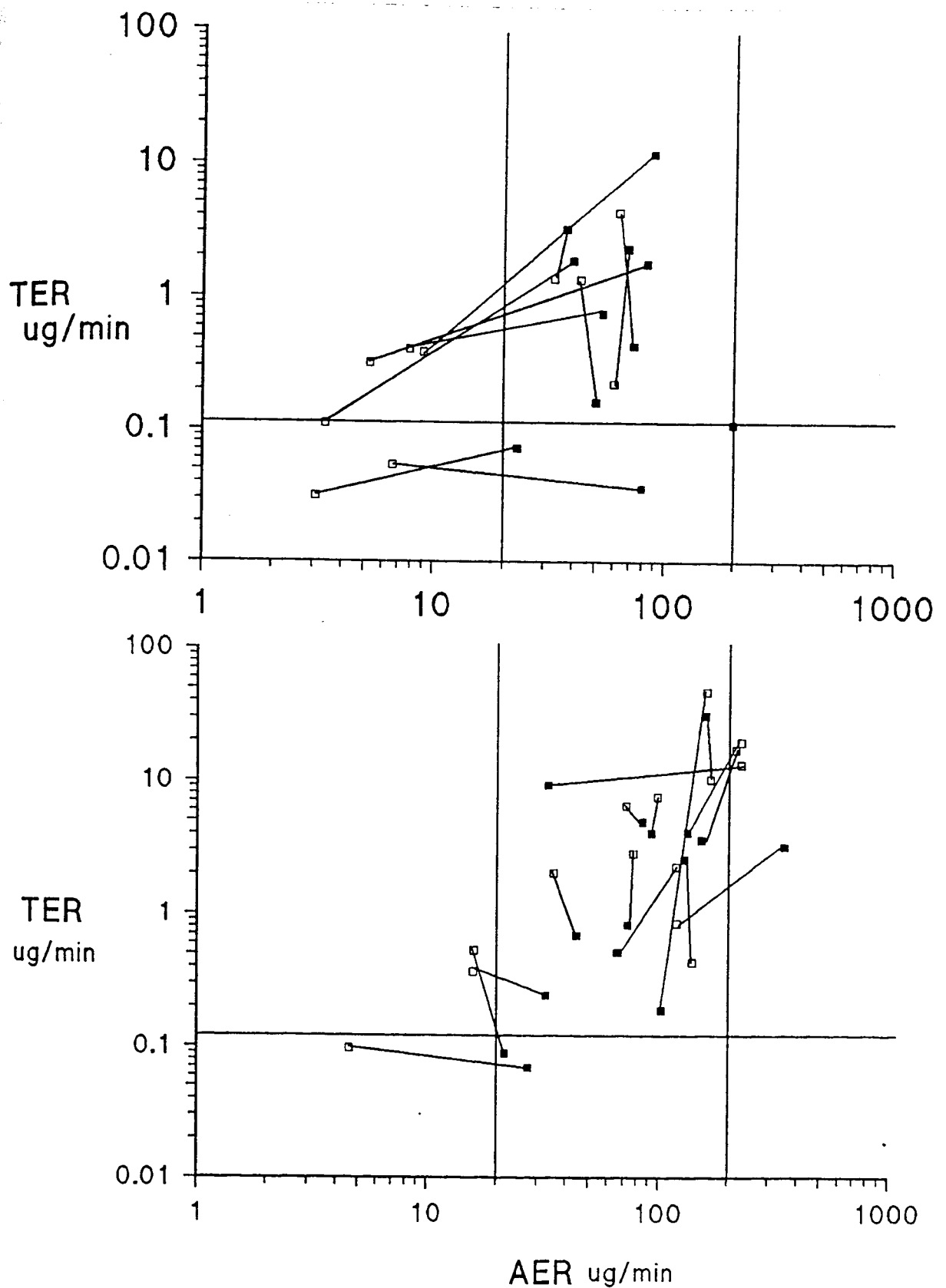


FIGURE 8.3.

Transferrin [TER] and albumin [AER] excretion rates in normotensive microalbuminuric diabetic patients treated with lisinopril [upper panel] or placebo [lower panel]. Closed square represents start of study, open square represents value after 50 weeks treatment.

sex, weight and treatment of diabetes [Table 8.1].

At entry to the study 10 lisinopril treated and 15 placebo treated patients had elevated transferrin excretion rates [TER > 0.11 $\mu\text{g min}^{-1}$, as previously defined]. Excretion rates rose in 12 placebo treated and 3 lisinopril treated patients [Fig 8.3] although median transferrin excretion rate did not alter appreciably in either group [Table 8.2].

Mean [\pm SD] albumin excretion rate fell progressively in the lisinopril group from 58.4 [26.7] $\mu\text{g min}^{-1}$ at baseline to 26.8 [26.8] $\mu\text{g min}^{-1}$ [P < 0.01] after 48 weeks treatment and rose in the placebo group [102.7 [79.9] $\mu\text{g min}^{-1}$ vs. 112.9 [76.7] $\mu\text{g min}^{-1}$, P = NS]. This represented a change in mean albumin excretion rate of -34.3 [30.9] $\mu\text{g min}^{-1}$ for the lisinopril group and +12.8 [88.4] $\mu\text{g min}^{-1}$ for the placebo group. After 48 weeks treatment 7 lisinopril treated patients were normoalbuminuric and 5 were microproteinuric; 3 placebo treated were normoalbuminuric, 9 microalbuminuric and 3 were macroproteinuric [Fig 8.3].

Transferrin and albumin excretion rates did not correlate in either group at the beginning of the study but did correlate at visit 7 [r = 0.53, P < 0.05], in the placebo group.

8.3.2. Hypertensive Patients

Fourteen patients were randomised to each treatment. Analysis of transferrin results is based on 12

Table 8.3.

Patient characteristics at entry to study of
hypertensive macroproteinuric diabetics.

	Lisinopril n=14	Nifedipine n=14
M:F	12:2	10:4
Age [years]	53.3 [11.2]	50.5 [19.2]
Weight [kg]	84.3 [20.8]	83.0 [18.5]

=====

Age & weight presented as Mean [SD].

Table 8.4.

Transferrin excretion rates [median, range] for
lisinopril and nifedipine treated patients during study of
hypertensive diabetics.

VISIT	LISINOPRIL {n=12}	NIFEDIPINE {n=12}
1	15.3, 0.31-54.69	14.3, 6.27-98.48
6	10.4, 0.07-32.84	19.2, 2.40-67.77
7	11.2, 0.05-39.46	31.5, 1.51-74.32

=====

There were no significant differences between any groups during
the study.

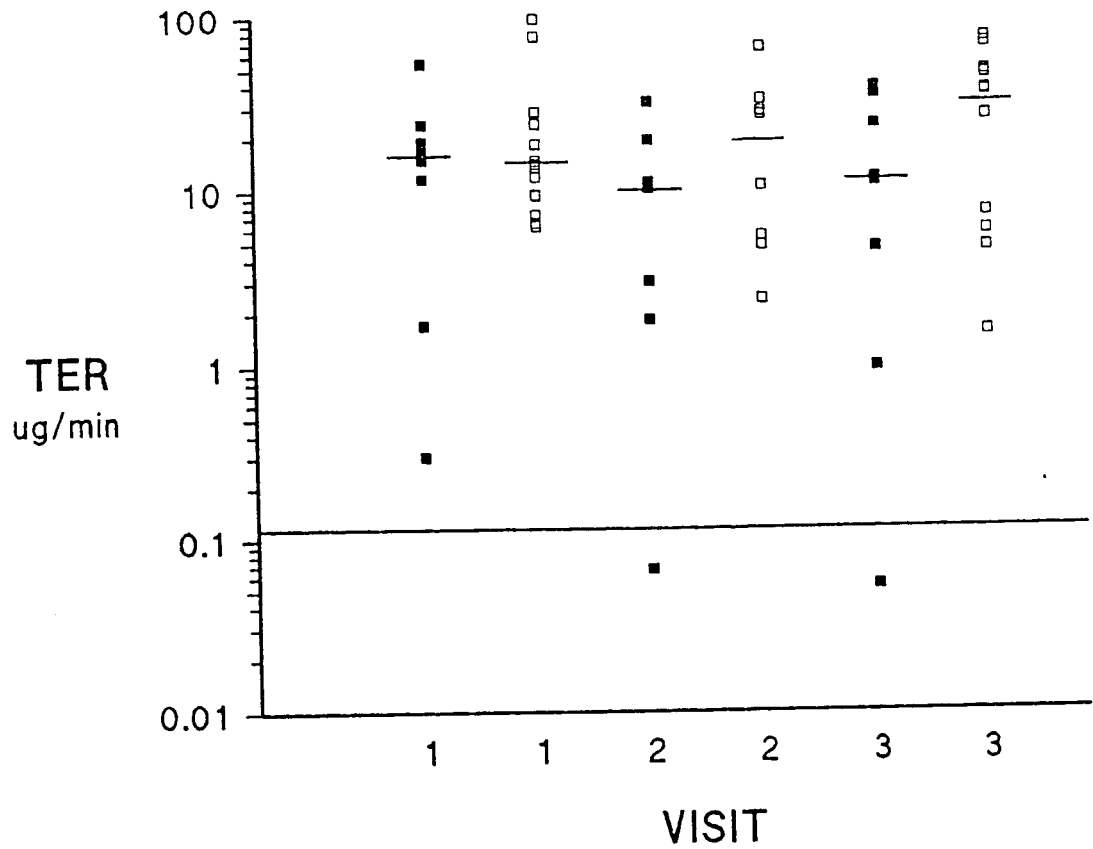


FIGURE 8.4.

Transferrin excretion rates [TER] by visit for lisinopril [closed square] and nifedipine [open square] treated hypertensive macroproteinuric diabetic patients.

patients from each group for whom there were complete data. The groups were well matched for age, weight, and sex [Table 8.3].

No patient had normal transferrin excretion rate at the start of the study but one lisinopril treated patient had normal transferrin excretion rate at the end of the study. Median transferrin excretion rate fell from 15.3 $\mu\text{g min}^{-1}$ to 11.2 $\mu\text{g min}^{-1}$ [P = NS] for lisinopril treated patients but rose from 14.3 $\mu\text{g min}^{-1}$ to 31.5 $\mu\text{g min}^{-1}$ [P = NS] for those receiving nifedipine [Table 8.3]. There were no significant differences in transferrin excretion rate between lisinopril and nifedipine treated groups at any visit [Fig 8.4].

Albumin excretion rate fell [from 738.7 \pm 635.2 $\mu\text{g min}^{-1}$ to 644.6 \pm 965.2 $\mu\text{g min}^{-1}$] between visits 2 and 7 in the lisinopril treated group. albumin excretion rate rose from 981.2 \pm 1022.2 $\mu\text{g min}^{-1}$ to 1072.5 \pm 908.5 $\mu\text{g min}^{-1}$ in the nifedipine treated group. There was, however, no significant difference between groups.

There was no correlation between transferrin excretion rate and albumin excretion rate at any time in the lisinopril treated group but there were strong correlations at visits 6 and 7 [$r=0.98$, $r=0.99$, respectively, $P < 0.01$] in nifedipine treated patients.

There was no correlation between transferrin excretion rate and systemic blood pressure nor between change

Table 8.5.

Changes in mean systolic [SBP] and diastolic [DBP]

blood pressures for lisinopril [L] and nifedipine [N]

treated hypertensive groups.

		Mean change from		Treatment	95% CI	P
		baseline		difference		
		L	N			
SBP	-20.4	-16.6	-3.8	-18.7 to 11.0	0.6	
	[mm Hg]					
DBP	-10.2	-13.7	3.5	-6.1 to 13.1	0.46	
	[mm Hg]					

Blood pressure

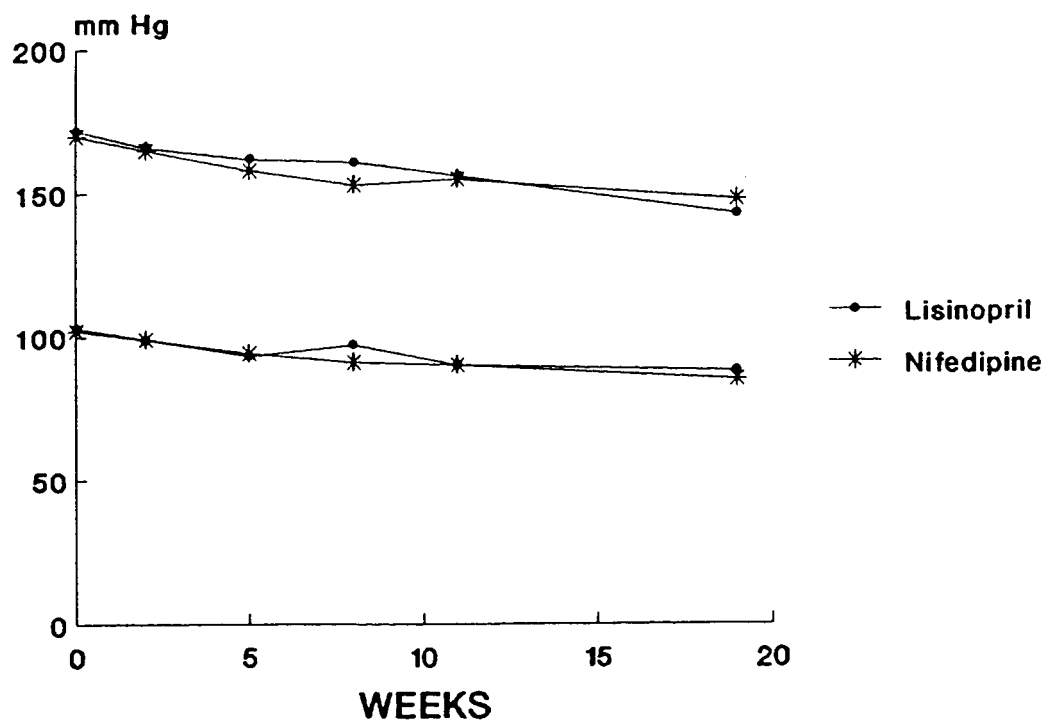


FIGURE 8.5.

Systolic and diastolic blood pressures during study for lisinopril and nifedipine treated patients.

in transferrin excretion rate and change in blood pressure in either group.

Blood pressure [mean \pm SD] was lower in both groups at visit 7 compared with visit 2 [143/88 \pm 17/13 mm Hg vs 166/99 \pm 23/9 mm Hg for lisinopril treated, and 148/85 \pm 25/10 mm Hg vs. 165/99 \pm 21/7 mm Hg for nifedipine treated, Table 8.5]. Blood pressures were not significantly different between groups throughout the study [Fig 8.5].

8.4. DISCUSSION

I have performed two studies of the effects of angiotensin converting enzyme [ACE] inhibition on urinary transferrin excretion. These addressed the questions of whether ACE inhibition has an effect on transferrin excretion in normotensive diabetic patients; whether lowering blood pressure in hypertensive diabetic patients affects transferrin excretion; whether the effect, if present, is specific to ACE inhibitors; and whether transferrin and albumin behave in the same way in diabetic patients.

For the normotensive patients the lisinopril and placebo treated groups were well matched for age, sex, weight and treatment of diabetes at entry to the study. Taking the upper limit of normal for urinary transferrin excretion as 0.11 $\mu\text{g min}^{-1}$ I found, as in previous studies, that the majority of diabetic patients had elevated excretion rates.

During the intervention studies median values for transferrin excretion did not alter significantly in either group although they showed a tendency to rise in placebo treated patients and to fall in those treated with lisinopril. These data suggest that transferrin excretion can be modified by **ACE** inhibition in this patient population. Similarly, there were differences between albumin excretion rates in placebo and lisinopril treated groups. A correlation between transferrin excretion and albumin excretion rate, however, was seen only in the placebo group. A correlation between transferrin and albumin excretion rates was noted in the cross-sectional data from the screening study of diabetic patients. The absence of a correlation between these proteins in patients taking **ACE** inhibitors suggests that the drug has in some way altered the renal handling of one or the other to a greater extent. The mechanism[s] by which transferrin excretion is altered by **ACE** inhibition remains to be defined but is probably the same as that responsible for reducing albuminuria i.e. reduction of intra-glomerular capillary pressure.

As in the normotensive diabetic patients median transferrin excretion fell in lisinopril treated hypertensive diabetic patients although again the change in excretion rate was not significant. Conversely there was a tendency for transferrin excretion to rise, insignificantly, in nifedipine treated patients. Similar changes were found for albumin. There were significant falls in systemic blood

pressure in both lisinopril and nifedipine treated groups. There was, however, no correlation between reduction in transferrin excretion and reduction of systemic blood pressure. These data suggest that, although there is a tendency for transferrin excretion to fall in **ACE** inhibitor treated patients when blood pressure is reduced, reduction of transferrin excretion depends upon a specific intra-renal effect of **ACE** inhibition [reduction of intra-glomerular capillary pressure]. Nifedipine appears not to be useful in this setting.

THE KIDNEY IN DIABETES MELLITUS:

PART II

THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM,
HYPERTENSION,
AND THE ROLE OF
ANGIOTENSIN CONVERTING ENZYME INHIBITORS
IN THERAPY

CHAPTER 9

BLOOD PRESSURE IN DIABETIC NEPHROPATHY:

1. ACTIVITY OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

9.1. INTRODUCTION

There have been varying reports of the activity of the Renin-Angiotensin-Aldosterone system in patients with diabetes mellitus. Depending upon whether the patient has renal impairment or hypertension the activity has been variously reported as normal [O'Hare et al 1988, Feldt-Rasmussen et al 1987], increased [De Chatel et al 1977, Burden and Thurston 1979] or decreased [Tuck et al 1979, Christlieb et al 1976a]. There is evidence from animal studies that angiotensin II, a component of the Renin-Angiotensin-Aldosterone system, may affect renal vascular tone and play a role in proteinuria [Yoshioka et al 1986]. I investigated the relationship of activity of the Renin-Angiotensin-Aldosterone system to proteinuria in type 1 diabetic patients.

9.2. Patients and Protocol

Patients were studied as part of the screening of type 1 diabetic subjects as described in Chapter 4. They attended at 09.00h having taken their usual breakfast and insulin. All were taking their usual diet and none was taking a diet restricted in salt or protein. Those taking diuretic or antihypertensive drugs were asked to stop them 2 weeks prior to the study day. Patients were instructed to empty their bladders and a cannula was inserted into an antecubital vein. They remained recumbent for the next 2h and were given 200 ml water per hour to drink. All urine passed during this time was collected and aliquots saved and

frozen at -20 °C for later estimation of albumin and electrolytes. A fresh sample of urine was sent for bacteriological examination.

After at least 30 min rest 10 ml blood was drawn from the indwelling catheter into vacuum tubes containing heparin and immediately placed on ice. these were spun at 2000g for 30 min at 4 °C and the plasma frozen at -70 °C for later estimation of renin activity and aldosterone content. Further samples were drawn for estimation of plasma glucose, glycated haemoglobin [**HbA₁**], electrolytes and creatinine.

After a further period of rest supine blood pressure was recorded twice in the right arm and the mean of these recorded.

9.3. RESULTS

In this study 52 [71%] patients were normoalbuminuric, 13 [18%] microalbuminuric, and 8 [11%] macroalbuminuric. All groups were comparable for age, plasma glucose, **HbA₁**, and creatinine but disease duration was longer in the macroalbuminuric group [Table 9.1].

Median plasma renin activity was increased ,in patients with micro- or macro-albuminuria compared with those with normoalbuminuria [Table 9.2]. There was no correlation between plasma renin activity and albumin excretion in any group.

Median plasma aldosterone was similar in each group [Table 9.2]. Plasma and urinary electrolytes and

Table 9.1.

Clinical characteristics of patients studied for Renin-Angiotensin-Aldosterone Activity.

	Normoalbuminuric [n = 52]	Microalbuminuric [n = 13]	Macroalbuminuric [n = 8]
Albumin excretion rate [$\mu\text{g min}^{-1}$]	6.3 \pm 4.7	75.6 \pm 46.7	1214.8 \pm 893.9
Age [years]	32 \pm 14	34 \pm 18	32 \pm 17
Duration of Diabetes [years]	13 \pm 11	11 \pm 9	22 \pm 11 *
Glucose [mmol l^{-1}]	11.7 \pm 6.4	10.4 \pm 4.4	8.4 \pm 4.3
HbA _{1c} [%]	9.8 \pm 2.7	10.0 \pm 3.0	8.8 \pm 3.3
Creatinine [$\mu\text{mol l}^{-1}$]	87 \pm 23	105 \pm 40	86 \pm 19
=====			
HbA _{1c} - Glycated haemoglobin, reference range 5.0 - 9.0%			
* P < 0.05 compared with other groups			

Table 9.2.

Plasma Renin Activity [PRA], Aldosterone concentration, Serum [S] and Urinary [U] electrolytes, and Systolic Blood Pressure [SBP] in water replete, recumbent type 1 Diabetic Patients.

	Normoalbuminuric [n = 52]	Microalbuminuric [n = 13]	Macroalbuminuric [n = 8]
PRA [$\text{nmol l}^{-1} \text{ h}^{-1}$]	1.0 [0.2-4.5]	2.3 [0.7-7.1]*	2.2 [0.5-8.2]**
Aldosterone [pmol l^{-1}]	298.9 \pm 156.8	369.3 \pm 342.5	310.1 \pm 394.9
SBP [mm Hg]	116 \pm 13	121 \pm 16	141 \pm 27***
Sodium[S] [mmol l^{-1}]	138 \pm 3	138 \pm 3	140 \pm 4
Potassium[S] [mmol l^{-1}]	4.3 \pm 0.3	4.1 \pm 0.3	4.2 \pm 0.5
Sodium[U] [mmol l^{-1}]	76 \pm 47	97 \pm 57	73 \pm 41
Potassium[U] [mmol l^{-1}]	34 \pm 23	51 \pm 35	40 \pm 11
Sodium[U] excretion rate [mmol min^{-1}]	155 \pm 92	137 \pm 88	196 \pm 176
PRA - median [range], other variables as mean \pm SD			
* P < 0.001, ** P < 0.05, *** P < 0.01 compared with normoalbuminuric group			

urinary sodium excretion rates were not significantly different between groups.

Mean systolic blood pressure was higher in the macro- than normo- group [141 ± 27 vs 116 ± 13 mm Hg, $P < 0.01$] but not different from the microalbuminuric [121 ± 16 mm Hg, $P = \text{NS}$]. There were no inter group differences for diastolic blood pressure. Overall there was a weak correlation between systolic blood pressure and albumin excretion rate [$r = 0.23$, $P < 0.05$].

9.4. DISCUSSION

Compared with the normoalbuminuric group those with elevated albumin excretion rates had elevated plasma renin activity but there was no correlation between the two variables. Overall there was a weak correlation between systolic blood pressure and albumin excretion rate but not with plasma renin activity. These observations although on a small number of patients suggest that altered plasma renin activity is not responsible for the hypertension seen in diabetic nephropathy.

In a similar study Feldt-Rasmussen et al [1987] reported no difference in plasma active renin levels between groups with different albumin excretion rates although there was a higher plasma inactive renin level in macroproteinuric patients. No patient in my study was

taking a drug known to influence plasma renin activity but it is possible that a biological effect persisted in those who had stopped medication. This is unlikely, however, since significant differences in plasma renin activity between normo- and microalbuminuric groups persisted even after those who had been taking medication were excluded from the analysis [data not shown]. No similar comparison involving macroproteinuric patients was possible since all had been taking drugs known to alter plasma renin activity.

In a study of 31 diabetic patients with proliferative retinopathy more than 50% had microalbuminuria [Drury and Bodansky 1985]. The median plasma renin activity in the albuminuric group was raised compared with those with normal albumin excretion rates, and the former group had lost the normal inverse relationship between blood pressure and plasma renin activity. Leutscher et al [1985] found that plasma inactive renin was elevated in patients with proteinuria but they did not correlate either variable with blood pressure.

In my study neither plasma nor urinary electrolyte concentrations nor urinary sodium excretion rate differed between groups. Total body sodium, a factor important in determining plasma renin activity, was not measured but is increased in diabetes mellitus [De Chatel et al 1977, Feldt-Rasmussen et al 1987]. Despite normal plasma biochemistry there may have been inter-group differences for exchangeable sodium. There were no significant inter group

differences for plasma aldosterone and all levels were within the laboratory's normal reference range. Others have reported comparable levels of aldosterone in diabetic patients and normal control subjects and an apparent separation between renin and aldosterone secretion [De Chatel et al 1977, Feldt-Rasmussen et al 1987]. Insulin is known to promote renal tubular reabsorption of sodium [De Fronzo 1981]. This may explain why type 1 diabetic patients [with high levels of exogenous insulin] have normal or low levels of plasma aldosterone in the face of a raised exchangeable body sodium. Hyporeninaemic hypoaldosteronism has been reported in diabetic nephropathy [Tuck et al 1979]. This was not observed in any patient in the present study despite the fact that several had a protein leak of several grams per day. None, however, had biochemical evidence of renal decompensation.

Angiotensin II levels were not measured but would be expected to be elevated in parallel with plasma renin activity. Angiotensin II may promote proteinuria by altering intraglomerular haemodynamics [Yoshioka et al 1986]. Other work has shown increased systemic vasopressor responsiveness to infused angiotensin II in patients with uncomplicated type 1 diabetes [Drury et al 1984]. Thus there is a pathway whereby increased plasma renin activity, and consequently angiotensin II, may in turn promote not only proteinuria but also an accompanying rise in systolic blood pressure.

CHAPTER 10

BLOOD PRESSURE IN DIABETIC NEPHROPATHY:

2.THE BLOOD PRESSURE RESPONSE TO EXERCISE IN PATIENTS WITHOUT EVIDENCE OF COMPLICATIONS

10.1. INTRODUCTION

Hypertension is common in type 1 diabetes mellitus and may relate to the development of nephropathy, although it is still unclear whether it precedes or is secondary to this complication [Mathiesen et al 1990]. An exaggerated systolic blood pressure response to exercise in type 1 diabetes mellitus has been reported and related to the degree of albuminuria [Christensen 1984], but might also result from abnormalities of catecholamines, plasma renin activity [PRA] or atrial natriuretic peptide [ANP]. Total body sodium is increased and resting catecholamines may be suppressed in type 1 diabetes mellitus [Feldt-Rasmussen et al 1987]. Studies of plasma renin activity in type 1 diabetes mellitus are conflicting. Activity has been reported as low [Christlieb et al 1976, Tuck et al 1979], normal [Feldt-Rasmussen 1987, O'Hare et al 1988] and high [De Chatel et al 1977]. I have found increased plasma renin activity in diabetic patients with raised albumin excretion rates [Chapter 9]. Others have reported low plasma renin activity and raised levels of ANP in diabetic patients with poor glycaemic control [Bell et al 1989].

The aims of this study were to determine the blood pressure response to exercise in uncomplicated diabetes and to investigate the relationship between such response and the above biochemical parameters.

10.2. SUBJECTS AND METHODS

Eight men with type 1 diabetes mellitus for at

least five years and eight sex and age matched healthy control subjects were studied. The diabetic subjects were normoalbuminuric [urinary albumin excretion rate less than 20 $\mu\text{g min}^{-1}$ confirmed by at least two timed urine collections], normotensive, and had no retinopathy [judged by ophthalmoscopy through dilated pupils] or clinical evidence of neuropathy. None had autonomic neuropathy [normal heart rate responses to standing, deep breathing and the Valsalva manoeuvre and blood pressure response to standing]. None of the control subjects had a family history of diabetes and no evidence of retinopathy, autonomic or peripheral neuropathy.

All subjects underwent a preliminary progressive exercise test to determine maximal workload capacity. The test commenced at a workload of 50w using an electromagnetically braked cycle ergometer [Rodley Elektronik 820, Sweden]. The workload was then increased by 20w min^{-1} until exhaustion. Not less than 1 week and no more than 2 months after the maximal test subjects performed a steady state sub-maximal test at 50% maximal workload. Data from the steady state tests are presented here.

On the study day subjects attended between 1300-1600 hours having eaten as normal at midday. Diabetic subjects took their usual insulin. At the start of the study period subjects emptied their bladders and drank 200 ml water. They then rested recumbent **for** 1h after which urine was collected for estimation **of** sodium excretion. Venous

blood was drawn for measurement of glycated haemoglobin, plasma electrolytes, urea, glucose, albumin, atrial natriuretic peptide, noradrenaline, adrenaline, dopamine and plasma renin activity. Subjects then exercised for 20 min at 50% maximal workload. Further blood and urine samples were collected immediately after exercise and following one hour's rest. To maintain fluid balance during the study subjects were given 200ml water plus a volume equivalent to their previous hour's urine output to drink per hour.

Blood pressure and heart rate were measured with a computerised exercise testing system [Magna 88, PK Morgan, Rainham, Kent] at the end of the initial rest period and then at five minute intervals throughout exercise.

All blood was immediately centrifuged [2000g, 4 °C] for 10 min. Plasma and urine were frozen and stored at -20 °C for later analysis.

10.3. RESULTS

Workloads for steady state exercise were higher in the control [260, 150-370w] than diabetic group [210, 150-290w, $p<0.05$].

Table 10.1 shows subject characteristics and biochemistry at the start of the study.

Table 10.2 shows heart rate and blood pressure responses to exercise. Resting and post-exercise heart rates were similar in both groups. Resting systolic blood pressure was slightly higher in the diabetic than control group [NS] and showed an exaggerated response to exercise [$p<0.01$]. Diastolic blood pressure was higher in the diabetic group at rest and following exercise [$p<0.05$].

Table 10.3 lists the plasma and urine electrolytes and Table 10.4 the changes in catecholamines, plasma renin activity and ANP during the study. Resting urinary sodium excretion was raised in the diabetic group and fell during exercise [$p<0.05$]. Plasma albumin was lower in diabetic than control subjects [$p<0.05$] but rose significantly in both groups during exercise [$p<0.05$]. There were no significant changes in adrenaline or dopamine levels in either group during the study. Resting noradrenaline levels were lower in the diabetic group [$p<0.02$] and showed a marginally greater response to exercise overall [NS]. This difference was accounted for by the marked rise in noradrenaline levels [178 and 307%] during exercise in the two diabetic patients with

the highest rise in systolic blood pressure. Plasma renin activity levels did not differ significantly between the two groups at rest or following exercise in the two groups. Resting atrial natriuretic peptide levels were lower in the diabetic group [$p<0.02$]. Levels rose significantly during exercise in both diabetic [$p<0.03$] and control [$p<0.05$] groups. There was a significant positive correlation between atrial natriuretic peptide levels and increasing duration of diabetes [$r=0.71$, $p<0.05$]. No correlation was found between any of the other variables studied.

Table 10.1

Subject characteristics and biochemistry at the start of study.

Age [yrs]	Disease Duration [yrs]	Glucose [mmol l ⁻¹]	HbA _{1c} * [%]	Albumin [g l ⁻¹]	Creatinine [μmol l ⁻¹]
<u>Patients</u>					
20	11	3.9	8.8	37	85
20	10	11.0	11.8	32	82
26	13	6.7	9.2	34	86
26	18	14.1	6.5	34	108
31	7	24.0	12.8	37	105
32	19	13.6	9.8	34	97
34	18	10.3	8.8	40	97
35	17	3.9	9.4	41	104
<u>Controls</u>					
20	-	3.7	5.5	42	92
23	-	5.4	3.8	37	95
24	-	6.1	4.9	43	96
27	-	3.5	4.5	43	111
30	-	7.0	6.1	38	102
31	-	7.5	3.3	39	93
32	-	2.0	5.2	37	126
35	-	5.6	4.3	43	87

*Reference range 5-9%

Table 10.2

Blood pressure and heart rate responses to exercise.

	Diabetic		Control	
	Pre exercise [0']	Immediate Post exercise [20']	Pre exercise [0']	Immediate Post exercise [20']
Blood Pressure [mm Hg]				
Systolic	123 [98-151]	#187 [163-217]	112 [100-145]	#*153 [138-178]
Diastolic	81 [62-96]	85 [69-144]	**69 [64-72]	**65 [57-95]
Heart rate [bpm]	81 [60-89]	#163 [143-168]	83 [52-118]	#170 [143-180]
** P < 0.05 diabetic vs control * P < 0.01 diabetic vs control # P < 0.01 from baseline value				

Values given as median [range].
 Resting and post-exercise heart rates were similar in both groups.
 Resting systolic blood pressure was slightly higher in the diabetic group [NS] and compared with the control group showed an exaggerated response to exercise [P < 0.01]. Diastolic blood pressure was higher in the diabetic group at rest and following exercise [P < 0.05].

Table 10.3

Plasma sodium, glucose and albumin, and urinary sodium excretion during the study.

	Diabetic			Control		
	Pre exercise [0']	Immediate Post exercise [20']	1h Post exercise [80']	Pre exercise [0']	Immediate Post exercise [20']	1h Post exercise [80']
<u>Plasma</u>						
Na [mmol l ⁻¹]	138[135-147]	139[135-143]	138[134-143]	141[139-144]	142[137-143]	141[136-143]
Glucose [mmol l ⁻¹]	12.0[3.9-24]	7.0[2.9-17.8]	7.6[1.8-14]	5.5[2.0-7.5]	5.0[2.6-6.4]	5.3[4.7-6.0]
Albumin [g l ⁻¹]	37 [32-41]	*41 [38-45]	39 [33-42]	*41 [37-43]	#45 [39-47]	42 [36-45]
<u>Urine</u>						
Na [µg min ⁻¹]	147[74-244]	†73[48-264]	143[21-195]	109[83-151]	82[23-92]	94[35-159]

Values given as median [range].

* P < 0.05 diabetic vs control

† P < 0.05 from baseline value

P < 0.01 from baseline value

Table 10.4

Plasma renin activity, atrial natriuretic peptide, and catecholamines during the study

	Diabetic			Control		
	Pre exercise [0']	Immediate Post exercise [20']	1h Post exercise [80']	Pre exercise [0']	Immediate Post exercise [20']	1h Post exercise [80']
PRA [nmol l ⁻¹ h ⁻¹]	2.9 [1.3-4.5]	**5.1 [2.7-8.0]	3.3 [1.2-5.4]	2.5 [2.0-4.6]	**4.2 [2.5-6.4]	2.5 [2.2-4.2]
ANP [pmol l ⁻¹]	10.1 [4.3-16.9]	#25.9 [5.2-38.9]	10.8 [4.3-19.9]	*16.0 [9.5-22.9]	#28.6 [17.3-47.2]	11.7 [61.0-42.4]
Nor-A [nmol l ⁻¹]	1.66 [0.55-3.92]	3.99 [1.29-5.09]	2.20 [0.54-4.21]	*2.96 [2.04-4.49]	3.86 [2.65-7.19]	3.12 [1.71-6.03]
Adrenaline [nmol l ⁻¹]	<0.2 [<0.2-0.49]	<0.32 [<0.2-0.82]	<0.2 [<0.2-0.92]	<0.2 [<0.2-1.46]	<0.46 [<0.2-1.31]	<0.30 [<0.2-1.12]
Dopamine [nmol l ⁻¹]	<0.2 [<0.2-0.42]	<0.2 [<0.2-0.39]	<0.2 [<0.2-0.53]	<0.2 [<0.2-1.02]	<0.2 [<0.2-1.40]	<0.2 [<0.2-0.5]

Values given as median [range].

* P < 0.02 diabetic vs control

** P < 0.01 from baseline value

P < 0.05 from baseline value

PRA - plasma renin activity

ANP - atrial natriuretic peptide

Nor-A - nor-adrenaline

10.4 DISCUSSION

The higher resting diastolic and exaggerated rise in systolic pressure on exercise in this patient group supports a previous report [Torffvit et al 1987], but is at variance with another [Kelleher et al 1987]. In the latter study, however, subjects exercised for only 6 minutes, whereas in the present study the differences were only apparent in the last 5 minutes of exercise. Autonomic dysfunction is unlikely to account for the results since the diabetic and control groups showed no significant difference in heart rate response and there was no evidence of autonomic neuropathy on formal testing. One previous study, however, did report impairment of heart rate response to exercise in teenage patients with only short duration of disease [Johanssen et al 1987]. Another possible explanation for the **BP** rise is the enhanced noradrenaline response to exercise reported in type 1 diabetes mellitus [Tamborlane et al 1979] and noted in some patients in this study. Increased vascular sensitivity to catecholamines has been demonstrated in type 1 diabetes mellitus by an augmented blood pressure response to noradrenaline infusion. This in turn may be normalised by chlorthalidone suggesting that sodium retention contributes to the increased catecholamine sensitivity and hypertension in type 1 diabetes mellitus [Weidmann et al 1979]. Exercise-induced hypertension may be the first manifestation of this hypersensitivity. Indeed, type 1 diabetes mellitus is associated with increased total body sodium, which is not explained by changes in aldosterone, angiotensin II or

catecholamines [Feldt-Rasmussen 1987].

There was no difference in basal urinary sodium excretion between the groups. The diabetic group, however, showed a significant reduction following exercise. This may be due to increased activity of the renin-angiotension-aldosterone system although the rise in plasma renin activity following exercise was only marginally higher in the diabetic than the control group. Increased vascular sensitivity to angiotensin II in type 1 diabetes mellitus has been demonstrated, however, even in the presence of normal plasma renin activity levels [Christlieb et al 1976b]. Increased sensitivity to aldosterone may account for the decreased sodium excretion seen in this diabetic group. The similar plasma renin activity response to exercise in both groups indicates that the exaggerated systolic blood pressure response was not due to increased renin secretion. It may, however, reflect enhanced sensitivity to angiotensin II.

Reduced resting atrial natriuretic peptide levels in uncomplicated diabetes have not been reported previously. There was, however, an appropriate response to exercise in this study. A previous study showed basal ANP levels to be similar in type 1 diabetes mellitus and controls, although 5 of the 7 diabetic subjects had microvascular or neuropathic complications [Donckier et al 1989]. In another study of uncomplicated subjects with type 2 diabetes basal ANP levels were not significantly lower than

controls [McKnight et al 1989]. Increased **ANP** levels have been reported in a group of poorly controlled patients with type 2 diabetes mellitus [Bell et al 1989] and may be secondary to sodium retention and hypertension [Dodson and Horton 1988]. Confirmation of the finding of reduced **ANP** levels in the diabetic group might suggest that **ANP** has a contributory role in the development of increased body sodium and diastolic hypertension in type 1 diabetes mellitus.

This study confirms previous reports of low resting noradrenaline levels and reduced plasma albumin in type 1 diabetes mellitus, the causes of which are unclear, the latter being unrelated to plasma volume or urinary albumin excretion [Kelleher et al 1987].

In conclusion, exercise provokes an exaggerated systolic blood pressure response and reduction in urinary sodium excretion in normoalbuminuric type 1 diabetic patients. Both findings may reflect increased sensitivity to the renin-angiotensin-aldosterone system despite normal plasma renin activity levels. The highest blood pressure changes were associated with an enhanced noradrenaline response despite lower basal levels in the diabetic group. Reduced atrial natriuretic peptide levels may stimulate sodium retention and increased diastolic blood pressure in early type 1 diabetes mellitus. The presence of these changes in a group of otherwise uncomplicated type 1 diabetic subjects suggests they are a consequence of the diabetic state rather than the development of micro- or macrovascular

complications. Assessment of the possible contribution of these abnormalities to the development of hypertension in type 1 diabetes awaits the results of prospective studies.

CHAPTER 11

STUDIES OF ANGIOTENSIN CONVERTING ENZYME [ACE] INHIBITION ON RENAL FUNCTION AND PROTEINURIA IN DIABETES MELLITUS

1. OVERT NEPHROPATHY

11.1. INTRODUCTION

As noted previously, up to **40%** of type 1 [insulin dependent] diabetic patients may develop nephropathy [Andersen et al 1983, Parving et al 1988a]. Comparable studies have not been made in type 2 [non-insulin dependent] diabetes but a population based study in North America reported a prevalence of 24.6% after twenty years' disease duration [Ballard et al 1988]. In the United Kingdom there are an estimated 600 cases of end stage renal failure as a consequence of diabetes mellitus each year [Joint Working Party on Diabetic Renal Failure 1988].

Lowering elevated blood pressure is the only manoeuvre which has been shown reliably to slow the progression of diabetic nephropathy [Mogensen 1976, 1982; Parving et al 1983, 1989]. A wide variety of agents [diuretics, vasodilators, β -adrenergic blockers, calcium channel blocking drugs, and angiotensin converting enzyme [ACE] inhibitors] have been used to treat hypertension in diabetic nephropathy but few studies have compared drugs. Calcium channel blockers have been advocated for the treatment of hypertension in diabetes but ACE inhibitors, because of their renal effects, especially lowering of intra-glomerular pressure, may be particularly suitable [Parving et al 1988b]. A recent study, however, found no difference between these classes of drugs [Baba et al 1989]. To investigate this further I conducted a prospective randomised double blind study to compare the effects of an ACE inhibitor

[lisinopril] with those of a calcium channel blocker [nifedipine] on renal function and blood pressure in hypertensive, macroproteinuric diabetic patients.

11.2. SUBJECTS AND METHODS

Male and female patients aged 18-70 years with type 1 or type 2 diabetes mellitus were recruited from the diabetic-renal clinic. Patients had Albustix positive proteinuria and all had persistent macroproteinuria [albumin excretion rate [AER] $\geq 200 \mu\text{g min}^{-1}$] confirmed in at least two timed overnight urine collections during a six month period. All had resting diastolic blood pressure 90-115 mm Hg off treatment. Macroproteinuric patients taking antihypertensive medication were eligible for entry to the study if their blood pressure was elevated after 1 month without treatment.

Exclusion criteria were: malignant or accelerated hypertension, or secondary hypertension of any aetiology; heart failure, aortic outflow obstruction, unstable angina, a history of myocardial infarction in the preceding 3 months; known renal disease other than diabetic nephropathy, plasma creatinine $\geq 200 \mu\text{mol l}^{-1}$; low protein diet; systemic malignancy; clinically significant abnormality of hepatic, haemopoietic or endocrine function; women of childbearing potential not using a medically acceptable method of birth control; a history of hypersensitivity to ACE inhibitors or nifedipine. Patients taking any of the following medications

were excluded; aldose reductase inhibitors, myoinositol, steroids, gold, penicillamine, non steroidal anti-inflammatory agents, monoamine oxidase inhibitors, appetite suppressants, lithium, vasopressor nasal decongestants, cimetidine. Patients fulfilling the inclusion criteria gave informed consent and then followed the study protocol as detailed in Figure 11.1.

All patients entered the two week placebo run in period to assess compliance [Visit **1**]. Tablets were counted at visit 2 and patients whose compliance was less than 80% were excluded from final data analysis. Patients were randomised double blind to receive lisinopril or nifedipine according to separate schedules for type 1 and type 2 diabetes. Those randomised to receive lisinopril were given a test dose of 2.5 mg with placebo matching slow release nifedipine 10mg twice daily. They were reviewed the following day and if the test dose had been tolerated without side effects were prescribed lisinopril 5 mg daily with placebo matching slow release nifedipine 10 mg twice daily [Visit **3**]. Patients randomised to receive nifedipine were given a test dose of slow release nifedipine 10 mg twice daily and placebo matching 2.5 mg lisinopril. If on review they had no side effects they were prescribed slow release nifedipine 10 mg twice daily and placebo matching lisinopril 5 mg once daily.

All patients were reviewed on three further occasions at three week intervals. At each visit, after at least 10 minutes rest, blood pressure was measured twice in

		Lisinopril 2.5mg od		Lisinopril 5mg od		Lisinopril 5/10mg od		Lisinopril 5/10/20mg od	
Placebo Lisinopril 10mg od									
Placebo Nifedipine 10mg od		SR Nifedipine 10mg od		SR Nifedipine 10/20mg od		SR Nifedipine 10/20/40mg od			
Visit:		SR Nifedipine 10mg od							
Weeks:	1	2	3	4	5	6	7		
	0	2 + 1 day		5	8	11	19		
Symptom Qucestionnaire		x							x
Medical history/ coexisting diseases	x								
Physical examination	x								
BP/HR	x	x		x	x	x			x
Tablet count		x		x	x	x			x
Haematology		x		x		x			x
Biochemistry		x		x	x	x			x
Body weight		x							x
Metabolic control	x	x		x	x	x			x
Proteinuria	x	x				x			x
GFR/RBF		x							x

FIGURE 11.1.

Study protocol for hypertensive macroproteinuric diabetic patients treated with lisinopril or nifedipine.

the right arm at heart level using a Hawksley random zero sphygmomanometer. The mean of these readings was calculated and with the aim of achieving a diastolic blood pressure ≤ 90 mm Hg [Korotkoff phase 5] the dose of lisinopril was increased stepwise to 10 mg [Visit 4] or 20 mg daily [Visit 5] and that of nifedipine to 20 mg twice daily [Visit 4] or 40mg twice daily [Visit 5]. Antihypertensive medication was continued for a further eight weeks at which time the study was terminated [Visit 7]. At each visit mean arterial blood pressure [MAP] was calculated.

11.2.1. Renal Function.

Glomerular filtration rate [GFR] and renal blood flow [RBF] were measured at visits 2 and 7 as the rates of disappearance from plasma of $^{51}\text{Cr-EDTA}$ and $^{125}\text{I-Hippuran}$ after simultaneous single injections of each isotope as described previously.

11.2.2. Proteinuria

Timed overnight urine collections were made at visits 1, 2, 6, and 7. Albumin concentration was measured by radioimmunoassay and the albumin excretion rate [AER] was calculated using the formula

$$\text{AER} = \frac{CV}{T}$$

[where C is the urinary albumin concentration, V is the volume of urine passed during the collection and T is the time of the collection]. Fractional albumin clearance was calculated as the ratio urinary clearance albumin:GFR.

11.2.3. Haematology and Biochemistry

Blood was taken at visits 2, 4, 6, and 7 for measurement of haemoglobin and haematocrit, creatinine, albumin, urea, sodium and potassium. Blood was drawn at visits 2, 4, 5, 6, and 7 for measurement of plasma glucose and glycated haemoglobin [**HbA_{1c}**, reference range 5.0-9.0%].

11.2.4. Statistical analysis.

For blood pressures, GFR, RBF, ERPF, FF, and RR changes from baseline [visit 7 minus visit 2] were analysed using the analysis of covariance [ANCOVA] technique: the baseline [visit 2] values were further included in the model as the covariate as this was found to substantially contribute to the models and hence improve the precision of the estimates of treatment difference. The assumptions underlying the ANCOVA technique were checked against these data and were not found to be violated. For AER and quantitative protein, however, the changes from baseline were found to violate the normality assumption underlying the ANCOVA technique as they were heavily skewed with extreme outliers even after logarithmic transformation. Therefore the non parametric Mann Whitney U test was applied to the untransformed changes from baseline for these endpoints. All analyses were performed using the SAS statistical package on an IBM mainframe computer.

11.3. RESULTS

Fourteen patients were randomised to each group.

Table 11.1.

Patient characteristics at entry to study.

	Lisinopril n=14	Nifedipine n=14
M:F	12:2	10:4
Age [years]	53.3 ± 11.2	50.5 ± 19.2
Weight [kg]	84.3 ± 20.8	83.0 ± 18.5
Insulin dependent	6	5
Non-insulin dependent	8	9

Age & weight presented as Mean ± SD.

Two subsequently withdrew from the lisinopril group [one with a history of ischaemic heart disease suffered a fatal myocardial infarction, one developed ankle oedema] hence results in treatment comparisons are based on 12 lisinopril versus 14 nifedipine treated patients. The groups were well matched for age, weight, sex distribution, and diabetic treatments [Table 11.1]. The mean age of the females [38.8 years] was less than that of the males [55.5 years]. The sex imbalance between groups, however, was consistent so it was unnecessary to adjust for this in the statistical analyses.

11.3.1. Plasma biochemistry and glycaemic control

Only small changes within each group and small differences between groups were observed for plasma levels of sodium, potassium, albumin, creatinine, or urea. Random blood glucose and HbA_{1c} were constant in both groups throughout the study and there were no differences between the groups [Table 11.2].

Table 11.2.

Plasma electrolytes, albumin, urea, creatinine, glucose and glycated haemoglobin [HbA_{1c}]*

for lisinopril and nifedipine treated groups during study.

VISIT	2	4	6	7
<u>LISINOPRIL</u>	[n=14]	[n=13]	[n=12]	[n=12]
Sodium [mmol l ⁻¹]	136.2 ± 4.4	136.5 ± 2.6	136.4 ± 2.5	137.1 ± 2.2
Potassium [mmol l ⁻¹]	4.5 ± 0.5	4.5 ± 0.5	4.9 ± 0.7	4.7 ± 0.6
Albumin [g l ⁻¹]	33.9 ± 5.1	34.2 ± 6.3	33.6 ± 5.0	34.1 ± 5.5
Urea [mmol l ⁻¹]	7.5 ± 2.2	7.7 ± 3.0	8.7 ± 2.5	9.9 ± 4.3
Creatinine [μmol l ⁻¹]	117 ± 29	114 ± 24	111 ± 20	127 ± 34
Glucose [mmol l ⁻¹]	11.7 ± 7.1	10.9 ± 8.0	9.9 ± 4.8	11.2 ± 3.6
HbA _{1c} [%]	9.8 ± 3.0	9.7 ± 3.9	10.5 ± 3.8	9.2 ± 2.4
<u>NIFEDIPINE</u>	[n=14]	[n=14]	[n=14]	[n=14]
Sodium [mmol l ⁻¹]	137.6 ± 1.6	137.9 ± 2.5	137.5 ± 2.6	137.7 ± 2.6
Potassium [mmol l ⁻¹]	4.2 ± 0.4	4.4 ± 0.4	4.2 ± 0.4	4.2 ± 0.3
Albumin [g l ⁻¹]	31.6 ± 2.8	32.1 ± 4.3	32.1 ± 4.5	32.6 ± 3.6
Urea [mmol l ⁻¹]	7.0 ± 3.1	6.9 ± 2.6	7.1 ± 2.4	7.5 ± 3.3
Creatinine [μmol l ⁻¹]	111 ± 34	116 ± 46	116 ± 41	125 ± 53
Glucose [mmol l ⁻¹]	11.8 ± 5.0	11.0 ± 4.0	12.7 ± 6.0	11.0 ± 6.1
HbA _{1c} [%]	9.7 ± 3.2	9.8 ± 3.5	9.9 ± 3.6	9.3 ± 3.2

* All given as mean ± SD.

HbA_{1c} - Reference range 5.0 - 9.0%

11.3.2. Haematology

Haemoglobin fell in the lisinopril group from $143.2 \pm 17.2 \text{ g L}^{-1}$ to $138.2 \pm 15.5 \text{ g L}^{-1}$ at the end of the study. Haematocrit did not alter [0.43 ± 0.04 and 0.41 ± 0.04 respectively]. Neither haemoglobin nor haematocrit altered appreciably in the nifedipine group [$139.0 \pm 16.6 \text{ g L}^{-1}$ and $137.8 \pm 20.7 \text{ g L}^{-1}$, and 0.42 ± 0.05 and 0.42 ± 0.06].

11.3.3. Blood pressure.

The mean [\pm SD] dose of lisinopril being taken at visit 7 was $15.0 [6.7]$ mg and of nifedipine was $25.7 [13.4]$ mg. Blood pressure [mean \pm SD] was lower in both groups at visit 7 compared with visit 2 [$143/88 \pm 17/13$ mm Hg vs $166/99 \pm 23/9$ mm Hg for lisinopril treated, and $148/85 \pm 25/10$ mm Hg vs. $165/99 \pm 21/7$ mm Hg for nifedipine treated, Table 11.3]. Blood pressures were not significantly different between groups at the beginning or end of the study [Fig 11.2].

11.3.4. Proteinuria.

The quantitative urinary protein excretion fell [from $551.7 \pm 768.7 \text{ mg L}^{-1}$ to $473.1 \pm 660.8 \text{ mg L}^{-1}$] as did albumin excretion rate [from $738.7 \pm 635.2 \text{ } \mu\text{g min}^{-1}$ to $644.6 \pm 965.2 \text{ } \mu\text{g min}^{-1}$] between visits 2 and 7 respectively in the lisinopril treated group. The nifedipine treated group showed slight increases in quantitative urinary protein excretion [$498.4 \pm 465.2 \text{ mg L}^{-1}$ vs. $554.6 \pm 521.9 \text{ mg L}^{-1}$] and albumin excretion rate [$1072.5 \pm 908.5 \text{ } \mu\text{g min}^{-1}$ vs. $981.2 \pm 1022.2 \text{ } \mu\text{g min}^{-1}$] between visits 2 and 7 [Fig 11-3]. The difference

Table 11.3.

Changes in mean systolic [SBP] and diastolic [DBP] blood pressures for
lisinopril [L] and nifedipine [N] treated groups.

	Mean change from baseline		Treatment difference	95% CI	P
	L [n=12]	N [n=14]			
SBP [mm Hg]	-20.4	-16.6	-3.8	-18.7 to 11.0	0.60
DBP [mm Hg]	-10.2	-13.7	3.5	-6.1 to 13.1	0.46

Table 11.4.

Median changes in quantitative protein excretion, albumin excretion rates [AER],
and fractional albumin clearances
[FAIb_c] for lisinopril [L] and nifedipine [N] treated groups at end of study.

	Median change from baseline		Treatment difference	95% CI	P
	L [n=12]	N [n=14]			
Quantitative protein [mg l ⁻¹]	-41.1	9.0	-49.2	-352.5 to 258.0	0.55
AER [µg min ⁻¹]	-132.8	2.9	-308.7*	-895.4 to 204.0	0.34
FAIb _c	-0.045	0.258	0.3024*	-0.107 to 0.712	0.13

* Because of non-parametric method used for these variables the treatment differences
are not the arithmetic sum of changes from baseline for each group individually.

between treatments, however, was not statistically significant for change in AER or for change in quantitative urinary protein excretion [Table 11.4].

The fractional albumin clearance [ratio urinary albumin clearance:glomerular filtration rate] rose between visits 2 and 7 from 0.62 ± 0.83 to 0.36 ± 0.41 in the nifedipine treated group and fell from 0.22 ± 0.26 to 0.35 ± 0.41 in the lisinopril treated group. The difference between treatment groups for change from baseline was not found to be significant [Table 11.4].

11.3.5. Renal Function.

GFR fell from $105.2 \pm 57.5 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ to $72.1 \pm 39.4 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ for lisinopril treated, and from $108.9 \pm 50.0 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ to $82.9 \pm 53.9 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ for nifedipine treated groups. RBF fell from $446.8 \pm 217.9 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ to $435.1 \pm 243.3 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ for lisinopril treated, and from $473.0 \pm 216.4 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ to $419.0 \pm 278.6 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ for nifedipine treated groups between visits 2 and 7 respectively. ERPF fell from $253.4 \pm 118.7 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ to $251.6 \pm 133.3 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ for lisinopril treated, and from $253.7 \pm 97.1 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ to $240.9 \pm 143.9 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ for nifedipine treated groups between visits 2 and 7 respectively. The differences between groups were not significant [Table 11.5]. Filtration fraction [GFR/ERPF] fell in the lisinopril treated group [from 0.44 ± 0.14 to

Blood pressure

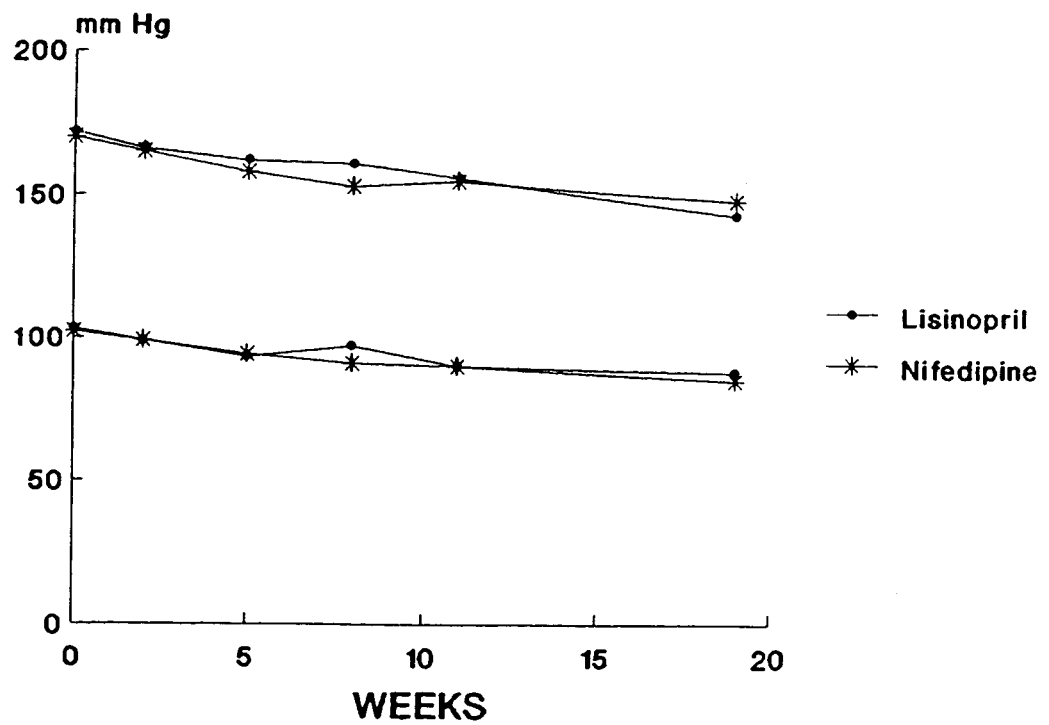


FIGURE 11.2.

Systolic and diastolic blood pressures during study for lisinopril and nifedipine treated patients.

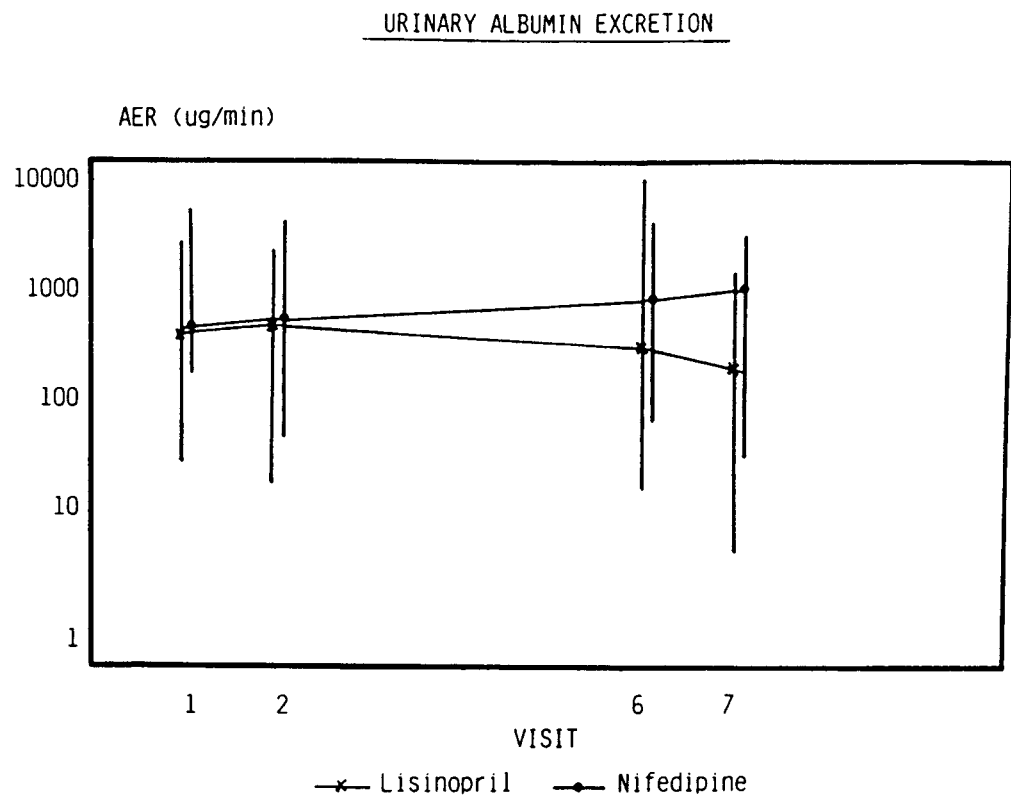


FIGURE 11.3.

Changes in albumin excretion rate [AER] during study for lisinopril and nifedipine treated patients.

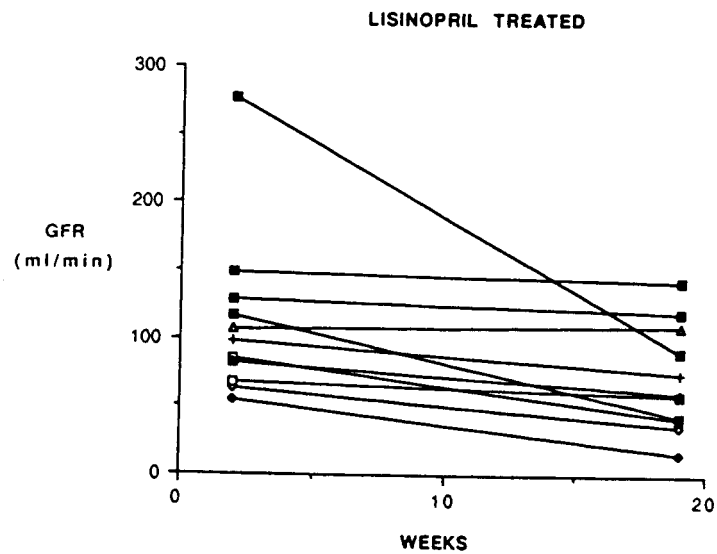
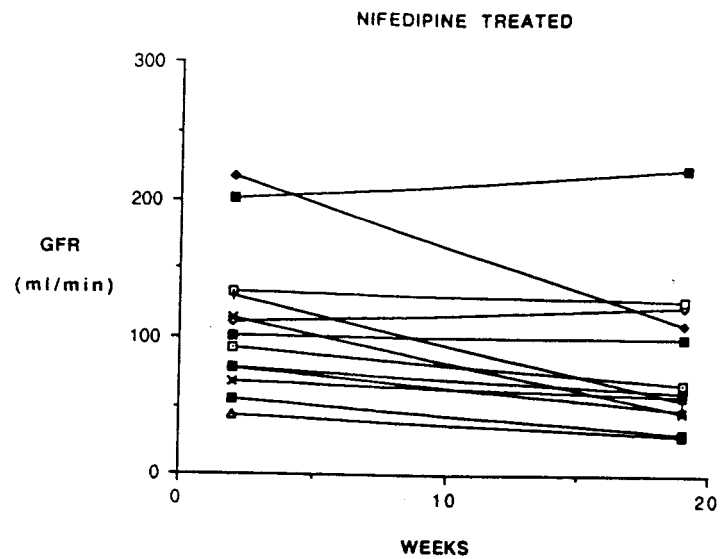


FIGURE 11.4.

Changes in glomerular filtration rate [GFR] during study for lisinopril and nifedipine treated patients.

Table 11.5.

Mean change from baseline for glomerular filtration rate [GFR], renal blood flow [RBF], renal plasma flow [RPF], filtration fraction [FF], and renal resistance [RR] in lisinopril [L] and nifedipine [N] treated groups at end of study.

	Mean change from baseline L [n=12] N [n=14]		Treatment difference	95% CI	P
GFR [ml min ⁻¹ 1.73m ⁻²]	-39.2*	-27.3‡	-11.9	-43.9 to 20.0	0.45
RBF [ml min ⁻¹ 1.73m ⁻²]	-43.9	-63.0‡	19.1	-161.2 to 199.4	0.83
RPF [ml min ⁻¹ 1.73m ⁻²]	-16.3	-31.2‡	14.9	-92.4 to 122.3	0.78
FF	-0.13*	0.05+	-0.08	-0.21 to 0.06	0.24
RR [mm Hg ml ⁻¹ min ⁻¹]	-0.00	0.10+	-0.11	-0.39 to 0.18	0.45

* n=11, + n=12, ‡ n=13.

0.31 \pm 0.12 at visit 7] and did not alter in the nifedipine treated group [0.43 \pm 0.14 and 0.38 \pm 0.17 at visits 2 and 7 respectively]. Total renal resistance [MAP/ERPF] was unaltered in the lisinopril treated group [0.60 \pm 0.0.30 mm Hg ml⁻¹ min⁻¹ at visit 2 vs. 0.55 \pm 0.31 mm Hg ml⁻¹ min⁻¹ at visit 7] but rose in the nifedipine treated **group** [0.57 \pm 0.30 mm Hg ml⁻¹ min⁻¹ at visit 2 to 0.64 \pm 0.47 mm Hg ml⁻¹ min⁻¹ at visit 7]. Inter-group differences for FF and RR were not significant.

11.4. DISCUSSION

Several studies have demonstrated that treatment of hypertension delays the rate of deterioration of renal function in diabetic nephropathy [Mogensen 1982, Parving et al 1983, 1988b, 1989a]. Some of the commonly prescribed classes of antihypertensive agents may not, however, be suitable in patients with diabetic renal disease. Diuretics and β -blockers may worsen glucose tolerance and have unfavourable effects on serum lipid profiles as well as provoking impotence. Calcium channel blockers and ACE inhibitors have better side effect profiles and some studies suggest that ACE inhibitors have beneficial effects on renal function in man and animals, lowering intra-renal pressures, reducing proteinuria and ameliorating histological changes in diabetes [Anderson et al 1986, Zatz et al 1986, Cooper et al 1989, Björck et al 1986, Marre et al 1988]. A recently published multicentre study reported enalapril to be better than metoprolol in reducing AER and preserving renal function

and suggested an intrinsic renal action for **ACE** inhibitors [Björck et al 1992]. There are, however, few other studies which have compared drugs in this condition.

11.4.1. Size of study and design

I have performed a randomised double blind study comparing the effects of an **ACE** inhibitor, lisinopril, and a calcium channel blocker, nifedipine, on blood pressure and renal function in diabetic patients with hypertension and macroproteinuria. Patients were selected carefully to exclude those with renal disease of non-diabetic origin and those taking medications or diets known to interfere with renal function.

Prolonged tight glycaemic control may prevent the development of diabetic nephropathy [Feldt-Rasmussen et al 1991] but there is no evidence of benefit in established renal disease. In this study there was no effect of either drug on glycaemic control.

11.4.2. Blood Pressure

I used staged increases in the doses of antihypertensive agents as recommended in a recent consensus document [Working Group on Hypertension in Diabetes Mellitus 1987]. Both drugs caused significant reductions in systolic and diastolic blood pressures at the end of the study and mean diastolic blood pressure was less than 90 mm Hg in both treatment groups.

11.4.3. Reduction in Proteinuria

Protein excretion was reduced in the lisinopril treated group but the nifedipine treated group showed a slight increase at the end of the study. Reductions in albumin excretion rate have been reported in normotensive and hypertensive diabetic patients during ACE inhibitor treatment [Marre et al 1988, Björck et al 1990, Mathiesen et al 1991]. In a short, open cross-over study Holdaas et al [1991] found reduced albumin excretion rate in lisinopril treated patients but no change in nifedipine treated patients. Alterations in proteinuria are thought to depend upon changes in intra-renal haemodynamics [Anderson et al 1986]. ACE inhibitors act primarily on the efferent renal arteriole and reduce intra glomerular pressure; this in turn reduces filtration pressure across the glomerular capillary basement membrane and so reduces protein filtration.

11.4.4. Renal Haemodynamics

GFR fell in both groups but the data in the lisinopril treated group were heavily skewed by one patient's results which fell from $277 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$ to $91 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$. Some of the patients must have been entering the stage of nephropathy when renal function begins to deteriorate and since pre-trial GFRs were not measured it is impossible to tell whether antihypertensive medication influenced rates of decline. Baba et al [1989] reported no change in GFR or ERPF in a study comparing nicardipine with enalapril in similar patients.

There was no change in renal resistance in the lisinopril treated group although there was a small rise in

the nifedipine treated group, perhaps explained by the greater fall in RBF. The filtration fraction fell in lisinopril but not nifedipine treated patients compatible with the facts that ACE inhibitors exert their predominant intra-renal effect on the efferent glomerular arteriole whilst calcium channel blockers act at the afferent arteriole [Lutzenhiser and Epstein 1985]. Fractional albumin clearance fell in the lisinopril treated group despite the reduction in GFR. Although the fall in GFR in the nifedipine treated group was of comparable magnitude there was an increase in fractional albumin clearance. This suggests that lisinopril may exert a specific intra-renal effect on protein handling independent of alterations in renal haemodynamics.

11.4.5. Conclusions

In this study both the ACE inhibitor lisinopril and the calcium channel blocker nifedipine reduced blood pressure equally with no significant intergroup differences in renal haemodynamics or proteinuria. There were, however, large confidence intervals for the observed changes in each of the measured variables. There was a trend for albumin excretion rate to fall in the ACE inhibitor group and rise in the calcium blocker group and the two groups appeared to be diverging at the end of the study. This may be the consequence of differing intra-renal actions of the two classes of drug. Reduced fractional albumin clearance in the ACE inhibitor group suggests that this drug may have an

intra-renal effect distinct from its effects on haemodynamics. Longer studies with larger patient numbers are required to answer this question.

CHAPTER 12

STUDIES OF ANGIOTENSIN CONVERTING ENZYME [ACE] INHIBITION ON RENAL FUNCTION AND PROTEINURIA IN DIABETES MELLITUS

2. INCIPIENT NEPHROPATHY

12.1.INTRODUCTION

The rate of decline of renal function in patients with established diabetic nephropathy may be slowed by control of hypertension using a variety of drugs [Mogensen 1982, Parving et al 1987]. Recent data, however, suggest that angiotensin converting enzyme [ACE] inhibitors may be particularly suitable agents for control of blood pressure in diabetic renal disease. Animal studies have shown that ACE inhibitors decrease proteinuria and may ameliorate the histological changes of diabetic nephropathy [Anderson et al 1986, Zatz et al 1986] and studies in human diabetic nephropathy [albumin excretion rate [AER] $\geq 200 \mu\text{g min}^{-1}$] suggest that ACE inhibition is beneficial, perhaps having a specific intra-renal effect [Mathiesen et al 1991, Björck et al 1992, Marre et al 1988].

Before entering the stage of frank nephropathy [AER $> 200 \mu\text{g min}^{-1}$ and systemic arterial hypertension] patients pass through a phase of incipient nephropathy [AER $\geq 20 \mu\text{g min}^{-1}$ but $\leq 200 \mu\text{g min}^{-1}$] when they are still normotensive. Interest has focussed recently on the intra renal effects of ACE inhibitors and their potential use in this phase of diabetic nephropathy. Two studies have reported protection of renal function with ACE inhibitors in such patients [Mathiesen et al 1991, Marre et al 1988]. Renal hyperfiltration in the early stages of diabetic nephropathy is well recognized and may depend in part upon intra renal prostaglandins [Esmatjes et al 1985, Gambardella et al 1988]

although this view has been challenged [Barnett et al 1987, Jenkins et al 1989]. Mathiesen et al [1988] and Hommel et al [1987], however, have demonstrated altered urinary prostaglandin excretion in established and incipient diabetic nephropathy. Both these workers, and earlier Barnett et al [1984], demonstrated that diabetic proteinuria could be reduced by drugs which interfere with prostaglandin synthesis. I have studied the effects of ACE inhibition with lisinopril on renal function, urinary prostaglandin excretion and albumin excretion rate in patients with incipient diabetic nephropathy.

12.2. SUBJECTS AND METHODS.

Male and female patients aged 18-70 years with type 1 or Type 2 diabetes mellitus and incipient diabetic nephropathy were recruited from the diabetic-renal clinic. All had resting diastolic blood pressure \leq 90 mm Hg.

Exclusion criteria were the same as the previous study [Chapter 11.2] except for the following: hypertension of any aetiology: plasma creatinine \geq 140 $\mu\text{mol l}^{-1}$. Patients fulfilling the inclusion criteria gave informed consent and then followed the study protocol as detailed in Figure 12.1.

All patients entered the two week placebo run in period to assess compliance [Visit 1]. Tablets were counted at visit 2 and those whose compliance was less than 80% were excluded from final data analysis. Patients were then randomised double blind to receive lisinopril or

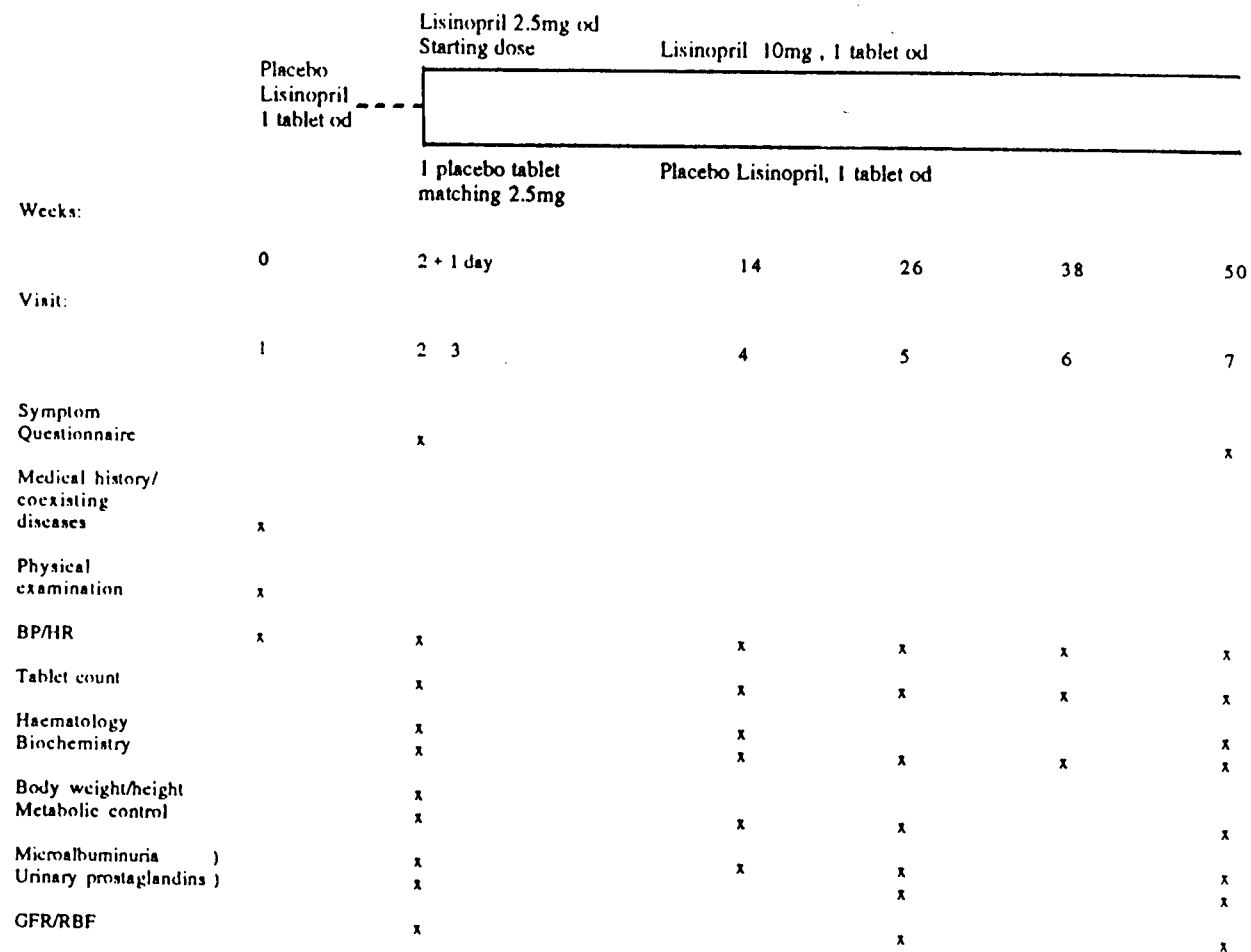


FIGURE 12.1.

Study protocol for normotensive microalbuminuric diabetic patients treated with lisinopril or placebo for 50 weeks.

placebo according to separate schedules for type 1 and type 2 diabetes. Patients randomised to receive lisinopril were given a test dose of 2.5 mg. All patients were reviewed the following day and if the test dose had been tolerated the lisinopril group continued on 10 mg daily and the placebo group with matching placebo for the duration of the study [48 weeks].

Patients were reviewed on four further occasions at 12 week intervals. At each visit, after at least 10 minutes rest, blood pressure was measured twice in the right arm, at heart level, using a Hawksley random zero sphygmomanometer and the mean of these readings was recorded. There was provision in the study protocol for open treatment with nifedipine of patients who developed diastolic blood pressure ≥ 95 mm Hg. One patient in each group required this intervention. At each visit mean arterial blood pressure [MAP] was calculated as diastolic blood pressure + pulse pressure/3. The radial pulse was counted for one minute to measure heart rate.

12.2.1. Renal Function.

Glomerular filtration rate [GFR] and renal blood flow [RBF] were measured at visits 2, 5 and 7.

12.2.2. Proteinuria

Timed overnight urine collections were made on the three consecutive days immediately prior to visits 1, 2, 4, and 7. Albumin concentration was measured in the

three samples and AER was calculated using the formula;

$$\text{AER} = \frac{CV}{T}$$

[where C is the urinary albumin concentration, V is the volume of urine passed during the collection and T is the duration of the collection].

The mean of the three values for each visit was calculated.

12.2.3. Urinary Prostaglandins

Aliquots of urine were taken from the third of the timed overnight collections, frozen at -70 xC and assayed in a single batch by radio-immunoassays for content of prostaglandin-Fl α [PGFl α] and thromboxane-B 2 [TXB 2] using commercially available kits [Amersham].

12.2.4. Haematology and Biochemistry

Blood was taken at visits 2 and 7 for measurement of haemoglobin and haematocrit, and at visits 2, 4, 5 and 7 for measurement of albumin, creatinine, urea, sodium, and potassium, plasma glucose and glycated haemoglobin [HbA 1 , reference range 5.0-9.0%].

12.2.5. Statistical analysis.

Primary end points for the study were changes in AER, urinary prostaglandins, GFR and RBF after 48 weeks treatment. Secondary end points were changes in heart rate, blood pressure and metabolic control. Changes from baseline [visit 2] to the end of randomised treatment [visit 7] were analysed using the analysis of covariance [ANCOVA] technique.

The baseline values were further included in the model since this was found to contribute substantially to the model and hence improve the precision of the estimate of treatment difference. The assumptions underlying the ANCOVA technique [normality and homoscedasticity] were checked against the data and found not to be violated. Patients whose compliance was less than 80% were excluded from the final analyses. All analyses were performed using the SAS statistical package on an IBM mainframe computer.

12.3. RESULTS

Thirty-two patients entered the study: 15 were randomised to lisinopril and 17 to placebo. Three lisinopril treated patients were withdrawn, 2 because of adverse effects [cough, skin rash] and 1 because of failure to attend. Two placebo treated patients were withdrawn, 1 because of adverse effects [diabetic keto-acidosis and pulmonary oedema] and 1 for failure to attend. Hence, 27 patients completed the study and descriptive data are presented for these. Four patients violated the study protocol hence statistical analyses of treatment differences were performed on data from the remaining 23 [11 lisinopril, 12 placebo]. The groups were well matched for age, sex, weight and treatment of diabetes [Table 12.1].

12.3.1. Proteinuria.

Mean [\pm SD]AER fell in the lisinopril groups from $57.6 \pm 25.7 \mu\text{g min}^{-1}$ [n=15] at baseline to $26.8 \pm 26.7 \mu\text{g}$

Table 12.1.

Patient characteristics at entry to the study.

	Lisinopril n=15	Placebo n=17
M:F	11:4	12:5
Age [years]	48.3 ± 13.0	49.1 ± 16.0
Weight [kg]	80.8 ± 16.1	76.6 ± 17.4
Height [cm]	163.9 ± 8.4	168.6 ± 7.0
Insulin dependent	11	12
Non-insulin dependent	4	5

Age, weight & height presented as mean±SD.

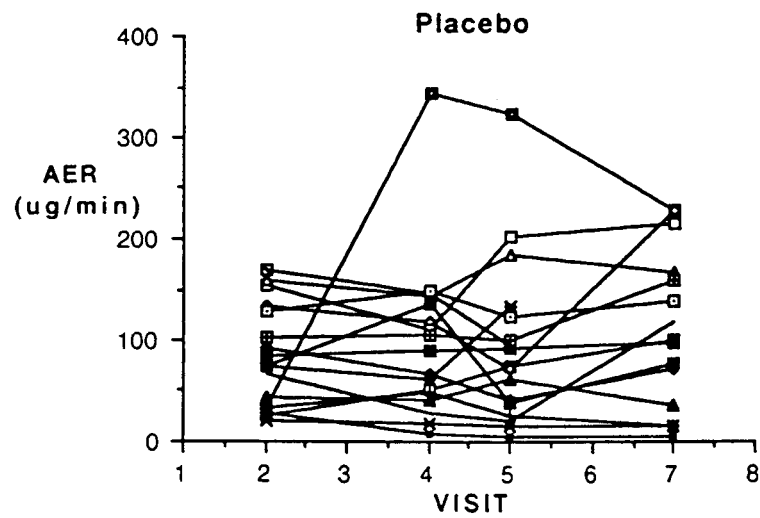
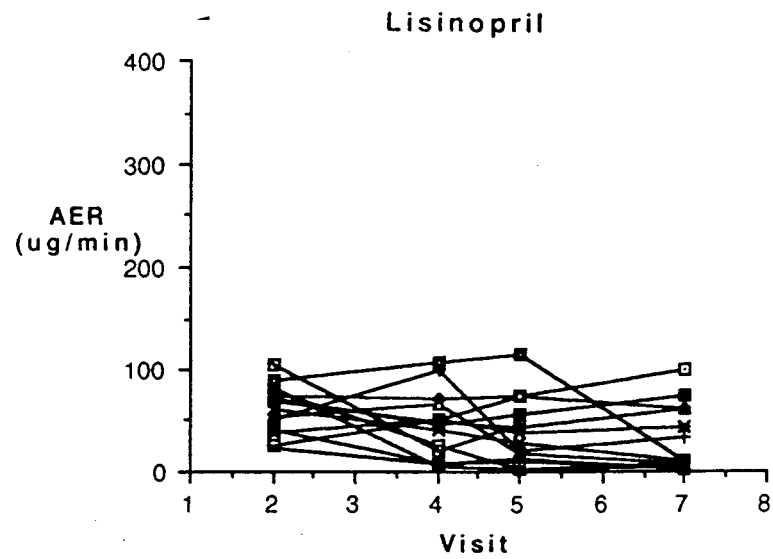


FIGURE 12.2.

Changes in albumin excretion rate [AER] for lisinopril and placebo treated normotensive diabetic patients during study.

Table 12.2.

Urinary excretion [mean \pm SD] of Prostaglandin-F_{1 α} [PGF_{1 α}] and Thromboxane-B₂ [TXB₂] during the study.

VISIT	2	5	7
<u>LISINOPRIL</u>			
PGF _{1α} [pg min ⁻¹]	303.6 \pm 211.1	223.7 \pm 140.1	203.7 \pm 115.1
TXB ₂ [pg min ⁻¹]	543.4 \pm 264.5	459.2 \pm 214.3	379.5 \pm 198.4
<u>PLACEBO</u>			
PGF _{1α} [pg min ⁻¹]	275.4 \pm 137.9	212.9 \pm 114.6	222.4 \pm 164.7
TXB ₂ [pg min ⁻¹]	551.4 \pm 297.4	433.9 \pm 227.7	489.4 \pm 398.9

min^{-1} [n=12] after 48 weeks treatment but was unaltered in the placebo group $119.2 \pm 116.6 \mu\text{g min}^{-1}$ [n=17] vs. $113.7 \pm 77.0 \mu\text{g min}^{-1}$ [n=15]. There was a least squares mean change from baseline of $-30.1 \mu\text{g min}^{-1}$ for the lisinopril and $+37.5 \mu\text{g min}^{-1}$ for the placebo groups giving a treatment difference of $-67.6 \mu\text{g min}^{-1}$ [95%CI, -115.0 to -20.2 , $P < 0.01$] in favour of lisinopril. After 48 weeks treatment 7 lisinopril treated patients were normoalbuminuric and 5 were microproteinuric; 3 placebo treated were normoalbuminuric, 9 microalbuminuric and 3 were macroproteinuric [Fig 12.2].

12.3.2. Urinary Prostaglandins

Urinary excretion of both $\text{PGF}_{1\alpha}$ and TXB_2 fell during treatment in both groups although the fall was greater for lisinopril [Table 12.2]. The treatment differences between lisinopril [n=9] and placebo [n=8] groups, however, were insignificant [$-18.9 \mu\text{g min}^{-1}$, 95% CI -138.6 to 100.9 , $P = 0.74$ for $\text{PGF}_{1\alpha}$ and $-105.4 \mu\text{g min}^{-1}$, 95% CI -385.2 to 174.4 , $P = 0.43$ for TXB_2].

12.3.3. Renal Function.

GFR fell in both groups during the study from 126.9 ± 46.7 [n=15] to $100.8 \pm 27.4 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ [n=12] for lisinopril treated patients and from 110.8 ± 34.0 [n=17] to $103.1 \pm 38.3 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ [n=15] for placebo treated patients [Fig 12.3]. There was a $15.7 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ [95% CI -41.5 to 10.1 , $P = 0.22$] larger mean decrease in the lisinopril group [n=11] than in the placebo group [n=12] but

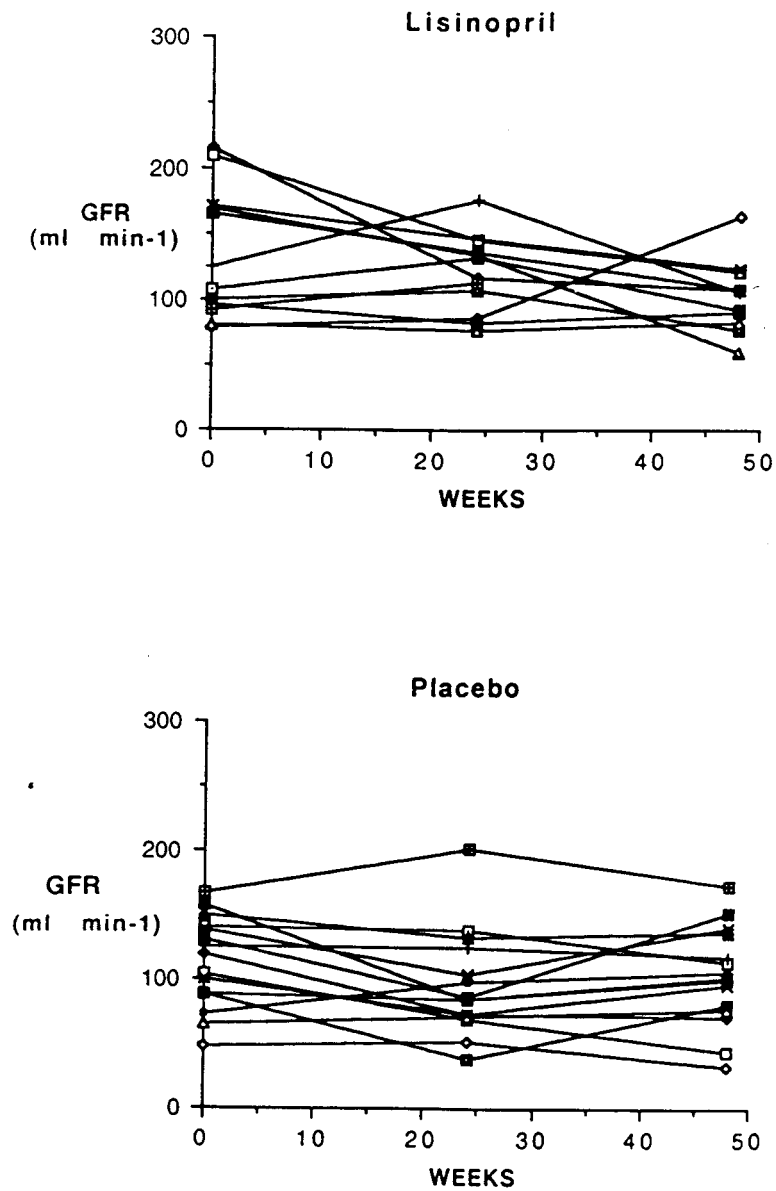


FIGURE 12.3.

Changes in glomerular filtration rate [GFR] for lisinopril and placebo treated normotensive diabetic patients during study.

this was not statistically significant.

ERPF fell in the lisinopril group from 323.1 ± 120.4 [n=15] to $304.7 \pm 115.1 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ [n=12] and rose in the placebo group from 312.6 ± 135.8 [n=17] to $349.6 \pm 189.4 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ [n=14]. There was a treatment difference of $-68.5 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ [95%CI -173.6 to 36.7, P = 0.19] in favour of lisinopril treated patients.

Filtration fraction fell slightly in both groups [from 0.40 ± 0.09 [n=15] to 0.35 ± 0.10 [n=12] for lisinopril, and from 0.38 ± 0.11 [n=17] to 0.33 ± 0.11 [n=14] for placebo] but there was no difference between the groups [treatment difference 0.00, 95% CI -0.09 to 0.10, P = 0.91].

Renal resistance did not alter in either group $0.34 \pm 0.13 \text{ mm Hg ml}^{-1} \text{ min}^{-1}$ [n=15] and $0.34 \pm 0.12 \text{ mm Hg ml}^{-1} \text{ min}^{-1}$ [n=12] for lisinopril, $0.37 \pm 0.18 \text{ mm Hg ml}^{-1} \text{ min}^{-1}$ [n=17] and $0.35 \pm 0.20 \text{ mm Hg ml}^{-1} \text{ min}^{-1}$ [n=14] for placebo. There was no difference between groups [treatment difference 0.04, 95% CI -0.06 to 0.13, P = 0.42].

12.3.4. Heart rate and Blood Pressure

Heart rate did not alter significantly from baseline in either group $178.5 \pm 13.9 \text{ beats min}^{-1}$ [n=15] at visit 2 and $80.5 \pm 11.8 \text{ beats min}^{-1}$ [n=12] at visit 7 for lisinopril treated and $80.9 \pm 10.4 \text{ beats min}^{-1}$ [n=17] and $79.9 \pm 8.4 \text{ beats min}^{-1}$ [n=15] for placebo treated]. There was no difference between groups [$1.3 \text{ beats min}^{-1}$ 95% CI -7.7 to 10.3, P = 0.77]. Systolic blood pressure fell in the

lisinopril group from 136 ± 20 mm Hg [n=15] to 125 ± 19 mm Hg [n=12] but did not alter in the placebo group, 136 ± 26 mm Hg [n=17] and 138 ± 23 mm Hg [n=15]. The treatment difference was not significant [12 mm Hg, 95% CI -27 to 3, $P = 0.1$]. Diastolic blood pressure did not alter appreciably in either group [from 78 ± 10 mm Hg at visit 2 to 78 ± 13 mm Hg at visit 7 for lisinopril, and from 77 ± 9 mm Hg to 74 ± 15 mm Hg for placebo groups].

12.3.5. Biochemistry and Glycaemic Control

Random glucose fell by 3.3 mmol l^{-1} in the lisinopril group [n=11] and rose in the placebo group [n=9] by 3.9 mmol l^{-1} giving a treatment difference of -7.2 mmol l^{-1} [95% CI -**13.3** to -1.2 , $P < 0.02$] in favour of lisinopril. GHb fell by 2.6% in the lisinopril [n=11] group and 0.8% in the placebo group [n=11] but this -1.8% difference [95% CI -4.0 to 0.4, $P = 0.11$] was not significant. There were no large differences within or between groups for any other variable [Table 12.3].

12.3.6. Haematology

Haemoglobin and haematocrit did not alter appreciably in either group between visits 2 and 7 [from 147 ± 17 g L^{-1} and 0.44 ± 0.04 , to 144 ± 15 g L^{-1} and 0.43 ± 0.05 for lisinopril, and from 142 ± 20 g L^{-1} and 0.42 ± 0.06 to 140 ± 20 g L^{-1} and 0.42 ± 0.06 for placebo].

Table 12.3.

Plasma electrolytes, albumin, urea, creatinine, glucose and glycated haemoglobin [HbA_{1c}]*

for lisinopril and placebo treated groups during study.

VISIT	2	4	5	7
<u>LISINOPRIL</u>	[n=13]	[n=13]	[n=13]	[n=12]
Sodium [mmol l ⁻¹]	136.4 ± 3.2	136.4 ± 4.6	135.8 ± 6.0	135.8 ± 2.6
Potassium [mmol l ⁻¹]	4.3 ± 0.4	4.7 ± 1.0	4.6 ± 0.5	4.6 ± 0.6
Albumin [g l ⁻¹]	34.8 ± 4.1	36.0 ± 2.7	35.0 ± 0.0	36.7 ± 3.4
Urea [mmol l ⁻¹]	6.7 ± 2.1	6.6 ± 2.0	7.1 ± 2.7	6.3 ± 1.9
Creatinine [μmol l ⁻¹]	94 ± 8	97 ± 10	100 ± 10	100 ± 8
Glucose [mmol l ⁻¹]	13.2 ± 6.1	10.3 ± 4.8	11.3 ± 6.0	10.4 ± 5.4
HbA _{1c} [%]	9.1 ± 3.4	8.7 ± 2.3	7.6 ± 2.1	6.9 ± 1.9
<u>PLACEBO</u>	[n=17]	[n=17]	[n=15]	[n=15]
Sodium [mmol l ⁻¹]	137.1 ± 2.4	136.6 ± 3.1	137.5 ± 2.6	136.5 ± 2.8
Potassium [mmol l ⁻¹]	4.3 ± 0.2	4.5 ± 0.3	4.4 ± 0.4	4.4 ± 0.4
Albumin [g l ⁻¹]	35.8 ± 4.2	36.2 ± 2.9	35.0 ± 4.1	36.6 ± 2.6
Urea [mmol l ⁻¹]	5.9 ± 2.1	6.1 ± 1.9	6.2 ± 2.2	6.7 ± 3.2
Creatinine [μmol l ⁻¹]	97 ± 19	104 ± 21	102 ± 20	108 ± 26
Glucose [mmol l ⁻¹]	11.5 ± 4.4	15.0 ± 5.2	13.7 ± 4.1	14.5 ± 5.4
HbA _{1c} [%]	9.9 ± 2.4	8.7 ± 2.0	7.6 ± 2.1	6.9 ± 3.0

*All given as mean ± SD.

HbA_{1c} reference range 5.0-9.0%.

12.4. DISCUSSION

Results from studies in animals and humans have suggested that ACE inhibitors exert specific intra-renal effects in diabetic nephropathy [Anderson et al 1986, Zatz et al 1986, Mathiesen et al 1991, Marre et al 1988]. Two studies have reported that treatment with ACE inhibitors postpones the development of overt diabetic nephropathy. Marre et al [1988] found that after 12 months treatment with enalapril 5 patients remained microalbuminuric and 5 were normoalbuminuric whereas 3/10 patients taking placebo had developed diabetic nephropathy. Mathiesen et al [1991] reported an open multicentre study in which nephropathy developed in 7/23 patients given placebo for 4 years but, in none of the 21 patients treated with captopril and thiazide. I performed a double blind placebo controlled single centre study involving 27 patients and found that 7 lisinopril treated patients became normoalbuminuric and 5 remained microalbuminuric; none developed nephropathy. Three placebo treated patients became normoalbuminuric, 9 remained microalbuminuric and 3 developed nephropathy. Hence I can confirm the renal protective effect of ACE inhibition in incipient diabetic nephropathy. The reduction in proteinuria in the lisinopril treated group appears to be independent of alterations in systemic blood pressure and this observation supports other workers' reports of a specific intra-renal effect of ACE inhibitors [Anderson et al 1986, Zatz et al 1986, Mathiesen et al 1991, Marre et al 1988]. The mechanism whereby ACE inhibitors exert this effect is thought to be efferent arteriolar dilatation causing a reduction in

intraglomerular hypertension [Anderson et al 1986, Zatz et al 1986].

Glomerular filtration rate fell in both groups. In patients eligible for analysis of treatment difference the average fall was $-29 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ for lisinopril treated patients and $-13.4 \text{ ml min}^{-1} 1.73\text{m}^{-2}$ for those on placebo. These falls are larger than reported by Marre et al [1988] or Mathiesen et al [1991] and may relate to methodological differences or the different population under study. It is perhaps noteworthy that 5 patients in the lisinopril group but only 1 in the placebo group were hyperfiltering [$\text{GFR} > 160 \text{ ml min}^{-1} 1.73\text{m}^{-2}$] at entry to the study and the greatest reductions in GFR were seen in these [Fig 12.3]. It is known that hyperfiltering diabetic patients experience a fall in GFR as their disease progresses. My patients may have been at this stage of their disease and the apparent difference between groups may have been a consequence of this rather than a drug effect. I could demonstrate no benefit of ACE inhibition over placebo on any other aspect of renal haemodynamics. A similar lack of benefit from ACE inhibition on renal function has been described in hyperfiltering normoalbuminuric diabetic children treated with enalapril [Drummond et al 1989].

It has been reported that both specific thromboxane synthetase inhibition and treatment with indomethacin, a cyclooxygenase inhibitor, may reduce

proteinuria in established and incipient diabetic nephropathy in association with altered urinary prostaglandin excretion [Barnett et al 1984, Hommel et al 1987, Mathiesen et al 1988]. The precise mechanism underlying this reduction is unclear but it may be a consequence of prostaglandin mediated alterations in intra renal haemodynamics or on glomerular mesangial cells reducing the surface area available for filtration. I found no difference between groups for urinary excretion of **prostaglandin-F_{1α}**, a metabolite of the vasodilator prostacyclin, or of **thromboxane-B₂**, the metabolite of vasoconstrictor **thromboxane-A₂**. Although the confidence intervals for the differences between placebo and active treatment groups are wide these data suggest that the effects of ACE inhibition on proteinuria are not prostaglandin mediated.

In summary, I have demonstrated a renal protective effect of ACE inhibition in patients with incipient diabetic nephropathy in a single centre double blind placebo controlled study. The observed reductions in proteinuria appear to be independent of changes in systemic blood pressure. Alterations in urinary protein excretion do not depend upon changes in renal prostaglandin activity. In these patients lisinopril appeared to be no more renal protective than placebo. The study population, however, was small and a larger number of patients is required to provide a definitive answer.

CHAPTER 13.

CONCLUSIONS
AND SUGGESTIONS FOR
FURTHER RESEARCH

CONCLUSIONS - PART I

Initial data from several workers suggested that urinary transferrin may be a useful marker for complications of diabetes mellitus and a predictor of diabetic renal disease. I have examined these claims in a series of cross-sectional, longitudinal and interventional clinical studies.

13.1. PREVALENCE OF PROTEINURIA

I have examined a defined population of type 1 diabetic patients and established that the prevalences of diabetic renal disease [defined by albumin excretion rate] and associated complications are similar to those reported by other investigators. There is an increased prevalence of retinopathy with increasing albuminuria and, similarly, an increased prevalence of hypertension. I could not confirm previous workers' reports of an association between cigarette smoking and diabetic nephropathy; this may relate to the crude method chosen to measure smoking habit.

13.2. PREVALENCE OF TRANSFERRINURIA

I established a reference range for urinary transferrin excretion based upon a group of 28 non-diabetic subjects. Transferrin excretion appears to be unrelated to sex or age in this group. Study of the patient population defined in chapter 4 established that transferrin and albumin are handled differently in the diabetic kidney; **85%** of diabetic patients had elevated transferrin excretion compared with only **38%** who had elevated albumin excretion rate. All diabetic patients with elevated albumin excretion rate had

elevated transferrin excretion.

13.3. EFFECTS OF GLYCAEMIC CONTROL ON TRANSFERRINURIA

Newly diagnosed diabetic patients are known to have increased albumin excretion rates. I have demonstrated that they also have increased urinary transferrin excretion rates. The transferrin excretion rates are correlated with markers of tubular function suggesting that the renal tubules may be important in the handling of transferrin in diabetes. I could not confirm other workers observations of an association between increased urinary transferrin excretion and the presence of diabetic complications.

13.4. EFFECT OF EXERCISE ON TRANSFERRINURIA

I have shown that urinary transferrin excretion may be provoked by moderate exercise in type 1 diabetic patients with no evidence of complications. This observation is novel. The mechanism for the increased proteinuria remains to be elucidated. It may, however, relate to increased glomerular leak rather than altered tubular handling of transferrin since there was no alteration in other markers of renal tubular function in this study. The data suggest that even patients with apparently uncomplicated diabetes mellitus may have abnormal renal function.

13.5. THE EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITION ON TRANSFERRINURIA

I have demonstrated in two studies that

angiotensin converting enzyme [ACE] inhibition in diabetic patients may influence transferrin excretion. The first was a study of normotensive patients with microalbuminuria. Patients treated with an ACE inhibitor tended to have a reduction in transferrin excretion rates. The second was a study of hypertensive macroalbuminuric patients. Those treated with an ACE inhibitor had reduced transferrin excretion which was independent of reductions in systemic blood pressure. The reduction in excretion is probably accomplished by lowering of intraglomerular pressure.

CONCLUSIONS - PART II

13.6. ACTIVITY OF RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Patients with elevated albumin excretion rates have elevated plasma renin activity but there is no correlation between the two variables. Overall there is a weak correlation between systolic blood pressure and albumin excretion rate but not with plasma renin activity. These observations although on a small number of patients suggest that altered plasma renin activity is not responsible for the hypertension seen in diabetic nephropathy.

13.7. RESPONSE OF RENIN-AGIOTENSIN-ALDOSTERONE SYSTEM AND BLOOD PRESSURE TO EXERCISE

Exercise provokes an exaggerated systolic blood pressure response and reduction in urinary sodium excretion in normoalbuminuric type 1 diabetic patients. Both

findings may reflect increased sensitivity to the renin-angiotensin-aldosterone system despite normal plasma renin activity levels. The highest blood pressure changes were associated with an enhanced noradrenaline response despite lower basal levels in the diabetic group. Reduced atrial natriuretic peptide levels may stimulate sodium retention and increased diastolic blood pressure in early type 1 diabetes mellitus. The presence of these changes in a group of otherwise uncomplicated type 1 diabetic subjects suggests they are a consequence of the diabetic state rather than the development of micro- or macrovascular complications.

13.8. ANGIOTENSIN CONVERTING ENZYME INHIBITION IN OVERT DIABETIC NEPHROPATHY

In a randomised double blind single centre study comparing an ACE inhibitor, lisinopril, with a calcium channel blocker, nifedipine, for 6 months in diabetic patients with hypertension and macroproteinuria both drugs reduced blood pressure equally. There were no significant intergroup differences in renal haemodynamics or proteinuria. There was, however, a trend for albumin excretion rate to fall in the ACE inhibitor group and rise in the calcium blocker group and the two groups appeared to be diverging at the end of the study. This may be the consequence of differing intra-renal actions of the two classes of drug. Reduced fractional albumin clearance in the ACE inhibitor group suggests that this drug may have an intra-renal effect distinct from its effects on haemodynamics.

13.9. ANGIOTENSIN CONVERTING ENZYME: INHIBITION IN INCIPIENT DIABETIC NEPHROPATHY

In a randomised double blind single centre study comparing an ACE inhibitor, lisinopril, with placebo for 12 months in diabetic patients with normotension and microproteinuria glomerular filtration rate fell in both groups. No lisinopril treated patients progressed to macroproteinuria but three placebo treated did. Hence it appears that ACE inhibition offers some renal protection in this patient group. The observed reductions in proteinuria appear to be independent of changes in systemic blood pressure and not to depend upon renal prostaglandin activity.

13.10. SUGGESTIONS FOR FURTHER RESEARCH

In the cross sectional study **85%** patients had elevated transferrin excretion rates and all patients with elevated albumin excretion rates had elevated transferrin excretion rates. The high prevalence of elevated transferrin excretion rate [as presently defined] makes this a poor predictor of diabetic nephropathy. It is proposed to follow the original patient population longitudinally to assess changes in albumin and transferrin excretions. This will allow the identification of new cases of elevated albumin excretion and perhaps permit identification of an initial transferrin excretion rate which is predictive of nephropathy.

Some data suggest that renal handling of transferrin is dependent upon tubular rather than glomerular function. To elucidate the true mechanism it is proposed to

observe alterations in urinary transferrin excretion after blocking renal tubular re-uptake with intravenously infused lysine.

Whilst they did not show a clear difference the results of the study comparing the effects of nifedipine and lisinopril in patients with overt nephropathy suggested that there may have been some difference between the two after six months. A longer term study using a similar protocol may resolve this question.

The question of whether or not **ACE** inhibitors are truly renal protective in diabetes remains unresolved and long term studies [5 to 10 years duration] are required to answer this definitively.

ADDENDA

ADDENDUM I.

ABBREVIATIONS

ACE	Angiotensin converting enzyme
AER	Albumin excretion rate
ANCOVA	Analysis of covariance
ANP	Atrial natriuretic peptide [factor]
A₁M	Alpha-1-microglobulin
BP	Blood pressure
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra-acetic acid
ERPF	Effective renal plasma flow
FF	Filtration fraction
GFR	Glomerular filtration rate
HbA₁	Glycated haemoglobin
MAP	Mean arterial pressure
NAG	N-acetyl-β-D-glucosaminidase
PGE₂	Prostaglandin E₂
PGF_{1A}	Prostaglandin F₁ALPHA
PGF_{2A}	Prostaglandin F₂ALPHA
PGI₂	Prostaglandin I₂ [Prostacyclin]
PRA	Plasma renin activity
RAAS	Renin angiotensin aldosterone system
RBF	Renal blood flow
RIA	Radio-immunoassay
RR	Renal resistance
SD	Standard deviation
SNGFR	Single nephron glomerular filtration rate

TER	Transferrin excretion rate
Tm _G	Tubular maximum reabsorption capacity for glucose
TXB ₂	Thromboxane B ₂

ADDENDUM II.

Reprints of published original articles.

University of Birmingham Research Archive
e-theses repository

Due to copyright restrictions, we are unable to publish the reprinted articles of Addendum II in electronic format. We have instead provided references for each of the articles, below, so that they may be consulted in their original format.

M.J. O'Donnell, P. Martin, C.M. Florkowski, M.J. Toop, C. Chapman, R. Holder and A.H. Barnett [1991] Urinary Transferrin Excretion in Type 1 (Insulin-dependent) Diabetes Mellitus. *Diabetic Medicine* (1991), 8: 657-661.

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