ASPECTS OF THE PATHOPHYSIOLOGY OF EARLY DIABETIC NEPHROPATHY

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ABSTRACT

In this thesis, I have focussed on a number of clinical aspects of the pathophysiology of early diabetic nephropathy which have previously received little attention.

a) Early diabetic nephropathy, hypertension and vascular disease

1. Patients with IDDM, both with normal albumin excretion and microalbuminuria, have no evidence of a defect of urinary dopamine excretion. This genetically determined association with essential hypertension does not, therefore, explain the link between early diabetic nephropathy and hypertension.

2. In hypertensive patients with IDDM, captopril and nifedipine retard have a similar effect in reducing blood pressure and albumin excretion after eight weeks treatment. Captopril, however, retains an acute effect on renal haemodynamics, resulting in a decreased filtration fraction, and this may be of specific importance in the management of patients with diabetic nephropathy.

3. In newly-presenting patients with NIDDM, 26% had abnormal urinary albumin excretion, this correlating with age, glycaemic control, systolic blood pressure and generalised vascular disease. Over the year following diagnosis, 16% of patients had persistent microalbuminuria.

b) Tubular function in early diabetic nephropathy

Markers of tubular enzymuria and low molecular weight proteinuria

were measured in patients with IDDM, both with and without microalbuminuria. Only 8 (38%) of 21 patients with a detectable urinary beta-thromboglobulin concentration had concurrent abnormal albumin excretion. Urinary excretion of N-acetyl-beta-D-glucosaminidase was increased in 22 (63%) of patients with microalbuminuria and also correlated with glycaemic control and smoking. Although tubular dyfunction is commonly found in patients with microalbuminuria, the relatively poor correlation of currently available markers would seem to limit their value in the detection of early nephropathy.

c) Hypoglycaemia and diabetic nephropathy

In patients with IDDM and normal albumin excretion, acute hypoglycaemia causes a number of changes in renal function, notably a significant fall in both effective renal plasma flow and glomerular filtration rate (GFR). The fall in GFR is more marked than in non-diabetic subjects. It is possible that hypoglycaemia may have a role in the aetiology or progression of diabetic nephropathy.

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SCOPE AND AIMS OF THESIS

Introduction

Nephropathy complicating diabetes mellitus is a major cause of morbidity and mortality. Until the last few years, it was only possible to diagnose diabetic nephropathy at an advanced and irreversible stage of the disease process. However, a much earlier stage of diabetic renal disease has now been identified, characterised by normal renal function but an increase in urinary albumin excretion, in comparison with normal subjects. This early phase of diabetic nephropathy has been termed 'microalbuminuria'. Microalbuminuria has been shown to be a strong predictor, if untreated, of progression to advanced renal disease and patients with microalbuminuria also have a significantly increased prevalence of generalised macrovascular disease and higher blood pressure than control subjects with normal urinary albumin excretion.

Studies to date suggest that intervention at the stage of microalbuminuria may slow, or even prevent, progression to more severe nephropathy, characterised by persistent proteinuria, with its inexorable decline towards end-stage renal disease, although long-term follow-up data is still awaited. The association between microalbuminuria, hypertension and generalised vascular disease is still, however, poorly understood and this, in addition to a number of other areas of potentially considerable clinical importance in the field of early diabetic nephropathy, have to date received little attention. In this thesis, I have attempted to identify and explore further some of these areas.

Aims of thesis

I have focussed on three major areas of research and attempted to answer a number of specific questions.

Early diabetic nephropathy, hypertension and vascular disease

a) Does an abnormality of dopamine excretion, a genetically determined association with essential hypertension, provide an explanation for the link between microalbuminuria and increased blood pressure?

b) In hypertensive patients with insulin-dependent diabetes (IDDM), do different classes of antihypertensive drugs (namely angiotensin converting enzyme inhibitors and calcium antagonists) have differing, and possibly specific, effects on blood pressure, urinary albumin excretion and renal haemodynamics?

c) In newly-diagnosed patients with non-insulin-dependent diabetes (NIDDM), what is the prevalence and natural history of abnormal urinary albumin excretion and its associations, particularly with blood pressure and macrovascular disease?

Tubular function in early diabetic nephropathy

Is tubular enzymuria and increased excretion of low molecular weight proteins, indicative of tubular dysfunction, of value in the detection of early diabetic nephropathy, possibly at an earlier stage of the disease process than that identified by the presence of microalbuminuria?

Hypoglycaemia and diabetic nephropathy

Could hypoglycaemia, which is commonly experienced by most patients with IDDM, be of importance in the aetiology or progression of diabetic nephropathy?

CHAPTER 1

INTRODUCTION

EARLY DIABETIC NEPHROPATHY

1.1 PREVALENCE AND IMPLICATIONS OF DIABETIC NEPHROPATHY

Renal disease (nephropathy) is a major cause of morbidity and mortality in patients with IDDM. This is particularly so in those developing the disease at a young age. Diabetes diagnosed before age 31 years has been reported to be associated with nephropathy in over 35% of patients, with 31% of deaths being due to renal disease (Deckert et al, 1978), excess mortality in patients developing nephropathy being 3 - 4 fold compared with those who do not. Another epidemiological study, finding a similar prevalence of nephropathy of approximately 40% in patients with IDDM, has suggested that this is an even more important cause of mortality, death from renal disease being reported in over 50% of affected patients (Andersen et al, 1983). In terms of resource implications, diabetic nephropathy currently accounts for over a quarter of all patients entering dialysis or transplantation programmes for end-stage renal disease in the United States (Vollmer et al, 1983; Eggers et al, 1984), with diabetic patients undergoing haemodialysis and transplantation spending much longer periods in hospital than patients with non-diabetic renal disease (Shyh & Bayer, 1985). In the United Kingdom, it has been estimated that around 20% of patients with renal failure sufficiently severe to merit renal replacement therapy have diabetes (Berisa et al, 1989), although a recent survey suggests that a third of such patients die without receiving renal support treatment (Joint Working Party on Diabetic Renal Failure, 1989).

1.2 CLINICAL DIAGNOSIS OF DIABETIC NEPHROPATHY

The hallmark of clinical (frank) diabetic nephropathy is excessive urinary excretion of protein, especially albumin (Parving et al, 1982), characteristically detected on standard dip-stick urine testing, eg Albustix. This usually indicates a protein excretion in excess of 0.5 g/24 h (Abuelo, 1983) and most commonly occurs 14 - 19 years after onset of diabetes, new cases of nephropathy being relatively uncommon after 25 - 30 years of diabetes (Andersen et al, 1983; Borch-Johnsen et al, 1985). Once clinical proteinuria develops, arterial blood pressure rises and glomerular function declines progressively and at a constant rate which varies considerably from patient to patient (Jones et al, 1979; Viberti et al, 1983a). Renal failure usually occurs 4 - 6 years after the onset of proteinuria (Rutherford et al, 1977; Andersen et al, 1978).

1.3 PATHOLOGICAL DIAGNOSIS OF DIABETIC NEPHROPATHY

Characteristic pathological changes in the kidneys of patients with IDDM have long been recognised. Nodular glomerulosclerosis was described by Kimmelstiel and Wilson over 50 years ago and was considered to be pathognomonic of diabetic renal disease (Kimmelstiel & Wilson, 1936). Other less specific changes are also well recognised, including diffuse glomerulosclerosis and mesangial cell proliferation. More recent pathological studies have shown that the most consistent finding in diabetic nephropathy is thickening of the glomerular basement membrane (Bergstrand & Bucht, 1959). Although

glomerular hypertrophy may be present at diagnosis, progressive thickening of the glomerular basement membrane appears to occur with duration of disease (Mogensen et al, 1979a; Mauer et al, 1984). Excessive deposition of glomerular basement membrane-like material causes mesangial expansion (Osterby, 1973) and encroachment on the subendothelial space may compromise the glomerular capillary lumen and blood flow, eventually causing capillary occlusion (Mauer et al, 1981). Although only around 40% of IDDM patients will develop clinical diabetic nephropathy, most patients will have some pathological abnormalities within a few years of diagnosis of the disease (Deckert & Poulsen, 1981). The extent of the pathological changes does not invariably correlate closely with either duration of disease or the clinical picture, but it is clear that clinical nephropathy manifested by frank proteinuria does not generally become manifest until pathological changes are well advanced (Mauer et al, 1984; Thomsen et al, 1984).

1.4 THE CONCEPT OF EARLY DIABETIC NEPHROPATHY (MICROALBUMINURIA)

It is evident from Section 1.2 that identification of diabetic nephropathy at the stage of frank proteinuria is too late to significantly influence morbidity and mortality.

In the last few years, it has been noted that, before the onset of frank proteinuria and progressive deterioration in renal function, there is a silent phase, when renal function remains normal, characterised by increased urinary albumin excretion (Mogensen et al, 1983). In normal subjects, albumin comprises approximately 11% of the

total urinary protein excretion (Viberti et al, 1983b) but this proportion rises progressively with increased renal damage. As mentioned above, standard dip-stick methods of testing for proteinuria detect only concentrations of protein in excess of approximately 500 mg/l (Abuelo, 1983), of which, at this stage of the disease process, some 50% is albumin (Viberti et al, 1983b). However, the presence of much lower concentrations of albuminuria also predicts the later development of frank nephropathy. In 1982, Viberti reported that, in a group of patients with IDDM who were followed up for 14 years, a urinary albumin excretion rate (AER) in excess of 30 ug/min predicted the later development of dip-stick positive proteinuria (Viberti et al, 1982a). Seven out of eight patients in this category went on to develop frank nephropathy, compared with only two out of 55 patients with a lower initial AER. A further series of studies published in the next two years (Parving et al, 1982; Mathiesen et al, 1984; Mogensen & Christensen, 1984) examined cut-off concentrations of albuminuria between 15 and 70 ug/min, patients being followed up for varying periods of time from upwards of six years. In each study the presence of the higher concentration of albuminuria was highly predictive of the development of proteinuria.

This increased concentration of urinary albumin excretion, between normality and that detectable by standard dip-stick testing, has been given the label 'microalbuminuria'. This has been defined more specifically, largely to standardise research in the field, by a concensus of a predominantly Scandinavian group of investigators (Mogensen, 1987). Thus microalbuminuria is diagnosed on the basis of a 24-hour urinary albumin excretion of 30 - 300 mg (20 - 20 ug/min)

in two out of three samples collected over a 6-month period, this latter proviso being made on account of the considerable day to day variation which can occur in urinary albumin excretion, depending on various factors, including exercise and state of hydration (Chachati et al, 1987). This day to day variation is reported to be as much as 47% in diabetic patients both with and without abnormal albumin excretion (Feldt-Rasmussen & Mathiesen, 1984).

In addition to being a reliable predictor of frank diabetic nephropathy in IDDM, microalbuminuria also clearly correlates with the presence of retinopathy (Barnett et al, 1985) and closely predicts the majority of early deaths in IDDM, not only from end-stage renal disease but also from cardiovascular causes (Jensen et al, 1987; Gatling et al, 1988a). Similarly, in NIDDM, the presence of microalbuminuria predicts the development of established nephropathy, albeit to a lesser extent than in the IDDM population. In one study (Mogensen, 1984) 22% of patients with microalbuminuria went on to develop persistent proteinuria over a nine year period. Microalbuminuria in the NIDDM population is also a strong predictor of increased mortality (Jarrett et al, 1984; Mogensen, 1984), with more than 50% of this excess being due to ischaemic heart disease (Mogensen et al, 1984; Schmitz & Vaeth, 1988). There is additional evidence that an association between increased urinary albumin excretion and cardiovascular disease is demonstrable in the non-diabetic population (Yudkin et al, 1988), lending weight to the theory that albuminuria may be a marker of a more generalised vascular process (See Section 1.5).

1.5 THE PATHOGENESIS OF MICROALBUMINURIA

The pathogenesis of microvascular disease in diabetes is not well understood and many theories have been put forward. With specific reference to early nephropathy several possible influential factors have been suggested.

1. Genetic Factors

There are a number of pointers to a possible genetic role in the aetiopathogenesis of diabetic nephropathy. The first relates to individual susceptibility to this particular complication. As discussed above (Section 1.1), nephropathy affects no more than 40% of patients and, moreover, development of nephropathy is extremely rare after 25 - 30 years of the disease. This only partly relates to metabolic control and exposure to hyperglycaemia, suggesting a genetic influence. Support is also lent to the concept of a hereditary factor by the evidence that diabetic nephropathy occurs in familial clusters (Seaquist et al, 1989).

A link between diabetic nephropathy and the HLA antigen B8 has been suggested (Barbosa et al, 1976) but the most interesting genetic data pertains to the association between nephropathy and hypertension (see also Chapter 3). Although it has traditionally been regarded that hypertension in patients with nephropathy relates directly to renal damage, the finding of a higher blood pressure in patients with microalbuminuria, and apparently normal glomerular function, than in control subjects with normal albumin excretion (Mathiesen et al,

1984; Wiseman et al, 1984a; Christensen & Mogensen, 1985) suggests the possibility of a more fundamental association. That this association may have a genetic component, with a predisposition to hypertension contributing to susceptibility to nephropathy, has been suggested by the finding that parents of patients with IDDM who have proteinuria have a significantly higher blood pressure than those of patients with normal protein excretion (Viberti et al, 1987). Furthermore, research into the erythrocyte sodium-lithium counter-transport system, which is apparently a genetically determined marker of hypertension (Semplicini et al, 1989), has shown higher rates of activity in diabetic patients with nephropathy compared with patients having a similar duration of disease but normal protein excretion (Krolewski et al, 1988; Mangili et al, 1988). This system is thought to reflect the activity of the physiological sodium-hydrogen antiport, which has an important role in the renal reabsorption of sodium and bicarbonate, and is also involved in the control of cell growth and replication (Mahnensmith & Aronson, 1985). As discussed in Section 1.3, glomerular hypertrophy and mesangial expansion are characteristic pathological findings in diabetic nephropathy.

Further clues to a genetic influence in the aetiology of diabetic nephropathy are provided by the ethnic variations in the prevalence of this complication, seen particularly in the population with NIDDM. The incidence of nephropathy is higher in a number of ethnic populations, including Japanese and American Indians (Nelson et al, 1989), although the fact that the latter tend to develop diabetes at an early age and the former are relatively protected from coronary heart disease (and therefore are more likely to survive for sufficiently long to develop nephropathy) may be of importance.

Nonetheless, there do seem to be some genuine differences between ethnic groups and, in the UK, Asian Indians have a higher prevalence of microalbuminuria than comparable patients of European extraction (Allawi et al, 1988).

2. Haemodynamic Factors

The glomerular filtration rate is approximately 25% higher in patients with short-term IDDM than in non-diabetic control subjects (Christiansen et al, 1981a) and is also significantly elevated in patients with longstanding disease who do not have frank proteinuria (Mogensen, 1972). Even at initial diagnosis an increase in glomerular filtration rate of some 40% compared with controls has been reported (Mogensen, 1971). This is accompanied by an elevation in renal plasma flow, which is a major determinant of glomerular filtration rate, accounting for around 50 - 60% of the observed increase (Mogensen & Andersen, 1973; Christiansen et al, 1981b). There is evidence that these haemodynamic abnormalities are related to the structural changes seen early in the course of the disease, with kidney size and glomerular filtration rate being positively correlated (Mogensen & Andersen, 1973). Micropuncture studies in rats have shown this to be associated with an elevated transglomerular pressure gradient (Hostetter et al, 1981a) and further animal studies have shown that such haemodynamic changes may cause alterations in glomerular selective permeability, resulting in increased albumin excretion (Olson et al, 1982). Nephrectomy results in compensatory haemodynamic mechanisms, with relative hyperfiltration in the reduced population of nephrons in the remnant kidney (Hostetter et al, 1981b).

Nephrectomised rat and dog models (Steffes et al, 1978 & 1982) have been shown to have more rapid development and progression of diabetic nephropathy than control animals. An anecdotal case report, suggesting the importance of haemodynamic factors in the human kidney, described the post-mortem findings in a diabetic patient with unilateral renal artery stenosis, who only had classical pathological changes of diabetic nephropathy affecting the kidney supplied by the normal renal artery (Berkman & Rifkin, 1973).

3. Metabolic Factors

Glycaemic control has long been considered important in the aetiology of diabetic complications, including nephropathy and an association is certainly supported by a number of studies (Mogensen et al, 1983; Hasslacher et al, 1985). However, at the stage of persistent proteinuria, the degree of proteinuria and renal impairment does not appear to relate to prevailing glycaemia and most authors have found that improvement in blood glucose control at this stage does not influence the rate of progression of the nephropathic process (Tamborlane et al, 1982; Viberti et al, 1983c). This is discussed further in Section 1.7.

In patients with microalbuminuria, there does seem to be a correlation between albumin excretion and glycaemic control (Wiseman et al, 1984a) and this may at least partly operate through the haemodynamic changes discussed above. In diabetic patients, a positive correlation certainly exists between prevailing plasma glucose concentrations and glomerular filtration rate (Wiseman et al,

1984b) and, even in non-diabetic subjects, infusion of glucose results in an increase in glomerular filtration rate (Brochner-Mortensen, 1973; Christiansen et al, 1981c).

Disturbances of glucagon and growth hormone secretion also occur in poorly controlled diabetic patients with serum concentrations of both of these hormones being increased. Infusion of both glucagon (Parving et al, 1977 & 1980) and growth hormone (Christiansen et al, 1981d & 1982) may, per se, increase glomerular filtration rate. The magnitude of the haemodynamic changes caused by infusion of these hormones is, however, less than is observed in IDDM, particularly in the early stages following diagnosis, and these metabolic factors are, therefore, unlikely to be the sole cause of the increased glomerular filtration rate observed in diabetic patients.

The Steno Hypothesis

A recent theory put forward by Deckert and his colleagues in the Steno Memorial Hospital in Gentofte, Denmark ties together a number of the possible pathogenetic factors discussed above and also attempts to provide an explanation for the clear association between increased urinary albumin excretion and evidence of vascular (notably coronary artery) pathology discussed in Section 1.4 (Deckert et al, 1989).

The heparan sulphate proteoglycan is the major glycosaminoglycan component of the basement membranes of the glomeruli (Kanwar & Farquhar, 1979; Wu et al, 1987), mesangium (Kanwar et al, 1983; Wu et al, 1987) and endothelial plasma membranes (Robinson & Gospodarowicz,

1984). In the kidney, heparan sulphate proteoglycan inhibits the glomerular filtration of albumin (Rosenzweig & Kanwar, 1982; Groggel et al, 1988) and contributes to the pore size of the glomerular basement membrane (Tarsio et al, 1988), in addition to inhibiting mesangial cell growth (Castellot et al, 1985). Elsewhere in the body, heparan sulphate proteoglycan has important antithrombogenic properties (Marcum et al, 1984; Pejler et al, 1987), binds lipoprotein lipase (Williams et al, 1983) and inhibits arterial smooth muscle proliferation (Castellot et al, 1984). A decreased glycosaminoglycan component within the glomerular basement membrane has been demonstrated in diabetic patients with nephropathy (Parthasarathy & Spiro, 1982; Shimomura et al, 1987), and loss of heparan sulphate from the glomerular basement membrane leads to the loss of anionic sites and consequently albuminuria (Rosenzweig & Kanwar, 1982; Cotran & Rennke, 1983; Groggel et al, 1988). In addition, its widespread effects elsewhere, which are likely to promote the development of atherogenesis (Marcum et al, 1984; Gallagher et al, 1986; Stender & Hjelms, 1987), may at least partly explain the association between albuminuria and extrarenal vascular abnormalities.

Deckert proposes that there is a genetically determined polymorphism of enzymes involved in the metabolism of heparan sulphate proteoglycan, this having been demonstrated in at least one rat model (Eriksson et al, 1986). He hypothesises that patients who develop albuminuria are characterised by enzymes which are particularly vulnerable to poor glycaemic control. In these patients a critical reduction in normal heparan sulphate proteoglycan may occur, resulting in microalbuminuria, glomerular expansion and other

extrarenal microvascular and macrovascular complications. It is postulated that such a genetic polymorphism may account for the fact that only a certain percentage of diabetic patients develop nephropathy and also might explain why even patients with persistently poor glycaemic control do not invariably develop renal disease or other specific vascular complications.

1.6 SCREENING FOR MICROALBUMINURIA

The recognition that a subclinical increase in urinary albumin excretion (undetectable on standard dip-stick testing) is highly predictive of later frank nephropathy and other vascular complications has led to the development of the concept of microalbuminuria, as discussed in Section 1.4. The wide range in the timing of the urine samples collected in the initial studies, and variety of assays used, meant that early definitions of microalbuminuria were inconsistent. As described earlier, in an attempt to standardise this, 'The Gentofte Convention on Microalbuminuria and Incipient Diabetic Nephropathy' in 1985 reached the consensus definition of microalbuminuria as a urinary albumin excretion of 20 - 200 ug/min (30 - 300 mg/24 h) in two out of three timed collections during a 6-month period (Mogensen, 1987).

Even the lower limit of this definition is considerably higher than the normal albumin excretion rate in healthy subjects. This has been quoted in different studies as between 2.5 and 26.0 mg/24 h (1.7 - 17.4 ug/min), with a mean of 9.5 mg/24 h (6.4 ug/min) (Viberti et al, 1984) and between 2.3 and 8.3 ug/min, with a mean of 4.3 ug/min

(Mogensen, 1984).

The recognition that albuminuria also increases with exercise has led to attempts to use a standardised test to unmask an even earlier stage of diabetic nephropathy and to exaggerate the increased albumin excretion in patients with early nephropathy, with a possibly greater predictive value (Viberti et al, 1978; Viberti & Keen, 1984; Mogensen et al, 1979b; Vittinghus & Mogensen, 1982). Nonetheless, the consensus definition at present remains the gold standard for the diagnosis of microalbuminuria. As for the timing of the urine sample, a full 24-hour collection probably remains the standard against which other screening methods must be compared. A timed overnight collection may be an equally valuable measurement, with a reduced risk of the problem of incomplete sampling which invalidates many 24-hour urine collections, although direct comparative data is lacking.

Many studies have attempted to identify a simple test for microalbuminuria which does not require patients to collect timed urine samples at home and which would therefore be more practical for large-scale screening. The albumin concentration in a random urine specimen does not, however, correlate particularly well with timed albumin excretion rates. A correlation co-efficient of 0.45 with the albumin excretion rate in a timed overnight collection has been reported (Gatling et al, 1985) and random urinary albumin concentrations of >25 mg/l and >26 mg/l have been shown in different studies to have a sensitivity of 56% and 100% and a specificity of 81% and 85% respectively in predicting an albumin excretion rate of >30 ug/min (Gatling et al, 1985; Watts et al, 1986). Clearly this method of screening results in too many false positive results.

The normal increase in albumin excretion which occurs with exercise, mentioned above, is exaggerated in patients with diabetes (Mogensen & Vittinghus, 1975) and this may partly account for the unacceptably low sensitivity and specificity of random spot urine albumin measurements in predicting microalbuminuria. On account of this, the predictive value of the albumin concentration in early morning urine specimens has been assessed. Such a measurement appears to correlate better with both the 24-hour urinary albumin excretion (Cowell et al, 1986) and the albumin excretion rate calculated from a timed overnight urine collection, a correlation co-efficient of 0.90 having been reported with the latter (Hutchison et al, 1988). An albumin concentration of >20 mg/l in an early morning urine specimen has a sensitivity of 86 - 91% and a specificity of 74 - 97% of predicting an overnight albumin excretion rate of >30 ug/min (Gatling et al, 1985; Marshall & Alberti, 1986).

The accuracy of such measurements of albumin excretion in early morning urine samples can be further refined by the measurement of the urinary creatinine excretion and the calculation of an albumin:creatinine ratio. Such a correction improves the predictive value even in random specimens of urine, but in early morning urine samples, calculation of this ratio is of even greater benefit. The albumin:creatinine ratio in an early morning urine sample has a correlation co-efficient of 0.74 with the 24-hour albumin excretion (Cowell et al, 1986) and 0.91 with the overnight albumin excretion rate (Gatling et al, 1985). An albumin:creatinine ratio of >3.5 mg/mmol has been reported to have a sensitivity of 88 - 100% and a specificity of 95 - 99% of predicting an overnight albumin excretion rate of >30 ug/min (Gatling et al, 1985 & 1988b). A further study

found that a cut-off of 2.5 mg/mmol in the albumin:creatinine ratio allowed clear separation of groups of subjects with microalbuminuria and normal albumin excretion (Cohen et al, 1987a).

Although the different cut-off values used in the various published studies, and the variable results reported, makes firm conclusions difficult, the available evidence does point to the measurement of the albumin:creatinine ratio in an early morning urine specimen being the most useful simple screening test for microalbuminuria, suitable for mass use in the clinical setting.

1.7 TREATMENT OF MICROALBUMINURIA

It is central to the whole concept of microalbuminuria that intervention at this stage of the nephropathic process may prevent progression to frank proteinuria and inevitable deterioration of renal function. Three major strategies have been found to be of benefit in such patients.

1. Improvement of Glycaemic Control

The potential role of poor glycaemic control in the causation of diabetic nephropathy is discussed in Chapter 1.5 (Mogensen et al, 1983; Hasslacher et al, 1985) and hyperglycaemia has been considered to be integral in the causation of the glomerular hyperfiltration which is an early feature of diabetic nephropathy (Hostetter et al, 1982). At the stage of incipient nephropathy, there is some evidence

of an independent correlation between prevailing glycaemia and albumin excretion, patients with microalbuminuria having poorer glycaemic control than those with normal albumin excretion (Wiseman et al, 1984a).

The potential therapeutic benefit of improving glycaemic control in patients with frank nephropathy is a matter of some debate. One study has estimated that glycaemic control accounts for approximately one-third of the progression of established renal disease over time, at the stage of a reduced glomerular filtration rate (Nyberg et al, 1987). Others, however, have shown that improved glycaemic control at this stage of the disease process does not have a significant effect in influencing deterioration of renal function (Tamborlane et al, 1982; Viberti et al, 1983c).

In patients with microalbuminuria, the evidence that improved glycaemic control has an important role appears to be more encouraging. Most studies have focussed on the use of the technique of continuous subcutaneous insulin infusion (CSII), which enjoyed a spell of popularity in the early 1980's, to tighten glycaemic control. Using this technique, a reduced rate of both increase in albumin excretion with time and progression to frank nephropathy has been demonstrated (Deckert et al, 1984; Feldt-Rasmussen et al, 1986a). Indeed, an actual fall in albumin excretion over a period of up to 4 years has been reported in patients with microalbuminuria treated with CSII to achieve improved glycaemic control (Kroc Collaborative Study Group, 1984; Feldt-Rasmussen et al, 1986b; Dahl-Jorgensen et al, 1988). A reduction in kidney size during treatment with CSII has also been shown in patients with microalbuminuria

(Feldt-Rasmussen et al, 1986b). Even in diabetic patients with normal albumin excretion, hyperfiltration, which may predispose to later nephropathy, appears to be reduced during treatment with CSII (Beck-Nielsen et al, 1985; Wiseman et al, 1985; Christensen et al, 1987).

The use of CSII has largely fallen out of favour because of poor patient acceptability, the rapid development of ketosis in the event of pump malfunction and the problem of hypoglycaemia unawareness associated with excessively tight glycaemic control. The use of multiple insulin injections to improve glycaemic control, albeit to a lesser extent than achieved using CSII, did not result in an obvious reduction in urinary albumin excretion over a 4-year period, compared with a conventionally-treated control group, in one study (Dahl-Jorgensen et al, 1988). Nonetheless, evidence that glycaemic control is important in the early stages of the nephropathic process is probably sufficiently compelling that optimization of glycaemic control should be a therapeutic goal in all such patients with IDDM.

Evidence from previous studies with regard to the importance of glycaemic control in patients with NIDDM and microalbuminuria is lacking and this is considered further in Chapter 5.

2. Reduced dietary protein intake

Low protein diets are widely used in the management of patients with chronic renal failure of all aetiologies. Prospective studies have been conducted in patients with a reduced creatinine clearance and elevated serum creatinine concentration, treated with a dietary

protein intake of 0.4 - 0.6 g/kg body weight/day for at least 18 months. Compared with control subjects on unrestricted diets, there was a significant slowing in the reduction in glomerular filtration rate and other parameters of deteriorating renal function, with fewer patients developing end-stage renal failure (Rosman et al, 1984; Ihle et al, 1989).

Relatively few studies have assessed the value of protein restriction specifically in patients with nephropathy secondary to IDDM. Short-term use of a 40 g protein diet over a 3-week period in diabetic patients with persistent proteinuria reduced both the plasma urea concentration and the urinary protein excretion (Bending et al, 1988). Longer-term studies have also been conducted using protein restriction of a similar degree in patients with established diabetic nephropathy. A highly significant fall in the rate of decline in glomerular filtration rate and in the albumin excretion rate was demonstrated after a mean period of 33 months of protein restriction to 0.67 g/kg body weight/day, compared with a similar period of treatment on a normal protein diet (Walker et al, 1989). A further small American study showed similar results in a group of patients with IDDM, proteinuria and reduced creatinine clearance, treated with a 40 g protein diet over a 12-month period (Evanoff et al, 1987).

Even at the stage of microalbuminuria, protein restriction appears to be potentially advantageous, at least in the short-term. During a 3-week crossover study on a 47 g protein diet and on a normal diet containing approximately twice that amount of protein, albumin excretion rate and glomerular filtration rate fell significantly during protein restriction, with no concomitant changes in blood

pressure or glycaemic control over the same period (Cohen et al, 1987b). Protein restriction reduces glomerular filtration and filtration fraction even in diabetic patients with no evidence of renal disease (Wiseman et al, 1987) and the effect of this form of treatment in diabetic patients with nephropathy may be specifically by its influence on renal haemodynamics, resulting in a reduction in glomerular hyperfiltration.

Although the available evidence does point to a potential benefit of protein restriction, even in early diabetic renal disease, from a practical point of view compliance may prove a problem in a group of patients who are asymptomatic and in whom dietary intake is already significantly limited.

3. Antihypertensive Therapy

Because of poor patient acceptability of dietary protein restriction and the difficulties which are often experienced in trying to optimize glycaemic control, this is probably the most important therapeutic option in the treatment of early diabetic nephropathy.

Hypertension and diabetic renal disease are inextricably linked and in patients with frank nephropathy hypertension is a common finding, with one study reporting that 51% of proteinuric diabetic patients had a diastolic blood pressure of >95 mm Hg (Parving et al, 1983). Debate continues as to whether this is a direct consequence of kidney damage caused by the nephropathic process, an alternative theory being that the hypertension and nephropathy have a common determinant, perhaps of a genetic nature (see Section 1.5).

Whatevever the precise mechanism of the association, treatment of hypertension in diabetic patients with frank nephropathy has been clearly shown in several studies to reduce the rate of decline of the glomerular filtration rate, although it does not stop the progression in renal impairment completely (Mogensen, 1982; Bjorck et al, 1986; Parving et al, 1987).

Diabetic patients with microalbuminuria also have a higher blood pressure than matched controls with normal albumin excretion, even although only a minority have hypertension as defined by conventional criteria (Mathiesen et al, 1984; Wiseman et al, 1984a; Christensen & Mogensen, 1985). Even throughout the range of albumin excretion within the defined limits of microalbuminuria, the blood pressure appears to rise significantly as the albumin excretion increases (Wiseman et al, 1984a).

There is increasing evidence of the value of antihypertensive therapy in patients with microalbuminuria. In one of the earlier studies, the albumin excretion rate was seen to fall by more than 50% over a period of up to 5 years in a group of patients with microalbuminuria and minimally elevated blood pressure, who were given antihypertensive treatment in the form of metoprolol, with or without diuretics (Christensen & Mogensen, 1987). Subsequent studies have focussed largely on the angiotensin converting enzyme inhibitors, predominantly because of the theoretical effect of this group of drugs in reducing intraglomerular pressure. This might be expected to be particularly beneficial in diabetic nephropathy, in which glomerular hyperfiltration is a prominent early feature (see Section 1.5) and, at least in diabetic rats, these drugs do seem to reduce

intraglomerular hypertension at the same time as reducing proteinuria (Zatz et al, 1986). As a result, it has been suggested that angiotensin converting enzyme inhibitors may be useful in the treatment of diabetic nephropathy even in the absence of hypertension. In studies in diabetic humans with microalbuminuria and a supine blood pressure of <160/95, a reduction in the albumin excretion rate, with a concomitant rise in glomerular filtration rate, has been demonstrated after a 6-month period of treatment with enalapril, using a placebo controlled, cross-over design (Marre et al, 1987). Indeed, even in patients with IDDM who had normal albumin excretion and normotension, there was a significant decline in filtration fraction, albumin excretion rate and fractional albumin excretion following a 3-month treatment period with enalapril (Mau Pedersen et al, 1988). It is still unclear, however, if the results of these studies are explicable on the basis of a specific action of angiotensin converting enzyme inhibitors, or are simply a non-specific effect of reducing blood pressure. The use of different antihypertensive agents in the treatment of hypertension in early diabetic diabetic nephropathy is discussed further in Chapter 3.

Although it is undoubtedly premature, on the basis of one study (Mau Pedersen et al, 1988), to advocate treating all patients with IDDM with antihypertensive drugs, increasing evidence would suggest that strict control of blood pressure in patients with microalbuminuria can normalise urinary albumin excretion. It is as yet too early to predict, however, whether this represents an actual reversal of the nephropathic process.

CHAPTER 2

METHODS

2.1 INTRODUCTION

The research presented in the following chapters takes the form of a series of clinical studies. The majority of investigations which have been conducted during the course of these studies are routine and well-established laboratory tests. In view of this, a brief description of methodology has been included in the relevant chapters, with acknowledgement being given to the laboratories performing the various investigations. In this chapter, I have confined myself to the more detailed description of techniques which are either not performed as standard laboratory investigations or are particularly important to one or more of the studies.

2.2 MEASUREMENT OF URINARY ALBUMIN EXCRETION

- Screening for the Presence of Microalbuminuria

This is relevant to all the studies described in the subsequent chapters and is clearly of major importance to this thesis.

The background to screening for microalbuminuria has been discussed in some detail in Section 1.6. Three major techniques are commonly used for the quantitative measurement of urinary albumin excretion, all of which have been well validated and are widely accepted. These are radioimmunoassays (Keen & Chlouverakis, 1963; Miles et al, 1970; Woo et al, 1978; Jury et al, 1985), enzyme-linked immunosorbent assays (ELISA) (Fielding et al, 1983; Mohamed et al, 1984) and immunoturbidimetric assays (Spencer & Price, 1979; Teppo, 1982).

Overall, radioimmunoassay and ELISA tend to have greatest sensitivity but can be difficult to automate. Immunoturbidimetry has a slightly lesser degree of sensitivity, but this is still perfectly adequate to detect abnormal levels of albumin excretion within the microalbuminuric range and the relative ease of automating immunoturbidimetric assays makes this method particularly suitable for clinical departments where large numbers of samples are processed.

The method used in the studies described here is an immunoturbidimetric assay developed by Dr Richard Spooner, Principal Clinical Biochemist, Gartnavel Hospital, Glasgow, based on previously published work by Spencer and Price (1979). Urine samples were assayed within four days of collection and during the period of storage were kept at $4^{o}C$, with one drop (50 ul) of sodium azide having been added to each 5 ml of urine.

The principle of the immunoturbidimetric assay involves the reaction of albumin in the test sample with a specific antibody, this resulting in a turbid solution, the absorbance of which is proportional to the albumin concentration in the sample. The necessary reagents were obtained from the AlbuSure QNT kit (Cambridge Life Sciences, Cambridge, UK). This kit comprises Anti-Human Serum Albumin clarified antiserum, polyethylene glycol buffer and six vials containing standards (with varying concentrations of albumin from 0 - 160 mg/l) for incorporation in each assay run to enable the calculation of a standard curve. Absorbance of individual samples was read using a Cobas Bio Analyser, firstly after addition of buffer alone and secondly 30 min after incubation with the antibody reagent.

The absorbance difference for each sample was calculated and the albumin concentration read from the standard curve.

The method described has a sensitivity of 5 mg/l in determining urinary albumin concentration over the range of 0 - 250 mg/l. Samples were tested in duplicate and the co-efficient of variation (CV) for the assay was calculated on repeat analysis control data, randomly selected specimens being analysed again on the next assay run. The inter-assay CV during the relevant period of time was 5.1 - 5.9%.

For the studies described here, albumin excretion was determined on at least two 24-h urine collections within a 6-month period, thereby enabling the diagnosis of microalbuminuria using the most clearly defined criteria (Mogensen, 1987). The exception to this are the studies described in Chapter 5 and 6, in which the large number of subjects and, in the case of Chapter 5, the repeated nature of the sampling, made the collection of timed urine collections unrealistic logistically. In these studies, the albumin excretion in early morning urine samples was corrected for creatinine excretion and an albumin:creatinine ratio was calculated. A ratio of >2.5 mg/mmol was considered abnormal, this cut-off having been shown in a previous study to enable clear separation of patients with a urinary albumin excretion above and below 30 ug/min (Cohen et al, 1987a).

2.3 MEASUREMENT OF RENAL HAEMODYNAMICS

- Renal Plasma Flow and Glomerular Filtration Rate

These techniques were used in the studies described in Chapters 4 and 7.

Clearance studies to measure renal plasma flow and glomerular filtration rate were pioneered over half a century ago and have been well validated over the years. In spite of the introduction of newer and more advanced methods with which to assess aspects of renal function, these older clearance methods remain an invaluable tool for studying renal physiology in human subjects. Haemodynamic parameters are estimated by measuring the clearance from plasma of stable inert marker substances which have been infused into the circulation. Following a suitable equilibration period, the clearance (C) can be calculated by a standard formula:-

$$C = U / P \times (\text{Urine Flow Rate})$$

in which U and P represent the urine and plasma concentrations respectively of the marker substance.

Inulin (or the very similar compound polyfructosan) remains the best marker substance for measuring glomerular filtration rate. It is a small enough molecule to filter freely through the glomerulus and it is excreted only by the kidney with no active tubular secretion or reabsorption occurring. A marker substance for renal plasma flow must have the additional property of near complete extraction from plasma during one pass through the kidney. Para-amino hippuric acid (PAH) approaches this ideal, with an extraction ratio of approximately 0.9.

Because this extraction ratio is less than unity, however, renal plasma flow measured by this technique is a slight underestimate, conventionally described as the effective renal plasma flow.

1. Effective Renal Plasma Flow

In the protocol described in Chapter 4, an initial intravenous loading bolus dose of PAH (0.45 g) was given, followed by a standard infusion of 8.5 g/l in 0.9% sodium chloride solution at a rate of 2 ml/min throughout the individual studies. In Chapter 7, a different protocol was used because of anticipated marked acute changes in effective renal plasma flow occurring during individual studies and potential problems with the collection of frequent urine samples during induced hypoglycaemia. Here the clearance of an intravenous bolus injection of PAH in a dose of 10 mg/kg body weight was measured at appropriate points in individual studies. Blood samples were withdrawn before and 5, 7, 10, 15, 20, 30 and 40 min after each injection of PAH. The effective renal plasma flow was calculated, assuming two-compartment kinetics, from the area under the curve of plasma PAH concentrations after each bolus injection.

In both cases, PAH concentrations were measured by a modification of the colorimetric method of Bratton and Marshall (1939), adapted for use with a Technicon Autoanalyser (Harvey & Brothers, 1962). Samples, following appropriate dilution, were first dialysed to allow transfer of PAH but not proteins into the dialysate. Sodium nitrite was added to diazotise the PAH and the colour was produced by coupling with N-(1-napthyl) ethylenediamine hydrochloride. The absorbance, measured

spectrophotometrically, is proportional to the PAH concentration in the sample, which can be calculated from a standard curve.

2. Glomerular Filtration Rate

The glomerular filtration rate was calculated by measuring the clearance of polyfructosan (Inutest; Laevosan, Linz, Austria), a synthetic multimer of fructose. In all studies this was administered as an intravenous infusion, at a concentration of 10 g/l in 0.9% sodium chloride solution and a rate of 2 ml/min, following an initial loading bolus injection of 3.5 g. After an equilibration period of at least 60 min, glomerular filtration rate was calculated during individual studies from regular measurements of polyfructosan in blood and urine specimens.

The method used was a modification of that described by Heyrovsky (1956), adapted for use on a Technicon Autoanalyser (Dawborn, 1965). Hydrochloric acid was added to samples, which were heated at 60^{o}C to hydrolyse the polyfructosan to fructose. Dialysis was used to remove protein and further heating to 60^{o}C, after mixing of the dialysate with hydrochloric acid and air, produces a colour which can be measured spectrophotometrically, the peak absorbance corresponding to the polyfructosan concentration in the sample.

2.4 MEASUREMENT OF URINARY FREE DOPAMINE

This is of particular relevance to Chapter 3 and urinary free dopamine excretion was also measured in the studies described in Chapter 7.

24-h urine collections for measurement of dopamine excretion were collected into a bottle containing 25 ml of 5 mol/l hydrochloric acid, to lower the urine pH to <3 in order to prevent oxidation of dopamine. Aliquots from the sample were then stored at -40^{0}C until assay.

It is likely that only free dopamine is physiologically active but up to 80% of urinary dopamine may be in conjugated form (Kuchel et al, 1979). Free dopamine was therefore extracted with alumina, the process being based on the method of Anton and Sayre (1964). Dopamine standards were extracted following the same procedure as for the test samples, the recovery of dopamine from the extraction process (mean (SD)) being 69.7 (0.5)%, compared against unextracted standards. Free dopamine was then measured by high performance liquid chromatography (HPLC) with electro-chemical detection, using epinine (N-methyl dopamine) as an internal standard (Jeffrey et al, 1987). Intra-assay and inter-assay co-efficients of variation were 4% and 6% respectively. Urinary free dopamine results were expressed as nmol/l and the sensitivity of the assay was 5 nmol/l.

CHAPTER 3

EARLY DIABETIC NEPHROPATHY AND HYPERTENSION (1)

URINARY DOPAMINE EXCRETION AND MICROALBUMINURIA

3.1 INTRODUCTION

The evidence for a genetic factor playing an important role in the pathogenesis of diabetic nephropathy is discussed in Section 1.5. Some of the most interesting data pertains to the close association between diabetic renal disease and hypertension, and there is some evidence of increased activity of the erythrocyte sodium-lithium countertransport system (a genetically determined marker of hypertension) in diabetic patients with nephropathy (Krolewski et al, 1988; Mangili et al, 1988). In this study, the possible role of renal dopamine, another factor which apparently has a genetically determined link with hypertension, has been examined in the context of early diabetic nephropathy.

Endogenous renal dopamine is believed to participate in sodium homeostasis and appears to function, under physiological conditions, as a natriuretic hormone (Kuchel et al, 1978). In normotensive patients with normal renal function, the urinary excretion of dopamine correlates directly with sodium output under normal conditions and responds appropriately to salt loading and depletion (Alexander et al, 1974; Carey et al, 1981).

In patients with advanced diabetic nephropathy, salt and water overload, in conjunction with hypertension, are characteristic features. With increasing impairment of renal function, urinary dopamine excretion progressively declines, concurrent with the glomerular filtration rate. At the same time, the response to salt loading is attenuated and it has been suggested that a relative deficiency of dopamine is an important factor in this abnormal sodium

handling by the kidney (Casson, 1984). Abnormal sodium retention has also been recognised at a much earlier stage in the natural history of IDDM, before the appearance of overt nephropathy (de Chatel et al, 1977). It was therefore of interest to determine whether a defect in the mobilisation of renal dopamine may be of importance in the pathophysiology of early diabetic nephropathy. To assess this, the relationship between sodium and dopamine excretion was examined in a group of normal male subjects and in two groups of normotensive males with IDDM, one with normal urinary albumin excretion and the other with microalbuminuria.

3.2 PATIENTS AND METHODS

A cohort of 48 male patients with IDDM (mean age 33, range 25 - 38 yr) was recruited from the Diabetic Clinic of Edinburgh Royal Infirmary. None of these patients was hypertensive by WHO criteria (World Health Organisation, 1986), all having a supine blood pressure of <160/95. All patients also had a creatinine clearance of >80 ml/min, calculated on a 24-h urine collection. 37 patients (GROUP 1) had consistently normal urinary albumin excretion, but 11 patients (GROUP 2) had microalbuminuria, with an elevated urinary albumin:creatinine ratio (>2.5 mg/mmol) on two or more occasions in the previous year, in conjunction with an albumin excretion of 30 - 300 mg in at least one 24-h urine collection. A control group (GROUP 3) comprised 40 healthy normotensive males of comparable age, with no history of renal disease and normal creatinine clearance.

Following careful instruction, all subjects collected a 24-h urine

sample for measurement of urinary sodium and dopamine excretion. No dietary restrictions were enforced but subjects were asked to refrain from alcohol during the collection. Blood pressure was measured using a standard mercury sphygomanometer, after resting for 5 min, and the diabetic patients were further assessed for retinopathy (by direct ophthalmoscopy after mydriasis) and prevailing glycaemic control, glycated haemoglobin (HbA_1) being measured using commercially available agar plates (non-diabetic reference range 6.0 - 8.0%). The characteristics of the diabetic and control groups are shown in Table 1. There was a trend towards the diabetic patients with microalbuminuria (GROUP 2) having a longer duration of diabetes, poorer glycaemic control and lower creatinine clearance than those with normal albumin excretion (GROUP 1), although none of these differences reached statistical significance.

Details of the assays used in the measurement of urinary albumin and dopamine excretion are given in Chapter 2 (Sections 2.2 and 2.4). Urinary sodium was measured by flame photometry, and plasma and urinary creatinine by an automated modification (Chasson et al, 1961) of the Jaffe method (Jaffe, 1886).

Statistical Methods

The sodium and dopamine data were log-transformed before analysis. Correlation co-efficients were calculated by least squares and regression lines compared by F-ratios. Results are expressed as mean (SD) unless otherwise specified.

Table 1

Characteristics of 48 males with IDDM (37 with normal urinary albumin excretion (GROUP 1) and 11 with microalbuminuria (GROUP 2)) and 40 non-diabetic controls with normal renal function (GROUP 3).

	GROUP 1	GROUP 2	GROUP 3
n	37	11	40
Age (yr)	33 (25 - 38)	31 (19 - 44)	26 (20 - 39)
Blood pressure (mm Hg)	128 (11)/ 77 (7)	124 (16)/ 78 (8)	126 (10)/ 74 (8)
Creatinine clearance (ml/min)	122 (25)	102 (19)	110 (12)
Duration of diabetes (yr)	12 (2 - 24)	15 (6 - 28)	
HbA_1 (%)	9.8 (1.6)	10.8 (0.9)	
Retinopathy			
None	11	2	
Background	23	5	
Proliferative	3	4	
Albumin excretion (mg/24 h)	10 (3 - 22)	141 (44 - 248)	

Results are given as mean (range or SD) or absolute number, as appropriate

3.3 RESULTS

Results of 24-h urinary sodium and free dopamine excretion are given in Table 2. It is evident from the sodium excretion data that the study was performed under salt replete conditions, and both urinary sodium and dopamine excretion were comparable in the three groups. Log-transformed sodium and dopamine data for the individual subjects in the three groups, with regression lines, are shown in Figures 1 - 3. There was, in both the control group and the two diabetic groups, a significant correlation between sodium and dopamine excretion but there were no significant differences between the slopes of the regression lines (Table 2).

Table 2

24-hour urinary sodium and dopamine excretion in diabetic patients with normal urinary albumin excretion (GROUP 1) and microalbuminuria (GROUP 2) and in non-diabetic control subjects (GROUP 3).

	GROUP 1	GROUP 2	GROUP 3	p
n	37	11	40	
Urinary sodium (mmol/24 h)	159.5 (57.6)	164.9 (54.7)	172.9 (54.3)	NS
Urinary dopamine (nmol/24 h)	1632 (437)	1450 (294)	1572 (453)	NS
Correlation co-efficients	r=0.45*	r=0.80**	r=0.50***	
Regression slope	0.38	0.55	0.46	NS
Regression intercept	2.40	1.95	2.17	NS

Results are given as mean (SD)

Significance: * $p<0.01$; ** $p<0.005$; *** $p<0.002$; NS $p>0.05$

Figure 1

Correlation between urinary dopamine and sodium excretion in diabetic patients with normal urinary albumin excretion (GROUP 1).

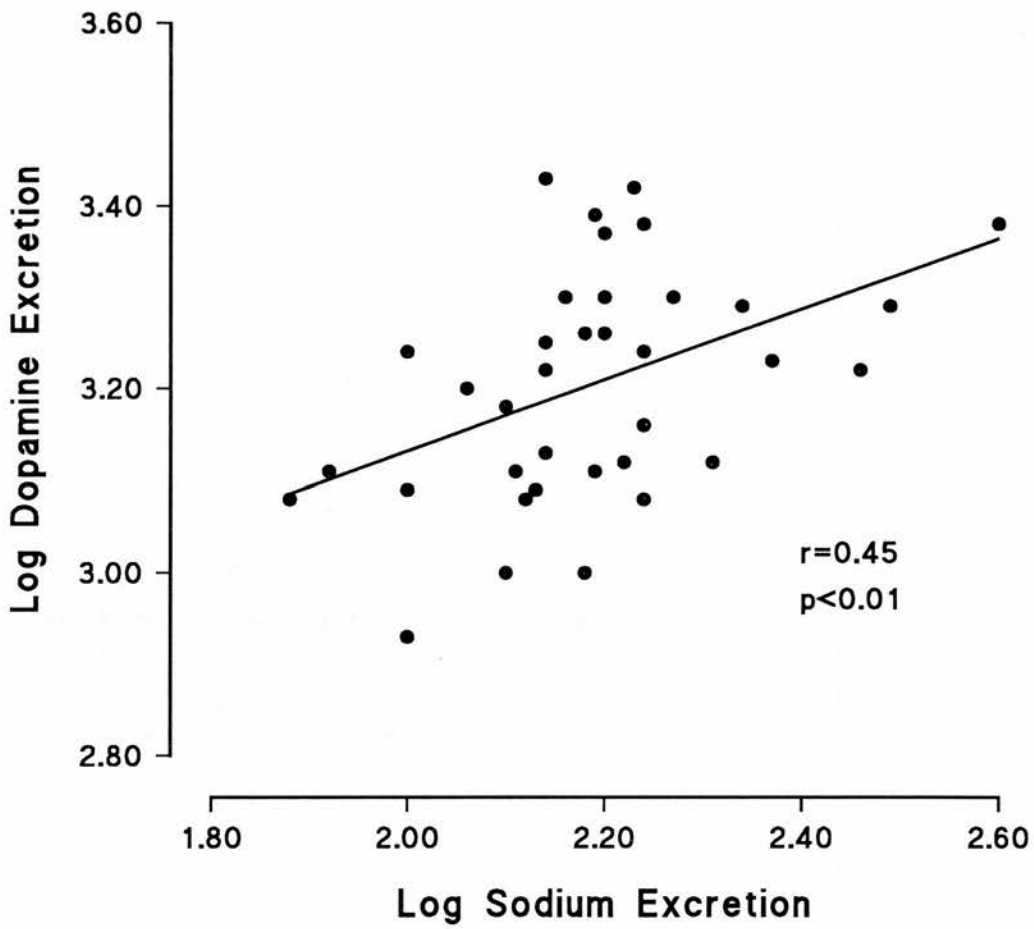


Figure 2

Correlation between urinary dopamine and sodium excretion in diabetic patients with microalbuminuria (GROUP 2).

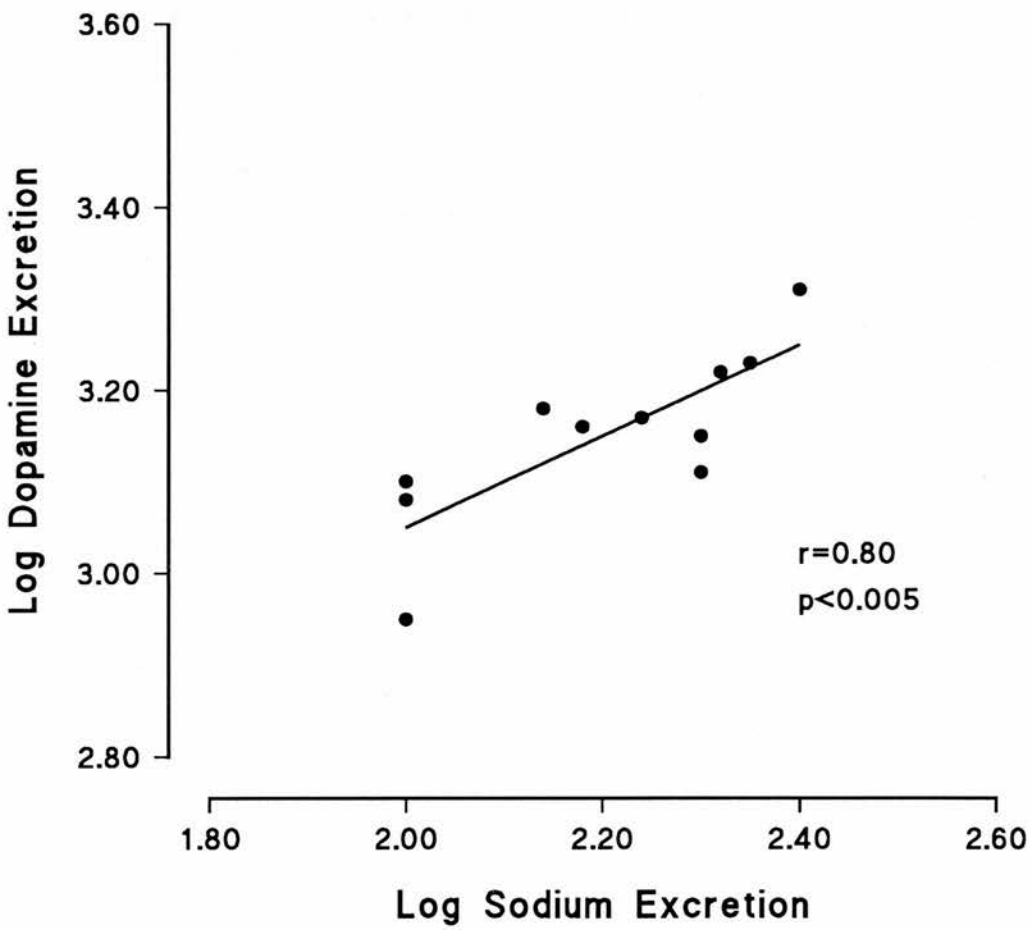
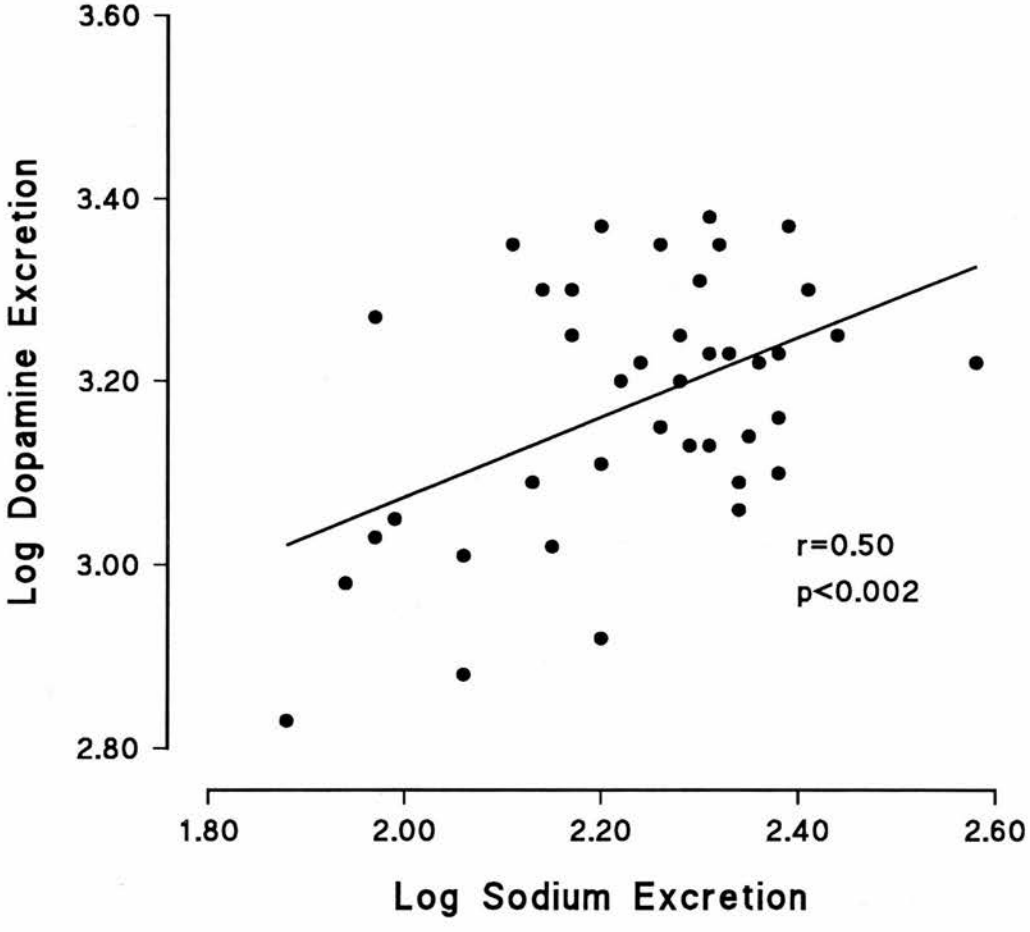


Figure 3

Correlation between urinary dopamine and sodium excretion in non-diabetic control subjects (GROUP 3).



3.4 DISCUSSION

The positive correlation between urinary dopamine and sodium excretion in normal subjects has stimulated research into defining a physiological role for endogenous renal dopamine. It contributes to the complex feedback mechanism which is responsible for preserving sodium homeostasis and the renal synthesis of dopamine is linked to prevailing salt status, apparently acting to promote sodium excretion (Kuchel et al, 1978). An inability to mobilise dopamine, appropriate to salt status, has been described in established essential hypertension (Harvey et al, 1984), a central feature of which may be a defect in the ability of the kidney to excrete sodium at normal perfusion pressures (de Wardener & MacGregor, 1982). In addition, in normotensive first-degree relatives of hypertensive patients (Saito et al, 1986) and in normotensive negroes (Critchley et al, 1987), both groups at a high risk of developing essential hypertension, the direct correlation between urinary sodium and dopamine is lost, suggesting that an abnormality of dopamine control, presumably genetically determined, antedates the development of essential hypertension.

In patients with IDDM, recent research into the erythrocyte sodium-lithium countertransport system has suggested that there may be a genetic linkage between the predisposition to hypertension and diabetic nephropathy (see Section 1.5). Previously, however, a possible role for a genetically determined defect in renal dopamine has not been considered in the pathophysiology of early diabetic nephropathy. A number of observations suggest that patients with IDDM do not handle sodium normally. A greater prevalence of hypertension

in diabetic patients, with or without nephropathy, is well recognised (Fuller, 1985), and blood pressure has also been reported to be higher in patients who have microalbuminuria than in those who have normal albumin excretion (Mathiesen et al, 1984; Wiseman et al, 1984a; Christensen & Mogensen, 1985), although no significant difference was observed between the small groups in this study. In addition, exchangeable sodium is increased in patients with IDDM before the development of overt nephropathy or hypertension (Feldt-Rasmussen et al, 1987), with a correlation between exchangeable sodium and blood pressure suggesting that sodium retention may play a major role in the pathogenesis of the rise in blood pressure observed in the early stages of diabetic renal disease. Reduced sodium excretion has been demonstrated in patients with IDDM in response to volume expansion (O'Hare et al, 1986), and tubular sodium retention occurring at an early stage in the diabetes process could be implicated in the development of nephropathy and hypertension.

The results of this study, however, do not suggest that these changes in sodium handling are associated with a defect in the mobilisation of dopamine, at least at a stage prior to the development of hypertension or frank nephropathy. Dopamine output over 24 h was comparable in all of the groups and the correlation between urinary sodium and dopamine excretion was maintained in the diabetic patients, even in the presence of microalbuminuria. The similar regression slopes in the three groups also suggest that the sensitivity of the dopamine response to salt intake and excretion was unaltered.

In conclusion, a defect in the mobilisation of dopamine does not

appear to predate the onset of frank nephropathy, and abnormalities demonstrated in patients with established renal disease are likely to represent a secondary phenomenon related to the underlying disease process.

CHAPTER 4

EARLY DIABETIC NEPHROPATHY AND HYPERTENSION (2)

A COMPARISON BETWEEN CAPTOPRIL AND NIFEDIPINE IN HYPERTENSIVE MALE PATIENTS WITH IDDM

4.1 INTRODUCTION

The association between microalbuminuria and hypertension, and the increasing evidence of the value of antihypertensive therapy in patients with early nephropathy, is discussed in Section 1.7. Because of the theoretical benefit of angiotensin converting enzyme inhibitors in lowering intraglomerular pressure, most studies have focussed on this group of drugs and, at least in rats, their use seems to reduce intraglomerular hypertension and proteinuria (Zatz et al, 1986). In diabetic humans, angiotensin converting enzyme inhibitors have been shown to reduce proteinuria in the presence of frank nephropathy (Bjorck et al, 1986; Parving et al, 1988 & 1989), microalbuminuria (Marre et al, 1987 & 1988) and even normal albumin excretion (Mau Pedersen et al, 1988). It remains unclear, however, whether these findings reflect a specific action of this class of drugs or simply an effect of blood pressure reduction. Certainly other antihypertensive agents do appear to have a potentially beneficial effect in early diabetic nephropathy and the beta blocker, metoprolol, has been shown to reduce urinary albumin excretion by more than 50% in a group of patients with IDDM and nephropathy (Christensen & Mogensen, 1987). A study in hypertensive patients with NIDDM has suggested that the calcium antagonist, nicardipine, has a similar effect on protein excretion to captopril (Baba et al, 1989) although in a further report of normotensive patients with IDDM, another calcium antagonist, nifedipine, appeared to actually increase urinary albumin excretion (Mimran et al, 1988).

To date, however, there is a real lack of carefully controlled

comparative studies of different antihypertensive agents in patients with IDDM. This study was therefore conducted to assess and compare both the acute and chronic effects of captopril and nifedipine retard in a group of hypertensive patients with IDDM.

4.2 PATIENTS AND METHODS

Patients

Ten male patients with IDDM were recruited from the Diabetic Clinic of Edinburgh Royal Infirmary. Their mean age (range) was 41 (32 - 50) yr and duration of diabetes was 21 (7 - 32) yr. All had a body mass index less than 26 kg/m^2 and a creatinine clearance (measured in a 24-h urine collection) of >80 ml/min. Six of the patients were receiving regular antihypertensive drugs at the time of recruitment. The remaining 4 patients had not previously received specific antihypertensive therapy but all had at least mild hypertension, which for the purpose of the study was defined as a supine blood pressure (after 5-min rest) of >150 (systolic) and/or 90 (diastolic), on at least three occasions. None of the patients was receiving any regular medications apart from insulin and antihypertensive drugs. Based on the urinary albumin excretion in a 24-h urine collection at the time of recruitment, 2 of the patients had frank diabetic nephropathy (albumin excretion 630 and 2418 mg/24 h) and 3 had definite microalbuminuria (albumin excretion 30 - 114 mg/24 h). Of the remaining 5 patients, all but one had a 24-h albumin excretion of >10 mg, outwith two standard deviations of the mean for the normotensive,

non-diabetic population, measured in the Clinical Chemistry Department of Edinburgh Royal Infirmary. Five patients had background retinopathy and 2 had previously had photocoagulation therapy for proliferative changes.

Study Protocol

The protocol followed a randomised, double-blind, cross-over design. During a four-week run-in period only placebo tablets were administered and those patients previously treated with antihypertensive agents discontinued these drugs at the start of this run-in period. Thereafter, patients were randomised to receive either captopril (Squibb Ltd, Hounslow, UK) in a dose of 25 mg twice daily or nifedipine retard (Bayer Ltd, Newbury, UK) 20 mg twice daily, for a period of eight weeks. Placebo treatment was then re-introduced for four weeks prior to the second active eight-week treatment limb of the study with either captopril or nifedipine retard. Throughout the 24-week period of the study, patients were seen at two-weekly intervals for measurement of blood pressure, using a Hawksley random zero sphygmomanometer, after resting for at least 5 min. At four-weekly intervals, 24-h urine samples were collected for measurement of urinary albumin excretion and venous blood was withdrawn for estimation of plasma renin activity.

At the beginning and end of each active treatment limb patients were admitted and the acute effects of a single dose of the drug (either captopril 25 mg or nifedipine retard 20 mg) on renal function were assessed.

Measurements of Renal Function

Effective renal plasma flow and glomerular filtration rate were calculated by measuring the clearance of para-amino hippuric acid (PAH) and polyfructosan, administered by a constant intravenous infusion after an initial loading dose, as described in Section 2.3. These measurements of renal haemodynamics were made during two 30-min periods before the administration of the drug and four consecutive 30-min periods thereafter. The basal effective renal plasma flow and glomerular filtration rate were calculated as the mean of the two clearance measurements before drug adminstration and the acute effect of the drug on renal haemodynamics was assessed by calculating the mean of the measurements during the third and fourth periods thereafter (between 60 and 120 min after drug administration). Throughout the acute studies, patients were given 200 ml tap water orally every hour in order to maintain a diuresis.

In order to exclude any effect of the prevailing plasma glucose concentration on renal haemodynamics, euglycaemia was maintained throughout the acute studies using a Biostator (Miles Laboratories, Slough, UK), which incorporates a glucose sensor and computer-controlled glucose delivery system. All patients were admitted at 0800 h having omitted their usual morning insulin and taken only soluble insulin on the previous evening. Following admission, a constant infusion of soluble insulin (Human Actrapid, Novo Nordisk, Crawley, UK) in 0.9% sodium chloride solution was commenced in a dose of 0.35 mU/kg/min and the Biostator was programmed to maintain a plasma glucose throughout the period of the study of 5.0 mmol/l, by means of a variable infusion of a 20% glucose solution. Renal

haemodynamic measurements were not commenced until the plasma glucose concentration was stable at the required level.

Other Laboratory Methods

Urinary albumin excretion was measured on 24-h samples collected at four-weekly intervals throughout the 24-week period of the study and also on the timed urine collections during the acute studies. An immunoturbidimetric assay was used as described in Section 2.2. Plasma renin activity was assessed using a commercially available radioimmunoassay kit, involving the generation of angiotensin I under standard conditions (Campagne Oris, Gif-Sur-Yvette, France). Glycated haemoglobin (HbA_1) was measured, at the end of the run-in period and each of the active treatment limbs, using commercially available agar plates, the laboratory non-diabetic reference range being 6.0 - 8.0%.

Statistical Methods

Results, unless stated otherwise, are given as mean (SE). Blood pressure and urinary albumin excretion data during the two treatment limbs were compared by paired t tests. The albumin data was log-transformed before analysis because of the wide spread of results which was not normally distributed. During the acute studies, data from before and after drug administration were also compared by paired t tests, after first determining the presence of any significant trends by performing two-way analysis of variance.

4.3 RESULTS

After breaking the randomisation code at the completion of the study it was found that six patients had commenced treatment with nifedipine retard and four with captopril. One patient was withdrawn from the study having had a transient ischaemic attack during the first week of the second period of placebo therapy between the first and second active treatment limbs, although his blood pressure at this time was only slightly elevated. Glycaemic control for the group did not change significantly during the course of the study, the mean glycated haemoglobin being 10.6 (0.5)% at the end of the run-in period on placebo treatment, 10.0 (0.5)% at the end of the eight-week period on nifedipine retard and 10.1 (0.5)% following the captopril limb of the study. Plasma renin activity was similar during the first and second placebo periods and did not rise during treatment with nifedipine retard. As would be expected, there was a significant rise to 4.3 (6.0) ng-Ang I/l whilst taking captopril (compared with 1.1 (0.3) ng-Ang I/l on nifedipine retard; $p<0.05$).

Chronic effects of nifedipine retard and captopril

Blood pressure data is shown in Table 1. The effect of the two drugs was similar and at the end of the nifedipine retard limb of the study the mean blood pressure for the group was 132 (4)/ 85 (3), compared with 131 (5)/ 86 (2) at the end of the period of treatment with captopril (p=NS). Nifedipine retard did not exert its maximum anti-hypertensive effect until after the fourth week of therapy, however, and at the mid-point of the two treatment limbs blood pressure was

significantly lower on captopril (130 (5)/ 84 (2) vs 139 (6)/ 91 (3) on nifedipine retard, mean arterial pressure 99 (2) vs 105 (3); $p<0.05$). A tendency for blood pressure to fall over time was also evident and at the end of the second placebo period the systolic pressure was significantly lower that that measured at the end of the run-in period (138 (4)/ 95 (3) vs 147 (6)/ 99 (4); $p<0.01$ for difference between systolic values).

Urinary albumin excretion was closely similar during the two active treatment limbs, the median (range) being 18.5 (8 - 1785) mg/24 h at the end of the period on nifedipine retard and 17 (12 - 1665) mg/24 h on captopril (p=NS). Albumin excretion was lower during both active treatment limbs than in the run-in period, when the median value was 30 (7 - 2418) mg/24 h, although this trend, which is likely to reflect the reduction in blood pressure, did not attain statistical significance.

Table 1

Changes in blood pressure (BP) during the run-in period on placebo (Placebo (1)), on treatment with captopril and nifedipine retard and at the end of the second period of placebo therapy between the active treatment limbs (Placebo (2)).

	Placebo (1)	Captopril		Placebo (2)	Nifedipine Retard	
		Week 4	Week 8		Week 4	Week 8
Systolic BP	147 (6)	131 (6)*	130 (5)*	138 (4)*	139 (6)*	132 (4)*
Mean BP	116 (3)	99 (2)* +	102 (3)*	109 (2)	105 (3)* +	100 (3)*
Diastolic BP	99 (4)	84 (2)*	86 (2)*	95 (3)	91 (3)*	85 (3)*

* indicates a value significantly lower than that at the end of the run-in period ie Placebo (1) ($p<0.05$)

+ indicates a significant difference between captopril and nifedipine retard treatment limbs

Acute effects of nifedipine retard and captopril

Following the first dose of both nifedipine retard and captopril there was an acute fall in blood pressure, this being greater after captopril. At the end of the eight-week period of treatment with each drug, a small acute reduction in mean arterial pressure was still observed after nifedipine retard, but not after captopril (Figure 1). Effective renal plasma flow increased significantly after both the first and last doses of captopril but not nifedipine retard (Figure 2). There was, however, no difference in the baseline measurements of effective renal plasma flow between the first and last doses of either drug and therefore the antihypertensive therapy *per se* did not influence this aspect of renal haemodynamics chronically. Changes in glomerular filtration rate varied considerably between individuals, but there was a non-significant trend towards an acute reduction in this parameter following the first and last doses of both drugs (Figure 3). The filtration fraction fell significantly after the first dose of both drugs but, at the end of the eight-week treatment period, an acute change in the filtration fraction only occurred after administration of captopril (Figure 4). Albumin excretion did not change significantly during any of the acute studies.

Figure 1

Acute changes in mean arterial pressure (MAP) in response to captopril (o) and nifedipine retard (•), following first dose of drug (a) and after eight weeks treatment (b).
* indicates $p<0.05$ compared with basal and + indicates $p<0.05$ between the two drugs.

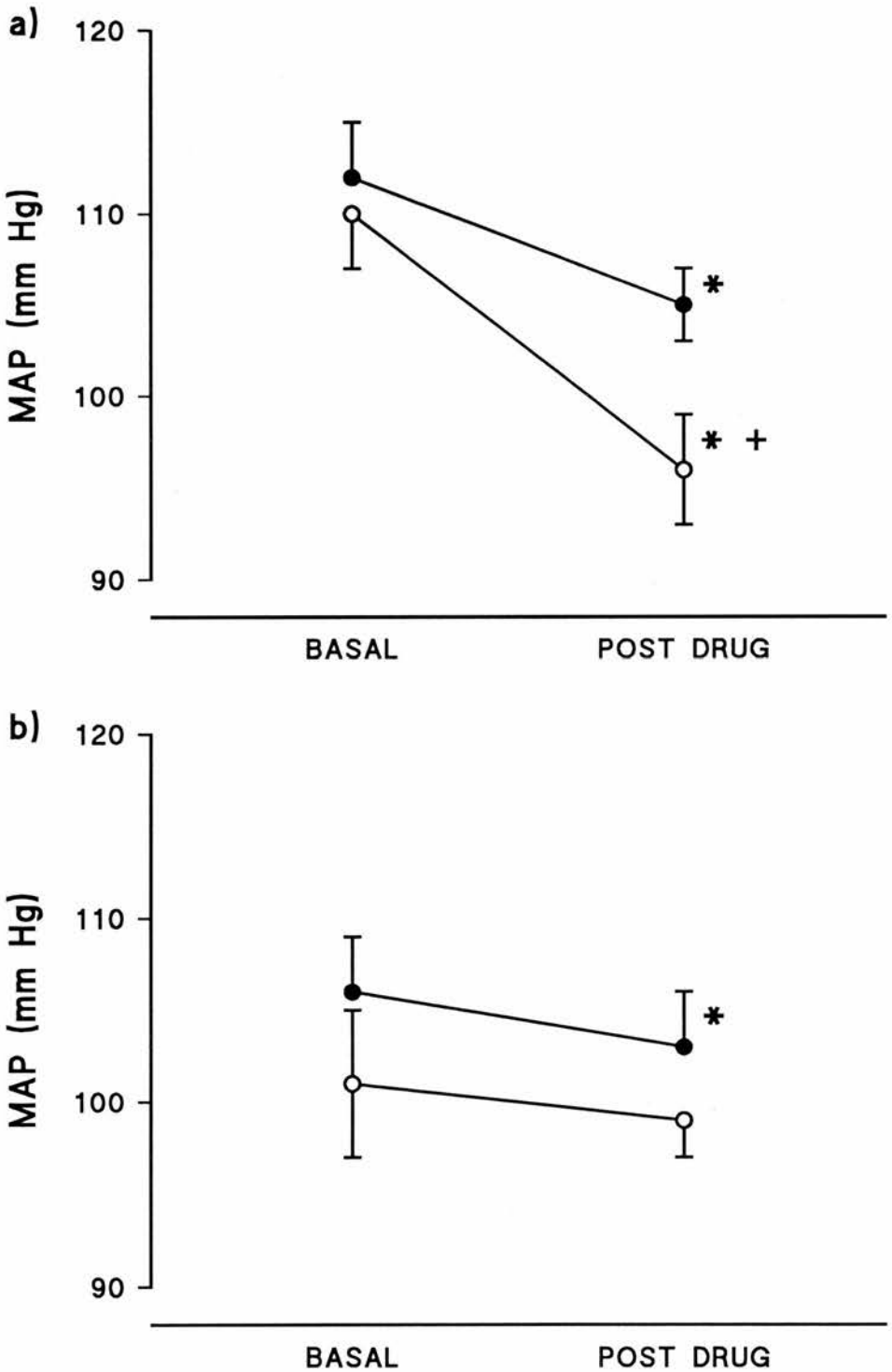


Figure 2

Acute changes in effective renal plasma flow (RPF) in response to captopril (o) and nifedipine retard (•), following first dose of drug (a) and after eight weeks treatment (b).
* indicates $p<0.05$ compared with basal.

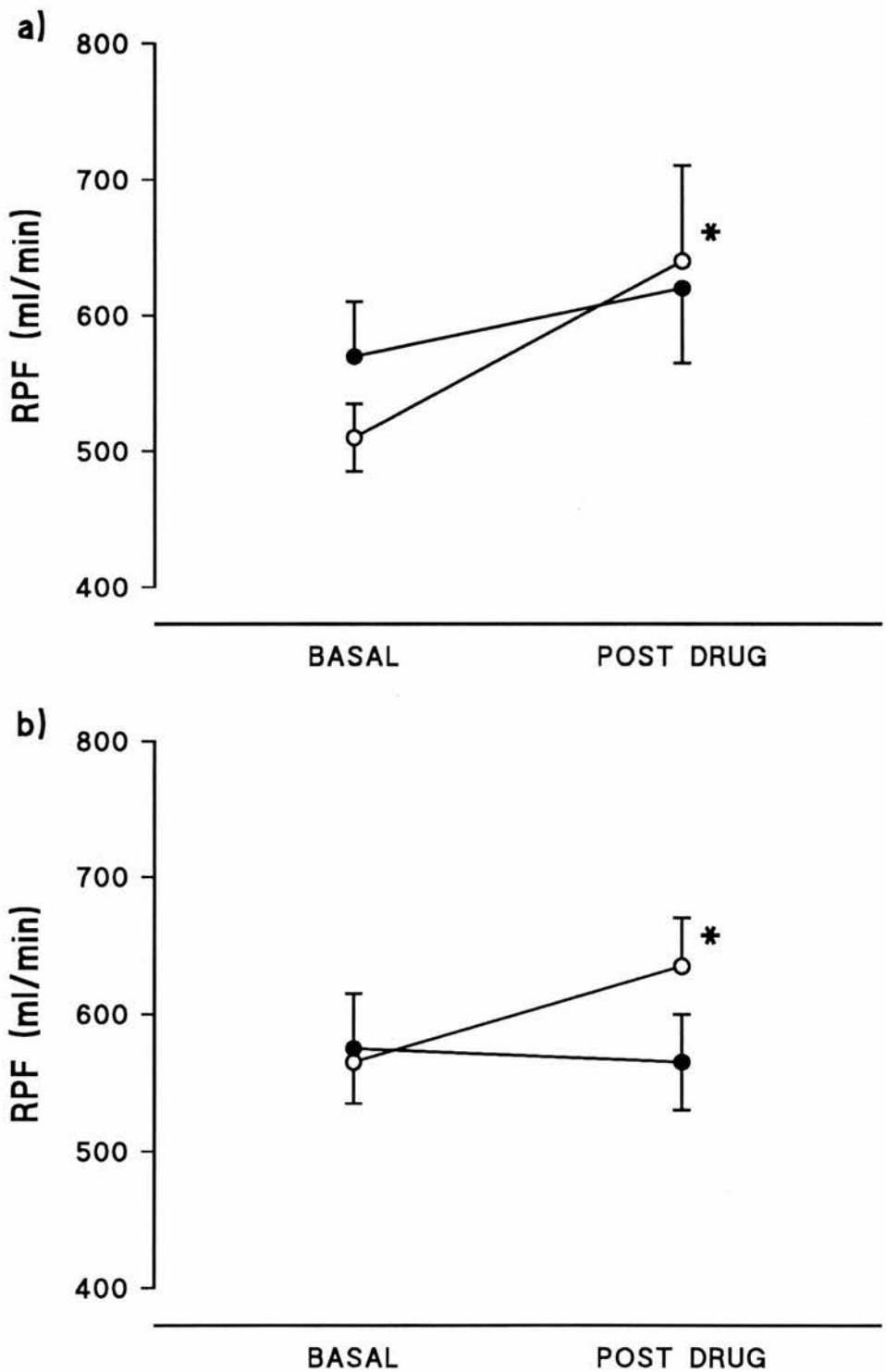


Figure 3

Acute changes in glomerular filtration rate (GFR) in response to captopril (o) and nifedipine retard (●), following first dose of drug (a) and after eight weeks treatment (b).

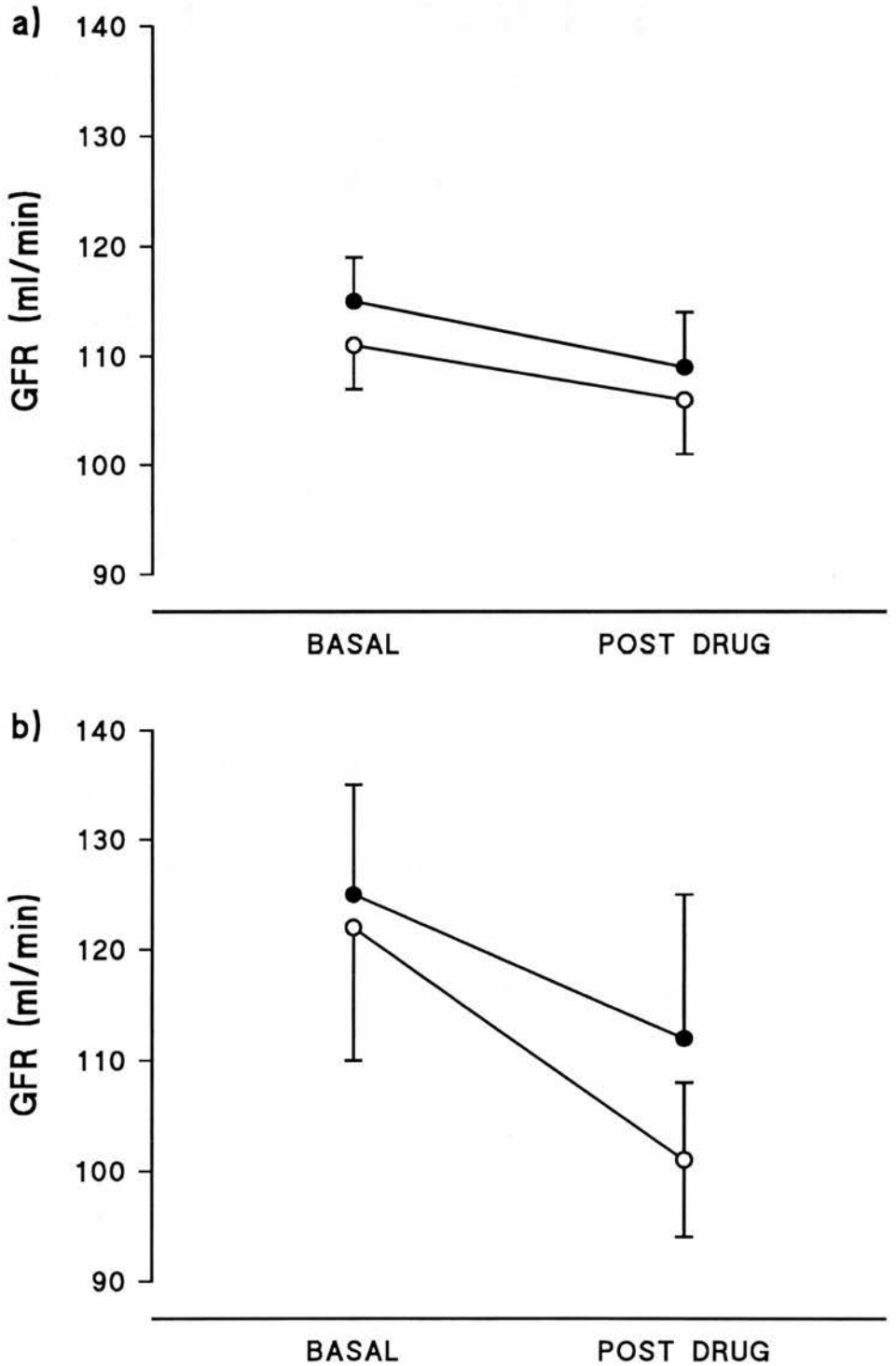
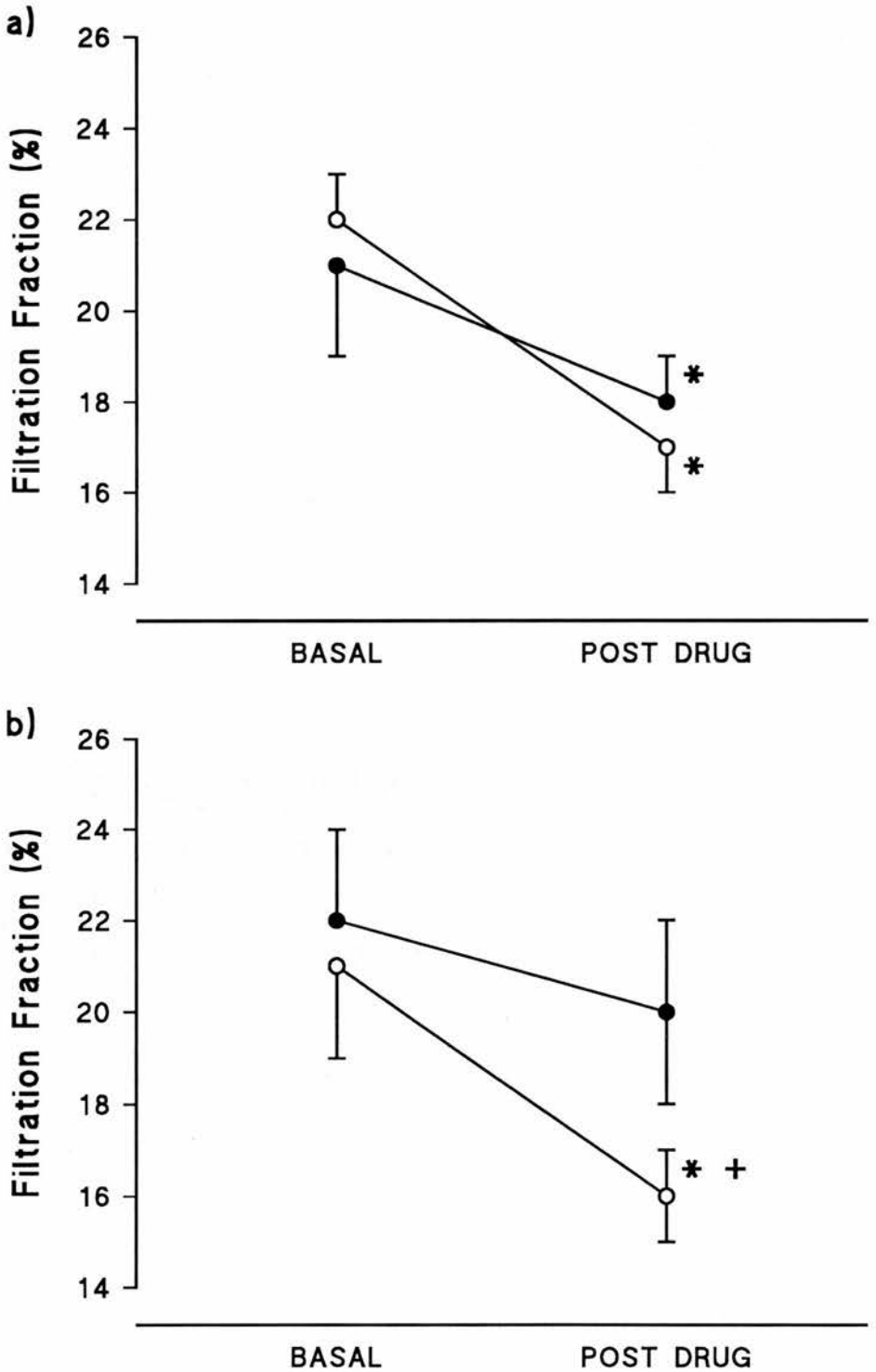


Figure 4

Acute changes in filtration fraction in response to captopril (o) and nifedipine retard (●), following first dose of drug (a) and after eight weeks treatment (b).
* indicates $p<0.05$ compared with basal and + indicates $p<0.05$ between the two drugs.



4.4 DISCUSSION

In this study, captopril and nifedipine retard were found to have a similar effect, after an eight-week period of adminstration, in lowering blood pressure in a group of hypertensive males with IDDM and varying degrees of albuminuria secondary to diabetic nephropathy. Nifedipine retard, however, was slower to exert its maximum antihypertensive effect than captopril. There was also a tendency for both drugs to reduce urinary albumin excretion compared with pre-treatment values, with no difference being found between the two in this respect. This latter finding contrasts with a previous report in a small group of normotensive patients with IDDM and microalbuminuria (Mimran et al, 1988), which did not employ a cross-over design. In this study, increased urinary albumin excretion (by an average of 40%) was reported following treatment for six weeks with nifedipine, although captopril reduced the albumin excretion rate. The only other directly comparative study between angiotensin converting enzyme inhibitors and calcium antagonists in diabetic subjects was conducted in patients with NIDDM (Baba et al, 1989), similar effects on renal function being reported following four-week treatment periods with enalapril and nicardipine.

In an attempt to more closely assess the comparative action of the two drugs on the kidney, acute studies were incorporated at the beginning and end of each active treatment limb, during which the effects of a single dose of the drug on renal haemodynamics was measured. The acute antihypertensive effect of both nifedipine retard and captopril was much greater after the first dose of each drug than at the end of the eight-week period of treatment, when the effect of

captopril on blood pressure was not significant. Both drugs had a similar effect in tending to reduce the glomerular filtration rate, this finding contrasting with that of the only previous study to assess haemodynamic responses to these two drugs (Mimran et al, 1988). Unlike nifedipine retard, however, captopril had an acute renal vasodilating effect, with a rise in effective renal plasma flow persisting even at the end of the period of treatment on the drug. As a result of these changes, a significant fall in the filtration fraction was still evident as an acute reponse to the final dose of captopril and this was the most striking difference between the action of the two drugs on renal haemodynamics. These changes in filtration fraction are comparable to those found in the small group of patients studied by Mimran et al (1988). Although caution is required in using the filtration fraction as a direct indicator of intraglomerular pressure (Carmines et al, 1987), this finding would support the theory that angiotensin converting enzyme inhibitors have a specific action in reducing intraglomerular hypertension, which is thought to be an important feature in the early stages of diabetic renal disease (see Section 1.5). This theory certainly has support from research in rats (Zatz et al, 1986) and a direct intrarenal action of captopril is particularly suggested in this study by the fact that the acute reduction in the filtration fraction at the end of the captopril treatment limb occurred without a significant coincidental fall in systemic blood pressure. Such an effect of systemic blood pressure may, however, have accounted for the changes in renal haemodynamics observed following the first dose of both captopril and nifedipine retard.

A number of previous studies have suggested that angiotensin

converting enzyme inhibitors may have a specific reno-protective action, thought to be secondary to their putative effect on intraglomerular pressure. In diabetic rats, captopril was more effective than combined therapy with three other antihypertensive agents (hydralazine, reserpine and a thiazide diuretic) in preventing the development of albuminuria and histological changes of diabetic nephropathy (Anderson et al, 1989). In studies in non-diabetic humans with hypertension and chronic renal disease, captopril has been reported to be more effective than a number of other commonly used antihypertensive agents, including nifedipine, in slowing both decline in renal function and reducing protein excretion, in spite of achieving blood pressure control comparable with the other agents (Ikeda et al, 1989; Ruilope et al, 1989). A number of studies in diabetic patients with varying degrees of nephropathy have also found a fall in urinary albumin excretion in response to angiotensin converting enzyme inhibitors and have suggested that this is related to a reduction in intraglomerular hypertension (Taguma et al, 1985; Mimran et al, 1988; Mau Pedersen et al, 1988; Parving et al, 1989). However, in this study, no significant difference in albumin excretion was found between captopril and nifedipine retard, during eight-week treatment periods with each drug, in spite of the fact that only captopril was associated with changes in renal haemodynamics suggestive of a direct effect on intraglomerular pressure. It would seem, therefore, that further evidence is required before attributing the effects of angiotensin converting enzyme inhibitors on urinary albumin excretion to this mechanism.

In conclusion, nifedipine retard and captopril have been shown to have a similar effect in reducing blood pressure in hypertensive

have a similar effect in reducing blood pressure in hypertensive patients with IDDM. After eight weeks treatment with each drug, captopril, but not nifedipine retard, retained an acute haemodynamic effect in reducing the filtration fraction, supporting the theory that angiotensin converting enzyme inhibitors may have a specific glomerular action. Although this could be important in protecting the kidney against progressive renal disease, in this study both captopril and nifedipine retard reduced urinary albumin excretion to a comparable extent, suggesting that the antihypertensive effect of the drugs *per se* may be the most important factor. It is acknowledged, however, that because of the relatively small number of patients in this study, there is the possibility of a Type 2 statistical error. The results, although largely in keeping with previous research in this field, therefore have to be interpreted with some degree of caution.

5.1 INTRODUCTION

Although most of the available data about microalbuminuria pertains to the population with IDDM, this is also an important complication of NIDDM, being a predictor of both frank nephropathy (Mogensen, 1984) and also premature death from ischaemic heart disease (Jarrett et al, 1984; Mogensen, 1984; Mattock et al, 1988, Schmitz & Vaeth, 1988) (see Section 1.4).

A prevalence of frank proteinuria of 12% has been reported in a large population of patients with NIDDM in the USA (Klein et al, 1988) and a cumulative incidence of proteinuria of 25% during the 20 years after the diagnosis of NIDDM has been estimated (Ballard et al, 1988). In contrast to patients with IDDM, abnormal protein excretion develops in many patients in the first few years after the development of NIDDM (Fabre et al, 1982; Marshall & Alberti, 1989) and a significant prevalence of microalbuminuria has been documented at the time of diagnosis (Damsgaard & Mogensen, 1986; Uusitupa et al, 1987; Ballard et al, 1988). It has been suggested that this is mainly a functional phenomenon, consequent upon the osmotic load associated with uncontrolled hyperglycaemia (Uusitupa et al, 1987; Schmitz et al, 1989), but previously the natural history of urinary albumin excretion following the diagnosis of NIDDM has not been reported in detail. In this study, the prevalence of microalbuminuria (and its association with other clinical parameters) was identified at diagnosis in a large group of patients with NIDDM and then assessed throughout the first year of treatment of hyperglycaemia.

5.2 PATIENTS AND METHODS

149 newly-diagnosed, previously untreated, patients with NIDDM were studied. They presented consecutively over a 9-month period to the Diabetic Clinic of Edinburgh Royal Infirmary and, in each case, a full history was obtained and clinical examination performed at the time of the initial clinic visit to determine the presence of macrovascular disease. Cerebrovascular disease was defined clinically as a history of stroke or transient ischaemic attack; ischaemic heart disease as a history of angina pectoris or myocardial infarction and/or ECG changes of myocardial ischaemia; and peripheral vascular disease as a history of intermittent claudication, rest pain, gangrene or amputation and/or the absence of both posterior tibial and dorsalis pedis pulses in at least one foot. Blood pressure was measured with a standard mercury sphygmomanometer after 5-min rest and retinopathy was assessed by direct ophthalmoscopy following mydriasis. Body mass index was calculated from measurements of height and weight. Exclusion criteria from the study were as follows: a history of preceding renal disease of any aetiology; plasma creatinine >150 umol/l; frank proteinuria; or urinary tract infection confirmed by culture of a midstream urine sample (collected on all patients). Early morning urine samples were collected and analysed to assess albumin excretion as described in Section 2.2, results being expressed as an albumin:creatinine ratio. Random plasma glucose and glycated haemoglobin (HbA_1) concentrations were measured on a venous blood sample, the former by a Yellow Springs glucose analyser and the latter using commercially available agar plates, the laboratory non-diabetic reference range being 6.0 - 8.0%.

regression models were fitted to correct for the effect of other variables, notably age. Linear regression was not appropriate in the analysis of trends in urinary albumin excretion over the 12 months of the study, as the majority of patients did not have a detectable trend. Instead, this data was assessed by calculating the Spearman rank correlation of the median albumin:creatinine ratio with the other continuous variables measured.

Table 1

Number of samples and median albumin:creatinine ratio (ACR) during the first year after presentation with NIDDM in patients defined as having persistent and intermittent microalbuminuria.

Persistent microalbuminuria group (n=21)				Intermittent microalbuminuria group (n=20)			
Patient	Samples (total)	Samples (abnormal)	Median ACR	Patient	Samples (total)	Samples (abnormal)	Median ACR
1	3	3	38.2	1	3	1	1.5
2	4	3	7.0	2	4	1	<1.0
3	4	4	6.3	3	3	1	<1.0
4	3	3	3.8	4	3	1	<1.0
5	4	4	4.1	5	3	1	<1.0
6	4	4	11.7	6	4	1	<1.0
7	3	3	4.6	7	4	1	2.1
8	3	3	10.8	8	3	1	1.8
9	3	3	5.9	9	3	1	<1.0
10	5	5	3.6	10	4	1	1.7
11	3	3	4.3	11	3	1	<1.0
12	3	3	4.9	12	3	1	<1.0
13	3	3	3.7	13	4	1	1.1
14	4	3	7.8	14	4	1	1.8
15	3	3	13.0	15	3	1	<1.0
16	5	4	4.9	16	3	1	<1.0
17	5	4	5.5	17	3	1	<1.0
18	3	3	3.5	18	3	1	1.8
19	3	3	16.8	19	4	1	1.2
20	3	3	11.2	20	3	1	1.5
21	4	4	27.4				

5.3 RESULTS

1. At Initial Presentation

At initial presentation, 110 (74%) of the 149 patients had a normal urinary albumin concentration (GROUP 1) but 39 (26%) had an albumin: creatinine ratio of >2.5 mg/mmol (GROUP 2). Clinical characteristics of the two groups are shown in Tables 2 and 3. Patients with microalbuminuria were older and had a higher random plasma glucose concentration, glycated haemoglobin and systolic blood pressure. There was also a strong association between elevated urinary albumin excretion at initial presentation and evidence of macrovascular disease, most strikingly peripheral vascular disease. Following correction for the effect of age (Tables 2 and 3), only the association with peripheral vascular disease clearly remained, although retinopathy and glycated haemoglobin fell only just short of significance as independently associated variables. Following further regression to correct for the combined effect of age, sex, blood pressure and glycated haemoglobin, there was still a strong trend towards an association between an abnormal urinary albumin:creatinine ratio at initial presentation and both peripheral vascular disease ($p=0.056$) and retinopathy ($p=0.090$).

Table 2

Clinical characteristics of patients with normal (GROUP 1) and abnormal (GROUP 2) urinary albumin excretion at initial presentation with NIDDM.

	GROUP 1	GROUP 2	p (unadjusted)	p (age adjusted)
n	110	39		
Sex (M:F)	64:46	23:16	1.000	0.985
Age (yr)	58 (11)	64 (11)	0.002	-
Body mass index (kg/m^2)	29.0 (4.9)	28.1 (6.0)	0.368	0.812
Random blood glucose (mmol/l)	12.3 (4.4)	14.4 (4.5)	0.014	0.155
Glycated Haemoglobin (%)	11.3 (2.7)	13.0 (3.1)	0.002	0.095
Systolic blood pressure (mm Hg)	140 (22)	149 (22)	0.021	0.248
Diastolic blood pressure (mm Hg)	83 (11)	84 (14)	0.721	0.613

Results are given as mean (SD) unless otherwise stated

Table 3

Diabetic complications in patients with normal (GROUP 1) and abnormal (GROUP 2) urinary albumin excretion at initial presentation with NIDDM.

	GROUP 1	GROUP 2	p (unadjusted)	p (age adjusted)
n	110	39		
Retinopathy	6 (6)	5 (13)	0.276	0.066
Treated hypertension	15 (14)	9 (24)	0.293	0.243
Peripheral vascular disease	9 (9)	13 (34)	0.001	0.014
Cerebrovascular disease	4 (4)	3 (8)	0.583	0.905
Ischaemic heart disease	16 (15)	6 (16)	1.000	0.519
Macrovascular disease (total)	24 (23)	18 (47)	0.009	0.207

Results are given as actual patient numbers (percentage of total)

2. After 12-month Follow-up Period

Clinical characteristics of the patients with consistently normal urinary albumin excretion (GROUP A) and those with persistent microalbuminuria (GROUP B) are shown in Tables 4 and 5. Eighteen (86%) of the group with persistent microalbuminuria also had an elevated albumin:creatinine ratio at initial presentation. A significant association with age was again observed, but following correction for this, there were no statistically significant associations with any of the other clinical characteristics assessed. There was, however, a strong trend towards an association between persistent microalbuminuria and macrovascular disease, 45% of the patients with persistent microalbuminuria having evidence of ischaemic heart disease, cerebrovascular disease or peripheral vascular disease, compared with only 25% of those with normal urinary albumin excretion. No association was found between urinary albumin excretion and the glycated haemoglobin concentration, either at initial presentation or after 6 months treatment. In contrast, in the 10 patients who had an initially elevated urinary albumin:creatinine ratio, but subsequently normal albumin excretion, the initial glycated haemoglobin was higher (14.4 (2.5)%) than in either of the 2 main groups ($p < 0.05$), indicating a greater hyperglycaemic stimulus (and presumably greater osmotic effect) prior to treatment of diabetes being instituted. Calculation of Spearman rank correlations failed to demonstrate any significant influence of the degree of microalbuminuria, as assessed by the median urinary albumin:creatinine ratio during the first year following presentation (Table 6).

Table 4

Clinical characteristics of patients with normal urinary albumin excretion (GROUP A) and persistent microalbuminuria (GROUP B) during the first year after diagnosis of NIDDM.

	GROUP A	GROUP B	p (unadjusted)	p (age adjusted)
n	88	21		
Sex (M:F)	48:40	14:7	0.445	0.190
Age (yr)	58 (10)	64 (7)	0.014	-
Body mass index (kg/m^2)	29.1 (4.8)	26.5 (5.1)	0.033	0.081
Random plasma glucose at presentation (mmol/l)	12.6 (4.5)	14.3 (5.2)	0.151	0.204
Glycated haemoglobin at presentation (%)	11.7 (2.8)	12.0 (3.0)	0.623	0.362
Glycated haemoglobin after 6 months treatment (%)	8.4 (1.5)	8.5 (1.4)	0.710	0.944
Systolic BP (mm Hg)	142 (23)	145 (22)	0.621	0.650
Diastolic BP (mm Hg)	84 (12)	82 (12)	0.675	0.964

Results are given as mean (SD) unless otherwise stated

Table 5

Diabetic complications in patients with normal urinary albumin excretion (GROUP A) and persistent microalbuminuria (GROUP B) during the first year after diagnosis of NIDDM.

	GROUP A	GROUP B	p (unadjusted)	p (age adjusted)
n	88	21		
Retinopathy	7 (8)	2 (10)	1.000	0.505
Treated hypertension	14 (16)	4 (20)	0.962	0.964
Peripheral vascular disease	10 (12)	5 (25)	0.243	0.537
Cerebrovascular disease	2 (2)	1 (5)	0.473	0.310
Ischaemic heart disease	11 (13)	6 (30)	0.127	0.087
Macrovascular disease (total)	21 (25)	9 (45)	0.125	0.230

Results are given as actual patient numbers (percentage of total)

Table 6

Spearman rank correlations for the median urinary albumin:creatinine ratio during the first year after diagnosis of NIDDM and other clinical features.

Age	0.18
Body mass index	-0.10
Systolic blood pressure	0.04
Diastolic blood pressure	0.04
Random plasma glucose at initial presentation	0.12
Glycated haemoglobin at initial presentation	0.05
Glycated haemoglobin after 6 months treatment	0.02

No correlation reached statistical significance

5.4 DISCUSSION

The prevalence of increased urinary albumin excretion at initial presentation of NIDDM is similar in this study to that previously reported from Finland (Uusitupa et al, 1987). The earlier study did not, however, find an association with age, glycaemic control, blood pressure or macrovascular disease, although the study population was younger than that reported here. An association between urinary albumin excretion and age has previously been noted in patients with longstanding NIDDM (Ballard et al, 1988; Schmitz & Vaeth, 1988), and the current findings would suggest that age may also be an important factor in the association between microalbuminuria and blood pressure which has been reported in patients with NIDDM of longer duration (Fabre et al, 1982; Schmitz & Vaeth, 1988; Marshall & Alberti, 1989).

An association between microalbuminuria and macrovascular disease in NIDDM has been recognised, with increased urinary albumin excretion apparently being an independent predictor (Mattock et al, 1988; Schmitz & Vaeth, 1988). In this study it is seen that such an association is evident even at the time of diagnosis, 47% of patients with an increased urinary albumin excretion at initial presentation having some clinical evidence of macrovascular disease, compared with only 23% of those with a normal urinary albumin excretion.

It has been suggested that abnormal urinary albumin excretion at the time of presentation with NIDDM is simply a manifestation of renal hyperfiltration secondary to uncontrolled hyperglycaemia (Uusitupa et al, 1987; Schmitz et al, 1989) and a fall in both glomerular filtration rate and urinary albumin excretion has been demonstrated

in a small group of patients following improved glycaemic control (Schmitz et al, 1989). In the larger study reported here, however, it was possible to identify different subgroups of patients who had increased urinary albumin excretion at initial presentation. In the 10 patients who reverted thereafter to normal urinary albumin excretion it seems reasonable to propose that a functional mechanism secondary to hyperglycaemia was operational. These 10 patients had higher glycated haemoglobin concentrations at presentation than either those with normal urinary albumin excretion throughout the year of study or those with persistent microalbuminuria, although similar glycaemic control was evident in all groups after 6 months of diabetic treatment. Eighteen patients, however, in addition to 3 who had an initially normal urinary albumin:creatinine ratio, had persistent microalbuminuria throughout the year of study. In these patients the degree of hyperglycaemia did not appear to be a major determining factor, with the glycated haemoglobin both at initial presentation and following 6 months of diabetic treatment being closely similar to that found in patients with a normal urinary albumin excretion throughout. Age was a definite associated factor and there was also a trend towards a higher prevalence of macrovascular disease (particularly peripheral vascular disease and ischaemic heart disease) in patients with persistent microalbuminuria from diagnosis. The relationship between increased urinary albumin excretion and generalised vascular disease is discussed further in Sections 1.4 and 1.5, and in Chapter 8.

In conclusion, persistent microalbuminuria is present from initial presentation in 16% of patients with NIDDM and in most cases this would appear to be a specific diabetes-related complication.

CHAPTER 6

TUBULAR FUNCTION IN EARLY DIABETIC NEPHROPATHY

ASSESSMENT OF URINARY EXCRETION OF BETA-THROMBOGLOBULIN AND N-ACETYL-BETA-D-GLUCOSAMINIDASE

6.1 INTRODUCTION

Microalbuminuria is essentially a measure of increased glomerular leakage and decreased selectivity (see Section 1.4), but tubular dysfunction is also an identifiable feature at the stage of early diabetic nephropathy. This has been demonstrated by the finding in diabetic patients of increased urinary concentrations of a number of small protein molecules, of a size freely filtered by the glomerular membrane but normally reabsorbed through the renal tubules. Proteins which have been suggested as potentially useful in the detection of early diabetic renal damage include beta-2-microglobulin (Hermansson & Ludvigsson, 1980; Poortmans et al, 1982; Ellis D et al, 1983), alpha-1-microglobulin (Walton et al, 1988; Martin et al, 1990), kappa light chains (Walton et al, 1988) and retinol-binding protein (Bernard et al, 1982; Gibb et al, 1989). One small protein which has not previously been evaluated as a potential marker for early diabetic nephropathy is beta-thromboglobulin (BTG). This is a platelet-specific protein which is located in the alpha granules and released during platelet activation (Ludlam & Cash, 1976). BTG is normally present only in very low concentrations in the urine, but increased urinary excretion has been reported in patients with IDDM (van Oost et al, 1983), particularly in those with frank nephropathy, in whom a correlation has been observed between plasma creatinine and urinary beta-thromboglobulin concentrations (Hopper et al, 1986).

A further urinary marker of tubular function, of possible value in the identification of early diabetic nephropathy, is N-acetyl-beta-D-glucosaminidase (NAG) (Whiting et al, 1979; Ellis EN et al, 1983;

Brouhard et al, 1985; Watts et al, 1988; Gibb et al, 1989; Martin et al, 1990), although its precise role remains to be defined. NAG is a lysosomal enzyme present in renal tubular cells. It is a much larger molecule than albumin and does not normally appear in the glomerular filtrate. Increased excretion of this protein therefore appears to be a manifestation of loss of integrity of the tubular membrane.

In this study, the urinary excretion of BTG and NAG was measured in patients with IDDM, both with normal albumin excretion and microalbuminuria. The association was examined between the excretion of these markers of tubular dysfunction, albumin excretion and a number of other clinical parameters.

6.2 PATIENTS AND METHODS

Patient Selection and Assessment

The study cohort comprised 132 patients with IDDM, recruited from the Diabetic Clinic of Edinburgh Royal Infirmary. None of the patients were hypertensive as defined by WHO criteria (World Health Organisation, 1986), all having a supine blood pressure of <160/95 mm Hg, measured using a standard mercury sphygmomanometer after 5-min rest, and all had a plasma creatinine concentration of <150 umol/l. None had a past history of known renal disease nor were taking any drugs which might cause proteinuria, and none had haematuria or proteinuria on standard dip-stick testing of urine. The presence of clinically apparent retinopathy was assessed in each patient by

direct ophthalmoscopy following mydriasis and if present was classified as background or proliferative. Proliferative retinopathy was defined as the presence of soft exudates (cotton wool spots), active neovascularisation or scarring from previous photocoagulation therapy; background retinopathy was identified by the presence of microaneurysms, haemorrhages or hard exudates. Details of drug therapy and smoking habit were obtained; those who were smoking five cigarettes or more daily at the time of the study were defined as 'smokers'.

97 of the participating patients had no evidence of diabetic nephropathy (GROUP 1), with a urinary albumin:creatinine ratio, measured on at least three occasions during the previous two years in early morning specimens of urine, consistently being within the normal range (see Section 2.2). The 35 other patients (GROUP 2) had been identified previously as having microalbuminuria on the basis of an elevated urinary albumin:creatinine ratio of >2.5 mg/mmol in two or more early morning specimens of urine within the preceding year.

Clinical details of the two groups are given in Table 1. The patients with microalbuminuria (GROUP 2) were significantly older, had a longer duration of diabetes, a higher mean glycated haemoglobin concentration and a greater prevalence of retinopathy than those with normal urinary albumin excretion (GROUP 1).

Table 1

Clinical details of 97 patients with normal urinary albumin excretion (GROUP 1) and 35 patients with microalbuminuria (GROUP 2).

	GROUP 1	GROUP 2	p
Age (yr) (range)	34 (14 - 63)	41 (1 - 44)	<0.02
Duration of diabetes (yr) (range)	11 (0.5 - 48)	19 (1 - 44)	<0.001
Sex (M:F)	52:45	22:13	NS
Glycated haemoglobin (%)	9.5 (2.1)	11.2 (2.1)	<0.001
Plasma creatinine (mmol/l)	79.2 (12.4)	84.4 (13.3)	NS
Systolic BP (mm Hg)	120 (17)	127 (22)	NS
Diastolic BP (mm Hg)	74 (10)	74 (10)	NS
Smokers (n (%))	23 (24%)	6 (19%)	NS
Non-smokers (n (%))	72 (76%)	25 (81%)	
No retinopathy (n (%))	79 (81%)	14 (41%)	
Background retinopathy (n (%))	15 (15%)	14 (41%)	
Proliferative retinopathy (n (%))	3 (3%)	6 (18%)	
Retinopathy (total) (n (%))	18 (19%)	20 (59%)	<0.001

Results are given as mean (SD) unless otherwise stated
Data on smoking habit was missing for 6 patients and on retinopathy for 1 patient

Study Protocol and Laboratory Methods

All of the patients collected a sample of the first urine voided immediately after rising, which was subsequently divided into three aliquots. As described in Section 2.2, urinary albumin excretion was measured, within four days of collection, in one of these aliquots stored at $4^{o}C$. The other two samples, for determination of urinary BTG and NAG excretion, were stored until analysis at $-20^{o}C$. A venous blood sample was collected for estimation of glycated haemoglobin (HbA_1) and plasma creatinine concentrations. HbA_1 was measured using commercially available agar plates, the laboratory non-diabetic reference range being 6.0 - 8.0%.

Urinary BTG was measured by standard radioimmunoassay, using an adaptation of the technique for measurement of plasma BTG described by Bolton et al (1976). The normal urinary concentration of BTG is approximately 0.5% of the plasma concentration (Dawes et al, 1978) and the sensitivity of the assay can be improved by the use of a greater dilution of antiserum and radio-labelled tracer, with delayed addition of the tracer. Unextracted urine was incubated at $4^{o}C$ for 24 h with antiserum, raised in rabbits, against purified BTG. ^{125}I-labelled BTG (0.1 ng/ml) was then added, this concentration resulting in binding of approximately 50% of the tracer. After a further 24 h, antibody-bound BTG was separated from free BTG and the ^{125}I activity in the precipitate containing the antibody-bound BTG fraction was measured. The detection limit for the assay was 0.1 ng/ml, with the intra- and inter-assay co-efficients of variation both being approximately 14%.

Urinary NAG was measured using a commercially available colorimetric assay supplied by Thames Genelink, Deeside, UK. The assay uses the principle that a chromogenic substrate, 2-methoxy-4-(2-nitrovinyl) phenyl-2-acetamido-2-deoxy-beta-D-gluco-pyranoside, is hydrolysed by NAG to release 2-methoxy-4-(2-nitrovinyl) phenol which, on addition of an alkaline buffer, produces a colour which can be measured at a wavelength of 505 nm (Yuen et al, 1984). The inter-assay co-efficient of variation was less than 6% and results were expressed as a ratio of urinary NAG:creatinine, the reference range in normal individuals being 1.0 - 24 umol/mmol.

Statistical Analysis

Chi-square and t tests were used, where appropriate, to determine the association of urinary BTG and the urinary NAG:creatinine ratio with the other variables. Multiple regression analysis was performed to assess the relative importance of the associations between the urinary NAG:creatinine ratio and the urinary albumin:creatinine ratio, blood pressure, smoking status, retinopathy, glycaemic control, age and duration of diabetes.

6.3 RESULTS

In the early morning urine specimen provided for the study, only 21 of the patients with microalbuminuria (GROUP 2) actually had an albumin:creatinine ratio of >2.5 mg/mmol. This apparent disparity, in a group of patients already identified as having microalbuminuria, can be explained on the basis of the recognised day to day variability in urinary albumin excretion in individual patients with diabetes (Feldt-Rasmussen & Mathiesen, 1984; Chachati et al, 1987).

Urinary BTG was above the level of detection of the assay (0.1 ng/ml) in 21 patients in total, of whom 13 (62%) were in GROUP 2. However, only 8 patients with a detectable urinary BTG (38%) also had an elevated albumin:creatinine ratio in the specimen of urine provided for the study. A detectable urinary BTG was associated with a longer duration of diabetes, 15 (71%) of the 21 patients having had diabetes for 10 years or more. The number of patients with a detectable urinary BTG was too small to reasonably assess the presence or absence of an association with any of the other clinical parameters considered.

The urinary NAG:creatinine ratio was elevated in a total of 55 patients. 22 (63%) patients in GROUP 2 had an elevated urinary NAG:creatinine ratio, and this association with microalbuminuria was significant (chi-square=8.79, $p<0.01$). Individual and mean urinary NAG:creatinine values for GROUP 1 and GROUP 2 are shown in Figure 1. The association between the urinary NAG:creatinine ratio and an abnormal urinary albumin:creatinine ratio in the sample collected for the study was even stronger, 16 out of the 21 patients (73%) having a

result above the normal reference range (chi-square=12.25, $p<0.001$).

When other clinical parameters were considered, a significant correlation was observed between the urinary NAG:creatinine ratio and prevailing glycaemic control, estimated by the glycated haemoglobin concentration, as shown in Figure 2 ($r=0.48$, $p<0.01$). The urinary NAG:creatinine ratio was elevated in 20 (57%) of the 35 patients with background or proliferative retinopathy, compared with 35 (37%) of patients without retinopathy (Figure 3) and this association with retinopathy also attained significance (chi-square=4.3, $p<0.05$). Similarly, an increase in the prevalence of an abnormal NAG:creatinine excretion was observed in smokers, as shown in Figure 4 (chi-square=12.7, $p<0.001$). No such association was observed between smoking habit and elevation of the urinary albumin:creatinine ratio in this study. There was a trend towards the mean urinary NAG:creatinine ratio increasing with duration of diabetes, with the lowest mean concentration seen in patients with a duration of diabetes <2 yr and the highest in patients with a duration of 10 - 20 yr. This trend did not, however, achieve statistical significance. No significant association was determined between the urinary NAG:creatinine ratio and either age, sex, systolic or diastolic blood pressure.

Multiple regression analysis was performed to determine the most important associations between the above variables and the urinary NAG:creatinine ratio. The co-efficients of each term in the multiple regression are shown in Table 2 and give an estimate of the difference in the urinary NAG:creatinine ratio attributable to each variable, independent of the other variables. Smoking habit,

glycaemic control and microalbuminuria were all independently associated. The NAG:creatinine ratio was on average 18.2 umol/mmol greater in smokers than non-smokers and 13.9 umol/mmol greater in patients with microalbuminuria compared with those patients who had a normal urinary albumin excretion. For each incremental rise of 1% in the glycated haemoglobin concentration, the NAG:creatinine ratio rose by 3.35 umol/mmol. The presence of retinopathy just failed to achieve significance as an independently important variable, with age, duration of diabetes and blood pressure falling well short of statistical significance.

Figure 1

Urinary NAG excretion, expressed as the NAG:creatinine (NAG/Cr) ratio (showing mean value) in diabetic patients with normal albumin excretion (GROUP 1) and microalbuminuria (GROUP 2). Dashed line indicates upper limit of normal for the NAG/Cr ratio.

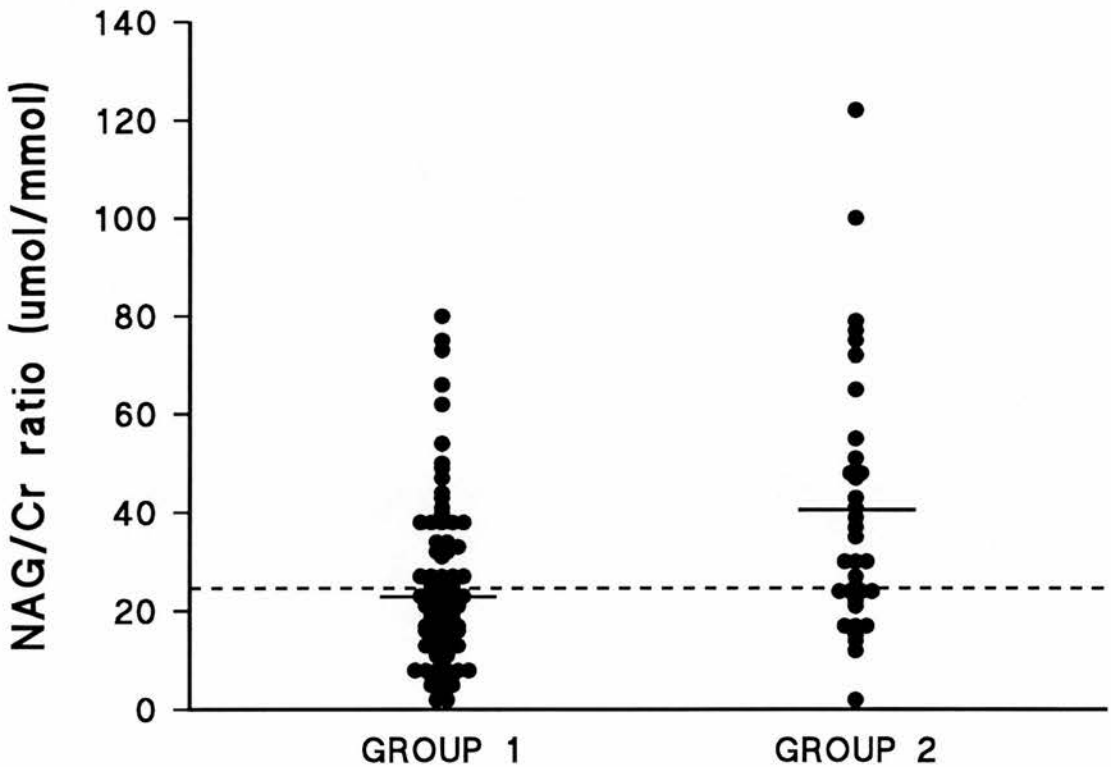


Figure 2

Relationship between urinary NAG excretion (NAG/Cr ratio) and plasma HbA_1 concentration.

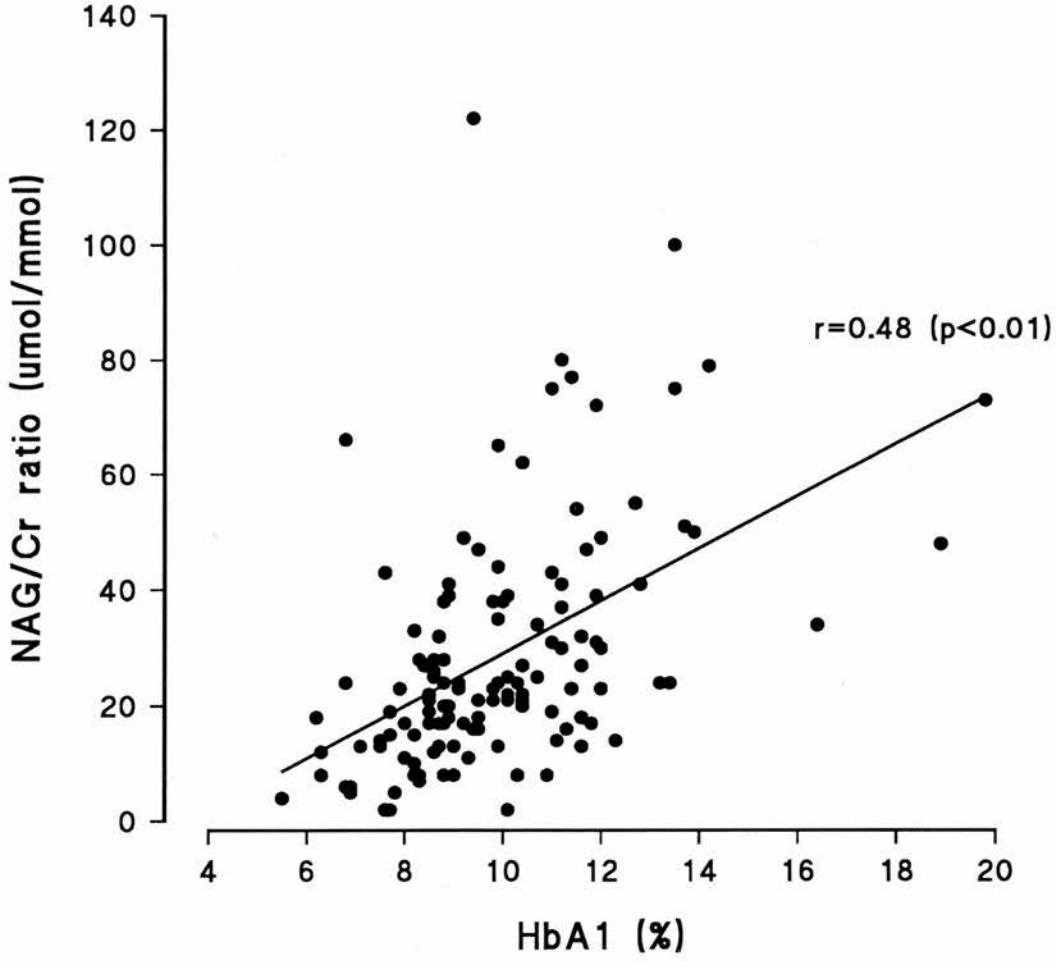


Figure 3

Relationship between urinary NAG excretion and retinopathy. Hatched areas indicate patients with a urinary NAG:creatinine ratio above the upper limit of normal.

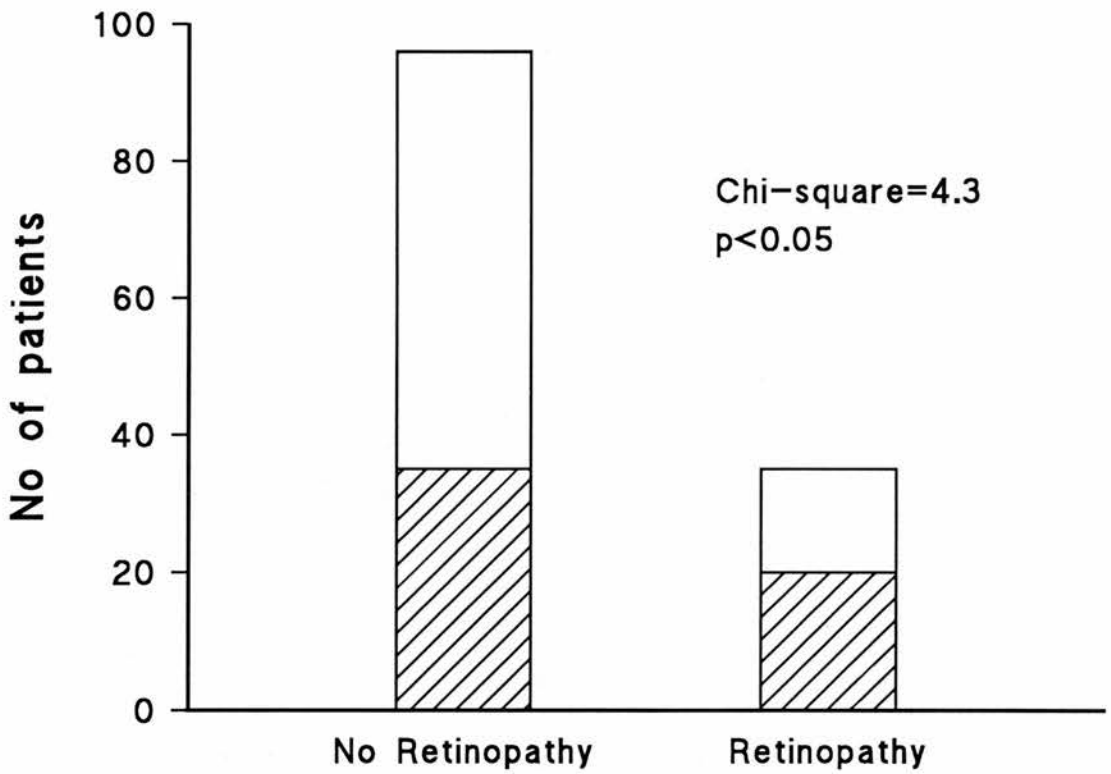


Figure 4

Relationship between urinary NAG excretion and smoking status. Hatched areas indicate patients with a urinary NAG:creatinine ratio above the upper limit of normal.

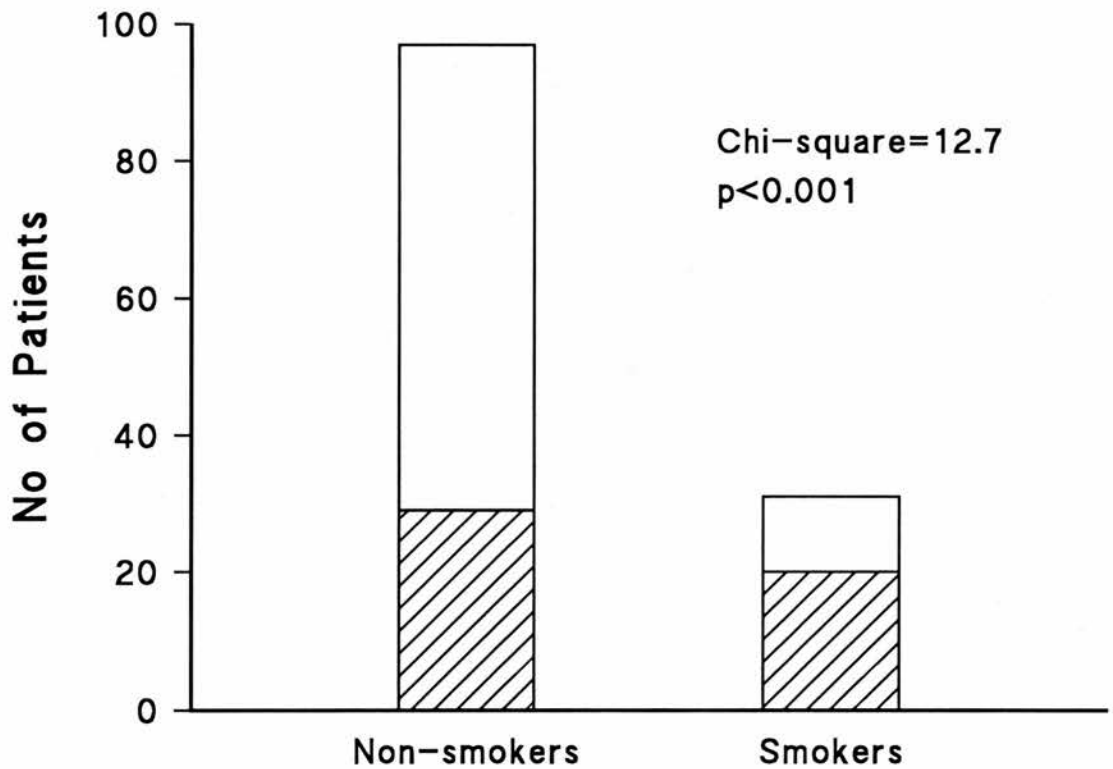


Table 2

Co-efficients, with 95% confidence limits, from multiple regression analysis, using the urinary NAG:creatinine ratio (umol/mmol) as the dependent variable.

Age (change per yr)	0.09	(-0.18, 0.36)
Duration of diabetes (change per yr)	-0.38	(-0.77, 0.01)
Systolic blood pressure (change per mm Hg)	0.01	(-0.17, 0.19)
Smoking habit (yes/no)	18.2*	(10.7, 267)
Retinopathy (yes/no)	5.40	(-2.53, 13.73)
Glycated haemoglobin (change per 1%)	3.35*	(1.92, 4.78)
Microalbuminuria (yes/no)	13.9*	(6.5, 21.3)

* represents variables having a statistically significant independent association with the urinary NAG:creatinine ratio

6.4 DISCUSSION

Determination of microalbuminuria, indicative of glomerular dysfunction, allows the identification of a stage of diabetic nephropathy sufficiently early to provide the possibilitity of therapeutic intervention to prevent progression of the nephropathic process (see Section 1.7). However, the evidence that abnormalities of tubular function are also a feature of nephropathy provides a possible alternative approach to identifying patients with diabetic renal disease at the earliest possible stage, when interventional measures are likely to have maximum impact. The urinary excretion of a number of proteins of small molecular weight, which are freely filtered by the glomerular membrane and are therefore markers of tubular reabsorption, have previously been studied as possible indicators of early diabetic nephropathy. An increased urinary excretion of beta-2-microglobulin has been reported in children and adolescents with IDDM (Hermansson & Ludvigsson, 1980), although other studies have failed to demonstrate a consistent abnormality in the excretion of this protein even in the presence of established diabetic nephropathy (Viberti et al, 1982b; Mathiesen et al, 1984). Beta-2-microglobulin has a low stability, both *in vitro* and *in vivo* (Bernard et al, 1982; Martin et al, 1990), which may be a factor in the discrepant results of these earlier studies, and its role as a potential marker of early tubular dysfunction therefore appears to be limited. Some of the other small protein molecules mentioned in the introduction to this chapter (Section 6.1), such as alpha-1-microglobulin, kappa light chains and retinol-binding protein, may be of greater diagnostic value in identifying early nephropathy, but

further evidence is clearly required before their use in routine clinical practice could be considered.

Beta-thromboglobulin (BTG) is a platelet-derived protein which usually occurs as a tetramer with a subunit molecular weight of 8,600 daltons and should therefore filter freely through the glomerular membrane. Reabsorption in the proximal tubule maintains a constant concentration of BTG excreted in the urine at approximately 0.5% of the plasma concentration (Dawes et al, 1978). BTG is released in response to platelet aggregation and activation (Ludlam & Cash, 1976) and, indeed, a previous study in diabetic patients used BTG as a marker to examine the role of platelet activation in microvascular complications of the disease (Hopper et al, 1986). Although that particular study did not find any clear correlation between the urinary BTG concentration and platelet survival, a strong association was observed between BTG excretion and both an increased plasma creatinine level and increased urinary beta-2-microglobulin excretion, suggesting a possible role for BTG in the detection of renal disease in patients with diabetes. A further study demonstrated an increase in urinary BTG excretion in non-diabetic hypertensive patients with renal impairment (Anderton et al, 1980), but the potential role of an increase in urinary BTG excretion as an indicator of early diabetic nephropathy had not been examined previously. The findings in this study, however, indicate that measurement of the urinary concentration of this protein does not have an adequate degree of sensitivity, using current assays, to have a useful role in screening for early nephropathy, even though urinary BTG excretion was increased predominantly in patients with microalbuminuria and in those with a long duration of diabetes. In

assessing tubular function, an alternative approach to measurement of the urinary excretion of low molecular weight proteins is the quantification in the urine of the enzyme, N-acetyl-beta-D-glucosaminidase (NAG). This enzyme, which has a molecular weight of 150,000 daltons, is too large to be filtered through the normal glomerular membrane although it cannot be excluded that, in the presence of severe glomerular damage, significant concentrations may appear in the urine via this route. In the main, however, the appearance of increased urinary concentrations of NAG is likely to predominantly reflect tubular damage, with loss of integrity of the tubular membrane, and increased urinary NAG excretion has previously been demonstrated in diabetic patients with nephropathy (Whiting et al, 1979; Ellis EN et al, 1983; Watts et al, 1988; Gibb et al, 1989).

In this study, a clear association between urinary NAG excretion and microalbuminuria has been demonstrated, significant correlations also being found with both glycaemic control and smoking habit. The association with glycated haemoglobin confirms the findings of previous studies (Ellis EN et al, 1983; Watts et al, 1988; Gibb et al, 1989) and research in experimental animals has given some insight into the possible mechanisms determining this association with glycaemic control. It has been demonstrated in dogs that urinary NAG excretion is related to the urinary glucose concentration (Rowe et al, 1985) and increased urinary flow rate (resembling the osmotic diuresis secondary to hyperglycaemia) has been shown in the rat to be associated with reduced tubular absorption of proteins (Chan & Straus, 1980). It seems likely that similar mechanisms may be operational in the human diabetic kidney. The observed association between urinary NAG excretion and smoking adds to the increasing body

between urinary NAG excretion and smoking adds to the increasing body of evidence that smoking in patients who have diabetes may have a pathogenic role in damaging the microcirculation of various organs, including the kidney (Muhlhauser, 1990), although the mechanism of this remains to be elucidated.

In conclusion, urinary excretion of BTG, in common with a number of other low molecular weight proteins, does not appear to be of value as a sensitive indicator of early diabetic nephropathy. Measurement of urinary NAG excretion may complement the quantification of urinary albumin excretion in the detection of early nephropathy but, in the light of current evidence, identification of microalbuminuria, rather than measures of tubular dysfunction, remains the 'gold standard' as an indicator of potentially reversible renal disease in diabetic patients. Further longitudinal studies will, however, be necessary to assess fully the true significance of abnormal urinary excretion of the various markers of tubular dysfunction.

CHAPTER 7

HYPOGLYCAEMIA AND RENAL FUNCTION

7.1 INTRODUCTION

In numerical terms, hypoglycaemia is an immense problem in patients with IDDM. Around one-half of insulin-treated patients will have at least one episode of hypoglycaemia in any month, and in any one year, even conservative estimates suggest that 8 - 13% of patients will experience severe hypoglycaemia, requiring external help to resuscitate them (Potter et al, 1982; Casparie & Elving, 1985; Matthews et al, 1986). Some patients, notably those who have lost the ability to perceive the early warning symptoms of hypoglycaemia (hypoglycaemia unawareness), have recurrent episodes of severe hypoglycaemia and often the blood glucose concentration has been in the hypoglycaemic range for a considerable length of time before they are effectively treated.

Acute insulin-induced hypoglycaemia in humans is a potent stimulus to autonomic neural activation and the secretion of a number of hormones, including catecholamines, angiotensin II (Trovati et al, 1988) and vasopressin (Fisher et al, 1989; Thompson et al, 1989). Profound haemodynamic and haemostatic changes occur during hypoglycaemia (Hilsted et al, 1980 & 1984; Frier et al, 1983) and the secretion of catecholamines in association with stimulation of the sympatho-adrenal system plays an important role in determining these (French et al, 1955; Frier & Hilsted, 1985). Both myocardial contractility and cardiac output increase significantly (Hilsted et al, 1984; Fisher et al, 1987) and overall there is a fall in vascular resistance. Considerable differences in regional blood flow have, however, been observed in several major organs and vascular systems,

including the skin (Hilsted et al, 1982), skeletal muscle (Middleton & French, 1974; Hilsted et al, 1984), brain (Neil et al, 1987) and hepato-splanchnic circulation (Bearn et al, 1952; Hilsted et al, 1984).

Several of these physiological responses to hypoglycaemia could be detrimental, particularly in those patients subjected to repeated prolonged and severe episodes, and may potentially have a role in the aetiology of the end organ damage which is a feature of the microvascular complications of diabetes. The possible effects of hypoglycaemia on the kidney have not, however, previously been studied. In this chapter, I have examined the changes in renal haemodynamics and other indices of kidney function during acute insulin-induced hypoglycaemia, both in patients with IDDM and in non-diabetic control subjects, in order to establish whether hypoglycaemia may in any way be implicated in either the causation or progression of diabetic nephropathy.

7.2 PATIENTS AND METHODS

Patient Selection

Eight male patients with IDDM were recruited from the Diabetic Clinic of Edinburgh Royal Infirmary. The mean age of the patients was 29 (range 24 - 40) yr and all had a normal body mass index (19 - 25 kg/m^2). Their mean duration of diabetes was 8 (range 2 - 15) yr and the mean (SD) glycated haemoglobin (HbA_1) at the time of study was

9.0 (2.4)%. None of the patients were hypertensive, or receiving antihypertensive medication. Similarly, none had evidence of any specific microvascular complications of diabetes, in particular nephropathy, with all having a normal plasma creatinine concentration (<100 umol/l) and normal 24-h urinary albumin excretion (<30 mg) in each of two collections. All of the patients were non-smokers and none were taking any medications apart from insulin. A control group comprised 8 healthy non-smoking, non-diabetic male volunteers, all of normal weight and with a mean age of 30 (range 23 - 39) yr.

Study Protocol

Subjects were admitted at 0800 h on the day of the study, having fasted since 2200 h on the previous evening. Ingestion of tea or coffee was not permitted for 12 h before the study. The diabetic patients administered only soluble insulin within the 24-h period prior to admission, with the last insulin dose being taken prior to an evening snack. On arrival in the laboratory, intravenous cannulae were inserted into both antecubital fossae, one for blood sampling and the other for administration of insulin and other drugs. In the diabetic patients, an intravenous infusion of 5% glucose solution was commenced, in addition to 50 ml of 0.9% sodium chloride solution containing 50 U soluble insulin (Human Actrapid, Novo Nordisk, Crawley, UK), administered via a 3-way connector. Infusion rates were adjusted as necessary to maintain the plasma glucose concentration within the range of 5.0 - 10.0 mmol/l during the hour following admission. The infusion was then discontinued and acute hypoglycaemia was induced by an intravenous bolus injection of Human Actrapid in a

dose of 0.125 U/kg body weight. In the control subjects, no glucose and insulin infusion was required to maintain euglycaemia, but an identical bolus dose of soluble insulin was administered to induce hypoglycaemia.

Following administration of the insulin bolus, heart rate, blood pressure and capillary blood glucose were monitored at 5-min intervals. The onset of an acute autonomic reaction to hypoglycaemia was identified by a sudden increase in heart rate, widening of the pulse pressure and the onset of sweating, in conjunction with a capillary blood glucose concentration of <2.5 mmol/l. Subsequent sampling of blood and urine was measured from this time point (designated 'time=R' in the subsequent results and figures), thus eliminating individual variability in the time from the administration of the insulin bolus to the onset of the acute autonomic reaction.

Measurements of Renal Function

Adequate hydration was ensured in all subjects by fluid loading with oral tap water (10 ml/kg body weight) at the time of admission, and then replacing the volume of urine passed subsequently during the study by giving an equivalent volume of tap water to drink. Fluid lost through sweating and insensible losses were not specifically replaced. In addition to this oral hydration, an intravenous infusion of a 20% mannitol solution was administered continuously at a rate of 50 ml/h throughout the study to maintain a diuresis, aiming for a urinary flow rate of 7 - 8 ml/min.

Effective renal plasma flow was estimated by the measurement of clearance of para-amino hippurate sodium (PAH; Merck, Sharp and Dohme, Hoddesdon, UK), as described in Section 2.3. An intravenous bolus injection of PAH was given at three time points (30 min prior to insulin administration, time=R and R+60 min) with blood samples for PAH measurement being taken before each injection and then on seven subsequent occasions up to 40 min. The plasma concentration of PAH was <0.3 g/l before each IV bolus injection. The intra-individual co-efficient of variation for measurement of effective renal plasma flow using this technique was 9.4%.

Glomerular filtration rate (GFR) was estimated from clearance of polyfructosan (Inutest; Laevosan, Linz, Austria), administered as a constant infusion after an initial intravenous bolus loading dose (see Section 2.3). Blood and urine samples were collected at four time points (30 min prior to insulin administration, 10 min after insulin administration, R+60 min and R+120 min) and measurement of polyfructosan concentrations in these specimens allowed calculation of GFR, (1) in the basal period; (2) over the time of the acute hypoglycaemic reaction; and (3) in the recovery period. The intra-individual co-efficient of variation using this technique was 13.4%.

Urinary concentrations of dopamine (see Section 2.4), albumin (see Section 2.2) and sodium (measured by flame photometry) were also calculated from each urine specimen.

Measurements of Hormone Excretion and Laboratory Methods

Blood samples were withdrawn before insulin was administered and at R, R+15 min, R+30 min, R+45 min, R+60 min, R+90 min and R+120 min for measurement of plasma glucose, plasma renin activity, adrenaline, noradrenaline and angiotensin II concentrations. Plasma glucose was measured by a glucose oxidase method using a Yellow Springs glucose analyser. Plasma renin activity was calculated using a commercially available radioimmunoassay kit method, involving the generation of angiotensin I under standard conditions (Campagne Oris, Gif-Sur-Yvette, France). Plasma adrenaline and noradrenaline (Ball et al, 1981) and plasma angiotensin II (Kappelgaard et al, 1976) were measured, using well-established radioenzymatic assays, in the MRC Blood Pressure Unit, Western Infirmary, Glasgow.

Statistical Analysis

Results, unless otherwise stated, are given as mean (SE). The data for all subjects were analysed by one-way analysis of variance, with paired t tests and the Wilcoxon Rank Sum test being used to compare basal values with those during the period of the autonomic reaction to hypoglycaemia and during the recovery phase.

7.3 RESULTS

All of the subjects experienced a typical autonomic reaction to hypoglycaemia. The onset of the reaction occurred at a mean of 35 (range 22 - 54) min after administration of the bolus insulin injection in the diabetic patients and 24 (range 22 - 28) min after insulin administration in the control group. In the control sujects, the nadir of plasma glucose occurred at time=R in each individual and this was also the case in three of the diabetic patients, with the nadir attained at R+15 min in the remaining five patients.

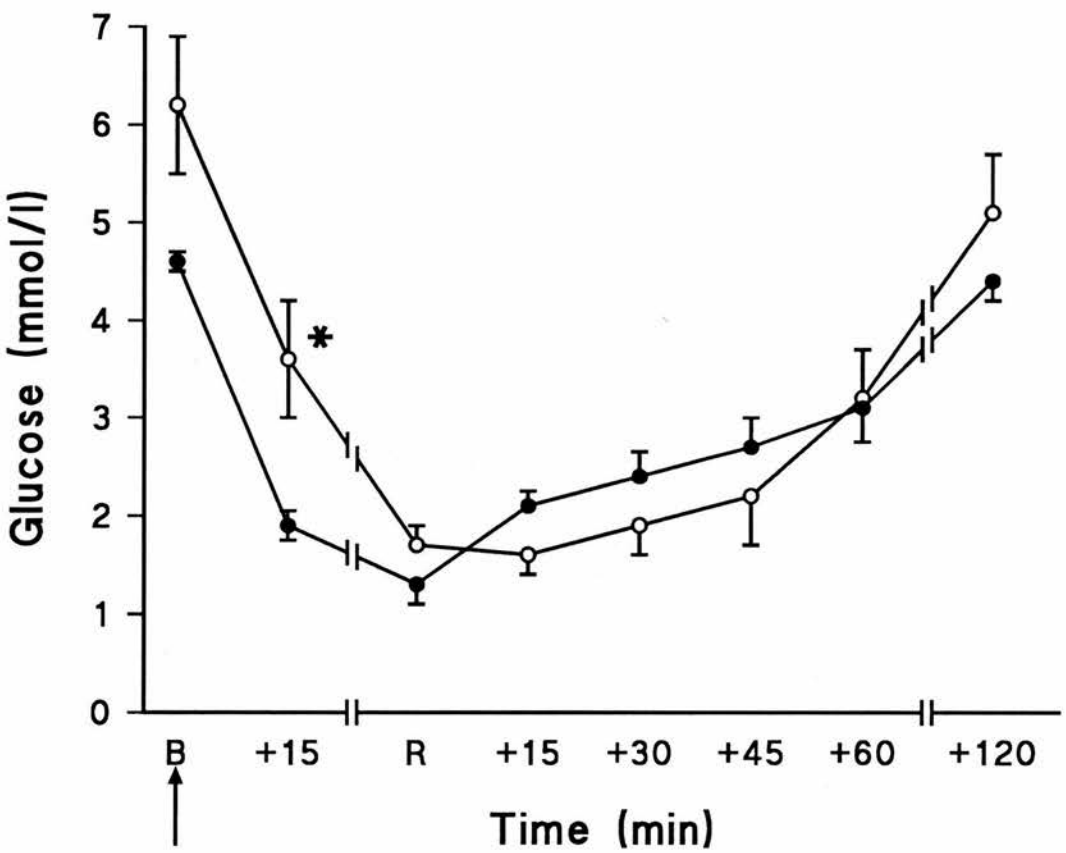
Metabolic and Hormonal Changes

Plasma Glucose

In the control subjects, the mean plasma glucose fell from 4.6 (0.2) mmol/l before insulin administration to 1.3 (0.2) mmol/l at time=R ($p<0.01$), with a characteristic biphasic recovery ensuing. By R=120 min the glucose concentration was restored to basal levels at 4.4 (0.2) mmol/l. In the diabetic patients, plasma glucose fell from 6.2 (0.7) to 1.7 (0.2) mmol/l at time=R ($p<0.001$) and 1.6 (0.2) mmol/l at R+15 min ($p<0.001$) with recovery thereafter to 5.1 (0.6) mmol/l at R+120 min (p=NS compared with basal value). There was no significant difference between the plasma glucose nadir in the two groups (Figure 1).

Figure 1

Plasma glucose response following administration of insulin (arrow) in diabetic (o) and non-diabetic (•) subjects. B=basal and R=onset of acute autonomic reaction.
* indicates $p<0.05$ between groups.



Plasma Adrenaline

In the control subjects, the mean plasma adrenaline concentration attained a peak of 6.6 (0.4) nmol/l at R+15 min, this representing a significant increase from basal values ($p<0.01$). In the diabetic patients, the peak value (again at R+15 min) was 1.7 nmol/l ($p<0.01$ vs basal), but compared with the non-diabetic group, this increase in response to hypoglycaemia was attenuated, with the adrenaline concentration being significantly lower between time=R and R+45 min (Figure 2).

Plasma Noradrenaline

In the diabetic patients, the minor increase in plasma noradrenaline from a baseline value of 1.3 (0.4) nmol/l to a peak level of 1.8 (0.3) nmol/l at R+15 min did not attain statistical significance. In the control subjects, noradrenaline concentrations were significantly higher than in the diabetic group at each time point and, in addition, the increase from basal (2.8 (0.30) nmol/l) to the peak value (4.6 (0.6) nmol/l) was also significant ($p<0.01$) (Figure 3).

Figure 2

Plasma adrenaline response following administration of insulin (arrow) in diabetic (o) and non-diabetic (●) subjects. B=basal and R=onset of acute autonomic reaction.
* indicates $p<0.05$ between groups.

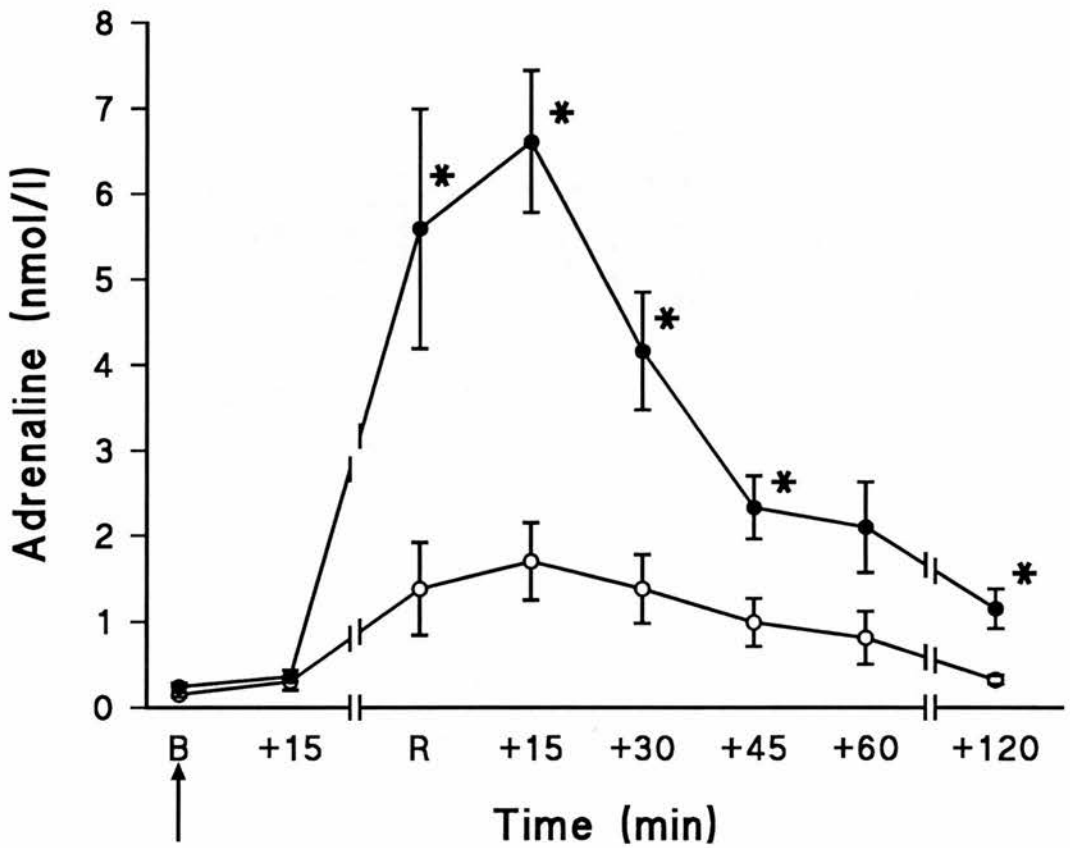
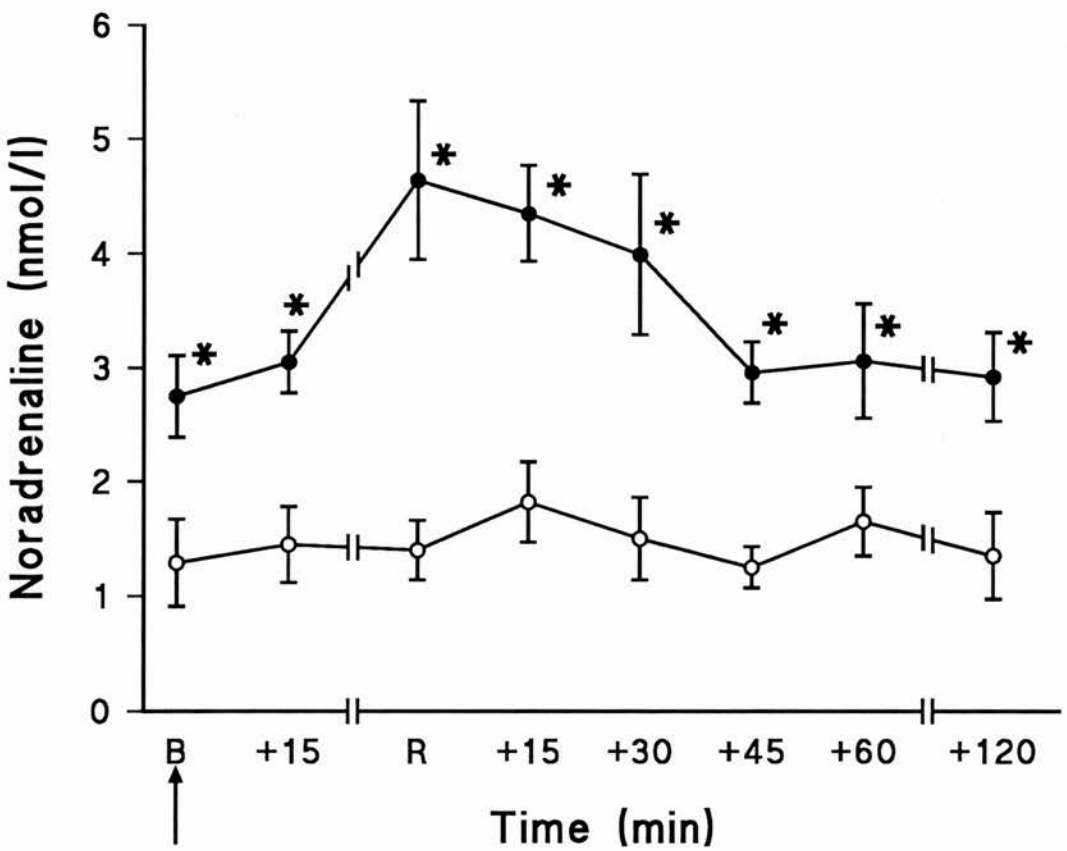


Figure 3

Plasma noradrenaline response following administration of insulin (arrow) in diabetic (o) and non-diabetic (●) subjects. B=basal and R=onset of acute autonomic reaction.
* indicates $p<0.05$ between groups.



Plasma Renin Activity

Plasma renin activity rose in the diabetic patients to a peak of 1.08 (0.17) ng-Ang I/l at R+15 min ($p<0.01$ vs basal), remaining significantly elevated until R+30 min and then returning to the basal value by R+120 min. In the control subjects, plasma renin activity remained significantly elevated ($p<0.01$) above baseline values until R+60 min and there was a non-significant trend towards concentrations being higher than in the diabetic group throughout (Figure 4).

Plasma Angiotensin II

Plasma angiotensin II concentrations did not rise significantly in the diabetic patients (peak value of 7.4 (1.5) ng/l at R+60 min vs a basal level of 5.1 (0.9) ng/l ($p=0.065$)), in contrast to the control group, in which an increase from 4.4 (0.4) ng/l to a peak of 15.1 (4.1) ng/l at R+30 min was observed ($p<0.01$), with a subsequent fall to basal values. Again there was a trend towards a greater response in the non-diabetic subjects, which just failed to reach significance because of the wide individual variation in responses (Figure 5).

Figure 4

Plasma renin activity (PRA) response following administration of insulin (arrow) in diabetic (o) and non-diabetic (●) subjects. B=basal and R=onset of acute autonomic reaction.

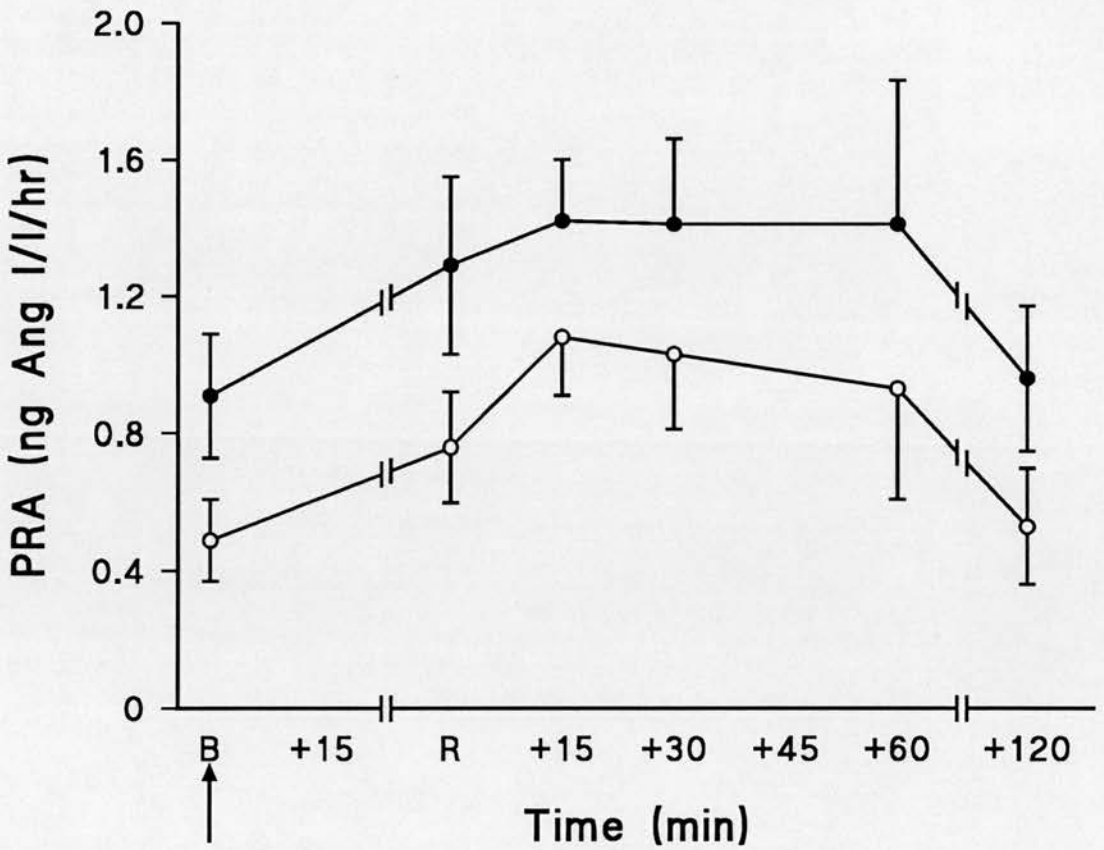
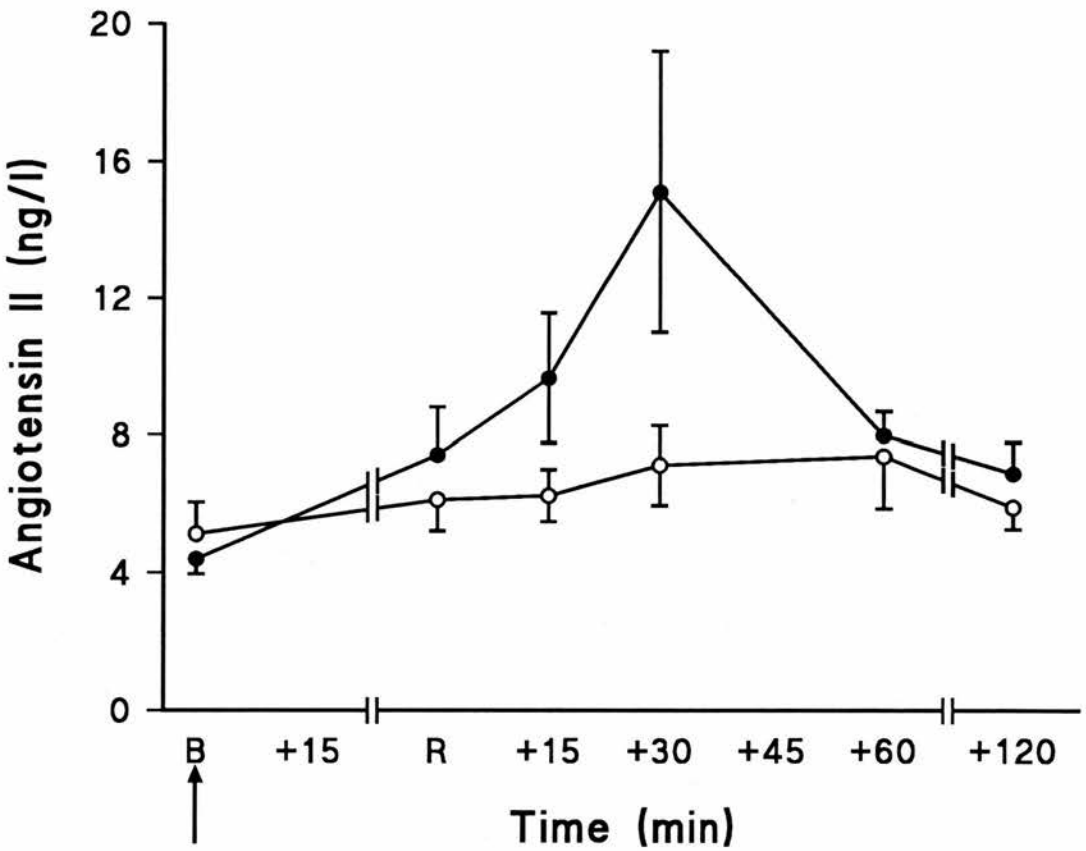


Figure 5

Plasma angiotensin II response following administration of insulin (arrow) in diabetic (o) and non-diabetic (●) subjects. B=basal and R=onset of acute autonomic reaction.



Renal Function

In the diabetic patients, effective renal plasma flow fell from 674 (106) ml/min in the basal period to 540 (198) ml/min during the period of hypoglycaemia ($p<0.01$), returning to normal in the recovery period. A similar response was observed in the control subjects, with a reduction in the effective renal plasma flow from 625 (38) to 485 (27) ml/min during hypoglycaemia ($p<0.01$). The values at each of the three time-points were lower in the control group, this difference reaching significance at the time of hypoglycaemia ($p<0.01$) (Figure 6). The magnitude of the fall in response to hypoglycaemia did not differ between the two groups (Figure 7).

Glomerular filtration rate (GFR) fell in the diabetic patients from a basal value of 143 (23) to 110 (36) ml/min during the period of hypoglycaemia ($p<0.02$), again returning to normal during the recovery phase. In the control subjects, the GFR values at each time point were significantly lower (Figure 6) and the overall fall in response to hypoglycaemia (from 118 (6) to 95 (4) ml/min) was also less than that observed in the diabetic group (Figure 7).

The correlation between GFR and effective renal plasma flow also differed between the two groups. In the diabetic patients, GFR and effective renal plasma flow were closely correlated both in the basal period ($r=0.75$, $p<0.05$) and during the period of hypoglycaemia ($r=0.96$, $p<0.001$). The changes in GFR and effective renal plasma flow occurring during hypoglycaemia were also well correlated ($r=0.78$, $p<0.05$). In contrast, in the non-diabetic control subjects, these correlations fell well short of significance.

Figure 6

Effective renal plasma flow (RPF) (a) and glomerular filtration rate (GFR) (b) during acute hypoglycaemia in diabetic (o) and non-diabetic (●) subjects.
* indicates $p<0.05$ between groups.

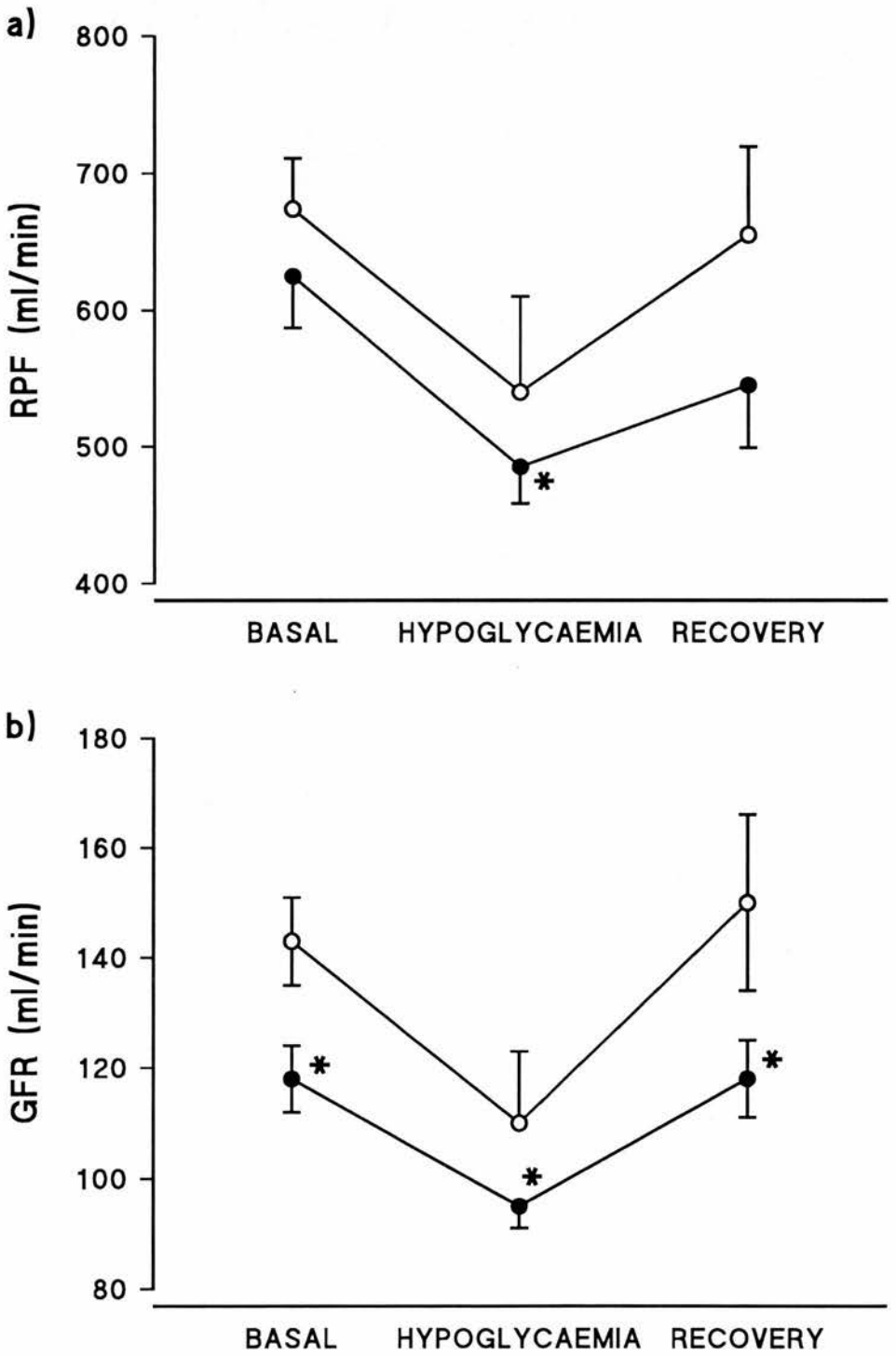
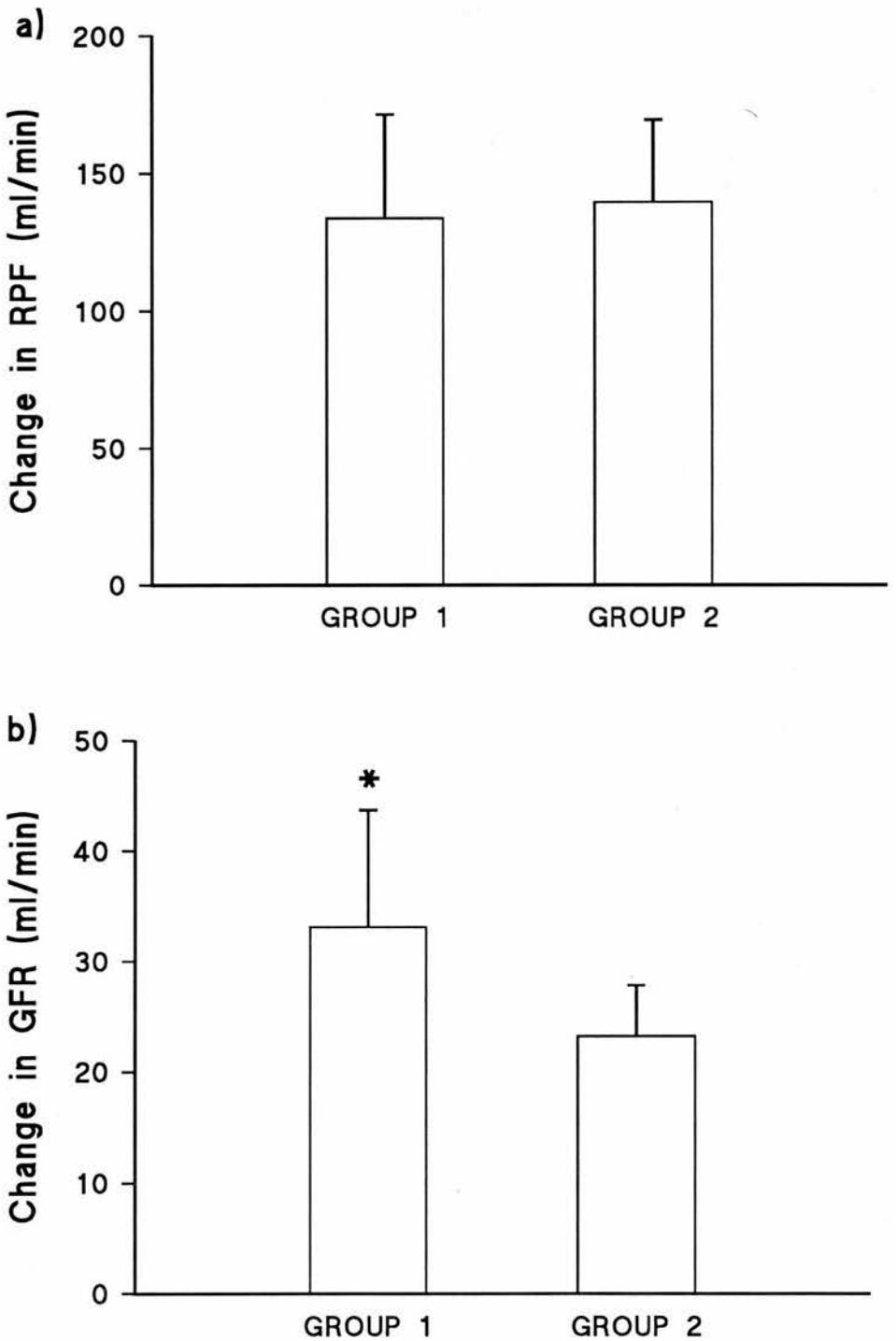


Figure 7

Change in effective renal plasma flow (RPF) (a) and glomerular filtration rate (GFR) (b) in response to acute hypoglycaemia in diabetic (GROUP 1) and non-diabetic (GROUP 2) subjects.
* indicates P<0.05 between groups.



The effects of acute hypoglycaemia on urine volume and urinary excretion of sodium, dopamine and albumin are shown in Table 1 for the diabetic patients and Table 2 for the control subjects. Both urinary volume and albumin excretion rate fell significantly during hypoglycaemia in the diabetic but not in the control group. Neither fractional nor absolute urinary sodium excretion changed significantly in the diabetic patients but a significant fall in urinary dopamine excretion was observed which did not correlate with urinary flow ($r=0.20$, $p=NS$) and was not dependent on changes in GFR, renal plasma flow or urinary sodium excretion. In contrast, in the non-diabetic control subjects, urinary sodium excretion fell during hypoglycaemia in parallel with dopamine excretion.

Table 1

Urine volumes and excretion of sodium, dopamine and albumin in response to hypoglycaemia in diabetic patients.

	Basal Period	Period of Hypoglycaemia	Recovery Period	p
Urine volume (ml/min)	10.6 (1.2)	4.7 (1.1)	7.7 (1.5)	<0.002
Absolute sodium excretion (umol/l)	262 (38)	250 (43)	303 (50)	NS
Fractional sodium excretion (%)	1.3 (0.2)	1.7 (0.3)	1.6 (0.2)	NS
Dopamine excretion (umol/min)	322 (37)	211 (29)	230 (25)	<0.005
Albumin excretion rate (ug/min)	46.2 (10.6)	26.0 (10.5)	42.6 (9.3)	<0.005

Results are given as mean (SE)

Significance is tested between time of hypoglycaemia and basal period

Table 2

Urine volumes and excretion of sodium, dopamine and albumin in response to hypoglycaemia in non-diabetic control subjects.

	Basal Period	Period of Hypoglycaemia	Recovery Period	p
Urine volume (ml/min)	7.6 (1.0)	6.7 (0.6)	5.9 (0.6)	NS
Absolute sodium excretion (umol/min)	403 (47)	273 (45)	203 (30)	<0.005
Fractional sodium excretion (%)	2.5 (0.3)	2.0 (0.8)	1.3 (0.6)	<0.01
Dopamine excretion (umol/min)	614 (198)	132 (29)	150 (29)	<0.02
Albumin excretion rate (ug/min)	33.1 (13.7)	15.5 (2.8)	18.9 (3.6)	NS

Results are given as mean (SE)

Significance is tested between time of hypoglycaemia and basal period

7.4 DISCUSSION

Activation of the autonomic nervous system and the hormonal secretion associated with acute hypoglycaemia in humans have a profound effect on the vascular perfusion and function of several organs (Bearn et al, 1952: Middleton & French, 1974; Hilsted et al, 1982 & 1984; Fisher et al, 1987; Neil et al, 1987) and the intravenous insulin bolus technique described in these studies is likely to maximally stimulate this neuro-hormonal activation. Patients with IDDM, who had no clinical evidence of nephropathy, had a fall in effective renal plasma flow and glomerular filtration rate of 20% and 23% respectively in response to acute hypoglycaemia, the reduction in glomerular filtration rate being significantly greater than that observed in non-diabetic control subjects. Both effective renal plasma flow and glomerular filtration rate were higher in the basal state in the diabetic patients, this being a well recognised feature of diabetes, particularly in the presence of poor glycaemic control (Mogensen, 1971 & 1972; Ditzel & Junker, 1972). In addition, a close correlation was identified in the diabetic patients, but not in the control subjects, between the changes in glomerular filtration rate and renal plasma flow during hypoglycaemia, suggesting that in IDDM the glomerular filtration rate may be more highly dependent on renal blood flow than in non-diabetic subjects.

Adrenaline and angiotensin II, two of the principal hormones which are known to affect renal blood flow, were stimulated to a lesser extent in the diabetic group than in the non-diabetic subjects. It is recognised that the catecholamine response to hypoglycaemia may be diminished in patients with IDDM of long duration (Hoeldke et al,

1982; Bolli et al, 1983; Adamson et al, 1984), which might at least partially explain the reduced adrenaline response. Both renal plasma flow and glomerular filtration rate fell significantly, however, in spite of these reduced hormonal responses. It may be, therefore, that direct stimulation of the efferent sympathetic nerves as part of the autonomic response to hypoglycaemia, rather than the specific effects of the hormones, is the major determinant of the changes in renal haemodynamics observed. The fall in plasma glucose per se may also be of importance. A significant fall both in glomerular filtration rate and renal plasma flow has been demonstrated in diabetic patients when the blood glucose concentration was reduced from the hyperglycaemic to the normoglycaemic range, without any simultaneous change in plasma catecholamine concentrations (Mogensen et al, 1978). This is apparently not explicable on the basis of changes in plasma volume or sympathetic activation occurring in response to hyperinsulinaemia (Mogensen et al, 1980; Christiansen et al, 1981b).

The secretion of vasopressin in response to acute hypoglycaemia is known to be markedly enhanced in IDDM (Fisher et al, 1989; Thompson et al, 1989) and the significant fall in urine volume observed in the diabetic patients during hypoglycaemia, but not in the control subjects, is likely to be explicable on the basis of this augmented vasopressin response to hypoglycaemia. The rapid reduction in urinary flow rate in response to hypoglycaemia may also underlie the significant fall in urinary albumin excretion rate in the diabetic group. The elevated basal urinary albumin excretion rate in both groups is probably related to the prevailing diuretic conditions, as it has been shown previously that albumin excretion rises significantly in response to water loading, falling again when the

urinary flow rate returns to normal (Viberti et al, 1982c).

Urinary dopamine excretion fell in response to hypoglycaemia, but a reduction in sodium excretion only occurred in the non-diabetic subjects. The absence of a fall in urinary sodium excretion in the patients with IDDM is perhaps surprising, as there is usually a close correlation between dopamine and sodium excretion (Alexander et al, 1974; see also Chapter 3). A reduced sodium excretion might also have been anticipated on the basis of the action of the insulin administered to induce hypoglycaemia, as insulin inhibits the renal excretion of electrolytes (Nizet et al, 1971; DeFronzo et al, 1975) and enhances sodium reabsorption in the renal tubules (Wiesmann et al, 1977). A possible explanation for this observed difference between diabetic and non-diabetic subjects is the absence of a significant rise in plasma noradrenaline in response to hypoglycaemia in the diabetic group. Noradrenaline promotes sodium retention, via alpha receptors in the proximal tubules (McMurray et al, 1988), this action being independent of any haemodynamic effects.

In conclusion, the studies described here have demonstrated reduced renal perfusion and glomerular filtration rate in response to acute hypoglycaemia, more marked in patients with IDDM. These changes in renal haemodynamics and function are superimposed upon the well-documented changes in plasma viscosity (Frier et al, 1983; Hilsted et al, 1984) and platelet activation (Hilsted et al, 1980; Dalsgaard-Nielsen et al, 1982) which also occur in response to hypoglycaemia. It is likely that these combined changes are potentially deleterious, with the overall effect being to initiate, or at least enhance, a tendency towards tissue ischaemia. Hypoglycaemia may therefore be a

factor in the causation, or possibly more importantly in the progression, of diabetic renal disease, particularly in those patients with hypoglycaemia unawareness who may be exposed to frequent and often prolonged hypoglycaemic episodes.

CHAPTER 8

GENERAL DISCUSSION AND FUTURE RESEARCH

It is not surprising, in such a major new area of potentially considerable clinical importance, that a substantial amount of research has been published in the time since the studies presented in this thesis were initiated. In this final chapter, I have attempted to discuss the findings of my own studies in the context of other relevant recently published work, where possible suggesting the future direction of research which may prove fruitful in enhancing our understanding of early diabetic nephropathy.

Early diabetic nephropathy, hypertension and vascular disease

In Chapter 3, I examined the possibility that a defect in dopamine excretion, which appears to have a genetically determined link with essential hypertension, may be a factor in the development of early diabetic nephropathy. The finding of a preserved relationship between urinary sodium and dopamine excretion in patients with microalbuminuria suggests that such a defect does not predate the onset of nephropathy in predisposed patients with IDDM. Although future research could increase the sensitivity of this study by measuring the dynamic response of dopamine excretion to sodium loading, it seems likely that it is necessary to look elsewhere to find a genetic factor which is of aetiological importance in the development of both diabetic nephropathy and hypertension. As discussed in Section 1.5, the sodium-lithium countertransport system has already received considerable attention as such a potential genetic link and research in this field has continued, although the most recent studies have done little to clarify the situation.

A higher sodium-lithium countertransport activity has been reported in parents of diabetic patients with nephropathy, when compared with parents of patients with normal urinary albumin excretion (Walker et al, 1990). In the same study, a significant correlation was noted between blood pressure in the patients with nephropathy and their parents. It was suggested that these findings constituted additional evidence that genetic factors associated with a predisposition to hypertension are relevant in determining susceptibility to diabetic nephropathy. However, a further Scandinavian study failed to show any difference in either blood pressure or sodium-lithium counter-transport activity between groups of parents of diabetic patients with and without nephropathy (Jensen et al, 1990). Indeed in this study, sodium-lithium countertransport activity did not differ even between the patient groups and the authors reached the opposite conclusion to that of Walker et al. It is perhaps of particular relevance that it has been very recently shown, on detailed examination of the kinetics of sodium-lithium countertransport, that the mechanism for increased activity in diabetes appears to be completely different from that found in essential hypertension, again suggesting that this genetic marker of hypertension does not provide the link with diabetes and diabetic nephropathy (Rutherford et al, 1992). The sodium-proton pump has also been the subject of recent attention and it has been suggested that measurement of the sodium-hydrogen exchange rate may be of more direct relevance in the field of diabetic nephropathy and hypertension than sodium-lithium countertransport activity (Barbe et al, 1992). Additional data in this area will doubtless follow in the next few years.

Further recent research has suggested that patients with early

diabetic nephropathy have a generalised increased sensitivity to the vasoconstrictive effects of noradrenaline and this could be relevant in the development of nephropathy (with enhanced vasoconstriction of efferent arterioles contributing to intraglomerular hypertension) and also systemic hypertension (Bodmer et al, 1992). The precise nature of this apparent hypersensitivity to endogenous vasoconstrictors remains to be elucidated but it may be genetically determined and further research in this field could prove fruitful in helping to explain the link between diabetic nephropathy, hypertension and generalised vascular disease.

In Chapter 4, the issue of antihypertensive therapy in hypertensive patients with IDDM was considered, captopril and nifedipine retard being shown to have a similar effect on reducing blood pressure and urinary albumin excretion over an eight-week period of treatment, although captopril maintained an acute effect on renal haemodynamics, independent of its action on systemic blood pressure. A number of other recent studies have now also examined the actions of different antihypertensive agents in the diabetic population. Treatment with captopril and thiazide diuretics in normotensive patients with microalbuminuria has been reported to reduce the albumin excretion rate in comparison with controls, without there being a significant effect on blood pressure (Mathiesen et al, 1991). This is suggested by the authors to be indicative of either a fall in intraglomerular pressure as a result of a direct effect of captopril on efferent arteriolar resistance or, alternatively, a specific action of the drug on the intrinsic selectivity of the glomerular membrane, resulting in a slow decline in the permeability of the glomerular

barrier to protein. A further study in hypertensive patients demonstrated that reduced protein excretion whilst taking angiotensin converting enzyme inhibitors was maintained over a 3-year treatment period (Hermans et al, 1992).

Exercise-induced microalbuminuria has been suggested as an earlier marker of diabetic nephropathy than increased urinary albumin excretion measured at rest (see Section 1.6) and captopril has also been shown to have an effect on reducing the rise in albumin excretion in response to exercise, again without having any significant effect on the systemic blood pressure response (Romanelli et al, 1989).

There have still been relatively few comparative studies between different antihypertensive agents in diabetic patients. In hypertensive patients with IDDM and advanced nephropathy, protein excretion was found to be less after an eight-week treatment period with captopril, compared to metoprolol, but the reduction in systemic blood pressure was also greater on captopril, making the interpretation of any specific intrarenal effect of the drug difficult (Bjorck et al, 1990). In a further group of patients with nephrotic syndrome secondary to diabetic nephropathy, lisinopril and diltiazem were reported to have similar effects in reducing protein excretion (Bakris et al, 1990). In patients with early nephropathy, a recently published multi-centre study from Australia found perindopril and nifedipine to have a comparable action in reducing blood pressure and urinary albumin excretion over a treatment period of 12 months and it was of note that, in both treatment groups, albumin excretion only fell in patients in whom there was also a

significant fall in blood pressure (Melbourne Diabetic Nephropathy Study Group, 1991).

It seems reasonable to conclude from the available data that the controversy of whether or not angiotensin converting enzyme inhibitors are specifically reno-protective, secondary to a direct intrarenal action over and above their effect on reducing systemic blood pressure, remains to be resolved.

There has recently also been increasing interest in 24-h ambulatory blood pressure monitoring. Early evidence suggests that, in diabetic patients with microalbuminuria, not only is the mean blood pressure higher than in controls with normal albumin excretion, but there is additionally a significant reduction in the normal nocturnal dip in blood pressure (Berrut et al, 1992; Hansen et al, 1992). This may be of particular importance as, certainly in non-diabetic patients with essential hypertension, there is evidence that loss of the nocturnal dip is associated with a greater left ventricular mass and an increased risk of cardiovascular complications (Pickering et al, 1990; Verdecchia et al, 1990). In the light of these findings it would seem that, to provide maximum information, further studies of different antihypertensive agents in diabetic patients will require to consider changes in 24-h blood pressure profiles, rather than simply alterations in one-off random measurements.

In Chapter 5, I have specifically focussed on patients with NIDDM, a group in which the link between microalbuminuria and generalised vascular disease has been shown to be particularly strong. 26% of patients had an elevated urinary albumin excretion rate at initial

presentation with NIDDM and this correlated strongly with both age and the presence of generalised macrovascular disease. Even after glycaemic control was achieved, 16% of patients had persistent microalbuminuria during a follow-up period of one year from presentation, presumably indicating established pathology. A further study in newly-presenting, normotensive patients with NIDDM has demonstrated an elevated glomerular filtration rate to be present in 45% (Vora et al, 1992), suggesting that hyperfiltration may be an important pathophysiological factor in the development of nephropathy in NIDDM, as has also been postulated to be the case in IDDM (Hostetter et al, 1982).

The close association between microalbuminuria in NIDDM and the development of generalised vascular disease has attracted most recent research interest, however. Patients with NIDDM who have increased urinary albumin excretion have been noted to have more atherogenic lipid profiles than normoalbuminuric controls, with higher plasma levels of VLDL-cholesterol and VLDL- and LDL-triglyceride, along with reduced HDL-cholesterol concentrations (Niskanen et al, 1990). Rheological studies have also demonstrated evidence of a hypercoagulable state, endothelial dysfunction and increased free radical activation in patients with microalbuminuria, all factors which have been suggested to be of potential importance in determining the excess of vascular disease in this group (Schmitz et al, 1990; Collier et al, 1992; Stehouwer et al, 1992).

The potential role of insulin in the pathophysiology of diabetic complications has also been the focus of some recent attention. Many patients with NIDDM have insulin resistance and hyperinsulinaemia,

and Reaven has proposed that diabetes per se may be just one part of a syndrome complex which includes obesity, hypertension, lipid abnormalities and a predisposition to vascular disease (Reaven, 1988). It has further been suggested that hyperinsulinaemia and abnormalities of lipoprotein metabolism may have a direct role in the aetiology of hypertension (Reaven, 1991; Pezzarossa et al, 1992). A recent preliminary study has additionally indicated that hyperinsulinaemia may be an important factor in the development of impaired natriuresis, hyperfiltration and microalbuminuria in patients with NIDDM (Sambarato et al, 1992).

Tubular function in early diabetic nephropathy

In Chapter 6, I have examined the possible role of markers of tubular dysfunction in the identification of early diabetic nephropathy, measuring urinary excretion of beta-thromboglobulin and N-acetyl-beta-D-glucosaminidase in groups of patients with IDDM, both with and without microalbuminuria. In common with other investigators, my findings suggest that, although abnormalities of tubular function are commonly found in the presence of microalbuminuria, the available markers may not be of adequate sensitivity to be of great value in the detection of diabetic nephropathy at the earliest possible stage.

In the last two years there has been only a limited amount of further published work in this field. It has, however, been suggested that one of the initial events in the development of diabetic nephropathy may be increased peritubular capillary leakage, leading to

interstitial oedema and early tubular dysfunction (Pinter & Atkins, 1990) and some very recent preliminary research lends support to this theory. Patients with IDDM and normal urinary albumin excretion, who had serum markers of endothelial dysfunction, also had evidence of increased free radical activation and abnormal tubular function, compared to both diabetic patients without evidence of endothelial dysfunction and also normal non-diabetic control subjects, although there was no difference in urinary albumin excretion between the three groups (Yaqoob et al, 1992a & 1992b). These findings suggest that endothelial dysfunction, possibly related to oxidant injury, may affect the postglomerular, peritubular capillaries at an early stage, initiating the nephropathic process and resulting in tubular enzymuria and increased excretion of low molecular weight proteins as a consequence of ischaemic damage and disruption of tubular cells. If further research supports these findings, there could be a considerable resurgence of interest in tubular function in diabetic patients.

Hypoglycaemia and diabetic nephropathy

In the final experimental studies described in Chapter 7, I have examined the effect of acute hypoglycaemia on renal function, both in patients with IDDM and in non-diabetic control subjects. The most striking findings during hypoglycaemia were a significant fall in both effective renal plasma flow and glomerular filtration rate, the reduction in the latter parameter being both greater and more directly dependent on renal blood flow in the diabetic group.

Hypoglycaemia is extremely common in patients with IDDM and recently increased attention has been given in the literature to the fact that many patients lose the warning symptoms of the onset of hypoglycaemia, putting them at risk of prolonged and more severe episodes. In one UK survey, reduced hypoglycaemia awareness was reported in 23% of insulin-treated patients attending a hospital diabetic clinic, predominantly in those with a long duration of diabetes and hence the highest prevalence of specific vascular complications of diabetes (Hepburn et al, 1990). Interest in this area has been enhanced by the suggestion that the use of human insulin may be implicated in the causation of altered hypoglycaemia awareness (Teuscher & Berger, 1987), resulting in an increased prevalence of severe hypoglycaemia (Egger et al, 1991). The debate which has ensued is outwith the scope of this thesis but what has become clear as a result of this increased publicity in both the medical and lay press is the fact that many insulin-treated diabetic patients experience frequent hypoglycaemia, which can often be asymptomatic and at other times severe and prolonged.

In view of its prevalence and associated physiological effects, it is perhaps surprising that the possibility that hypoglycaemia may be of importance in either the initiation or progression of specific microvascular complications of diabetes has not been researched more extensively. There seems little doubt that the reduced blood flow to a number of organs observed during hypoglycaemia, including the kidneys as described here, could be potentially damaging. This is particularly the case when considered in conjunction with the widespread rheological changes which occur during hypoglycaemia (see Section 7.4), all of which would tend to favour microvascular

thrombosis. With specific reference to the kidney, these potential detrimental effects of hypoglycaemia could be examined further by studying the renal responses to acute hypoglycaemia in patients who already have evidence of nephropathy and also by carefully assessing renal function in a group of patients who have experienced recurrent severe hypoglycaemia, in comparison with matched controls in whom hypoglycaemia has not been a problem.

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APPENDIX

PUBLICATIONS

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1990, *16*, 441-447

URINARY EXCRETION OF BETA-THROMBOGLOBULIN AND N-ACETYL-BETA-D-GLUCOSAMINIDASE IN TYPE 1 DIABETES : POTENTIAL INDICATORS OF EARLY NEPHROPATHY ?

Excrétion urinaire de Béta-Thromboglobuline et de N-acétyl-beta-D-glucosaminidase dans le diabète de type I : marqueurs potentiels de la néphropathie débutante ?

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RÉSUMÉ

Alors que la microalbuminurie indique une lésion glomérulaire dans la néphropathie diabétique, des anomalies tubulaires interviennent aussi dans la maladie rénale diabétique à son stade précoce. L'excrétion urinaire de la beta-thromboglobuline (BTG) et de la N-acetyl-beta-D-glucosaminidase (NAG) a été mesurée chez 132 patients diabétiques de type 1 (insulino-dépendants) normo-tendus, sans maladie rénale manifeste, parmi lesquels 35 avaient une microalbuminurie et les autres avaient une élimination urinaire de l'albumine normale. Sur les 21 patients qui avaient une concentration urinaire en BTG détectable, seulement 8 (38 %) avaient un taux d'albuminurie anormal associé. L'excrétion de la NAG était élevée chez 22 patients (63 %) sur les 35 avec microalbuminerie ; des associations significatives ont aussi été identifiées entre l'excrétion urinaire de NAG et le tabac ($x^2 = 12,7$, $p < 0.001$) et l'hémoglobine glycosylée ($r = 0,49$, $p < 0.01$). En conclusion, la mesure de la BTG urinaire n'est pas d'une sensibilité suffisante pour détecter une maladie rénale diabétique à son stade précoce, mais le taux de NAG urinaire pourrait être utile dans la détection de la néphropathie diabétique à un stade potentiellement réversible.

Mots clés : Diabète de type 1. Néphropathie diabétique. Microalbuminurie. Béta-thromboglobuline. N-acétyl-beta-D-glucosaminidase.

SUMMARY

While microalbuminuria indicates the glomerular damage of early diabetic nephropathy, tubular abnormalities also occur at an early stage of diabetic renal disease. Urinary excretion of beta-thromboglobulin (BTG) and N-acetyl-beta-D-glucosaminidase (NAG) was measured in 132 normotensive Type 1 (insulin-dependent) diabetic patients with no evidence of overt renal disease, of whom 35 had microalbuminuria and the remainder had normal urinary albumin excretion. Of 21 patients in whom there was a detectable urinary BTG concentration, only 8 (38 %) had a concurrently abnormal urinary albumin excretion. NAG excretion was elevated in 22 (63 %) of the 35 patients with microalbuminuria ; significant associations were also identified between urinary NAG excretion and smoking habit ($x^2 = 12.7$, $p < 0.001$) and glycated haemoglobin ($r = 0.49$, $p < 0.01$). It is concluded that measurement of urinary BTG is not of sufficient sensitivity to be of value in the detection of early diabetic renal disease, but measurement of urinary NAG may be of value in the detection of diabetic nephropathy at a potentially reversible stage.

Key Words : Type 1 diabetes. Diabetic nephropathy. Microalbuminuria. Beta-thromboglobulin. N-acetyl-beta-D-glucosaminidase.

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INTRODUCTION

Microalbuminaria, defined as a urinary albumin excretion rate of 20-200 ug min^{-1}, is an indicator of early diabetic renal disease, and predicts progression to clinical nephropathy (1-4). In a Type 1 diabetic population microalbuminuria has been associated with male gender, hypertension, advanced retinopathy and neuropathy, indicated by abnormal vibration sensation (5, 6). Increased urinary excretion of albumin is predominantly a measure of glomerular leakage, but tubular dysfunction is also a feature of early diabetic nephropathy. Increased urinary concentrations of small protein molecules, including beta-2-microglobulin (7-9), alpha-1-microglobulin (10, 11) and kappa light chains (10), which are freely filtered across the glomerular membrane, have been demonstrated in diabetic patients. It is possible that these proteins, and others such as retinol-binding protein (12, 13), may be of value in the early detection of diabetic renal damage.

Beta-thromboglobulin (BTG) is a platelet-specific protein which is located in the alpha granules and released during platelet activation. It occurs as a tetramer with a subunit molecular weight of 8 600 daltons and is normally present in very low concentrations in the urine. Urinary BTG was found to be increased in patients with Type 1 diabetes, when compared with normal controls (14), and its urinary excretion was significantly higher in patients who had nephropaty, with a correlation being observed with plasma creatinine and beta-2-microglobulin (15). The potential value of this urinary protein as a marker for early nephropathy has not been investigated previously.

Urinary N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme which is a much larger molecule than albumin and, although present in renal tubular cells, does not normally appear in the glomerular filtrate. Urinary NAG excretion has been studied previously in diabetic patients and may be of some value in identifying early diabetic nephropathy (11, 13, 16-19), although its precise role remains to be defined. In the present study, the urinary excretion of BTG and NAG was measured in a group of Type 1 diabetic patients with and without microalbuminuria. The association between urinary BTG and NAG and albumin excretion was examined and the relationship was determined between the urinary concentrations of these proteins and age, sex, duration of diabetes, blood pressure, glycaemic control, smoking status and presence of retinopathy in these diabetic patients.

METHODS

132 Type 1 diabetic patients were recruited from the Diabetic Clinic at the Royal Infirmary, Edinburgh. Patients were excluded if they had hypertension as defined by WHO criteria (> 160/95 mm Hg), a past history of renal disease, and haematuria or proteinuria on routine testing of urine with Multistix (Ames, Slough, UK), which identifies those with a urinary protein concentration of > 300 mg l^{-1} approximately. No patients had a plasma creatinine concentration > 150 umol l^{-1} or were taking any drug which might cause proteinuria. Of the participating patients, 97 (Group A) had no evidence of diabetic nephropathy, with at least three previous estimations of urinary albumin excretion consistently being within the normal range. The 35 other patients (Group B) had been identified previously as having microalbuminuria with an elevated urinary albumin/creatinine ratio of > 2.5 mg $mmol^{-1}$ in two or more first morning specimens of urine within the preceding year, this level having previously been shown (20) to predict an albumin excretion rate of > 30 ug min^{-1}.

The presence of clinically apparent retinopathy was assessed in each patient by direct ophthalmoscopy through dilated pupils and if present was classified as background or proliferative. Proliferative retinopathy was defined as the presence of soft exudates (cotton wool spots), active neovascularisation or scarring from previous photocoagulation therapy ; background retinopathy was identified by the presence of microaneurysms, haemorrhages or hard exudates. Seated blood pressure was recorded, after resting for 10 minutes, using a standard mercury sphygmomanometer. Details of drug therapy and smoking habit were obtained ; those who were smoking five cigarettes or more daily at the time ot the study were defined as « smokers ». No attempt was made to further quantify the number of cigarettes smoked. Clinical details of the two groups are given in Table 1. The patients in Group B were older, had a longer duration of diabetes, a higher mean glycated haemoglobin concentration and a greater prevalence of retinopathy.

On the day of attendance at the clinic, venous blood was withdrawn for estimation of glycated haemoglobin and plasma creatinine, and all patients collected a sample of the first urine voided immediately after rising, wich was subsequently divided into three aliquots. Urinary albumin excretion was measured, within 48 hours of collection, in one of these aliquots stored at 4 °C, with the other two samples, for determination of urinary BTG and NAG concentrations, being stored until analysis at −20 °C. Urinary albumin concentrations were measured by an immunoturbidometric assay (21) (Cambridge Life Sciences, Cambridge, UK) and urinary creatinine by the Jaffe reaction. BTG was measured by immunoassay (22), with the normal urinary concentration being less than 0.1 U ml^{-1}, and urinary NAG was determined by a colorimetric assay using a chromogenic substrate, 2-Methoxy-4-(2'-nitrovinyl) phenyl-2-acetamido-2-deoxy-Beta-D-gluco-pyranoside (23) (Thames Genelink, Deeside, UK). A urinary NAG/creatinine ratio of ⩾ 25 μmol h^{-1} $mmol^{-1}$ is considered abnormal, using this commercially available method. Glycated haemoglobin was estimated by electroendosmosis using agar plates (laboratory normal range 6.0-8.0 %).

Statistical Analysis

Chi squared and t-tests were used, where appropriate, to determine the association of BTG and the NAG/creatinine ratio with the other variables. Multiple regression analysis was also performed to assess the relative importance of the associations between urinary NAG/

TABLE I. — Clinical details of diabetic patients with normal urinary albumin excretion (Group A) and with microalbuminuria (Group B)

	Group A (n = 97)	Group B (n = 35)	Difference
Age (years and range)	34 (14-63 yrs)	41 (1-44 yrs)	$p < 0.02$
Duration of diabetes (years and range)	11 (6 mo-48 yrs)	19 (1-44 yrs)	$p < 0.001$
Sex	52 M : 45 F	22 M : 13 F	NS
Glycated haemoglobin (%)	9.5 (2.1)	11.2 (2.1)	$p < 0.001$
Plasma creatinine (mmol l^{-1})	79.2 (12.4)	84.4 (13.3)	NS
Systolic BP (mm Hg)	120 (17)	127.2 (22.2)	NS
Diastolic BP (mm Hg)	74 (10)	74 (10)	NS
* Smoking status (No (%) pts)			
— Smokers	23 (24 %)	6 (19 %)	NS
— Non-smokers	72 (76 %)	25 (81 %)	
* Retinopathy (No (%) pts)			
— Absent	79 (81 %)	14 (41 %)	
— Background	15 (15 %)	14 (41 %)	
— Proliferative	3 (3 %)	6 (18 %)	
— Retinopathy (Total)	18 (19 %)	20 (59 %)	$p < 0.001$

Mean (SD) where appropriate
* Data on smoking habit not available for 6 patients and on retinopathy for 1 patient.

creatinine ratio and urinary albumin/creatinine ratio, blood pressure, smoking status, retinopathy, glycaemic control, age and duration of diabetes.

RESULTS

Of the 35 patients previously identified as having microalbuminuria, only 21 had an albumin/creatinine ratio (ACR) of > 2.5 mg $mmol^{-1}$ in an early morning specimen of urine provided at the time of study. This apparent disparity can be explained by the recognised day to day variability in urinary albumin excretion in individual patients with diabetes (24, 25). Urinary BTG was above the level of detection of the assay (0.1 U ml^{-1}) in only 21 patients in total, 13 of whom (62 %) belonged to the group with established microalbuminuria (Group B), although only 8 of these patients (38 %) also had an elevated ACR in the specimen of urine provided for the study. Urinary BTG was associated with a longer duration of diabetes : 15 patients (71 %) with a detectable urinary BTG had diabetes for 10 years or more. The number of patients with a detectable urinary BTG was too small to establish an association with either retinopathy, smoking habit or glycated haemoglobin concentration.

The NAG/creatinine ratio was elevated in 55 patients. 22 patients (63 %) with microalbuminuria had an elevated NAG/creatinine ratio, including 16 (73 %) of those who had an elevated ACR at the time of study *(Fig. 1)*. A significant correlation was observed between the urinary NAG/creatinine ratio and glycated haemoglobin *(Fig. 2)*. Complete data on the NAG/creatinine ratio and retinopathy was not available on two patients and, for smoking habit, data was missing on six patients. However, an association was evident between urinary NAG/creatinine excretion and both of these parameters, with the NAG/creatinine ratio being elevated in 20 (57 %) of 35 patients with clinically apparent background or proliferative retinopathy, compared with 35 (37 %) of patients without retinopathy ($x^2 = 4.3$, $p < 0.05$). A similar increase in the prevalence of abnormal NAG/creatinine excretion was observed in smokers (20 (69 %) of 29 patients) compared with non-smokers (31 (32 %) of 97 patients) ($x^2 = 12.7$, $p < 0.001$). No association was observed between smoking habit and elevation of the urinary ACR. When the patients were grouped according to duration of diabetes *(Fig. 3)*, the mean urinary NAG/creatinine ratio was noted to rise progressively from the group with a duration of disease of less than 2 years to that with a duration of 10-20 years, although a correlation was not found between the NAG/creatinine ratio and the duration of diabetes considered as a continuous variable. No significant association was determined between the urinary NAG/creatinine ratio and either age, sex, systolic or diastolic blood pressure.

Multiple regression analysis was performed to determine the most important associations between the above variables and the NAG/creatinine ratio. The coefficients of each term in the multiple regression are shown in *Table II* and give an estimate of the difference in the NAG/creatinine ratio attributable to each variable independent of the other variables. Smoking habit, glycaemic control and the presence of microalbuminuria were all independently associated, but the presence of clinically apparent retinopathy just failed to achieve signi-

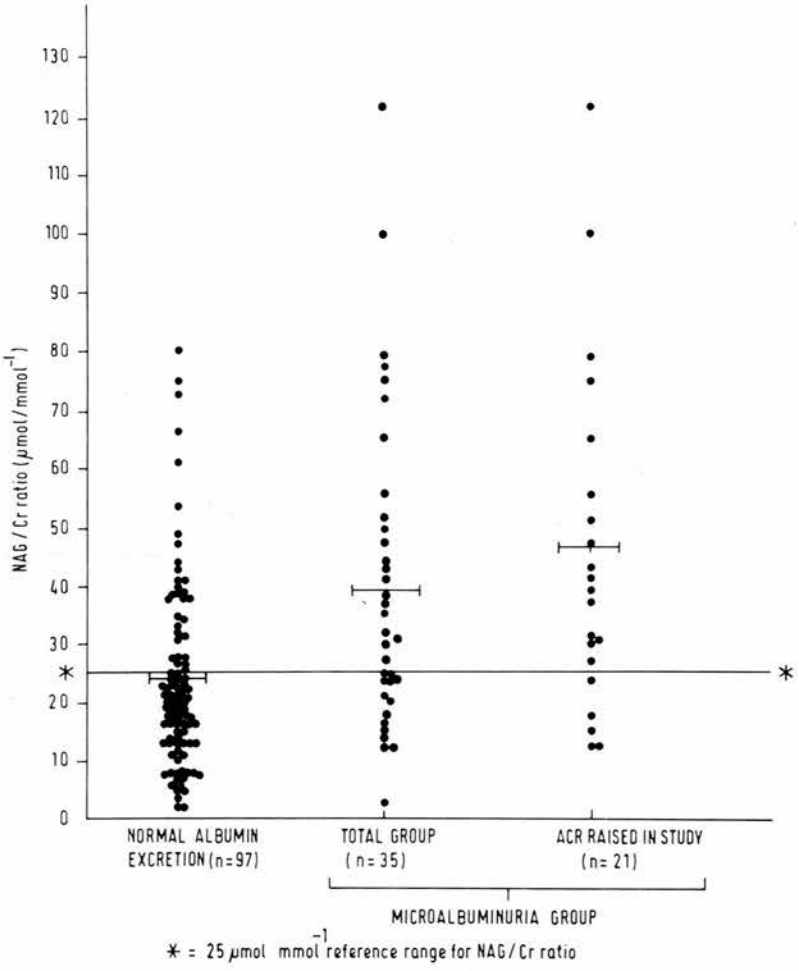

FIG. 1. — *Relationship between urinary excretion of NAG and albumin, both expressed as a ratio against urinary creatinine. Bars indicate mean values.*

ficance as an independently important variable. Thus the NAG/creatinine ratio was on average 18.2 umol h^{-1} $mmol^{-1}$ greater in smokers than non-smokers and 13.9 umol h^{-1} $mmol^{-1}$ greater in patients with microalbuminuria compared with those patients who had a consistently normal urinary albumin excretion. For each incremental rise of 1 % in the glycated haemoglobin, the NAG/creatinine ratio rose by 3.35 umol h^{-1} $mmol^{-1}$. The coefficients for age and duration of diabetes are given per year, and for blood pressure per mm Hg ; the association with these parameters was not statistically significant.

DISCUSSION

The ability to identify patients with early diabetic nephropathy, by the detection of microalbuminuria, has provided the possibility of earlier therapeutic intervention to delay progression of the renal disease, but the identification of the abnormal urinary excretion of other proteins has been pursued in an attemps to identify patients at an even earlier stage of diabetic renal disease than can be detected by microalbuminuria. Several proteins of small molecular weight have been studied as possible indicators of early diabetic nephropathy. An increased urinary excretion of beta-2-microglobulin has been reported in children and adolescents with Type 1 diabetes (9, 17), but other studies have failed to demonstrate an abnormality even in the presence of established diabetic nephropathy (26, 27). The low stability of beta-2-microglobulin (11, 12), both in vitro an in vivo, may partly explain these discrepant results, and the role of this protein as a potential marker of early tubular dysfunction appears to be limited. Alpha-1-microglobulin, kappa light chains and retinol-binding protein may be of greater diagnostic value, but further studies are required.

The platelet-derived protein, beta-thromboglobulin (BTG), has a low molecular weight and should therefore filter freely through the glomerular mem-

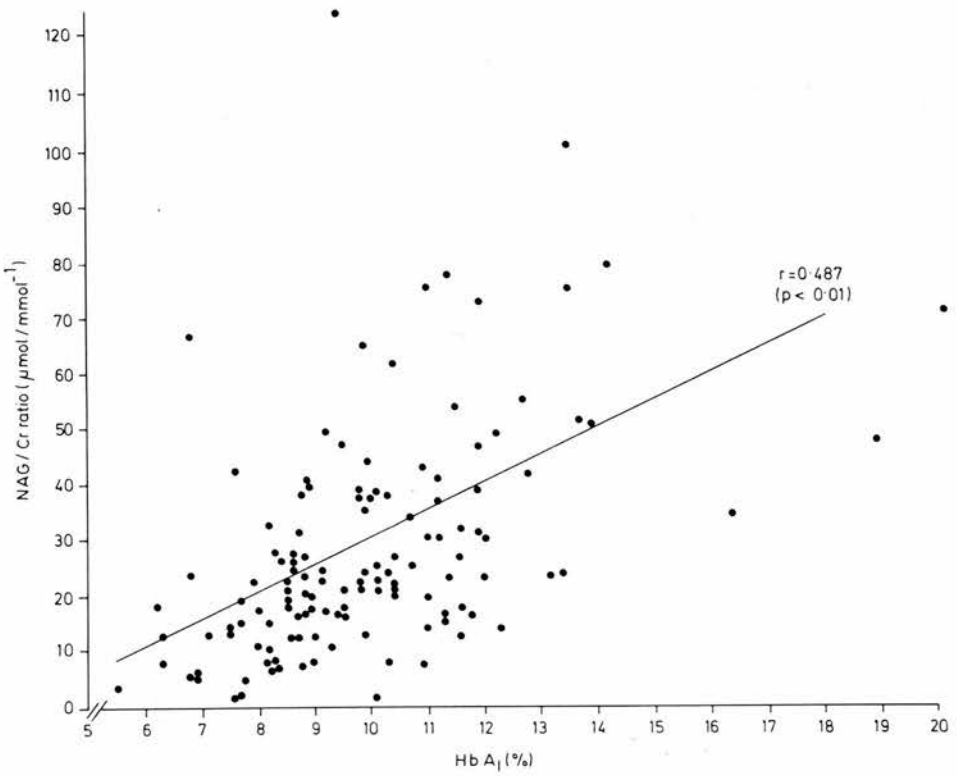

FIG. 2. — *Relationship between urinary NAG/creatinine ratio and HbA_1.*

TABLE II. — Coefficients, with 95 % confidence limits, from multiple regression analysis, using the NAG/creatinine rato as the dependent variable

Age (change per year)	0.09	(− 0.18, 0.36)
Duration of diabetes (change per year)	− 0.38	(− 0.77, 0.01)
Systolic blood pressure (change per mm Hg)	0.01	(− 0.17, 0.19)
Smoking habit (yes/no)	18.2	(10.7, 267)
Retinopathy (yes/no)	5.40	(− 2.53, 13.73)
Glycated haemoglobin (change per 1 %)	3.35	(1.92, 4.78)
Microalbuminuria (yes/no)	13.9	(6.5, 21.3)

brane. Reabsorption in the proximal tubule maintains a constant concentration of BTG excreted in the urine of approximately 0.5 % of the plasma concentration (28). BTG is released in response to platelet aggregation and activation (29), and a previous study of diabetic patients used BTG as a marker to examine the role of platelet activation in microvascular complications (15). Although that particular study did not find any correlation between urinary BTG and platelet survival, a strong correlation was observed with plasma creatinine and Beta-2-microglobulin, suggesting a possible role for BTG in the detection of renal disease in patients with diabetes. A further study demonstrated an increase in urinary BTG excretion in non-diabetic hypertensive patients with renal impairment (30) but to our knowledge the possible role of BTG as an indicator of early diabetic nephropathy has not been examined previously. In the present study, although urinary BTG excretion was elevated in a higher proportion of the diabetic patients with microalbuminuria and in those with a long duration of diabetes, the results do not suggest that measurement of the urinary concentration of this protein has an adequate degree of sensitivity to replace or even complement urinary albumin excretion as an indicator of early diabetic nephropathy.

An alternative approach to the identification of

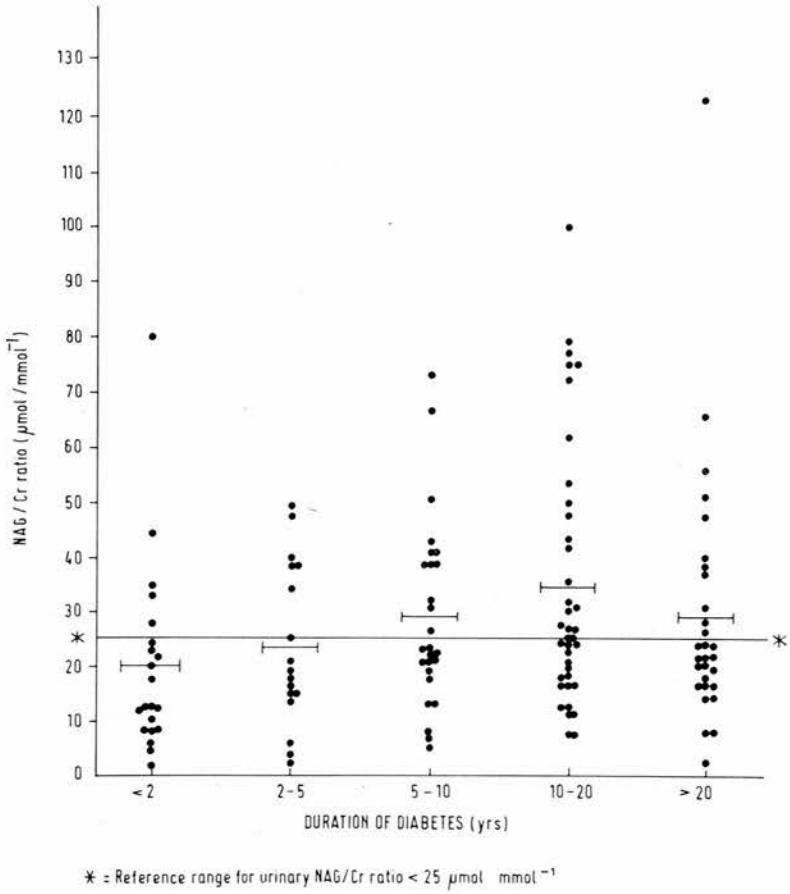

FIG. 3. — *Relationship between urinary NAG/creatinine ratio and duration of diabetes. Bars indicate mean values.*

early diabetic nephropathy is the measurement of the urinary enzyme, N-acetyl-beta-D-glucosaminidase (NAG). This enzyme, which has a molecular weight of 150 000 daltons, is too large to be filtered through the normal glomerular membrane although it is possible that in the presence of glomerular damage significant concentrations appear in the urine via this route. However, increased urinary excretion of NAG is likely to be predominantly caused by tubular damage, and increased urinary NAG concentrations have previously been demonstrated in diabetic patients who had either frank proteinuria or microalbuminuria (13, 16-18). The present study has confirmed this association, and similar correlations were found between the urinary NAG/creatinine ration and both glycaemic control and smoking habit. It was not possible to confirm an association with the presence of diabetic retinopathy because of the small number of patients affected by retinopathy detected clinically on direct ophthalmoscopy.

The association of the urinary NAG/creatinine ratio with glycated haemoglobin confirms the findings of previous studies (13, 17, 18). It has been demonstrated in dogs that urinary NAG excretion was related to the urinary glucose concentration (31) and, in the rat, increased urinary flow rate (resembling the osmotic diuresis secondary to hyperglycaemia) was associated with reduced tubular absorption of proteins (32). It is possible that similar mechanisms are operational in the human diabetic kidney wich would explain the observed association with glycaemic control.

The observed association between the urinary NAG/creatinine ratio and smoking adds to the increasing body of evidence that smoking may have a pathogenic role, in diabetic patients, in damaging the microcirculation of various organs, including the kidney (33). When the effects of smoking and glycaemic control were excluded by the use of multiple regression analysis, a significant correlation was still demonstrable between urinary NAG excretion and microalbuminuria.

The present study has shown that urinary BTG, in common with various other low molectular weight proteins, is not of value as a sensitive indicator of early diabetic nephropathy. Urinary NAG excretion, which can be estimated by a simple kit method,

may complement the measurement of urinary albumin excretion in the detection of early nephropathy, although further studies will be necessary before abnormalities of tubular function can be considered as a substitute for microalbuminuria as the most useful indicator of potentially reversible renal damage in diabetic patients.

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The Natural History and Associations of Microalbuminuria in Type 2 Diabetes During the First Year After Diagnosis

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The prevalence of microalbuminuria was assessed in 149 consecutive, newly-diagnosed and untreated patients with Type 2 diabetes, 129 of whom were followed up for 1 year, with at least three urine specimens being obtained during this period. At initial presentation, 39 (26 %) patients had a urinary albumin to creatinine ratio (ACR) of > 2.5 mg mmol^{-1} and compared with patients who had a normal ACR, they were older (64 (11) (SD) vs 58 (11) yr, $p < 0.002$), with higher random blood glucose (14.4 (4.5) vs 12.3 (4.4) mmol l^{-1}, $p < 0.02$) and glycosylated haemoglobin (13.0 (3.1) vs 11.3 (2.7) %, $p < 0.01$) concentrations. An elevated ACR was also associated with a higher systolic blood pressure (149 (22) vs 140 (22), $p < 0.05$) and the presence of macrovascular disease, particularly peripheral vascular disease ($p < 0.001$), with this association persisting after adjustment for the effect of age. Ten patients reverted to normal albumin excretion on improving blood glucose control, this group having a significantly higher glycosylated haemoglobin concentration at initial presentation than the group with a persistently elevated ACR (14.4 (2.5) vs 12.0 (3.0) %, $p < 0.05$). The 21 (16 %) patients with a persistently elevated ACR from diagnosis of Type 2 diabetes were older than those with normal albumin excretion throughout (64 (7) vs 58 (10) yr, $p < 0.02$) and it is probable that these patients have abnormal albumin excretion secondary to established renal pathology.

KEY WORDS **Type 2 diabetes Microalbuminuria Glycosylated haemoglobin Diabetic complications Vascular disease Nephropathy**

Introduction

Diabetic nephropathy is a major cause of mortality in Type 1 diabetes, particularly in those patients who develop the disorder early in life.[1] Although the relative risk of death from renal disease is apparently much lower in older patients,[2] between 32 % and 90 % of all diabetic patients undergoing dialysis therapy have Type 2 diabetes.[3,4]

Abnormal urinary excretion of protein has been reported in up to 50 % of patients with Type 2 diabetes,[5] and develops in many patients soon after diagnosis.[5,6] The presence of microalbuminuria in this group is associated with macrovascular disease,[7,8] and predicts the development of frank proteinuria[7] and increased mortality.[7,9] In contrast with a recent study of Type 1 diabetic patients,[10] a significant prevalence of microalbuminuria has been documented in Type 2 diabetes at diagnosis.[11–13] It has been suggested that this is a largely functional phenomenon, consequent upon the osmotic load presented by untreated hyperglycaemia,[12,14] but the presence of microalbuminuria may also indicate a long prodromal period of asymptomatic hyperglycaemia prior to diagnosis. The natural history of abnormal urinary albumin excretion following diagnosis has not previously been reported in detail in patients with Type 2 diabetes.

The aims of the current study were to assess the prevalence of microalbuminuria, and associations with other clinical features, in a cohort of patients with Type 2 diabetes, at initial presentation, and to examine the natural history of this phenomenon during the first year of treatment of hyperglycaemia.

Patients and Methods

The study group comprised 149 newly diagnosed, previously untreated patients (87 male, 62 female) presenting consecutively to the diabetic clinic of Edinburgh Royal Infirmary over a period of 9 months. In each case an initial diagnosis of Type 2 diabetes was made on the basis of clinical presentation, biochemical data, and the absence of ketosis. Details of current drug therapy were recorded, a full history was obtained and a careful clinical examination was performed to determine the presence of macrovascular disease. Cerebrovascular disease was defined as a history of stroke or transient ischaemic attacks; ischaemic heart disease as a history of angina pectoris or myocardial infarction and/or ECG evidence of ischaemia; and peripheral vascular disease as a history of intermittent claudication, rest pain, gangrene or amputation and/or the absence of the posterior tibial and dorsalis pedis pulses in at least one

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foot. Blood pressure was measured in the arm using a standard mercury sphygmomanometer after the patient had been seated for 5 min, and fundi were examined, using direct ophthalmoscopy after mydriasis, to assess retinopathy. This was defined as the presence of microaneurysms, haemorrhages, exudates or proliferative changes in one or both eyes. Weight and height were measured and body mass index (BMI) calculated. Exclusion criteria were known preceding renal disease, a plasma creatinine concentration of greater than 150 μmol l^{-1}, the presence of Albustix positive proteinuria, or urinary tract infection determined by culture of a midstream specimen of urine collected from all patients at their initial clinic visit.

The patients were contacted prior to attendance and asked to provide a specimen of the first urine voided, immediately after rising, on the morning of the clinic visit. This was stored at 4 °C until analysis for albumin was performed, within 72 h of collection. Urinary albumin concentration was determined by an immunoturbidimetric method[15] and urinary creatinine was measured by the Jaffe method. Results were expressed as a ratio of albumin to creatinine (ACR), and a level of greater than 2.5 mg $mmol^{-1}$ was considered abnormal, this level having previously been shown[16] to predict an albumin excretion rate of > 30 μg min^{-1}. Venous blood was withdrawn for estimation of random plasma glucose and glycosylated haemoglobin which was measured by electrophoresis using commercially available agar plates (laboratory normal range: 6.0–8.0 %).

Appropriate treatment was initiated to optimize blood glucose control, using dietary therapy supplemented by either sulphonylurea drugs or metformin where indicated. Patients were reviewed at 1–3 months, 6 months, and 12 months after diagnosis, and more frequently if indicated on clinical grounds. On each occasion an early morning sample of urine was collected for measurement of the ACR and venous blood was taken for estimation of glycosylated haemoglobin. During the study period, diabetic treatment was altered where necessary on the basis of the clinical response to management and the glycosylated haemoglobin results. Patients with at least two abnormal ACR measurements (excluding the initial specimen) in the year following diagnosis were defined as having 'persistent' microalbuminuria, and in practice all patients defined in this way had at least three abnormal results (Table 1). Those with only one abnormal result were defined as having 'intermittent' microalbuminuria and the remainder of the patients were considered to have 'normal' urinary albumin excretion for the purposes of this study.

Of the initial cohort of 149 patients, 15 were lost to follow-up within a year of commencing treatment, at least 2 having died during this period. A further 5 patients required conversion to insulin therapy within 3 months of their initial presentation and they were therefore excluded from further analysis as a diagnosis of Type 2 diabetes was in doubt. The remaining 129 patients were seen on at least a further three occasions during the year following diagnosis and urine collections were obtained

Table 1. Number of samples (total and abnormal) and median ACR level during the first year after diagnosis of Type 2 diabetes in patients defined as having persistent microalbuminuria and intermittent microalbuminuria

Persistent microalbuminuria group (n=21)				Intermittent microalbuminuria group (n=20)			
Patient	Samples (total)	Samples (abnormal)	Median ACR	Patient	Samples (total)	Samples (abnormal)	Median ACR
1	3	3	38.2	1	3	1	1.5
2	4	3	7.0	2	4	1	<1.0
3	4	4	6.3	3	3	1	<1.0
4	3	3	3.8	4	3	1	<1.0
5	4	4	4.1	5	3	1	<1.0
6	4	4	11.7	6	4	1	<1.0
7	3	3	4.6	7	4	1	2.1
8	3	3	10.8	8	3	1	1.8
9	3	3	5.9	9	3	1	<1.0
10	5	5	3.6	10	4	1	1.7
11	3	3	4.3	11	3	1	<1.0
12	3	3	4.9	12	3	1	<1.0
13	3	3	3.7	13	4	1	1.1
14	4	3	7.8	14	4	1	1.8
15	3	3	13.0	15	3	1	<1.0
16	5	4	4.9	16	3	1	<1.0
17	5	4	5.5	17	3	1	<1.0
18	3	3	3.5	18	3	1	1.8
19	3	3	16.8	19	4	1	1.2
20	3	3	11.2	20	3	1	1.5
21	4	4	27.4				

each time. On the basis of the subsequent ACR measurements, 88 patients (68 %) were defined as having normal urinary albumin excretion, 20 (16 %) as having intermittent microalbuminuria, and 21 (16 %) had persistent microalbuminuria. Median ACR levels and total number of specimens examined are given for the latter two groups in Table 1. All patients in the group defined as having normal albumin excretion had a median ACR level of < 1.0 mg mmol^{-1}. Within the group with persistent microalbuminuria, 18 patients (86 %) had also been shown to have an elevated ACR at the time of initial presentation, with only three having had a normal ACR at their first clinic visit. Of the remaining 21 patients with an initially elevated ACR, 8 were lost to follow-up (1 of whom was known to have died during the first year of treatment), 3 had intermittent microalbuminuria, and 10 had consistently normal albumin excretion thereafter.

Statistical Methods

Univariate comparisons between groups (with and without abnormal albumin excretion) were made using Student's *t*-tests for the continuous data and chi-squared tests (with Yates' correction) for the categorized data. Where the numbers in the table were small Fisher's exact test was used. In order to investigate the confounding effect of other variables (in particular age) on the above comparisons, logistic regression models were fitted.

Analysis of trends with the median ACR during the year following initial presentation was performed in two ways; by calculating the Spearman rank correlation of the median ACR with the continuous variables and also by dividing the ACR into four groups (< 1.0, 1.0–2.5, 2.5–5.0, and > 5.0 mg mmol^{-1}) and examining any trends across these groups. It is not appropriate to perform multiple linear regression on these data as 99 of the 129 patients followed up over 1 year had no detectable trend and therefore the distributional assumptions for multiple regression would not be satisfied.

Results

At Initial Presentation

Of the 149 patients, 39 (26 %) had an ACR of > 2.5 mg mmol^{-1} in the early morning specimen of urine provided at the time of their first clinic visit. The clinical characteristics of this group of patients, and those with a normal urinary albumin excretion at initial assessment, are shown in Table 2. Patients with microalbuminuria at presentation were older ($p = 0.002$) and had a higher initial random plasma glucose ($p = 0.014$) and glycosylated haemoglobin ($p = 0.002$). A significant association was also observed with systolic blood pressure ($p = 0.021$) but not with diastolic blood pressure, gender or BMI. The associations between the initial ACR and the presence of treated hypertension, retinopathy, and clinical evidence of macrovascular disease are shown in Table 3. A strong association was noted with macrovascular disease; 18 (47 %) of the microalbuminuria group had clinical evidence of macrovascular disease compared with 24 (23 %) of the remaining patients ($p = 0.009$). The most striking association was with peripheral vascular disease ($p = 0.001$).

Logistic regression was performed to correct for the effect of age and the adjusted *p* values are also shown in Tables 2 and 3. The strong association between an elevated ACR and peripheral vascular disease was retained ($p = 0.014$) but the associations with random plasma glucose and systolic blood pressure were lost and those with retinopathy ($p = 0.066$) and glycosylated haemoglobin ($p = 0.095$) fell just short of statistical significance. Further regression models were fitted to correct for the combined effect of age, sex, blood pressure, and glycosylated haemoglobin; peripheral vascular disease ($p = 0.056$) and retinopathy ($p = 0.090$) approached significance as having an association with an elevated initial ACR as an independent variable.

Table 2. Clinical characteristics of patients with normal and abnormal urinary albumin excretion at initial presentation with Type 2 diabetes, showing *p* values unadjusted and adjusted to correct for the effect of age

	Normal albumin excretion	Abnormal albumin excretion	*p* (unadjusted)	*p* (adjusted for age)
n	110	39		
Sex (M : F)	64 : 46	23 : 16	1.000	0.985
Age (yr)	58 (11)	64 (11)	0.002	–
BMI (kg m^{-2})	29.0 (4.9)	28.1 (6.0)	0.368	0.812
Random plasma glucose (mmol l^{-1})	12.3 (4.4)	14.4 (4.5)	0.014	0.155
Glycosylated haemoglobin (%)	11.3 (2.7)	13.0 (3.1)	0.002	0.095
Systolic BP (mmHg)	140 (22)	149 (22)	0.021	0.248
Diastolic BP (mmHg)	83 (11)	84 (14)	0.721	0.613

Mean (SD) unless otherwise stated.

Table 3. Presence of other complications in patients with normal and abnormal urinary albumin excretion at initial presentation with Type 2 diabetes, showing p values unadjusted and adjusted to correct for the effect of age

	Normal albumin excretion	Abnormal albumin excretion	p (unadjusted)	p (adjusted for age)
Retinopathy	6 (6)	5 (13)	0.276	0.066
Treated hypertension	15 (14)	9 (24)	0.293	0.243
Peripheral vascular disease	9 (9)	13 (34)	0.001	0.014
Cerebrovascular disease	4 (4)	3 (8)	0.583	0.905
Ischaemic heart disease	16 (15)	6 (16)	1.000	0.519
Total macrovascular disease	24 (23)	18 (47)	0.009	0.207

Number (%).

After Follow-up for 1 year

The clinical characteristics and associations of the two groups who were identified as having persistent microalbuminuria and normal albumin excretion during the year following diagnosis are shown in Tables 4 and 5. An age difference was again observed, the group with microalbuminuria being significantly older ($p = 0.014$). The only other clinical characteristic which achieved statistical significance was the BMI, the group with microalbuminuria having a lower BMI than the group with normal urinary albumin excretion ($p = 0.033$), but this association was lost when results were corrected for the effects of age. No difference was observed in the mean glycosylated haemoglobin concentrations between the two groups, either at initial presentation, or 6 months later. By contrast, in the 10 patients who initially had an elevated ACR, but reverted to a normal urinary albumin excretion on subsequent measurement, a trend was noted towards a higher random plasma glucose at presentation (14.9 (4.6) mmol l^{-1}) and the initial glycosylated haemoglobin was significantly higher at 14.4 (2.5) % (mean SD) than for either of these two groups ($p < 0.02$ vs group with normal urinary albumin excretion, and $p < 0.05$ vs group with persistent microalbuminuria). A greater number of patients with persistent microalbuminuria had evidence of macrovascular disease compared with the group with normal albumin excretion (45 % vs 25 %). The pattern was similar for peripheral vascular disease (25 % vs 12 %) and ischaemic heart disease (30 % vs 13 %), but because of the smaller number of patients, none of these results achieved statistical significance.

The effect of any trends in the actual value of the median ACR was examined by calculating Spearman rank correlations for the continuous variables. Results are shown in Table 6, with no statistically significant associations being demonstrated. Further analysis by

Table 4. Clinical characteristics of patients with normal urinary albumin excretion and persistent microalbuminuria during first year after diagnosis of Type 2 diabetes, showing p values unadjusted and adjusted to correct for the effect of age

	Normal albumin excretion	Persistent microalbuminuria	p (unadjusted)	p (adjusted for age)
n	88	21		
Sex (M : F)	48 : 40	14 : 7	0.445	0.190
Age (yr)	58 (10)	64 (7)	0.014	–
BMI (kg m^{-2})	29.1 (4.8)	26.5 (5.1)	0.033	0.081
Random plasma glucose at presentation (mmol l^{-1})	12.6 (4.5)	14.3 (5.2)	0.151	0.204
Glycosylated haemoglobin at presentation (%)	11.7 (2.8)	12.0 (3.0)	0.623	0.362
Glycosylated haemoglobin after 6 months treatment (%)	8.4 (1.5)	8.5 (1.4)	0.710	0.944
Systolic BP (mmHg)	142 (23)	145 (22)	0.621	0.650
Diastolic BP (mmHg)	84 (12)	82 (12)	0.675	0.964

Mean (SD) unless otherwise stated.

Table 5. Presence of other complications in patients with normal urinary albumin excretion and persistent microalbuminuria during first year after diagnosis of Type 2 diabetes, showing p values unadjusted and adjusted to correct for the effect of age

	Normal albumin excretion	Persistent microalbuminuria	p (unadjusted)	p (adjusted for age)
Retinopathy	7 (8)	2 (10)	1.000	0.505
Treated hypertension	14 (16)	4 (20)	0.962	0.964
Peripheral vascular disease	10 (12)	5 (25)	0.243	0.537
Cerebrovascular disease	2 (2)	1 (5)	0.473	0.310
Ischaemic heart disease	11 (13)	6 (30)	0.127	0.087
Total macrovascular disease	21 (25)	9 (45)	0.125	0.230

Number (%).

Table 6. Spearman rank correlations for the median ACR during the first year after diagnosis of Type 2 diabetes and age, body mass index, blood pressure, random plasma glucose, and glycosylated haemoglobin

Age	0.18
BMI	−0.10
Systolic BP	0.04
Diastolic BP	0.04
Random plasma glucose at presentation	0.12
Glycosylated haemoglobin at presentation	0.05
Glycosylated haemoglobin after 6 months treatment	0.02

No correlation reached statistical significance.

dividing the median ACR value into four groups also failed to reveal any clear trends, although the patient numbers in three of the groups were again small.

Discussion

Despite the accumulating information about abnormal urinary excretion of albumin in diabetic patients, the natural history of microalbuminuria in patients with Type 2 diabetes has not been examined prospectively from diagnosis. In the present study, the prevalence of increased urinary albumin excretion in newly diagnosed, untreated Type 2 diabetic patients was of similar magnitude to that reported previously by Uusitupa *et al.*[12] However, in contrast to this previous report,[12] the abnormal urinary albumin excretion at initial presentation was found to be associated with age, blood glucose control, systolic blood pressure, and evidence of macrovascular disease. The patients in the present cohort whose urinary albumin excretion was initially elevated had a mean age of 64 years and this was equivalent to the upper limit of Uusitupa *et al.*'s study population, which was restricted to patients within the age range 45–64 years.[12] The association with age may therefore be attributable to the older age group of the patients in the present study. Other studies of patients with longstanding Type 2 diabetes have demonstrated that microalbuminuria is more common in older patients.[9,13] Similarly the association with systolic blood pressure in the newly diagnosed patients reported in the present study has been described previously in association with Type 2 diabetes of longer duration,[5,6,9,17] although our findings would suggest that the effect of age may be an important factor in this association.

The relationship between increased urinary albumin excretion and macrovascular disease is now well recognized, not only in Type 2 diabetic patients,[8,9] but also in non-diabetic individuals.[18] Cardiovascular disease is the major cause of excess morbidity and mortality in Type 2 diabetes[19] and the presence of microalbuminuria appears to be an independent predictor of this.[8] In the present study, 47 % of the patients who had an increased urinary albumin excretion at initial presentation had some clinical evidence of macrovascular disease, compared with only 23 % of those who had a normal urinary albumin excretion. The most striking association was with peripheral vascular disease, which is consistent with a similar observation in Type 2 diabetic patients by Marshall and Alberti,[6] and this association was preserved even when an adjustment was made to correct for the influence of age.

Blood glucose control has been shown to correlate with the urinary albumin excretion rate in patients with Type 1 diabetes[20] and it has been suggested that the finding of an abnormal urinary albumin excretion at the time of presentation with Type 2 diabetes is predominantly a functional phenomenon, related to renal hyperfiltration occurring secondary to uncontrolled hyperglycaemia.[12,14] Schmitz *et al.* showed a fall in urinary albumin excretion and glomerular filtration rate in a small group of newly diagnosed Type 2 patients after a short period of improved blood glucose control,[14] and in larger groups of Type 1 diabetic patients near-normoglycaemia has also been demonstrated to reduce urinary albumin excretion.[21] The present study confirms an association between urinary

Relationship Between Urinary Excretion of Sodium and Dopamine in Type 1 Diabetic Patients with and without Microalbuminuria

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The relationship between urinary sodium and dopamine excretion was investigated in 40 normal males and in 48 normotensive, Type 1 diabetic males, 11 with microalbuminuria and 37 with normal albumin excretion. In all three groups a significant correlation was demonstrated and the regression lines were similar. Thus, no evidence was found that a defect in dopamine mobilization contributes to the early renal pathophysiological changes of Type 1 diabetes.

KEY WORDS **Type 1 diabetes** **Microalbuminuria** **Urinary sodium** **Urinary dopamine**

Introduction

Endogenous renal dopamine is believed to participate in sodium homeostasis and function under physiological conditions as a natriuretic hormone.[1] The urinary excretion of dopamine correlates directly with sodium output under normal conditions and responds appropriately to salt loading and depletion manoeuvres.[2,3]

Salt and water overload, and hypertension, are characteristic features of advanced diabetic nephropathy. With increasing chronic renal failure, urinary dopamine excretion progressively declines concurrent with glomerular filtration rate, and the response to salt loading is attenuated. It has been suggested that a relative deficiency of dopamine is a factor in the abnormal handling of sodium by the kidney.[4]

However, abnormal sodium retention has been recognized at a much earlier stage in the natural history of Type 1 diabetes, before the appearance of overt nephropathy.[5,6] It was therefore of interest to determine whether a defect in the mobilization of dopamine, relative to salt status, might provide an explanation for early pathophysiological changes in the condition. Therefore the relationship between sodium and dopamine excretion was examined in a group of normal males and in normotensive, Type 1 diabetic males both with and without evidence of early diabetic nephropathy (microalbuminuria).

Patients and Methods

A cohort of 48 normotensive, Type 1 diabetic males (age 33, range 25–38, years) was drawn from the Diabetic Department of the Royal Infirmary of Edinburgh. All had a creatinine clearance of >80 ml min^{-1} but 11 had evidence of microalbuminuria with an albumin/creatinine ratio, in an early morning urine specimen, of >3.0 g mol^{-1} on at least two occasions in the previous year. A control group consisted of 40 healthy normotensive males with normal renal function. The characteristics of the diabetic and control groups are given in Table 1. In each of the 11 patients defined as having microalbuminuria, a daily albumin excretion of >30 mg was confirmed on a 24-h urine collection. Although, in comparison with the diabetic patients who had normal albumin excretion, those patients with microalbuminuria tended to have a longer duration of diabetes, poorer blood glucose control, and lower creatinine clearance, none of these differences reached statistical significance.

All subjects were given careful instruction on the collection of a 24-h urine sample, at home, into a bottle containing 25 ml of 5 mol l^{-1} HCl to prevent oxidation of dopamine. No dietary restrictions were enforced but subjects were asked to refrain from alcohol during the collection. Blood pressure was measured, seated, by an experienced observer using a mercury sphygmomanometer, and hypertension was defined by WHO classification as a reading above 160/95 mmHg.

Glycosylated haemoglobin was measured using commercial agar plates, the normal range being 6.0–8.0 %. Retinopathy was assessed by ophthalmoscopy, after mydriasis, by two independent observers. Urinary albumin excretion was measured by an immunoturbidimetric method.[7] Urinary free dopamine was extracted onto alumina and assayed by high performance liquid chromatography with electrochemical detection.[8] Sodium was measured by flame photometry and plasma and urinary creatinine by the Jaffe method.

The sodium and dopamine data were log-transformed

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Table 1. Characteristics of 48 Type 1 diabetic males with and without evidence of early nephropathy (microalbuminuria) and 40 non-diabetic control subjects with normal renal function

	Diabetic (normal albumin excretion)	Diabetic (microalbuminuria)	Control
n	37	11	40
Age (years)	33 (25–38)	31 (19–44)	26 (20–39)
Blood pressure (mmHg)	128 (11)/77 (7)	124 (16)/78 (8)	126 (10)/74 (8)
Creatinine clearance (ml min^{-1})	122 (25)	102 (19)	110 (12)
Duration of diabetes (years)	12 (2–24)	15 (6–28)	
HbA_1 (%)	9.8 (1.6)	10.8 (0.9)	
Retinopathy			
None	11	2	
Background	23	5	
Proliferative	3	4	
Albumin excretion (mg 24-h^{-1})	10 (3–22)	141 (44–248)	

Mean (range or SD), or number.

before analysis. Correlation coefficients were calculated by least squares, and regression lines compared by F-ratios. Results are expressed as mean (SD) unless otherwise specified.

Results

It is evident from Table 2 that the study was performed under salt replete conditions and urinary sodium excretion was comparable in the three groups. Similarly, there was no significant difference in urinary dopamine output between diabetic patients and control subjects. There was, in both the control group and the two diabetic groups, a significant correlation between sodium and dopamine excretion (Table 2). The individual points and regression lines are shown in Figure 1 for the three groups. There was no significant difference between the slopes of the regression lines (Table 2).

Discussion

The positive correlation between urinary dopamine and sodium excretion in normal subjects has stimulated research into defining a physiological role for endogenous renal dopamine. It contributes, with other hormonal and neuronal influences, to a complex feedback mechanism which preserves sodium homeostasis. Thus the renal synthesis of dopamine is linked to prevailing salt status, and in turn acts to promote sodium excretion.[1] An inability to mobilize dopamine, appropriate to salt status, has been described in established essential hypertension,[9] a central feature of which may be a defect in the ability of the kidney to excrete sodium at normal perfusion pressures.[10] In addition, in a group of normotensive first-degree relatives of hypertensive patients, and in a normotensive population of Negroes, both groups at high risk of essential hypertension, the direct correlation between urinary sodium and dopamine was lost, suggest-

Table 2. Twenty-four hour urinary sodium and dopamine excretion in diabetic and control groups

	Diabetic (normal albumin excretion)	Diabetic (microalbuminuria)	Control	*p*
n	37	11	40	
Urinary sodium (mmol 24-h^{-1})	160 (58)	165 (55)	173 (54)	NS
Urinary dopamine (nmol 24-h^{-1})	1632 (437)	1450 (294)	1572 (453)	NS
Correlation coefficients	$r = 0.44$[a]	$r = 0.80$[a]	$r = 0.51$[b]	NS
Regression slope	0.38	0.55	0.46	NS
Regression intercept	2.40	1.95	2.17	NS

Mean (SD).
[a]$p < 0.01$; [b]$p < 0.001$.

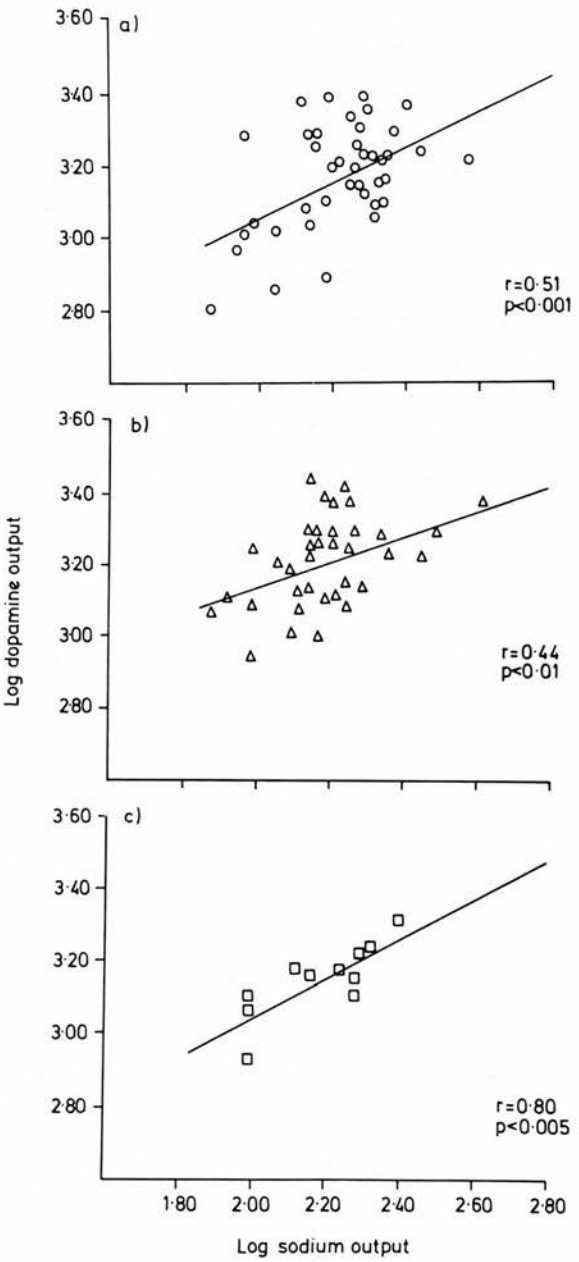

Figure 1. Correlation between urinary dopamine and sodium excretion with individual points and regression lines for the normal group (a), the diabetic group with normal albumin excretion (b), and the diabetic group with microalbuminuria (c)

ing that an abnormality of dopamine control antedates the development of essential hypertension.[11,12]

Recent research into the erythrocyte sodium-lithium counter-transport system has suggested that there may be a genetic linkage between the predisposition to essential hypertension[13] and diabetic nephropathy.[14] A number of observations suggest that patients with Type 1 diabetes do not handle sodium normally. It has been well demonstrated that there is a greater prevalence of clinical hypertension in diabetic patients,[15] and that blood pressure is significantly elevated in patients with early diabetic nephropathy (microalbuminuria) compared with those having normal albumin excretion,[16–18] although no difference in blood pressure was observed between the small groups of the present study. In addition, exchangeable sodium is elevated in patients with Type 1 diabetes even before the development of overt nephropathy or hypertension,[5,19] with a correlation between exchangeable sodium and blood pressure, suggesting that sodium retention may play a major role in the pathogenesis of the rise in blood pressure observed in the early stages of diabetic renal disease.[19] Reduced sodium excretion has been demonstrated in patients with Type 1 diabetes in response to volume expansion, and tubular sodium retention could be an early functional change in the diabetic kidney, possibly implicated in the development of nephropathy and hypertension.[20]

From our results, however, there is no evidence that a defect in the mobilization of dopamine exists prior to the development of hypertension or overt nephropathy. Dopamine output over 24 h was comparable in all the groups. The correlation between sodium and dopamine in urine was maintained in the diabetic patients, even in the presence of microalbuminuria, and the regression lines were similar in the three groups, suggesting that the sensitivity of dopamine response to salt intake and excretion was unaltered. Indirect evidence in support of these findings comes from the study of ter Wee and colleagues who demonstrated identical renal responsiveness to infused dopamine in insulin-dependent diabetic patients without clinical nephropathy or hypertension, and in normal control subjects.[21]

Other possible pathogenic mechanisms include defects in other vasoactive hormonal systems, such as the prostaglandin and kallikrein-kinin systems, and increased responsiveness to the renin-angiotensin system and catecholamines.[5]

Further sensitivity could be added to analysis of the relationship between sodium and dopamine excretion by dynamic studies assessing the dopamine response to sodium loading. On the present evidence, however, a defect in the mobilization of dopamine does not appear to predate the onset of clinical nephropathy, as indicated by the presence of frank proteinuria, and abnormalities demonstrated in patients with established renal disease are likely to represent a secondary phenomenon related to the underlying disease process.

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albumin excretion and the degree of preceding hyperglycaemia, but in the patients who initially had an increased ACR, examination of the level of urinary albumin excretion during the first year of treatment of diabetes revealed different categories of response. After management of diabetes was begun, one subgroup of 10 patients reverted to normal urinary albumin excretion, confirmed at each subsequent measurement. It could be postulated that a functional mechanism secondary to hyperglycaemia was the principal cause of the initially elevated urinary albumin excretion in these individuals. This premise would be supported by the observation that the glycosylated haemoglobin concentration at presentation was significantly higher in this group than in the patients who had normal urinary albumin excretion throughout the period of observation or in those patients who had persistent microalbuminuria. After 6 months of treatment, improved blood glucose control was achieved in all groups of patients with a mean glycosylated haemoglobin value of 8.5 %.

In the year following diagnosis of diabetes, a total of 21 patients were found to have persistent microalbuminuria, representing 16 % of the total group followed over this period of time. This compares with 8.2 % of Type 2 diabetic patients with persistently abnormal urinary protein excretion from diagnosis reported by Ballard *et al.*[13]. Although most (86 %) of these patients could be predicted by determining an abnormal ACR at diagnosis, the degree of hyperglycaemia did not appear to be the major determining factor in this group. Thus the glycosylated haemoglobin measurements at initial presentation and after 6 months of treatment were not significantly different from those of the group of patients who had normal urinary albumin excretion. However, a definite association with age was observed and a trend was discerned towards a greater prevalence of macrovascular disease, particularly ischaemic heart disease and peripheral vascular disease. The apparent association between macrovascular disease and urinary albumin excretion in newly diagnosed Type 2 diabetic patients is of interest in view of the recent suggestion that albuminuria is an indicator of widespread vascular damage, possibly reflecting a genetically determined abnormality of the heparan sulphate proteoglycan in the extracellular matrix and basement membrane.[22]

The present study indicates that persistent microalbuminuria is found in a significant number of Type 2 diabetic patients at the time of diagnosis. While factors such as blood pressure may be relevant in some patients,[23] in others this finding presumably indicates a specific diabetes-related complication, possibly related to the duration of exposure to the diabetic diathesis. Further study of this group may help to elucidate the pathophysiology of the vascular complications of diabetes.

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The Effects of Acute Insulin-induced Hypoglycaemia on Renal Function in Normal Human Subjects

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The effects of acute insulin-induced hypoglycaemia on renal function were studied in 8 normal male subjects. Plasma glucose (mean (SE)) fell from 4.6(0.2) to 1.3(0.2) mmol l^{-1}, the nadir being coincident with the acute autonomic reaction, and returned to the basal value over the following 120 min. Glomerular filtration rate declined from 118(6) to 95(4) ml min^{-1} at the glucose nadir ($p < 0.01$), and during the recovery phase returned to 118(7) ml min^{-1} (NS compared with basal). Renal plasma flow fell from 625(38) to 485(27) ml min^{-1} ($p < 0.01$), rising to 545(46) ml min^{-1} during recovery from hypoglycaemia (NS compared with basal). Following hypoglycaemia, urinary excretion of sodium and dopamine were reduced significantly, but the albumin excretion rate was unchanged. Plasma concentrations of adrenaline, noradrenaline, angiotensin II, and plasma renin activity increased in response to hypoglycaemia. These acute changes in renal function are probably caused by sympatho-adrenal activation and secretion of catecholamines, but other hormones, such as angiotensin II, may be contributory.

KEY WORDS **Insulin Hypoglycaemia Glomerular filtration rate Renal plasma flow Catecholamines Plasma renin activity Angiotensin II**

Introduction

Acute insulin-induced hypoglycaemia in human beings is a potent stimulus to autonomic neural activation and hormonal secretion. Stimulation of the sympatho-adrenal system provokes the secretion of catecholamines, which contribute to the profound haemodynamic and haemostatic changes associated with hypoglycaemia.[1,2] Myocardial contractility and cardiac output increase following hypoglycaemia,[3,4] and regional changes in blood flow have been studied in different vascular beds including those of the skin,[5] skeletal muscle,[3,6] the brain,[7] and the hepato-splanchnic system.[3,8] Although the overall vascular resistance declines rapidly it is evident that considerable regional differences in blood flow occur and the effect of hypoglycaemia on the perfusion of many organs and vascular systems is unknown. The aim of the present study was to examine the effect of acute insulin-induced hypoglycaemia on the human kidney by measuring changes in renal plasma flow, glomerular filtration rate, and other indices of renal function in normal subjects, and to relate these changes to the sympatho-adrenal activation and hormonal secretion which occurs in response to the hypoglycaemic stimulus.

Subjects and Methods

Subjects

The study was approved by the local Medical Ethical Advisory Committee and informed consent was obtained from all subjects. Eight healthy non-diabetic male volunteers were studied (age 30 (range 23–39) years), all of whom had a normal body mass index. None of the subjects was taking any medication, or had any intercurrent illness. No dietary restriction or modification was imposed prior to the study, but the ingestion of tea or coffee was prohibited overnight and smoking was not permitted during the study.

Protocol

Subjects were studied in a supine position after an overnight fast. Intravenous cannulae (Venflon, Viggo, Helsingborg, Sweden) were inserted into both antecubital fossae, one for venous blood sampling and the other for administration of drugs. Each subject was given ordinary tap water (10 ml kg^{-1}) to drink before commencing the study and thereafter the urinary fluid loss was measured and replaced by an equivalent volume of tap water (approximately 200 ml every 30 min) given orally. Fluid loss through sweating was not replaced. Mannitol 20 % (200 g l^{-1}) at a rate of 50 ml h^{-1} was given by IV infusion throughout the study period to maintain a urine flow rate of approximately 7–8 ml min^{-1}. Acute

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hypoglycaemia was induced by an IV bolus injection of soluble insulin (Human Actrapid, Novo, Basingstoke, UK) in a dose of 0.125 U kg-body-weight^{-1}. Heart rate, blood pressure, and capillary blood glucose were monitored at frequent intervals. The onset of the acute autonomic reaction (time = *R*) was indicated by a sudden increase in heart rate and accompanying changes in blood pressure, and sampling of blood and urine was timed from the start of the reaction to eliminate individual variability in the time taken to develop hypoglycaemia after the administration of insulin.

Laboratory Methods

Plasma glucose was measured by a glucose oxidase method using a Yellow Springs Analyser. Plasma renin activity was calculated by radioimmunoassay of angiotensin I generated under standard conditions (Campagne Oris, Gif-sur-Yvette, France). Catecholamines[9] and angiotensin II[10] were measured using radioenzymatic assays. The timing of drug administration and blood sampling is illustrated in Figure 1.

Measurements of Renal Function

Glomerular filtration rate (GFR) was estimated from inulin clearance. A constant infusion of polyfructosam (Inutest, Laevosam, Linz, Austria) in normal saline (10 g l^{-1} at a rate of 120 ml h^{-1}) was commenced after an initial IV bolus dose of 3.5 g, and a 60-min equilibration period was allowed. Inulin concentrations were measured by an auto-analyser after conversion to fructose.[11]

Effective renal plasma flow was estimated from the clearance of amino hippurate sodium (Merck, Sharp and Dohme, Hoddesdon, UK) using a 'single shot' technique. An IV bolus injection of amino hippurate sodium (PAH) in a dose of 10 mg kg-body-weight^{-1} was given at the start of each clearance period (Figure 1). Blood samples were taken at 5, 7, 10, 15, 20, 30, and 40 min after the injection and at the end of each clearance period. Plasma and urinary PAH were measured by the method of Bratton and Marshall, modified for use with an auto-analyser.[12] Plasma concentrations of PAH were <0.3 g l^{-1} before commencing each clearance period, and the area under the curve following each IV bolus injection was calculated assuming two-compartment kinetics.

Timed collections of urine were obtained at intervals of 30–60 min throughout the study and GFR and renal plasma flow were calculated: during the basal period; over the time of the acute autonomic reaction; and during the recovery phase (from *R* + 60 min to the conclusion of the study). The urinary excretion of dopamine, albumin, sodium, and kallikrein was measured from each specimen of urine collected. Urinary free dopamine was extracted onto alumina and assayed by HPLC with electrochemical detection.[13] Sodium was measured by plasma photometry and albumin by an immunoturbidimetric assay.[14] Kallikrein excretion was measured by the method of Amundsen *et al.*[15] and results were expressed as units of kallikrein activity per minute (nkat min^{-1}).

Control Studies

To ensure that any changes observed were related to hypoglycaemia alone, and not caused by any other component of the study protocol, separate control studies were performed in two of the subjects under identical conditions but without administration of insulin. Because of the obvious absence of hypoglycaemic symptoms, the nature of these control studies was not concealed from the subjects.

Statistical Analysis

Results, unless stated otherwise, are presented as mean (SE). The data for all subjects were analysed by one-way analysis of variance. The Wilcoxon Rank Sum test was applied to compare basal values with those at the time of the autonomic reaction and during the recovery phase, and a value $p < 0.05$ was regarded as significant.

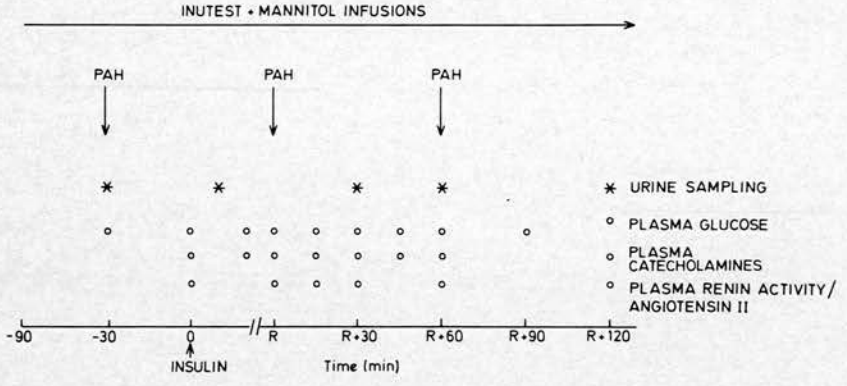

Figure 1. Protocol used to test the effect of acute hypoglycaemia on renal function. The timing of blood (○) and urine (*) sampling is indicated. Time = *R* indicates the onset of the autonomic reaction

Results

All subjects experienced a typical autonomic reaction to hypoglycaemia with acute onset of sweating, tremor, and other autonomic symptoms at a mean of 24 (range 22–28) min after the administration of insulin. In every case the autonomic reaction was concurrent with the nadir of plasma glucose. None of the subjects lost consciousness and administration of glucose was not required.

Metabolic and Hormonal Changes

The mean plasma glucose fell from 4.6(0.2) mmol l^{-1} to a nadir of 1.3(0.2) mmol l^{-1} at time = R ($p < 0.01$). Thereafter the recovery was biphasic with an initial rapid rise until $R + 15$ min, and a slower rise between $R + 15$ min and $R + 120$ min, by which time the plasma glucose was restored to basal concentrations at 4.4(0.2) mmol l^{-1} (Figure 2(a)).

Mean plasma adrenaline rose from a basal value of 0.2(0.04) nmol l^{-1} to a peak of 6.6(0.8) nmol l^{-1} at $R + 15$ min ($p < 0.01$). At $R + 120$ min plasma adrenaline was still significantly elevated at a concentration of 1.2(0.2) nmol l^{-1} ($p < 0.01$ vs basal). A rise in noradrenaline was also observed, from a basal value of 2.8(0.3) nmol l^{-1} to 4.6(0.6) nmol l^{-1} at time = R ($p < 0.01$), returning to the basal concentration by $R + 45$ min (Figure 2(b)). Plasma renin activity increased from a basal value of 0.91(0.18) ng-Angl l^{-1} to 1.42(0.18) ng-Angl l^{-1} at $R + 15$ min ($p < 0.01$) and remained elevated until $R + 60$ min, subsequently declining to basal values at $R + 120$ min (Figure 2(c)). Angiotensin II concentrations rose from 4.4(0.5) ng l^{-1} in the basal period to a peak of 15.1(4.1) ng l^{-1} at $R + 30$ min, and had returned to the basal value by $R + 120$ min (Figure 2(d)).

Renal Function

GFR fell from a basal value of 118(6) ml min^{-1} to 95(4) ml min^{-1} during the period of the hypoglycaemic reaction ($p < 0.01$), returning to 118(7) ml min^{-1} in the recovery phase. Examination of individual results revealed a consistent response in each subject (Figure 3(a)).

Renal plasma flow fell from a basal value of 625(38) ml min^{-1} to 485(27) ml min^{-1} during the period of the autonomic reaction to hypoglycaemia ($p < 0.01$), rising to 545(46) ml min^{-1} in the recovery phase (NS compared with basal). The rapid fall in renal plasma flow occurred in all 8 individual subjects following hypoglycaemia, but in 2 subjects a rise in renal plasma flow did not occur during the recovery phase (Figure 3(b)).

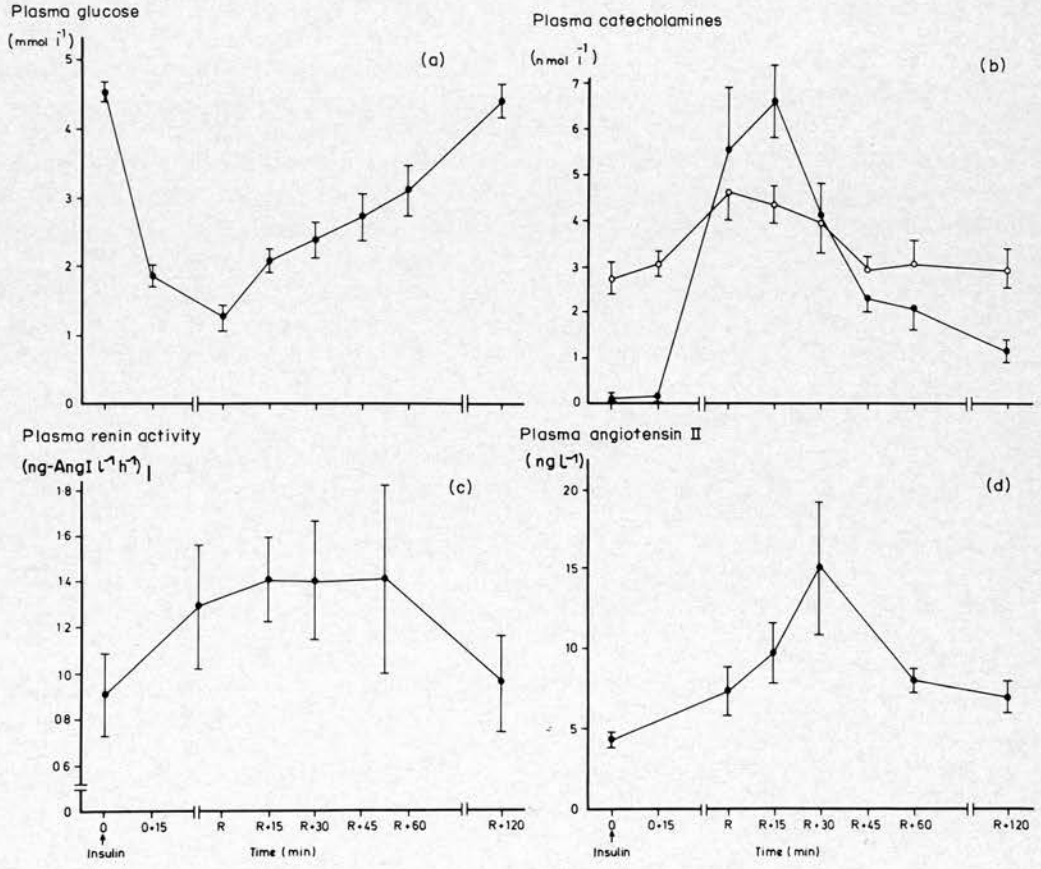

Figure 2. Mean response (SE) of (a) plasma glucose, (b) plasma adrenaline (●) and noradrenaline (○), (c) plasma renin activity, and (d) plasma angiotensin II following injection of intravenous soluble insulin (0.125 U kg^{-1}) at time 0. Time = R marks the onset of the autonomic reaction

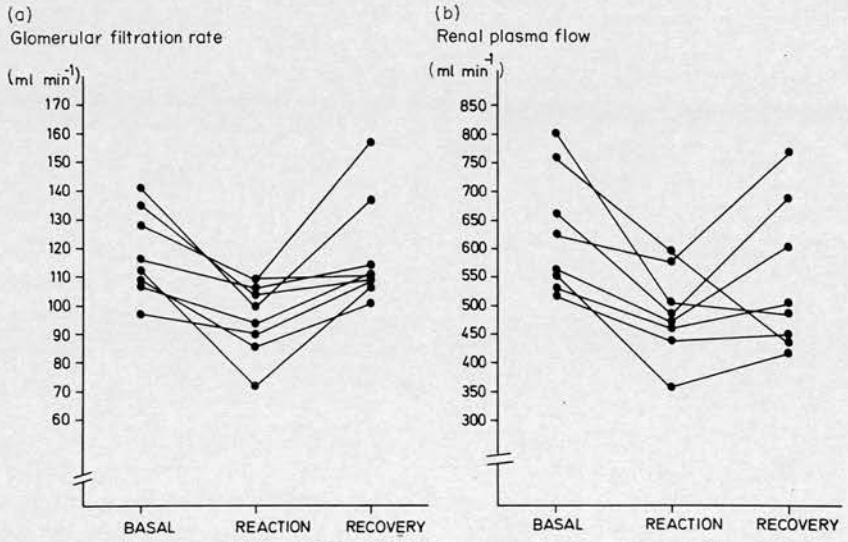

Figure 3. Changes in (a) GFR and (b) renal plasma flow following acute hypoglycaemia in the 8 subjects

The effect of acute hypoglycaemia on the other urinary measurements is shown in Table 1. Urine volumes did not fall significantly during the study and similarly there were no significant changes in the excretion rates of kallikrein and albumin. Urinary dopamine and both absolute and fractional sodium excretion were significantly reduced following hypoglycaemia and remained depressed during the recovery phase.

Control Studies

Blood glucose remained unchanged throughout, with no significant hormonal responses. Renal function was unaltered and no significant changes were observed in any of the other urinary measurements.

Discussion

Acute hypoglycaemia in humans has a pronounced effect on the vascular perfusion and function of several organs, which is associated with the activation of the autonomic nervous system and the stimulated secretion of various hormones. In the present study the effects of acute insulin-induced hypoglycaemia on the kidney were examined in normal human volunteers. During the period of hypoglycaemia, a transient but substantial reduction in GFR and renal plasma flow occurred (19 % and 22 %, respectively, from basal values). As the urine volumes did not fall significantly during the study, these changes are likely to represent a true phenomenon, and do not simply result from problems with bladder emptying or increased dead space wash-out.

Table 1. Urine volumes and urinary sodium, dopamine, kallikrein, and albumin excretion in response to acute hypoglycaemia

	Basal period	Time of hypoglycaemia	Recovery period	*p*
Urine volume (ml min^{-1})	7.6 (1.0)	6.7 (0.6)	5.9 (0.6)	NS
Absolute sodium excretion (μmol min^{-1})	403 (47)	273 (45)	205 (30)	< 0.005
Fractional sodium excretion (%)	2.5 (0.3)	2.0 (0.8)	1.3 (0.6)	< 0.01
Dopamine (μmol min^{-1})	614 (198)	132 (29)	150 (29)	< 0.02
Kallikrein (nkat min^{-1})	13.9 (1.4)	12.9 (2.4)	8.7 (0.8)	NS
Albumin excretion rate (μg min^{-1})	33.1 (13.7)	15.5 (2.8)	18.9 (3.6)	NS

Mean (SE).
Significance is tested between the time of hypoglycaemia and basal value.

The brisk rise in plasma catecholamines in response to acute hypoglycaemia had a close temporal relationship to the observed changes in renal function and may have been responsible for some of these effects, but direct stimulation of efferent sympathetic nerves *per se* may also be of importance.[16] Intravenous infusions of either noradrenaline or adrenaline have been shown to cause a pronounced reduction in renal blood flow in normal humans, although no significant changes in GFR were observed.[17] Many other hormones are released in response to hypoglycaemia which may have acute effects on renal function. Both renin and angiotensin II were secreted in response to hypoglycaemia and may have contributed to the observed changes in renal haemodynamics observed in the present study. Angiotensin II is a potent vasoconstrictor,[18] its effects including the enhancement of sympathetic tone[19] and facilitation of noradrenergic neurotransmission,[20] and the intrarenal infusion of angiotensin II has been shown to cause a significant reduction in both GFR and renal plasma flow in diabetic rats with hyperfiltration.[21] The increase in plasma renin activity in response to hypoglycaemia is probably associated with the stimulatory effect of elevated concentrations of plasma catecholamines as the rise of plasma renin activity can be prevented by β-adrenergic blockade.[22,23] However the release of renin following hypoglycaemia can occur independently of sympathoadrenal activation as shown in sympathectomized humans, in whom catecholamines are not released,[24] and can be stimulated by non-adrenergic mechanisms or haemodynamic changes.

The fall in plasma glucose *per se* may influence renal haemodynamics. Mogensen *et al.*[25] showed a 9 % reduction in GFR and a 13 % reduction in renal plasma flow when the blood glucose was reduced from 13.9 mmol l^{-1} to 6.5 mmol l^{-1} in 5 patients with Type 1 diabetes mellitus, without a rise in plasma catecholamine concentrations. In that study it was postulated that the changes in renal function may have resulted from increased sympathetic activity in response to a reduction in plasma volume induced by insulin. Subsequent studies, however, demonstrated that GFR and renal plasma flow remained unchanged in diabetic patients during a hyperinsulinaemic, euglycaemic clamp,[26,27] and that both these variables fell only when blood glucose was allowed to decline. Conversely, experimental induction of moderate hyperglycaemia in normal[28,29] and in diabetic human subjects[29] has been shown to cause a significant increase both in GFR and renal plasma flow. Although alteration of plasma volume has been postulated as a cause of this phenomenon,[30] similar changes could not be induced by a saline infusion.[29]

Insulin inhibits the rate of renal excretion of electrolytes,[31,32] so the reduction in urinary sodium excretion observed in the present study was not unexpected. This effect is partly independent of blood glucose concentration,[31] and in addition insulin is known to enhance the tubular reabsorption of sodium.[33] A close correlation between the urinary excretion of sodium and dopamine is recognized in the normal kidney,[34] and in the present study the changes in urinary dopamine excretion in response to hypoglycaemia paralleled those of sodium.

The lack of any significant changes in urinary albumin excretion following hypoglycaemia in normal subjects is of interest, as it has been suggested that albumin excretion is increased in Type 1 diabetic patients when the blood glucose is reduced by insulin infusion, particularly in those patients who have higher basal albumin excretion rates,[25,27] although Christensen *et al.*[35] have demonstrated a fall in albumin excretion in a group of patients with diabetes of long duration who had frank proteinuria. Considerable individual variation was observed in the present study in the basal urinary excretion of albumin, with a surprisingly high value being recorded in 2 subjects, but the albumin excretion rate remained low thereafter during acute hypoglycaemia and in the recovery period. It is not, however, justified to extrapolate this observation in normal subjects to diabetic patients, an investigation of whom is currently in progress.

Many patients with Type 1 diabetes are frequently exposed to hypoglycaemia and are presumably subjected to a similar transient modification of renal function. Diabetic patients who have nephropathy, with varying degrees of renal impairment, may be more vulnerable to the rapid changes in renal perfusion which are induced by hypoglycaemia. Furthermore the rise in haematocrit and plasma viscosity[3,36] and the enhancement of platelet activation and aggregation[37,38] which are precipitated by hypoglycaemia, may exacerbate pre-existing renal microangiopathy by potentially enhancing ischaemia.[2] Further studies are required to investigate renal function during acute hypoglycaemia in diabetic patients and the possible effects of hypoglycaemia on the pathogenesis and acceleration of diabetic nephropathy.

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Changes in Renal Function During Acute Insulin-induced Hypoglycaemia in Patients with Type 1 Diabetes

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The effect of acute hypoglycaemia on renal function was examined in eight male patients with Type 1 diabetes who had normal urinary albumin excretion. Insulin was given as a bolus intravenous injection (0.125 U kg^{-1}) and plasma glucose fell to a nadir of 1.6 (SE 0.2) mmol l^{-1}, with all patients experiencing an acute autonomic reaction. Renal plasma flow fell from 674 (106) to 540 (198) ml min^{-1} during hypoglycaemia ($p < 0.01$) and returned to 655 (181) ml min^{-1} (NS vs baseline). Glomerular filtration rate (GFR) declined from 143 (23) to 110 (36) ml min^{-1} during hypoglycaemia ($p < 0.02$), rising to 150 (44) ml min^{-1} in the recovery period (NS vs baseline). The urinary flow rate and urinary albumin excretion rate both fell significantly in response to hypoglycaemia (10.6 (1.2) to 4.7 (1.1) ml min^{-1}; $p < 0.002$, and 46.2 (10.6) to 26.0 (10.5) μg min^{-1}, respectively). Urinary dopamine excretion also declined, from 322 (37) to 211 (29) μmol min^{-1} ($p < 0.005$) but sodium excretion was unchanged. Plasma adrenaline concentration (0.2 (0.03) to 1.7 (0.4) nmol l^{-1}; $p < 0.01$) and plasma renin activity (0.49 (0.13) to 1.08 (0.17) ng-Ang I l^{-1} h^{-1}; $p < 0.01$) increased during hypoglycaemia, but changes in plasma noradrenaline and angiotensin II levels did not attain significance. These acute changes in renal function, observed during hypoglycaemia in diabetic patients, may result from direct stimulation of the efferent sympathetic nerves to the kidney, complemented by the hormonal changes induced by hypoglycaemia.

KEY WORDS **Insulin Hypoglycaemia Glomerular filtration rate Renal plasma flow Catecholamines Angiotensin II Sympathetic nervous system**

Introduction

Acute insulin-induced hypoglycaemia in humans stimulates autonomic neural activation and secretion of various hormones, including catecholamines, angiotensin II[1,2] and vasopressin.[3,4] Profound haemodynamic and haemostatic changes are associated with hypoglycaemia[5–7] and catecholamines play an important role in determining these changes. While there is an overall fall in vascular resistance, considerable differences in regional blood flow have been observed in several major organs and vascular systems.[2,7–13] In the kidney, a significant reduction in effective renal plasma flow and glomerular filtration rate has been demonstrated in response to acute hypoglycaemia in non-diabetic human subjects.[2] The aim of the present study was to examine the effect of acute insulin-induced hypoglycaemia on renal function in Type 1 diabetic patients who had normal urinary albumin excretion, a group not previously studied.

Patients and Methods

Patients

Eight male patients with Type 1 (insulin-dependent) diabetes were recruited from the diabetic clinic at the Royal Infirmary of Edinburgh. Their age was 29 (range 24–40) yr and all had a normal body mass index. Duration of diabetes was 8 (2–15) yr and total glycated haemoglobin at the time of study was 9.0 (SD 2.4) %, with a laboratory normal range of 5.0–8.0 %. The patients had no history or clinical evidence of hypertension, and had no specific diabetic complications. In particular, all had normal plasma creatinine levels and had a urinary albumin excretion of < 30 mg in each of two 24-h collections. Urinary tract infection was excluded in every patient by culture of a mid-stream specimen of urine. None of the patients smoked, were taking medication apart from insulin, or had any intercurrent illness at the time of study. Ingestion of tea or coffee was not permitted for 12 h before the study.

The protocol for the study had been approved by the local medical ethical committee and all participating patients gave their written consent.

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Protocol

The patients were admitted at 0800 h on the day of the study, having fasted since 2200 h on the previous evening. Only unmodified insulin had been administered during the 24-h period before the study, with the last insulin dose being administered prior to an evening snack. Intravenous cannulae (Venflon, Viggo, Helsingborg, Sweden) were inserted into both antecubital fossae, one for blood sampling and the other for administration of drugs. An intravenous infusion of 50 g l^{-1} glucose together with unmodified insulin (Human Actrapid, Novo Nordisk, Crawley, UK) was commenced to maintain the plasma glucose concentration in the 5.0–10.0 mmol l^{-1} range over the subsequent hour. In three patients with a plasma glucose on admission of > 10.0 mmol l^{-1} the insulin infusion was adjusted as necessary to reduce the concentration below this level. The infusion was then discontinued and acute hypoglycaemia was induced by an intravenous bolus injection of Human Actrapid in a dose of 0.125 U kg-body-weight^{-1}, this technique being chosen in order to achieve maximal autonomic and counter-regulatory hormone stimulation. Heart rate, blood pressure, and capillary blood glucose were monitored at frequent intervals during the study and the onset of the acute autonomic reaction (time *R*) was identified by the sudden increase in heart rate and sweating. Subsequent sampling of blood and urine was measured from this time to eliminate individual variability in the time taken from the administration of insulin to the onset of the acute autonomic reaction to hypoglycaemia. Fluid loading was carried out by giving the patients tap water to drink (10 ml kg^{-1}) at the time of admission, and the volume of urine passed subsequently during the study was replaced orally by an equivalent volume of tap water. An intravenous infusion of mannitol (200 g l^{-1}) was also administered continuously at a rate of 50 ml h^{-1} throughout the study to maintain a diuresis.

Measurements of Renal Function and Hormonal Concentrations

Effective renal plasma flow was estimated by the measurement of clearance of para-amino hippurate sodium (PAH; Merck, Sharp and Dohme, Hoddesdon, UK). An intravenous bolus injection of PAH in a dose of 10 mg kg-body-weight^{-1} was given at three time-points (30 min prior to insulin administration, time *R* and *R* + 60 min) with blood samples for PAH measurement being taken before each injection and then after 5, 7, 10, 15, 20, 30, and 40 min. The effective renal plasma flow was calculated, using two-compartment kinetics, from the area under the curve of plasma PAH concentrations following each IV bolus injection.

Glomerular filtration rate (GFR) was estimated from clearance of polyfructosan (Inutest, Laevosan, Linz, Austria), administered as a constant infusion in normal saline (10 g l^{-1} at a rate of 120 ml h^{-1}) after an initial intravenous bolus dose of 3.5 g. Measurements were commenced after a 60-min equilibration period, to allow attainment of steady state plasma levels of polyfructosan. Blood and urine samples were collected at four time-points (30 min prior to insulin administration, 10 min after insulin administration, time *R* + 60 min and time *R* + 120 min) and measurement of polyfructosan concentrations on these specimens allowed calculation of GFR in the basal period, over the time of the acute hypoglycaemic reaction and in the recovery period.

Urinary excretion of dopamine, albumin, and sodium were calculated from each urine specimen. Blood samples were withdrawn before insulin was administered and at time *R*, *R* + 15 min, *R* + 30 min, *R* + 45 min, *R* + 60 min, *R* + 90 min, and *R* + 120 min for measurement of plasma glucose, plasma renin activity, catecholamines, and angiotensin II.

Laboratory Methods

Plasma glucose was measured by a glucose oxidase method using a YSI analyser (Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma renin activity was calculated by radioimmunoassay of angiotensin I generated under standard conditions (Campagne Oris, Gif-Sur-Yvette, France). Plasma adrenaline and noradrenaline[14] and plasma angiotensin II[15] were measured using radioenzymatic assays. Blood and urine polyfructosan concentrations were measured by an autoanalyser after conversion to fructose,[16] and PAH was estimated by the method of Bratton and Marshall, modified for use with an autoanalyser.[17] Plasma concentrations of PAH were less than 0.3 g l^{-1} before each IV bolus injection. This technique for measurement of GFR has an intra-individual co-efficient of variation (CV) of 13.4 % and for measurement of renal plasma flow the intra-individual CV is 9.4 %. Urinary sodium was measured by plasma photometry and albumin by an immunoturbidimetric assay.[18] Urinary free dopamine was extracted onto alumina and assayed by HPLC with electrochemical detection.[19]

Statistical Analysis

Results, unless otherwise stated, are presented as mean (SE). Paired *t*-tests were applied to compare basal values with values during the period of the autonomic reaction to hypoglycaemia and during the recovery phase.

Results

All eight patients experienced a typical autonomic reaction to hypoglycaemia with the onset occurring at a mean of 35 (range 22–54) min after insulin administration. The nadir of plasma glucose occurred at time *R* in three patients and at time *R* + 15 min in five patients. Five patients required an intravenous bolus of 10 g glucose to accelerate recovery because significant neuroglycopenia

had developed and/or a plasma glucose level of < 2.0 mmol l^{-1} persisted after time $R + 30$ min.

Metabolic and Hormonal Changes

Plasma glucose fell from 6.2 (0.7) to 1.7 (0.2) mmol l^{-1} at time R ($p < 0.001$) and 1.6 (0.2) mmol l^{-1} at time $R + 15$ min ($p < 0.001$) with recovery thereafter to a level of 5.1 (0.6) mmol l^{-1} by time $R + 120$ min (NS compared with basal value) (Figure 1(a)).

Plasma adrenaline rose from a baseline value of 0.2 (0.03) nmol l^{-1} to a peak of 1.7 (0.4) nmol l^{-1} at time $R + 15$ min ($p < 0.01$), falling by time $R + 120$ min to 0.3 (0.05) nmol l^{-1} (Figure 1(b)). The minor increase in plasma noradrenaline from a baseline value of 1.3 (0.4) nmol l^{-1} to a peak level of 1.8 (0.3) nmol l^{-1} at time $R + 15$ min was not statistically significant.

Plasma renin activity rose from 0.49 (0.13) ng-Ang I l^{-1} h^{-1} in the basal period to a peak of 1.08 (0.17) ng-Ang I l^{-1} h^{-1} at time $R + 15$ min ($p < 0.01$), remaining significantly elevated until time $R + 30$ min and then returning to the basal value by time $R + 120$ min (Figure 1(c)). Plasma angiotensin II concentrations possibly rose from a basal value of 5.1 (0.9) ng l^{-1} to a peak of 7.4 (1.5) ng l^{-1} at time $R + 60$ min ($p = 0.065$), with a fall to 5.8 (0.6) ng l^{-1} by time $R + 120$ min (Figure 1(d)).

Renal Function

Effective renal plasma flow fell from 674 (106) ml min^{-1} in the basal period to 540 (198) ml min^{-1} during the period of hypoglycaemia ($p < 0.01$), rising to 655 (181) ml min^{-1} in the recovery period (NS vs baseline) (Figure 2(a)). GFR fell from a basal value of 143 (23) ml min^{-1} to 110 (36) ml min^{-1} during the period of the autonomic reaction to hypoglycaemia ($p < 0.02$), rising to 150 (44) ml min^{-1} in the recovery period (NS vs baseline) (Figure 2(b)). GFR and renal plasma flow were closely correlated both in the basal period ($r = 0.75$, $p < 0.05$) and during the period of hypoglycaemia ($r = 0.96$, $p < 0.001$). The changes in GFR and renal plasma flow were also well correlated ($r = 0.78$, $p < 0.05$).

The effects of acute hypoglycaemia on urine volume and urinary excretion of sodium, dopamine, and albumin are shown in Table 1. Both urine volume and albumin excretion rate fell significantly during hypoglycaemia. Neither fractional nor absolute urinary sodium excretion changed significantly but a significant fall in urinary dopamine excretion was observed which did not correlate with urinary flow ($r = 0.20$) and was not dependent on changes in GFR, renal plasma flow or urinary sodium excretion.

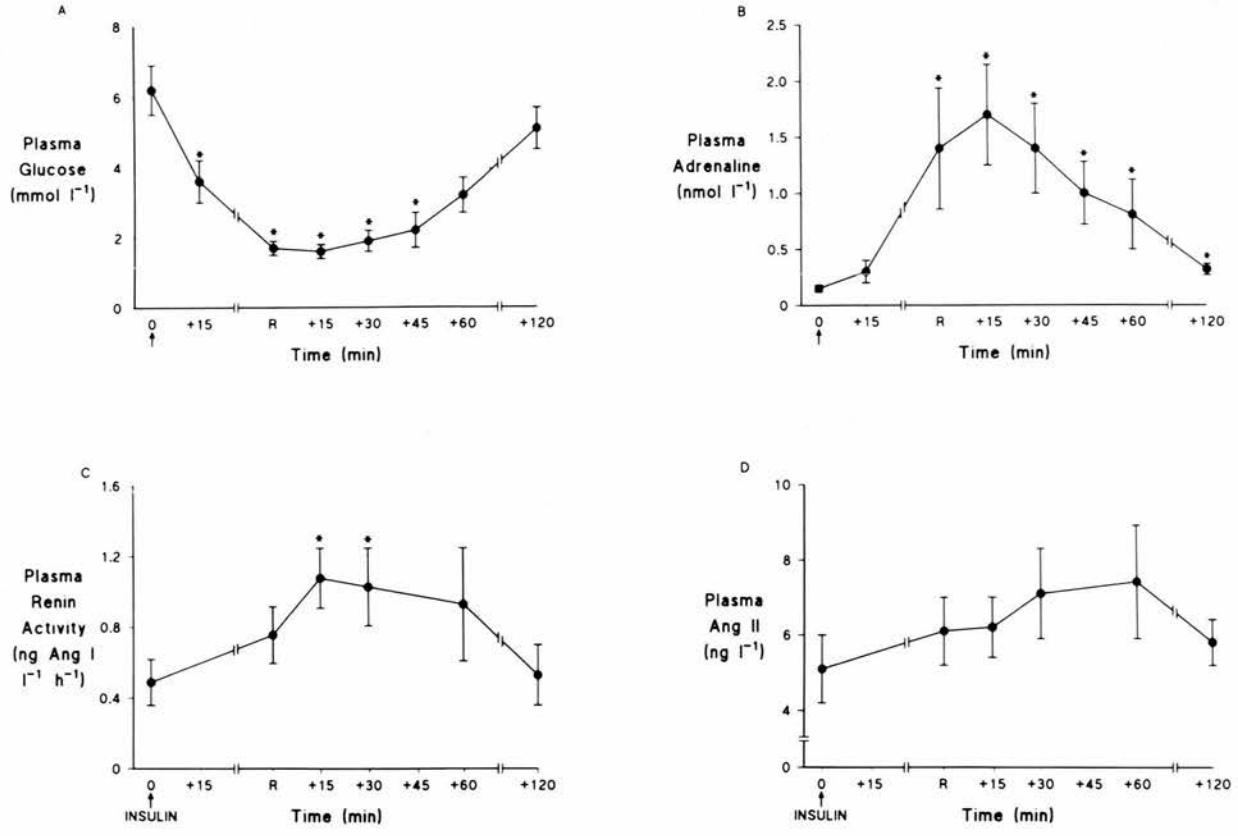

Figure 1. Mean response (SE) of (a) plasma glucose, (b) plasma adrenaline, (c) plasma renin activity, and (d) plasma angiotensin II, following intravenous injection of insulin at time 0. R denotes the onset of the acute autonomic reaction. A significant difference ($p < 0.05$) from the basal value is shown by ⋆

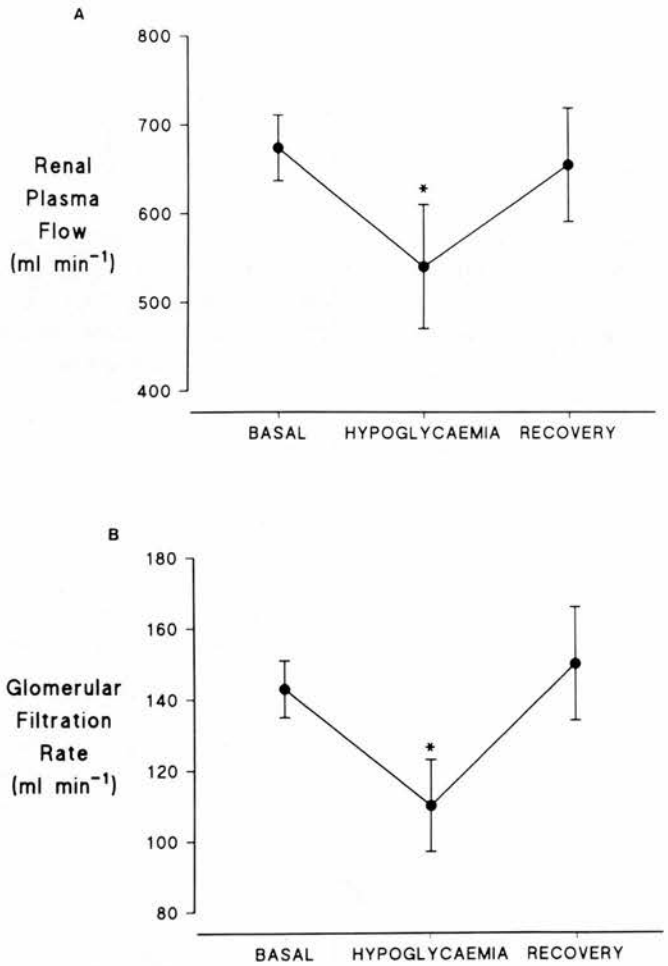

Figure 2. Changes in (a) renal plasma flow and (b) GFR in response to acute hypoglycaemia. A significant difference ($p < 0.05$) from the basal value is shown by ⋆

Discussion

In normal humans, the acute activation of the autonomic (particularly the sympatho-adrenal) nervous system and the hormonal secretion associated with acute hypoglycaemia have a profound effect on the vascular perfusion and function of several organs, including the heart,[12] the brain,[10] the spleen,[13] and the kidney.[2] Acute hypoglycaemia induced by an IV bolus injection of unmodified insulin, as in this study, is likely to maximally stimulate this neurohormonal activation, in contrast to stepped hyperinsulinaemic, hypoglycaemic clamp techniques, which are of greater value in studying threshold effects.

In the present study, Type 1 diabetic patients who had normal urinary albumin excretion were shown to have a fall in effective renal plasma flow and glomerular filtration rate, by 20 and 23 %, respectively, in response to hypoglycaemia. Both renal plasma flow and glomerular filtration rate were higher in the basal state in the diabetic patients than in a group of non-diabetic subjects previously studied,[2] which is well recognized to be associated with diabetes and may be maintained by hyperglycaemia.[20–22] In contrast to the findings reported in non-diabetic subjects,[2] a close correlation was identified between the changes in glomerular filtration rate and renal plasma flow. This suggests that in diabetic patients the glomerular filtration rate may be more highly dependent on renal blood flow than in non-diabetic subjects.

Two of the principal groups of hormones which are known to affect renal blood flow (catecholamines and angiotensin II) were stimulated to a lesser extent in the diabetic group in the present study than in the non-diabetic subjects previously investigated.[2] The catecholamine response to hypoglycaemia may be progessively diminished in patients with Type 1 diabetes of long duration,[23–25] which might partially explain this finding, despite a significant degree of hypoglycaemia being achieved in all subjects. Although the hormonal response was apparently attenuated, a reduction in renal plasma flow and glomerular filtration rate was still manifest in the group of diabetic patients. It is possible, therefore, that direct stimulation of the efferent sympathetic nerves is the major determinant of changes in renal haemodynamics occurring secondary to acute hypoglycaemia.

Table 1. Urine volumes and urinary excretion of sodium, dopamine, and albumin in response to hypoglycaemia

	Basal period	Period of hypoglycaemia	Recovery period	p
Urine volume (ml min^{-1})	10.6 (1.2)	4.7 (1.1)	7.7 (1.5)	<0.002
Absolute sodium excretion (μmol l^{-1})	262 (38)	250 (43)	303 (50)	NS
Fractional sodium excretion (%)	1.3 (0.2)	1.7 (0.3)	1.6 (0.2)	NS
Dopamine (μmol min^{-1})	322 (37)	211 (29)	230 (25)	<0.005
Urinary albumin concentration (mg l^{-1})	4.2 (1.0)	5.4 (1.1)	4.6 (1.1)	NS
Albumin excretion rate (μg min^{-1})	46.2 (10.6)	26.0 (10.5)	42.6 (9.3)	<0.005

Mean (SE).
Significance is tested between time of hypoglycaemia and basal period.
The basal period commenced 30 min before the insulin bolus was given and ended 10 min after insulin injection. The period of hypoglycaemia commenced at the onset of the acute autonomic reaction (time R), ending at time R + 60 min. The recovery period extended from time R + 60 min to time R + 120 min.

The potential role of the fall in plasma glucose *per se* does, however, deserve comment in this context. Mogensen *et al.*[26] previously demonstrated a significant fall both in GFR and in renal plasma flow, in a group of diabetic patients, when the blood glucose level was reduced from 13.9 mmol l^{-1} to 6.5 mmol l^{-1}, without a simultaneous rise in catecholamine concentrations. These changes do not appear to be explicable on the basis of changes in plasma volume or of concurrent sympathetic activation occurring in response to hyperinsulinaemia.[27,28]

Vasopressin is secreted in response to acute hypoglycaemia and this is markedly enhanced in diabetic patients.[3,4] The significant fall in urine volume observed in the diabetic patients during hypoglycaemia is likely to be explained by this augmented vasopressin response to hypoglycaemia, rather than simply bladder emptying problems, as such a reduction was not found in non-diabetic subjects using identical techniques.[2] The rapid reduction in urinary flow rate in response to hypoglycaemia may also underlie the fall in urinary albumin excretion rate. The unexpectedly elevated basal albumin excretion rate is probably related to the prevailing diuretic conditions, as it has been shown previously that the urinary albumin excretion rate rises significantly in response to water loading, falling again when the urinary flow rate returns to normal.[29] Changes in urine volume may have determined alterations in urinary albumin excretion which have been reported previously in response to insulin infusion,[30] although others have suggested that the urinary albumin excretion rate actually rises in response to a falling plasma glucose concentration in diabetic patients.[26,28]

Urinary dopamine excretion fell in response to hypoglycaemia but, in contrast to previous findings in non-diabetic subjects,[2] no significant change either in total or in fractional sodium excretion occurred in the diabetic patients. A close correlation has been observed between urinary dopamine and sodium excretion in the normal kidney,[31] and a fall in urinary sodium excretion might have been anticipated on the basis of insulin inhibiting the renal excretion of electrolytes[32,33] and enhancing sodium reabsorption in the renal tubules.[34] One possible explanation for this apparent difference between diabetic and non-diabetic subjects is the absence of a significant rise in plasma noradrenaline in response to hypoglycaemia in the diabetic group, as noradrenaline promotes sodium retention, via alpha receptors in the proximal tubules,[35] and this is independent of haemodynamic effects.

An overall reduction in the prevailing level of blood glucose control is one of the main therapeutic aims of diabetic management but a potential risk of such a strategy is to increase the frequency of hypoglycaemia. The present study has examined the effects of hypoglycaemia on the kidney in diabetic patients who have no clinical or biochemical evidence of nephropathy. If these changes in renal haemodynamics and function are superimposed upon the documented changes in plasma viscosity[6,7] and platelet activation[5,36] which are known to occur in response to hypoglycaemia, the combined effects are potentially deleterious,[37] particularly in patients who have established renal microangiopathy.

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DECLARATION

I confirm that this thesis has been composed by myself and that the experimental work described is my own, with technical assistance by others acknowledged in the text where appropriate. I further confirm that this thesis, or any part thereof, has not been submitted in candidature for any other degree, diploma or professional qualification.