Nephropathy, Retinopathy and Blood Pressure in Insulin Dependent Diabetes Mellitus

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Abstract

The relation of blood pressure to the renal and retinal complications of insulin dependent diabetes was examined in a cross-sectional analysis of 3250 clinic-attending patients from 31 centres in Europe (the EURODIAB IDDM Complications Study). In addition, the hypothesis that susceptibility to diabetic nephropathy can be identified by a parental history of hypertension was examined.

The relation between blood pressure and albumin excretion differed strikingly between patients with and without retinopathy. This original finding has not been reported before. In patients without retinopathy, albumin excretion was low even when blood pressure was high and glycaemic control poor. In patients with retinopathy, albumin excretion increased steeply with blood pressure above the median (120/75 mmHg). Thus albumin excretion tended to be high only in patients with both raised blood pressure and retinopathy.

The prevalence of retinopathy increased with blood pressure, but the association was prominent only in patients with raised albumin excretion, in whom proliferative retinopathy was two to three times more frequent when blood pressure was above 105/65 mmHg than below this level.

Both blood pressure and albumin excretion were higher in patients reporting a parental history of hypertension than in patients reporting no parental
hypertension. However, parental hypertension made no contribution to the relation between blood pressure and albumin excretion independent of blood pressure in the offspring.

These data suggest that patients with retinopathy are particularly vulnerable to the effect of raised blood pressure on the kidney. This offers a plausible explanation for the well known observation that nephropathy without retinopathy is rare, while retinopathy without nephropathy is common. The hypothesis that susceptibility to diabetic nephropathy is determined by a genetic disposition to hypertension is not supported by this study. Rather, it is suggested that patients at high risk of nephropathy can be distinguished by the presence of both retinopathy and raised (above median) blood pressure, irrespective of parental hypertension.
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1. Introduction

The subject of this thesis is the relation of blood pressure to the renal and retinal complications of insulin dependent diabetes (IDDM). The specific aims are set out after a brief description of IDDM and its complications. Further description of retinopathy and nephropathy follows, with emphasis on the role of blood pressure. The introduction ends with a summary of the relevant literature.

1.1 Insulin Dependent Diabetes Mellitus

IDDM results from autoimmune destruction of the insulin-producing cells of the pancreatic islets of Langerhans. The autoantibodies which destroy the $\beta$ cells are produced by genetically susceptible individuals in response to environmental agents which remain largely unidentified, although viral infection is probably important (Green and Gale, 1992). When $\beta$ cell destruction has progressed to a critical point, patients present subacutely with symptoms of insulin deficiency. Lifelong insulin replacement is then essential to prevent progressive hyperglycaemia, ketoacidosis, coma and death. Several incidence studies in Europe have indicated that IDDM is becoming more common and starting earlier in childhood (Bingley and Gale, 1989; Stewart-Brown et al. 1983; Metcalfe and Baum, 1991; Burden et al. 1989).

Diabetes is characterised metabolically by hyperglycaemia and clinically by
vascular disease. Chronic injury to large blood vessels (macroangiopathy) results in cardiovascular, cerebrovascular and peripheral vascular disease. The pathology and clinical consequences of macroangiopathy are similar in diabetic and non diabetic individuals (Jarrett et al. 1982). Specific diabetic complications result from damage to small blood vessels (microangiopathy) particularly in the eye and kidney where characteristic lesions develop at an early stage. Blindness and renal failure are the end results of diabetic eye disease (retinopathy) and renal disease (nephropathy). Visual loss can usually be prevented by photocoagulation therapy (Rohan et al. 1989) providing sight threatening retinopathy is detected in time, but diabetes remains one of the commonest causes of blindness in industrialised countries (Klein and Klein, 1983; Department of Health and Social Security, 1988). The safety and effectiveness of efforts to prevent diabetic renal failure are less well defined, and diabetes accounts for a high proportion of renal transplant and dialysis recipients in Europe (Brunner et al. 1988). Reduction of morbidity and mortality from these complications remains an important goal, whose achievement requires better understanding of risk factors for diabetic microangiopathy.

Virtually all IDDM patients develop retinopathy, (Klein et al. 1984) but only 35%-40% (Andersen et al. 1983; Krolewski et al. 1985a) develop nephropathy. The nephropathy data come from 292 IDDM patients who first attended the Joslin Diabetes Center between 1939 and 1959 (Krolewski et al. 1985a) and from 1475 patients at the Steno Memorial Hospital in Denmark who were diagnosed with
IDDM before 1953 (Andersen et al. 1983). These studies were conducted at a time when intervention was less common than it is now, and are often cited as natural history studies. More recent studies in the UK have found lower rates of proteinuria (McNally et al. 1990).

The challenge remains to distinguish, at an early stage, the subgroup of patients at risk of nephropathy from the remainder who are not at risk. Duration of diabetes and blood glucose control are established risk factors for retinopathy and nephropathy, (Leslie and Sperring, 1986; Raskin and Rosenstock, 1986; Chase et al. 1989) but leave much of the individual’s risk unexplained. Recent research has therefore focused on the role of blood pressure, as a potentially modifiable risk factor for retinopathy (Parving, 1991; Janka et al. 1989b) and nephropathy (Mogensen, 1989; Nosadini et al. 1991).

In addition, it has been suggested that susceptibility to nephropathy in IDDM is determined by a genetic predisposition to hypertension (Krolewski et al. 1988; Viberti et al. 1987). This hypothesis emerged from finding genetic markers of hypertension in IDDM patients with nephropathy (Mangili et al. 1988) and raised blood pressure in the parents of such patients (Viberti et al. 1987). If the hypothesis is correct, patients at greatest risk of nephropathy might be distinguishable by a parental history of hypertension.
1.2 Aim and scope of thesis

This thesis examines the relation between blood pressure and the renal and retinal complications of IDDM using data from a cross-sectional epidemiological study of 3250 IDDM patients from 31 centres in Europe. The specific aims are:

1) to describe quantitatively the relation of blood pressure to urinary albumin excretion and to retinopathy

2) to assess the importance of blood pressure as a risk factor for these complications.

3) to examine the hypothesis that a parental history of hypertension distinguishes patients at risk of nephropathy from those who are not at risk.

The term risk factor is commonly applied to an attribute or exposure associated with an increased probability of disease (Last, 1988). A causal relation is not necessarily implied by this definition, but elucidation of causation is a major goal of epidemiology. The best evidence of causality comes from randomised controlled trials of risk factor reduction or, failing that, from prospective observation of the incidence of disease in populations with different levels of risk. Since the data on which this thesis is based is cross-sectional, assessment of the
importance of blood pressure as a risk factor for diabetic microangiopathy and marker of susceptibility to nephropathy is limited to interpretation of cross-sectional associations. The extent to which the findings can be generalised to a wider population of IDDM patients is discussed later. Although the complications of diabetes are common to both IDDM and non insulin dependent diabetes (NIDDM), no attempt is made to generalise the findings to NIDDM.
1.3 Diabetic nephropathy

1.3.1 Epidemiology and Natural History.

Diabetic nephropathy is one of the most dreaded complications of diabetes. It is frequently complicated by cardiovascular disease, (Brunner et al. 1988) and 45% of insulin dependent patients die from renal failure or myocardial infarction before the age of 55 years (Krolewski et al. 1987b). Diabetes is the single commonest diagnosis in new dialysis patients in the USA (Teutsch et al. 1989) and accounts for a rapidly increasing proportion of transplant and dialysis recipients in Europe (Brunner et al. 1988). In the UK, the proportion of people accepted for renal replacement with diabetes increased from 1.2% in 1974 to 11.4% in 1985 (Brunner et al. 1988).

These data (which do not distinguish between IDDM and NIDDM) should not be interpreted as evidence that the incidence of diabetic renal failure is rising, because increased acceptance of diabetic patients for renal replacement undoubtedly reflects changes in medical attitudes towards patient suitability and greater resources. On the contrary, there is evidence from the Joslin Diabetes Center in Boston, Massachusetts (Krolewski et al. 1985a) to suggest that the age-specific incidence of nephropathy in IDDM has declined over the last few decades: those whose diabetes was diagnosed in the 1930's had twice the cumulative risk of persistent proteinuria by age 40 as those in whom IDDM was diagnosed in later
decades. Despite this favourable trend, diabetic nephropathy remains an important public health problem in Europe and the USA.

The development of diabetic nephropathy is associated with characteristic structural, histological, (Osterby, 1992) functional and biochemical changes (Viberti, 1988). The most easily detectable of these is raised urinary protein excretion, which is regarded as the hallmark of diabetic nephropathy. Urinary protein excretion in healthy non diabetic individuals rarely exceeds 10 μg/min. When protein (mainly albumin) excretion is about twenty times higher than this, it can be detected by semi-quantitative strip reagents, and is known as dipstick (usually Albustix) positive proteinuria. Although renal function is usually normal at this stage, proteinuria is an ominous sign which heralds a progressive decline in glomerular filtration rate (GFR) and hypertension, leading to end stage renal failure after a median of 7 to 10 years (Krolewski et al. 1985a; Andersen et al. 1983).

Two large retrospective cohort studies (Andersen et al. 1983; Krolewski et al. 1985a) have shown that the incidence of dipstick positive proteinuria rises most steeply in patients whose duration of IDDM is between 10 and 20 years, reaching a peak incidence of around 2.5% per year, and a peak prevalence of 20%. After this time, the incidence of proteinuria falls and the cumulative incidence after 40 years, which approaches a lifetime of IDDM, is around 35%-45%. It has been concluded from these natural history studies that susceptibility to nephropathy is
confined to a subset of patients (Krolewski et al. 1987b). Familial clustering of nephropathy in IDDM, (Seaquist et al. 1989) and racial differences in the incidence of diabetic renal failure (Rostand, 1989; Cowie et al. 1989) have stimulated the search for genetic risk factors (see below).

Diabetic nephropathy is often defined as persistent dipstick positive proteinuria in the absence of other renal disease, but some use the term as soon as urinary albumin excretion has risen above normal, while others reserve it to describe a triad of persistent proteinuria, falling GFR and hypertension. Mogensen has combined these definitions in a five-stage description of the natural history of renal disease in insulin dependent patients (Mogensen et al. 1983; Mogensen, 1989). Although several aspects need confirmation, it provides a useful framework for relating putative risk factors to renal histology or clinical events. The five stages are described below.

Stage 1 - Renal Hypertrophy and Hyperfiltration.

Even at diagnosis, kidney size and GFR are nearly always increased in IDDM (Christiansen et al. 1981; Mogensen et al. 1981). Glomerular volume and capillary surface area are greater than normal (Osterby and Gundersen, 1975) and albumin excretion rate may be raised. These abnormalities often reverse on starting insulin therapy, (Christiansen et al. 1982) but persistent hyperfiltration (increased GFR) is a well-recognised feature of early uncomplicated IDDM.
Single-nephron micropuncture studies in experimental diabetic rats indicate that hyperfiltration results from increased pressure within glomerular capillaries which leads to progressive renal damage (Mauer et al. 1981). It has therefore been hypothesised that patients with early hyperfiltration are more likely to develop nephropathy (Mogensen, 1984; Brenner, 1983). Some studies in humans support this hypothesis, (Mogensen, 1984; Mogensen, 1986) but others do not (Lervang et al. 1988; Jones et al. 1991). Further longitudinal data are needed to clarify the significance of hyperfiltration.

Stage 2 - Renal Lesions without Clinical Signs

Glomerular and tubular capillary basement membranes appear normal at diagnosis, but thicken significantly in nearly all patients over the next two to three years (Osterby, 1974). Glomerular mesangial volume also increases with deposition of proteinaceous materials, including albumin, IgG, fibrin, and platelet-degradation products. GFR remains elevated at this stage, but albumin excretion is not yet raised. Basement membrane thickening does not seem to predict development of proteinuria, but mesangial thickening, which occurs at the expense of capillary filtration surface, seems to initiate the decline in nephron function (Steffes et al. 1989). As mesangial expansion progresses, a process of scarring, shrinkage and loss of glomerular function known as "glomerulosclerosis" occurs. Nodular lesions, first described in 1936 by Kimmelstein and Wilson (Kimmelstein and Wilson, 1936) are considered to be pathognomonic of diabetic nephropathy, but
are much less common than diffuse glomerulosclerosis. Some degree of glomerulosclerosis is found in nearly all patients after ten years of IDDM, (Honey et al. 1962; Thomsen, 1965) although only 35%-40% of them will go on to develop proteinuria, hypertension and impaired renal function.

Stage 3 - Incipient Nephropathy

Conventional dipsticks cannot detect proteinuria until it is around twenty times higher than normal, but sensitive laboratory assays can detect the low concentration of albumin that is normally found in urine. The rate of albumin excretion can then be calculated from a timed urine collection. Excretion rates which are higher than normal i.e. above the limit found in healthy non-diabetics, and lower than that detected by dipsticks are referred to as microalbuminuria. Defining the limits of microalbuminuria, particularly the lower one, has been a subject of much debate. The main difficulty arises from the high biological variability of urinary albumin excretion. Clear definition of an abnormally high rate of albumin excretion is hindered by the wide diurnal and day-to-day variation in excretion rates, compounded by use of different urine collection periods, such as overnight, 12 hour and 24 hour. These differences are important because albumin excretion is considerably lower at night than during the day, due to changes in posture and exercise. Different researchers have therefore adopted a range of cutoff levels for microalbuminuria, from 15μg/min to 75μg/min.
One widely used definition of microalbuminuria, arrived at during a consensus meeting of experts (Mogensen et al. 1986) is "a urinary albumin excretion rate between 20 and 200 \( \mu g/\text{min} \) in an overnight or 24 hour sample on at least 2 or 3 occasions within a period of 6 months." The limit of 200 \( \mu g/\text{min} \) corresponds approximately to the lower limit of detection with Albustix. The term macroalbuminuria has therefore been applied to excretion rates above 200\( \mu g/\text{min} \), or Albustix positive proteinuria. For people with IDDM, the following definitions are commonly applied: normal albumin excretion (also called normoalbuminuria) \(<20 \mu g/\text{min} \text{ or } <30 \text{ mg/24 hours}; \) microalbuminuria \(20-200 \mu g/\text{min} \text{ or } 30-300 \text{ /24 hours and macroalbuminuria } >200 \mu g/\text{min} \text{ or } >300 \text{ mg/24 hours}.\) Using this definition, microalbuminuria is found in around 20%-25% of patients after seven to fifteen years of IDDM, (Parving et al. 1988) and is believed to be rare in patients with IDDM for less than five years (Microalbuminuria Collaborative Study Group, 1992). GFR may be normal or still somewhat elevated (Mathiesen et al. 1984) in microalbuminuric patients.

Four small studies (Mogensen, 1984; Viberti et al. 1982; Mathiesen et al. 1984; Parving et al. 1982) have reported that microalbuminuria in IDDM is highly predictive of progression to dipstick positive proteinuria. All were retrospective studies of patients whose albumin excretion rate had been measured on two occasions separated by several years. Patients with macroalbuminuria on the first occasion were not included. In each study, microalbuminuria was defined as the level which would optimally distinguish patients who progressed to
macroalbuminuria from those who did not. With the cutoff level ranging from 15μg/min to 75μg/min in the different studies, optimised sensitivity ranged from 70% to 100%, and specificity from 94% to 100%. Assuming the prevalence of microalbuminuria were 20%, the risk of progression, or predictive value of microalbuminuria would be 75% to 100%.

In view of the high within person variability in urinary albumin excretion, (Mathiesen et al. 1984) the test characteristics look impressive. However, these studies probably overestimated the risk of progression associated with microalbuminuria (Stephenson, 1991) because they failed to take account of differences in duration of IDDM between patients with microalbuminuria (mean 14, (Viberti et al. 1982) 19, (Mathiesen et al. 1984) and 19 (Parving et al. 1982) years) and those with normal albumin excretion (mean 9, 12 and 15 years respectively). Because the differences in mean duration span the period when the incidence of macroalbuminuria rises most rapidly, the high risk of progression in patients with microalbuminuria could in part be explained by their longer duration of diabetes. Further doubt about the predictive value of microalbuminuria is raised by cross-sectional studies (Parving et al. 1988; Orchard et al. 1990) showing that the prevalence of microalbuminuria continues to rise after more than 30 years of IDDM. Since the incidence of nephropathy declines markedly after 25 years, these findings imply that progression from microalbuminuria to proteinuria might not be the rule, at least in patients with longstanding disease. This conclusion is supported by a more recent prospective study of patients with
IDDM for more than 15 years, in which only five of eighteen (28%) microalbuminuric patients developed macroalbuminuria during a ten year follow up period (Forsblom et al. 1992).

Stage 4 - Clinical Nephropathy

Once persistent dipstick proteinuria has developed, GFR is no longer raised, and blood pressure is substantially higher than in patients without raised albuminuria (Parving et al. 1983; Mogensen et al. 1983). Retinopathy is almost always present, (Malins, 1968; Deckert et al. 1978; Parving et al. 1988) and cardiovascular morbidity (Jensen et al. 1987) and mortality (Krolewski et al. 1987a; Borch-Johnsen and Kreiner, 1987) are much higher than in normoalbuminuric patients. Unlike the earlier stages, which natural history studies have shown to be non-progressive in at least some patients, persistent proteinuria appears to be a point of no return (Siperstein, 1988). The decline in GFR which starts within a few years of the onset of persistent proteinuria reflects irreversible damage to glomeruli and loss of filtration area (Ellis et al. 1986). Although the decline in GFR is progressive, the rate of decline differs considerably between individuals, and appears to depend closely on systemic blood pressure (Hasslacher et al. 1985; Laffel et al. 1987).
Stage 5 - End Stage Renal Failure

Once nephropathy is established, GFR steadily declines and blood pressure continues to rise. Although the rate of decline in GFR varies considerably between individuals, (Jones et al. 1979) around 50%-75% of patients develop end-stage renal disease within ten years of the onset of proteinuria (Krolewski et al. 1985a; Andersen et al. 1983). Without intervention, this stage is reached after twenty to forty years of IDDM, when patients are still relatively young, typically in their forties (Mogensen, 1989). In the early days of renal replacement, diabetic patients were often excluded because of advanced atherosclerosis and high cardiovascular risk. Although this is no longer the case, many diabetic patients in need of renal support do not receive it, (Joint Working Party on Diabetic Failure of the British Diabetic Association, 1988; Joint working party on diabetic renal failure of the British Diabetic Association, 1989) and survival remains poorer in diabetic than non diabetic recipients (Broyer et al. 1988).

1.3.2 Risk factors for Diabetic Nephropathy

Blood glucose control

Established risk factors for diabetic nephropathy are duration of IDDM, as explained above, and poor blood glucose (glycaemic) control. Evidence linking glycaemic control with nephropathy comes from animal models and human
studies, both cross-sectional and longitudinal. In animal models, glomerular basement membrane thickening and mesangial expansion can be prevented by tight metabolic control (Rasch, 1979). Mesangial expansion, but not basement membrane thickening, can be reversed when glycaemic control is restored by islet cell transplantation (Mauer et al. 1974). In humans, pancreas transplantation with restoration of normoglycaemia halts the progression of early diabetic glomerular changes, particularly mesangial expansion, in renal allografts (Bilous et al. 1989).

In longitudinal epidemiological studies, poor glucose control is a strong predictor of developing clinical nephropathy in IDDM (Krolewski et al. 1985a; Andersen et al. 1983; Hasslacher et al. 1985). The mechanisms by which chronic hyperglycaemia causes microangiopathy are complex, but a number of biochemical pathways have been identified, including the polyol pathway, non-enzymatic glycation, glucose autoxidation and de novo synthesis of diacylglycerol leading to protein kinase C and phospholipase A₂ activation (Larkins and Dunlop, 1992).

Blood glucose levels can be maintained close to the normal range (near-normoglycaemia) through meticulous control of diet and subcutaneous infusion or multiple daily injections of insulin (DCCT Research Group, 1987). During the last decade, the pursuit of strict control became central to the care of insulin dependent patients, and was generally perceived as the best practical means of preventing or delaying the onset of long-term diabetic complications, (Siperstein et al. 1977) particularly retinopathy and nephropathy. Small randomised trials in
patients with microalbuminuria suggested that strict glycaemic control could slow or reverse the progression of albumin excretion (Kroc Collaborative Study Group, 1984; Feldt-Rasmussen et al. 1986) and conclusive evidence that improved glycaemic control reduces the incidence of proteinuria (The Diabetes Control and Complications Trial Research Group, 1993) has most recently been provided by the Diabetes Control and Complications Trial (DCCT, see below). Once persistent proteinuria has developed, strict glycaemic control is probably not effective in lowering protein excretion (Viberti et al. 1983). Whether sustained normalisation of microalbuminuria prevents disease progression, in histological terms, is an important unanswered question.

Concern about the adverse effects of tight control increased when short-term randomised trials reported transient deterioration of retinopathy (Kroc Collaborative Study Group, 1984; Lauritzen et al. 1983; Dahl-Jorgensen et al. 1985) and increased hypoglycaemia (Kroc Collaborative Study Group, 1984; Dahl-Jorgensen et al. 1986) in patients with improved control. Subsequent debate about the relative benefits and risks of strict control (Unger, 1982; Hanssen et al. 1986; Siperstein et al. 1977; DCCT Research Group, 1988) resulted in a definitive randomised controlled trial to test the hypothesis that intensive insulin treatment, aimed at achieving normoglycaemia, reduces the incidence or progression of complications in IDDM. In this trial, over 1400 patients with IDDM for less than 15 years and little or no evidence of microangiopathy were randomly allocated to either conventional or strict control. The primary endpoint of the trial is diabetic
retinopathy and the results were due to be reported in 1994. However, the trial was stopped before schedule and the results published in September 1993, (The Diabetes Control and Complications Trial Research Group, 1993) because it became clear that intensive treatment substantially reduced the risk of retinopathy, nephropathy and neuropathy.

Another important finding had previously emerged from the DCCT: compared with conventionally controlled patients, the incidence of severe hypoglycaemia was two to six times higher in the strictly controlled group (DCCT Research Group, 1991). Earlier fears (Unger, 1982) that achievement of near normoglycaemia might be accompanied by an increase in severe hypoglycaemia have therefore been borne out. As severe hypoglycaemia is a life-threatening and debilitating complication of insulin treatment, it imposes a serious limitation on the pursuit of strict glycaemic control.

**Blood Pressure and Genetics**

Since duration of IDDM is immutable, and chronic hyperglycaemia cannot be simply and safely normalised, interest has focused on blood pressure as a potentially modifiable risk factor for diabetic nephropathy. Hypertension in chronic renal failure is almost invariable, and raised blood pressure in diabetic nephropathy was initially assumed to be a late feature, (Watkins et al. 1972) secondary to deterioration in renal function (Anonymous, 1978). However,
subsequent studies found significantly higher blood pressure in patients with proteinuria and normal serum creatinine, compared to those without proteinuria, (Parving et al. 1983) and significantly higher blood pressure in patients with microalbuminuria, compared to those with normal albumin excretion (Wiseman et al. 1984; Christensen and Mogensen, 1985; Mathiesen et al. 1984). These reports of a cross-sectional association between blood pressure and early diabetic nephropathy gave rise to controversy about whether the rise in blood pressure was primary or secondary to the rise in urinary albumin excretion. Studies by Hostetter (Hostetter et al. 1982) and Brenner (Brenner, 1983) support the primary involvement of raised blood pressure by emphasising the importance of renal haemodynamic factors in the early development of diabetic nephropathy. Renal plasma flow and glomerular transcapillary hydraulic pressure are increased in the diabetic kidney. As duration of IDDM increases, these haemodynamic alterations lead to direct cellular injury, resulting in an increase in mesangial cells and matrix and finally glomerulosclerosis. In addition, these elevated pressures and flows may alter the permselective properties of the glomerular basement membrane, leading to abnormal loss of protein in the urine. While it is now agreed that a rise in blood pressure causes further renal damage in patients who already have microalbuminuria, (Mogensen et al. 1992) the role of blood pressure as an initiator of microalbuminuria remains controversial. One prospective study (Mathiesen et al. 1990) detected a rise in albumin excretion before any rise in blood pressure, but others have been less clear cut. In a six year follow up study (Chase et al. 1990) of patients with IDDM for at least five years, raised blood pressure was
detected before the onset of microalbuminuria in 26 patients, while microalbuminuria was found before blood pressure became raised in 27 patients. Since interpretation of such studies depends on the frequency of measurements and definition of raised blood pressure, it is not altogether surprising that both sequences of events have been detected.

Another approach to investigating the role of blood pressure in the early development of nephropathy has been to ask whether IDDM patients with a genetic predisposition to hypertension are more likely to develop nephropathy than patients with no family history of hypertension. One case control study (Viberti et al. 1987) found that the parents of IDDM patients with nephropathy had significantly higher blood pressures than parents of similarly aged IDDM patients without nephropathy. Another case control study (Krolewski et al. 1988) found that having a parent with hypertension tripled the risk of nephropathy. Furthermore, patients with diabetic nephropathy (Krolewski et al. 1988; Mangili et al. 1988; Walker et al. 1990) and their parents (Walker et al. 1990) have been found to have increased sodium lithium countertransport activity, which is believed to be a genetic marker of hypertension (Hasstedt et al. 1988; Canessa et al. 1980). These studies may indicate that even subtle or transient elevations in blood pressure have a damaging effect on the diabetic kidney. Alternatively, the family history of hypertension may be a marker of some other genetic factor that determines susceptibility to nephropathy in IDDM. In the first interpretation, the family history of hypertension is causal, in the second it is confounding. Since
these studies were not prospective, reverse causality offers a third explanation i.e. the stress associated with having diabetic offspring with renal disease might cause hypertension to develop in the parents (Mauer, 1988). One conflicting case control study should also be mentioned (Jensen et al. 1990) which found neither raised blood pressure nor increased sodium lithium countertransport activity in the parents of patients with diabetic nephropathy.

Many diabetologists favour a genetic theory to explain susceptibility to diabetic nephropathy, but extensive investigation of potential genetic markers has not revealed consistent associations between diabetic nephropathy and HLA antigens, or complement or immunoglobulin markers (Barnett and Pyke, 1986). Familial clustering of diabetic nephropathy implies shared risk factors, but these may be environmental or genetic. The view that susceptibility to nephropathy is determined by a genetic predisposition to hypertension is controversial (Mogensen et al. 1992). An alternative theory (Deckert et al. 1989) known as the "Steno hypothesis" proposes that susceptibility to diabetic nephropathy is due to genetic polymorphism of enzymes that determine the composition of the glomerular basement membrane and extracellular matrix. In patients with glomerulosclerosis, the density of heparan sulfate proteoglycan in the glomerular basement membrane is decreased, (Shimomura and Spiro, 1987) and this has been shown in rats to cause albuminuria (Van den Born et al. 1992). The theory proposes that diabetic patients with genetic defects in the regulation of heparan sulfate proteoglycan are at greater risk of developing functional and structural abnormalities that lead to
glomerulosclerosis and eventually clinical nephropathy (Deckert et al. 1992).

Several studies have examined the renal effects of blood pressure reduction at different stages of diabetic nephropathy. Three studies (Parving et al. 1987; Mogensen, 1982; Bjorck et al. 1986) are widely quoted as evidence that antihypertensive treatment is beneficial in patients with clinical nephropathy. In fact, all three were uncontrolled studies of 6, (Parving et al. 1987) 11 (Mogensen, 1982) and 14 (Bjorck et al. 1986) patients, in whom GFR was measured before and after introducing antihypertensive treatment. The rate of decline was reported to be significantly lower after intervention, but the lack of any independent control group makes valid interpretation of these studies difficult. There is also a conflicting report that patients with persistent proteinuria treated with antihypertensive medication in the early 1970s and untreated patients with hypertension had similar rates of decline in renal function (Laffel et al. 1987). Two retrospective studies have concluded that antihypertensive treatment has improved survival in diabetic nephropathy, (Parving and Hommel, 1989; Mathiesen et al. 1989) but these studies suffer from the inherent biases of non randomised comparison, compounded in one study (Parving and Hommel, 1989) by the use of historic control groups.

There is, however, little doubt that lowering blood pressure reduces the rate of albumin excretion in both proteinuric (Parving et al. 1989a; Hommel et al. 1986b) and microalbuminuric (Hommel et al. 1986a; Marre et al. 1987; Marre et al. 1987).
1988; Passa et al. 1987; Melbourne Diabetic Nephropathy Study Group, 1991; Mathiesen et al. 1991) patients. This has been shown consistently in randomised controlled trials of up to 12 months duration, and a nonrandomised trial has reported a sustained reduction in albuminuria in patients on antihypertensive treatment for five years (Christensen and Mogensen, 1987). Since diabetic nephropathy is rarely found without persistent proteinuria, (Klahr, 1991) which has been shown to induce glomerular injury in animals, (Remuzzi and Bertani, 1990; Williams et al. 1988) it may be correct to regard albuminuria as part of the disease process itself, but long-term studies are needed to determine whether sustained normalisation of albumin excretion can prevent the development of clinical nephropathy.

Despite the limitations of the available data on the risks and benefits of blood pressure reduction in diabetes, (Fletcher and Bulpitt, 1992) most working groups recommend reduction of blood pressure to less than 90mmHg diastolic, using antihypertensive drugs if simple non-drug measures fail (The Working Group on Hypertension in Diabetes., 1987; 1992). More aggressive guidelines (Oster et al. 1990) recommend antihypertensive therapy, presumably for life, in IDDM patients with microalbuminuria and blood pressure above 135/85mmHg.

One class of antihypertensive drug, angiotensin converting enzyme (ACE) inhibitors, has attracted particular interest in diabetic nephropathy (Mogensen, 1992). Unlike diuretics and beta blockers, ACE inhibitors lower intraglomerular
capillary pressure as well as systemic blood pressure and do not adversely affect glucose control, plasma lipids or male potency. There is, however, much debate about whether ACE inhibitors in practice lower albumin excretion more effectively than other antihypertensive drugs (Sawicki et al. 1990; Anderson, 1990). A recent trial in the USA (Lewis et al. 1993) has addressed this question by randomising 409 IDDM patients with clinical nephropathy (proteinuria ≥ 500 mg/day and serum creatinine ≥ 2.5 mg/dl), of whom 60% were already on antihypertensive therapy, to either captopril or captopril placebo. During a median follow-up of three years, the treatment goal was a defined reduction in blood pressure, and the primary endpoint was a doubling of baseline serum creatinine concentration. Captopril treatment was associated with a 48% reduction in risk of doubling of serum creatinine and a 50% reduction in the risk of the combined end points of death, dialysis and transplantation that was independent of the minor disparity in blood pressure between treatment groups. The authors concluded that captopril protects against deterioration in renal function and is significantly more effective than blood pressure control alone.

ACE inhibitors are not without side effects (Ferner, 1990), the most common being dry cough, but the most serious being rapid worsening of renal function in patients with pre-existing functional impairment (Speirs et al. 1988). It is also instructive to compare the cost of different antihypertensive drugs (Swales, 1990): a years’ supply of bendrofluazide costs approximately £2-£3; the figure for ACE inhibitors and calcium channel blockers is around £150-£200.
Dietary protein intake

In animal models, high protein feeding leads to increased intraglomerular pressure and GFR (Hostetter et al. 1986). Progressive albuminuria and diffuse glomerulosclerosis can be induced in diabetic rats by a high-protein diet, whereas protein restriction reduces both glomerular pressure and hyperfiltration (Hostetter et al. 1982). These changes are accompanied by reduced glomerular mesangial production of prostaglandins which are important in regulating intraglomerular haemodynamics.

In humans, severe protein restriction has been used for many years to control symptoms of advanced renal failure (Bergstrom, 1984). The rate of decline of renal function has been reported to be reduced in diabetic patients with renal failure (Barsotti et al. 1988) or nephropathy (Walker et al. 1989; Evanoff et al. 1989) following a low-protein diet, but these trials were small and essentially uncontrolled. In addition, GFR was often estimated indirectly as the reciprocal of serum creatinine (Evanoff et al. 1989), or creatinine clearance (Barsotti et al. 1988), which may not reflect changes in GFR accurately (Shemesh et al. 1985), particularly when protein intake is low (Gabriel, 1986). A more recent randomised controlled trial (Zeller et al. 1991), in which GFR was measured by iothalamate clearance in 35 insulin dependent patients with nephropathy over a mean follow up period of 35 months, found that the rate of decline in GFR was four times lower in the protein restricted group than in the control group. Another
randomised trial reported a significant reduction in albumin excretion in patients with nephropathy who were randomly assigned to a low protein diet; albumin excretion increased significantly in the same patients when protein intake was no longer restricted (Ciavarella et al. 1992). In insulin dependent patients with microalbuminuria, a small randomised cross-over trial found that moderate protein restriction (40g/day) reduced the rate of albumin excretion and GFR, independently of changes in blood glucose or blood pressure (Cohen et al. 1987). No trials have reported serious adverse effects associated with protein restriction. Protein restriction should therefore prove beneficial in insulin dependent patients with nephropathy, although compliance with low protein diets can be difficult (Locatelli et al. 1991). Whether protein restriction is effective in primary prevention of diabetic nephropathy is not known.

Other cardiovascular risk factors

In addition to high blood pressure, patients with diabetic nephropathy have high total and LDL-cholesterol, low HDL-cholesterol, increased fibrinogen, clotting factors and high platelet activity (Jensen et al. 1988; Winocour et al. 1987). Similar findings have been reported in patients with microalbuminuria (Jensen et al. 1988; Jones et al. 1989; Winocour, 1992). The high cardiovascular morbidity (Jensen et al. 1987) and mortality (Krolewski et al. 1987a; Borch-Johnsen and Kreiner, 1987) of patients with nephropathy, and the association between albuminuria and markers of endothelial cell injury, such as raised von Willebrand
factor and plasminogen activator (Jensen et al. 1989), have given rise to the view that albuminuria reflects generalised vascular disease in IDDM (Jensen, 1991). Less clear, however, is the effect of lipid and haemostatic abnormalities on renal function. Although some evidence suggests that hyperlipidaemia stimulates glomerulosclerosis and mesangial cell proliferation, (Keane et al. 1988) no studies in man have estimated the risk of clinical nephropathy associated with hyperlipidaemia, independent of other risk factors. Randomised trials of lipid reduction, or antiplatelet therapy with clinical endpoints are lacking. Some cross-sectional studies have suggested that smoking increases the risk of nephropathy (Muhlhauser et al. 1986; Christiansen, 1978; Telmer et al. 1984), but there is little prospective data to support this claim (Klein et al. 1991; Watts et al. 1991).
1.4 Diabetic retinopathy

1.4.1 Epidemiology and Natural History.

The annual incidence of blindness due to diabetes has been estimated to be 3.3 per 100,000 population in North America (Moss et al. 1988). Despite advances in photocoagulation and vitreoretinal surgery, diabetic retinopathy is still the leading cause of blindness in the working population in industrialised countries (Klein and Klein, 1983; Department of Health and Social Security, 1988). Most components of diabetic retinopathy, including endothelial proliferation, capillary closure and preretinal new vessels, occur in other diseases (Kohner et al. 1982). But the constellation of lesions, from early thickening of the basement membrane and loss of pericytes to microaneurysms, increased permeability and new vessel formation, is quite specific to diabetes. The great majority of patients develop background retinopathy, consisting of microaneurysms, dot-blot haemorrhages and hard exudates. Additional changes in background retinopathy, including venous beading, intra-retinal microvascular abnormalities (IRMA) and cotton wool spots, are associated with the development of a more severe form of retinal disease, proliferative retinopathy. This is recognised by the appearance of new retinal vessels which extend into the vitreous cavity, leading to haemorrhage and ultimately loss of vision. Maculopathy, or clinically significant macular oedema, is another important cause of visual loss in diabetic patients.
Although the causes of retinopathy are not well understood, the sequence of anatomical changes leading to visual loss in diabetes is fairly well defined (Merimee, 1990). The earliest anatomical changes are thickening of the basement membrane and selective loss of retinal capillary pericytes. Loss of these contractile cells, which can alter blood flow by changing the size of the vessel lumen, leads to impaired autoregulation. The overall effect of basement membrane thickening, decreased capillary perfusion and hyperglycaemia is capillary closure. The resultant ischaemia is a potent stimulus for increased retinal blood flow and new vessel formation (Patel et al. 1992). Features indicative of progression to proliferative disease in a patient with background retinopathy are those associated with widespread capillary non-perfusion, including multiple cotton-wool spots, venous beading, IRMA and deep retinal haemorrhages. Features which predict the development of maculopathy include hard exudates and multiple microaneurysms in the macular area. Long-standing oedema may cause permanent damage to photoreceptors so that vision rarely improves even if the oedema resolves.

Visual prognosis in proliferative retinopathy is poor, particularly in patients who have new vessels arising from the optic disc (Kohner et al. 1982). Early studies (Caird et al. 1968; Deckert et al. 1967; Kohner, 1977) found that 30% to 50% of patients with proliferative retinopathy were blind within five years of diagnosis. Fortunately, the outlook has been greatly improved by retinal photocoagulation and vitreoretinal surgery. From an overview of several randomised controlled trials,
(Hercules et al. 1977; British Multicentre Study Group, 1984; Diabetic Retinopathy Study Group, 1981a; British Multicentre Study Group, 1983) it has been estimated that photocoagulation can reduce the incidence of blindness from diabetic retinopathy by around 73% (Rohan et al. 1989).

Much of our knowledge of the epidemiology of diabetic retinopathy has come from the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) conducted by Klein and colleagues (Klein et al. 1989a). They have devised a detailed method for grading retinopathy, based on comparison of retinal photographs with standard photographs chosen to illustrate lesions of different severity. The method entails stereoscopic fundus photography of seven retinal fields and field-by-field, lesion-by-lesion evaluation of photographs of each eye, using a modification of the Airlie House classification of diabetic retinopathy (Diabetic Retinopathy Study Research Group: Report 7, 1981b). Prospective evaluation (Klein et al. 1984) has shown the method to be useful for characterising the overall severity of retinopathy and for assessing progression in a clinically meaningful way. In the WESDR, diabetic patients were divided according to the age at diagnosis, and those diagnosed before 31 years were assumed to be mostly insulin dependent. In this younger onset-group, the prevalence of retinopathy rose steeply with duration of diabetes, from around 1% at five years, to 97.7% after fifteen to twenty years of diabetes. Proliferative retinopathy, which was rare in patients with diabetes for less than ten years, increased steadily to reach a peak prevalence of 67% in those with diabetes for thirty five years or more (Klein et
al. 1984). Similar findings were reported by a population-based prevalence study in Pittsburgh (Orchard et al. 1990). Somewhat lower prevalences of retinopathy have been reported in population-based Scandinavian studies (Sjolie, 1985) in which retinopathy was determined by ophthalmoscopy, not retinal photography.

1.4.2 Risk factors for diabetic retinopathy

_Blood glucose control_

A strong relation between chronic hyperglycaemia and severe diabetic retinopathy has been observed in cross-sectional (Klein et al. 1984; Orchard et al. 1990) and prospective (Janka et al. 1989a; Teuscher et al. 1988; Klein et al. 1988) studies. In a four year follow up (Janka et al. 1989a) of 153 patients with IDDM for at least fifteen years and little or no background retinopathy at baseline, the risk of progression, relative to patients in the lowest baseline quartile of glycated haemoglobin, was three, ten and twenty-six times higher in patients in the second, third and quartiles respectively. Klein and others (Klein et al. 1988) found a particularly strong relation between hyperglycaemia and proliferative retinopathy: patients in the highest quartile of glycated haemoglobin had a four-year risk of developing proliferative retinopathy which was twenty-eight times higher than that of patients in the lowest quartile.

Since poor blood glucose control is a strong and modifiable risk factor for retinopathy, several randomised trials have compared the effects of strict versus
conventional control. Initially, it appeared that strict control neither reversed nor prevented progression of retinopathy. Three trials (Lauritzen et al. 1983; Dahl-Jorgensen et al. 1985; Kroc Collaborative Study Group, 1984) found that tight control of blood glucose was followed by a rapid, if transient, worsening of retinopathy, which, in some patients, resulted in retinal infarcts, increased microaneurysms and even proliferative changes. Another study (Ramsay et al. 1988) found that even long-term restoration of normoglycaemia by successful pancreatic transplantation failed to retard progression of retinopathy in patients with established disease.

However, as noted above, the DCCT has now shown that intensive treatment, with achievement of strict control, substantially reduces both the incidence and progression of diabetic retinopathy (The Diabetes Control and Complications Trial Research Group, 1993). Since continuous normoglycaemia was rarely achieved in the DCCT, it remains unclear whether long-term normoglycaemia would prevent the development of retinopathy in all IDDM patients. Failure of normoglycaemia to arrest microangiopathy in some individuals might be explained at a biochemical level by nonenzymatic glycosylation (Brownlee et al. 1988). This leads to cross-linkage between proteins, a process which contributes to the thickening of basement membranes and continues even after the concentration of ambient glucose is lowered to normal (Furth and Harding, 1989). In dogs with experimentally induced diabetes-like retinopathy, glycaemic control prevents retinopathy only if established within two months of the onset of diabetes.
Interestingly, DCCT showed an initial worsening of retinopathy in the intensively-treated group before reduced risk in that group became apparent. This agrees well with the findings of earlier trials and suggests that patients in those early trials were not followed up long enough to demonstrate the benefit that is now clear from the DCCT.

**Blood pressure and flow**

The first indication that haemodynamic abnormalities might contribute to the development of diabetic retinopathy was the observation that endothelial injury could result from an increase in shear stress alone (Fry, 1969). When retinal vessels are exposed to increased shear stress the endothelial cells become deformed and ultimately disappear. The structural changes and later disappearance of endothelial cells increase the permeability of vessel walls to proteins and other substances. The resulting leakage of fluid and protein gives rise to retinal exudates and oedema which leads, in the macular area, to diabetic maculopathy. Disturbances in the integrity of the blood-retinal barrier can in fact be detected before any anatomical lesions are visible (Cunha-Vaz and Lima, 1978). Barring a substantial change in intraocular pressure, any increase in systemic blood pressure will increase perfusion pressure in the retinal vasculature once autoregulation of blood flow is impaired. The increase in perfusion pressure enhances exudation from damaged vessels and increases shear stress on the endothelial layer. Support for the role of hyperperfusion in the pathogenesis of
retinopathy comes from the observation that raised intraocular pressure and unilateral carotid stenosis, both of which reduce retinal blood flow, tend to protect against diabetic retinopathy (Behrendt and Duane, 1970; Gay and Rosenbaum, 1966). The question then naturally arises: are patients with high blood pressure at greater risk of developing or worsening retinopathy?

Several studies have examined the association between blood pressure and retinopathy, (Bodansky et al. 1982; Klein et al. 1984; Parving et al. 1988; Teuscher et al. 1988; Krolewski et al. 1985b; Hasslacher et al. 1986; Janka et al. 1989a; Klein et al. 1989b; Janka et al. 1989b; Chase et al. 1990) but relatively few have examined the relation prospectively in large numbers of insulin dependent patients. Follow up of 153 patients from the Joslin Diabetes Center (Janka et al. 1989a) showed that patients with diastolic pressure above 70mmHg had a markedly higher risk of proliferative retinopathy than those with diastolic pressure below 70mmHg. Klein and others (Klein et al. 1989b) found a prospective association between diastolic blood pressure and progression of retinopathy in young onset patients that was only just statistically significant after controlling for other risk factors. Systolic blood pressure was a significant predictor of the incidence of any retinopathy, but the relationship was not strong; the authors estimated that an increase of 10mmHg in systolic blood pressure is associated with a 40% increase, or a relative risk of 1.4, in the four year incidence of retinopathy. Although this relation was independent of glycaemic control, it may have been confounded or modified by raised albumin excretion,
which is positively associated with both high blood pressure and retinopathy (see below). This possibility is supported by cross-sectional analyses (Krolewski et al. 1985b; Norgaard et al. 1991; Sjolie, 1985) which found little association between retinopathy and high blood pressure in the absence of proteinuria.

Another study which found a prospective association between systolic blood pressure and retinopathy, (Teuscher et al. 1988) suggested that antihypertensive treatment might have a beneficial effect because patients who were on treatment at baseline had a lower incidence of retinopathy than untreated patients with similar levels of blood pressure. Parving and others have examined the effect of antihypertensive treatment on blood retinal barrier permeability (Parving et al. 1989b) through measurement of fluorescein leakage across the retinal barrier before and after 7 months of antihypertensive treatment in nine hypertensive insulin dependent patients. Reduction in mean blood pressure from 152/97 to 134/82 was associated with a near 50% reduction in the rate of fluorescein leakage. However, no randomised controlled trials have examined the effect of blood pressure reduction on the incidence or development of retinopathy (Parving, 1991).

Endocrine factors

Two observations show that the course of diabetic retinopathy is influenced by the pituitary gland: regression of diabetic retinopathy after infarction of the pituitary
gland is well recognised (Poulson, 1953) and a trial of hypophysectomy in diabetic patients with severe retinopathy resulted in significantly less retinopathy in the hypophysectomised patients than the control group after five years (Lundbaek, 1976). A specific link between growth hormone and diabetic retinopathy is indicated by the conspicuous absence of microangiopathy in diabetic patients with dwarfism due to growth hormone deficiency (Merimee, 1978). Despite experimental data linking insulin-like growth hormone (IGF-I), which mediates many of the anabolic effects of growth hormone, with new retinal vessel formation, (Merimee et al. 1983) the pathogenetic role of endocrine factors remains unclear. The earliest physiologic and anatomical changes of diabetic retinopathy can be explained reasonably well by haemodynamic and biochemical disturbances. Growth hormone, IGF-I and other growth factors may modulate these processes, but are not essential for their development. Endocrine factors are more likely to contribute to the end stages of retinopathy, which are less well explained by haemodynamic and biochemical factors (Merimee, 1990).

*Other factors*

Although the studies of Klein and others indicate that virtually all diabetic patients are at risk of retinopathy, many do not develop proliferative retinopathy despite many years of diabetes and periods of poor control. In one case control study of genetic factors, (Dorman et al. 1982) the odds ratio for retinopathy associated with poor control was 6.7, the odds ratio associated with HLA-DR4 was 3.7 and the
odds ratio when both factors were present was 33.3. The authors therefore suggested that vulnerability to the effects of poor glycaemic control might be genetically determined. Another study has reported an excess of HLA-DR phenotypes in certain subgroups of IDDM patients with proliferative retinopathy, (Rand et al. 1985) but a third case control study was unable to find any association between HLA antigens and proliferative retinopathy (Groop et al. 1986). The importance of genetic factors in diabetic retinopathy remains unclear (Barbosa and Saner, 1984).

The effect of cigarette smoking on retinopathy has been examined in several studies. Some have found a positive association (Muhlhauser et al. 1986; Paetkau et al. 1977; Sjolie, 1985), others have not (Dornan et al. 1982; West et al. 1980a; West et al. 1980b; Rand et al. 1985; Klein et al. 1983). Failure to detect an association in cross-sectional studies might reflect survivor bias i.e. limited opportunity to examine smokers with retinopathy because of poor survival. However, incidence data (Moss et al. 1991) from the WESDR found no relation between smoking and the incidence or progression of retinopathy in younger-onset patients, after controlling for duration of diabetes and glycated haemoglobin. The authors concluded that smoking is unlikely to be an important risk factor for diabetic retinopathy.

Finally, an association between hyperlipidaemia and retinopathy has been noted, but it is not clear whether this persists in the absence of nephropathy. Whatever
its origin, hyperlipidaemia might be expected to increase the amount of lipid-containing hard exudates (King et al. 1963), but its effect on overall progression of retinopathy is unknown.
1.5 Association between retinopathy and nephropathy

Diabetic nephropathy and retinopathy have so far been described separately, but the association between the two gives important information about the likely extent of a common aetiology. As already noted, virtually all IDDM patients can expect to develop some degree of retinopathy during their life-time, but only 35% to 45% are at risk of nephropathy. It has long been recognised that diabetic nephropathy rarely occurs without any retinopathy; so much so that some diabetologists consider nephropathy in a diabetic patient with no retinopathy to be of non-diabetic origin. The converse (that retinopathy rarely occurs without nephropathy) clearly cannot be true, and retinopathy is commonly observed in patients who never develop nephropathy. Although the rate of albumin excretion tends to increase with severity of retinopathy, around 35% to 50% of patients with proliferative retinopathy have no evidence of nephropathy (Agardh et al. 1987; Marre et al. 1985; Sjolie, 1985).

One proposed explanation for the almost invariable presence of retinopathy in patients with nephropathy is that raised blood pressure secondary to nephropathy causes progression of retinopathy (Agardh et al. 1987). However, the implication that blood pressure is not raised in patients without nephropathy leaves proliferative retinopathy in these patients unexplained (Bodansky et al. 1982). Secondly, the temporal sequence of retinal and renal changes, on which the explanation depends, is not well defined. Whatever the correct interpretation of
the incomplete association between retinopathy and nephropathy in IDDM, it clearly cannot be explained by a single common aetiology.

1.6 Summary of relevant literature

IDDM is becoming more common in Europe and few patients escape its complications. The great majority have retinopathy after fifteen years of IDDM, and around 35%-40% are at risk of nephropathy. Despite the efficacy of retinal photocoagulation and vitreoretinal surgery in the treatment of advanced retinopathy, diabetes remains the leading cause of blindness in industrialised countries. It is also one of the commonest underlying diseases in renal dialysis and transplant recipients. Duration of diabetes and poor glycaemic control are well-established risk factors for both complications, but leave much of the individual’s risk unexplained. A definitive randomised controlled trial (the DCCT) has recently shown that intensive insulin treatment, associated with strict glycaemic control, substantially reduces the risk of retinopathy and nephropathy. However, strict glycaemic control also increases the risk of severe hypoglycaemia, and has little beneficial effect in patients with persistent proteinuria.

Raised blood pressure and progression of diabetic renal disease are closely related. It is not yet clear, however, whether a rise in blood pressure precedes or follows the earliest changes of nephropathy, or whether normalisation of albumin excretion through blood pressure reduction can prevent the development of clinical
nephropathy. Genetic factors are thought to be important in nephropathy, and it has been suggested that susceptibility to nephropathy is determined by a genetic predisposition to hypertension. Prospective studies have shown that the risk of retinopathy increases with blood pressure, but the extent to which this relation is confounded or modified by raised albumin excretion is not clear. Although there is ample evidence to implicate abnormal blood pressure and flow in the development of retinopathy, no trials have yet shown that reduction of blood pressure reduces the incidence or progression of retinopathy. Other putative risk factors, including smoking, hyperlipidaemia and haemostatic factors, have been investigated, but their effect on retinal and renal function is largely unknown.
2. Methods

2.1 The EURODIAB IDDM Complications Study.

The EURODIAB IDDM Complications Study is a cross-sectional study of diabetic complications in three and a quarter thousand IDDM patients from 31 centres in 16 European countries. The study was set up to measure the prevalence of complications, and to examine specific relations between putative risk factors (exposures) and diabetic complications. It was sponsored by the European Community as a Concerted Action Programme on the Epidemiology and Prevention of Diabetes. The study was co-ordinated at the Department of Epidemiology and Public Health, University College London, and the data were collected during 1990-1992. The full study protocol is given in the appendix and the relevant parts are summarised below.

2.2 Subjects and Methods

IDDM was defined as diabetes diagnosed before the age of 36 with continuous need for insulin after one year. At each centre (except Cagliari), a stratified random sample of up to 140 (see below) patients aged 15-60 years was selected from a sampling frame of all IDDM patients who had attended the diabetes clinic, or related clinics such as a renal clinic, at least once during the previous 12 months. Patients were stratified by sex, age in three groups (15-29, 30-44 and 45-
60 years) and duration in three groups (1-7, 8-14 and 15+ years) before randomly selecting up to 10 patients from each stratum. In Dusseldorf and in Northern France (Valenciennes and Paris), recruitment was shared by two independent centres; data from each of the two centres have been combined in this report. Data from four English centres (NW London, Sheffield, Wolverhampton, Manchester) who recruited smaller numbers of patients, have been combined as one centre. In Cagliari, all clinic attenders were aged below 30 years. These patients were stratified according to sex and duration as above before random selection.

At each centre, local ethical committee approval for the study and informed consent from all patients were obtained. Pregnant women, patients who were unrepresentative of local ethnic groups and those with IDDM for less than one year were not recruited into the study. Patients were invited to participate in the study in person, by phone and by letter. Fourteen percent of eligible patients could not be contacted. Of the remainder who responded to the invitation, 85% took part in the study, giving a total of 3250 insulin dependent patients (1668 male). Their mean (sd) age was 32.7 (10.7) years and their mean duration of diabetes was 14.7 (9.3) years. The number of patients studied in each centre and the distribution of stratification variables is shown in Table 1.
### Table 1. Age and duration of IDDM in men and women by centre.

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<th>Duration (mean±sd)</th>
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<td>59</td>
<td>34.4± 9.8</td>
</tr>
<tr>
<td>Leiden</td>
<td>71</td>
<td>65</td>
<td>33.9± 9.7</td>
</tr>
<tr>
<td>Lisbon</td>
<td>70</td>
<td>68</td>
<td>32.2± 9.8</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>61</td>
<td>55</td>
<td>31.2± 9.5</td>
</tr>
<tr>
<td>Milan</td>
<td>62</td>
<td>63</td>
<td>34.7± 9.3</td>
</tr>
<tr>
<td>Munich</td>
<td>73</td>
<td>74</td>
<td>32.3± 9.6</td>
</tr>
<tr>
<td>N. France</td>
<td>70</td>
<td>57</td>
<td>34.2±10.1</td>
</tr>
<tr>
<td>Padua</td>
<td>57</td>
<td>44</td>
<td>34.3±13.0</td>
</tr>
<tr>
<td>Perugia</td>
<td>61</td>
<td>47</td>
<td>33.0± 9.0</td>
</tr>
<tr>
<td>Pisa</td>
<td>71</td>
<td>71</td>
<td>32.3± 9.5</td>
</tr>
<tr>
<td>Rome</td>
<td>69</td>
<td>68</td>
<td>32.7± 9.9</td>
</tr>
<tr>
<td>Turin</td>
<td>55</td>
<td>51</td>
<td>32.8±10.2</td>
</tr>
<tr>
<td>Thessaloniki</td>
<td>49</td>
<td>56</td>
<td>29.8± 9.5</td>
</tr>
<tr>
<td>Verona</td>
<td>60</td>
<td>61</td>
<td>30.3± 9.9</td>
</tr>
<tr>
<td>Vienna</td>
<td>63</td>
<td>59</td>
<td>35.1± 9.9</td>
</tr>
<tr>
<td>Zagreb</td>
<td>70</td>
<td>70</td>
<td>32.6±10.1</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>1668</td>
<td>1582</td>
<td><strong>32.7±10.2</strong></td>
</tr>
</tbody>
</table>
2.3 Measurement of nephropathy

After attending regional training sessions, investigators followed standardised procedures described in a comprehensive manual of operations. The rate of urinary albumin excretion (AER) was calculated from a single timed 24 hour urine collection. Containers for collecting urine, without added preservative, were provided by the local centres. Patients were instructed to collect urine on the day before their scheduled clinic visit as follows: "On the morning that you start your collection, pass urine into the toilet in the usual way. Note the precise time that you do this and write it on the label attached to the container. For the rest of the day and following night pass all your urine into the container(s) provided. The next morning, if possible exactly 24 hours after you began collecting urine, pass urine into the bottle. Write down the time and the date on the label. The collection is now over. Do not pass any more urine into the bottle." Patients were advised to keep the urine in a fridge or cool place before bringing it to the clinic. These instructions were explained to the patient and written on labels attached to the containers.

On arrival at the clinic, urine from each container was screened for infection using test strips (Nephur-Test+Leuco, Boehringer Mannheim) which reveal the presence of nitrites and hence indirectly of nitrite-forming bacteria in the urine. A positive test result, detected by a colour change in the test strip, would indicate the need for further investigation for urinary tract infection. The patient would then be treated as appropriate and asked to repeat the urine collection approximately one week later. Following a negative nitrite test result, the volume of urine was measured to the
nearest 2ml using a measuring cylinder, or, preferably, weighed to the nearest gram using a sensitive balance. Labelled 2ml aliquots of urine were then frozen at or below -20 °C without delay. One aliquot was stored locally at or below -20 °C as a back-up sample. The remainder were transported frozen in dry ice to a central laboratory (Guy’s Hospital, London) for measurement of urinary albumin concentration.

At the central laboratory, the concentration of urinary albumin was measured by an immunoturbidimetric method (Kearney et al. 1987) using goat anti-human albumin antisera (Sanofi Diagnostics Pasteur Inc, Minnesota, USA) and human serum albumin standards (ORHA 20/21 grade HSA, Behring Diagnostics, Hoechst UK Ltd, Hounslow Middlesex UK). The coefficient of variation of the assay was 8.95% at 10.7 mg/l and 3.60% at 86.8 mg/l.

Information about the timing and volume of urine collected was sent directly from local centres to the Co-ordinating Centre where the rate of urinary albumin excretion was calculated as the concentration of urinary albumin (mg/l) multiplied by the urine volume collected (ml), divided by the collection period (minutes), and expressed as µg/min. Mean time between collection and measurement of urine samples was 179 ± 87 (standard deviation) days.

The normal range of AER determined by the central laboratory from a small sample (n=56) of people without diabetes is 1.5 to 18.0 µg/min, with a mean of 6.5 µg/min. Since AER is a continuous variable with no clear distinction between normal and
abnormal values, most analyses in this thesis treat AER as a continuous variable. However, AER is often categorised as normoalbuminuria, microalbuminuria or macroalbuminuria, for a combination of historical, methodological and operational reasons (see introduction). In this study, AER (also termed albuminuria) was measured from a single 24 hour urine collection and defined as normal below 20\(\mu g/min\) and raised at or above this level. Microalbuminuria was defined as AER between 20 - 200 \(\mu g/min\) and macroalbuminuria as over 200 \(\mu g/min\).

2.4 Measurement of Retinopathy

All but two centres (Bucharest and Krakow) took retinal transparencies with a wide angle camera after pupil dilation. Two retinal fields, disc-macula-temporal and disc-nasal were photographed in each eye, using Kodachrome 64 ASA diapositive film. A single observer graded all retinal transparencies by comparing them with two sets of standard transparencies taken with a 45° camera (Canon CF-60UV). The standards were carefully chosen to illustrate specific lesions at two levels of severity. For each field, the observer decided whether each type of lesion was less severe than the first standard (Grade 2), at least as severe as the first standard but less severe than the second (Grade 3), or at least as severe as the second standard (Grade 4). Each patient's level of retinopathy was determined by the worse eye. Retinopathy was categorised on 6 levels as follows:
<table>
<thead>
<tr>
<th>Level</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No retinopathy</td>
</tr>
</tbody>
</table>
| 1     | Any HMA graded 2, or  
      | any HE graded 2 or 3, or  
      | HE graded 4 in either nasal field |
| 2     | Any HMA graded 3, or  
      | HE graded 4 in either macular field |
| 3     | Any CWS graded 2, or  
      | any IRMA graded 2, or  
      | any VB graded 2, or  
      | any HMA graded 4 |
| 4     | Any CWS graded 3 or 4, or  
      | any IRMA graded 3 or 4, or  
      | any VB graded 3 or 4 |
| 5     | Any NVD NVE FPD FPE PRH VH, or  
      | panretinal photocoagulation |

**KEY**

- **HE** - hard exudates  
- **HMA** - haemorrhages or microaneurysms  
- **CWS** - cotton wool spots  
- **IRMA** - intraretinal microvascular abnormalities  
- **VB** - venous beading  
- **NVD** - new vessels on optic disc  
- **NVE** - new vessels elsewhere  
- **FPD** - fibrous proliferations on optic disc  
- **FPE** - fibrous proliferations elsewhere  
- **PRH** - pre-retinal haemorrhage  
- **VH** - vitreous haemorrhage

Background or nonproliferative retinopathy corresponds to levels 1 to 4. Proliferative retinopathy corresponds to level 5.
2.5 Measurement of Risk Factors

- **blood pressure**

A Hawksley random zero sphygmomanometer (Wright and Dore, 1970) was used to measure blood pressure in the sitting position after the patient had rested in a quiet location for at least five minutes and avoided strenuous exercise, smoking and caffeine consumption for 30 minutes. The measurement protocol was taken from the INTERSALT study (Elliott and Stamler, 1988) - an epidemiological study of the relation between blood pressure and urinary sodium excretion (Intersalt Co-operative Research Group, 1988). Blood pressure in the right arm was measured to the nearest 2 millimetres of mercury using a range of different sized cuffs to allow for differing arm circumferences. Diastolic pressure was recorded at the disappearance of sound (Korotkoff phase 5). The average blood pressure of two readings was used for analysis.

- **parental hypertension**

Patients were asked if their mother or father was being treated (if alive) or had been treated (if deceased) for high blood pressure. If either parent was receiving treatment, or if either deceased parent had received treatment, a positive history of parental hypertension was assumed. If neither parent (alive or deceased) had received treatment, a negative history was assumed.

- **glycaemic control**

HbA$_1c$ was measured centrally by an enzyme immunoassay (John et al. 1993) using
a monoclonal antibody raised against HbA\textsubscript{1c} (Dako Ltd, Ely, UK). The normal reference range for this method is 2.9% to 4.8% (John et al. 1993). The within batch coefficient of variation is 2.4% ± 0.2% at 6.4% HbA\textsubscript{1c} and 2.3% ± 0.3% at 11.4% HbA\textsubscript{1c}. Between batch coefficients of variation are 5% and 2.5% respectively (John et al. 1993).

- other factors

A venous blood sample was taken after an overnight fast for measurement of plasma lipids. Plasma was separated by centrifugation at 1500 G for ten minutes at ambient temperature. Aliquots of plasma were stored at -20 °C at the local centres before transport to the central laboratory, frozen in dry ice. Total cholesterol was assayed by the CHOD-PAP method (Boehringer Mannheim (UK) Ltd, East Sussex, UK) using a cobas-bio centrifugal analyzer (Roche, Welwyn Garden City, Herts, UK). Patients were classified as smokers or non-smokers from their response to the question "Do you now smoke cigarettes?"

2.6 Quality Control

- General aspects

In general, standardisation of methods and procedures was achieved through practical training sessions, backed up by a detailed operational guide covering all aspects of the conduct of the study. All centres had to successfully complete a "dry run" or pilot study including all procedures in at least four patients before proceeding with the main study. Site visits were also conducted to ensure that the study methods were
being followed in accordance with the protocol, and sort out any problems noted during data collection.

The interviewer-administered questionnaire was translated where necessary by the local investigators. The translated questionnaires were returned to the Co-ordinating Centre for back-translation into English so that accuracy and meaning could be verified. Completed questionnaires were photocopied locally; the original was sent to the Co-ordinating Centre and the copy retained by the local centres. At the Co-ordinating Centre, questionnaire responses, hard-copy laboratory results and retinal photography gradings were computerised using bespoke data entry programmes. All data were entered by two independent operators (double data entry). Another computer programme was used to check for discrepancies between first and second data entries. Discrepancies were resolved by checking with hard-copy data, or by contacting the local centres. The final dataset was prepared by reading 20 separate text files into a statistical software package (SAS Institute Inc., 1988), and then merging them into a permanent SAS dataset. The data was further "cleaned" by examining the distribution of each variable of interest (e.g. time and volume of urine collection, AER, blood pressure, HbA_{1c} etc. using SAS "proc univariate"). Extreme values, or "outliers" were checked with hard-copy data, and amended or deleted as appropriate. Any protocol violators (e.g. those aged below 15 years or with duration of diabetes of less than one year) were excluded at the final stage of data preparation.

- reliability of measurement of urinary albumin concentration

In any study involving the measurement of X as a response, the aim will be to
compare the distribution of X values between different groups of individuals. Ideally, one would like to use the true value for each individual, but in reality, one has only the observed values which are composed of the true value plus an error. The effect of the errors on the distribution of true values will depend on two things: the range of true values about their mean and the range of the errors about zero. For example, if the true values range from 80 to 220, one can afford a larger error than if they range from 95 to 105. This aspect of a measurement is called its reliability. Because reliability depends on the range of true values in the study it provides more information than the coefficient of variation of a biochemical assay; it may also vary appreciably from study to study.

This study was designed to allow estimation of the reliability of urinary albumin concentration as follows: hidden duplicate samples of urine were obtained from a 10% random subsample of patients i.e. two sets of urine aliquots were prepared from the same single urine collection, and each set was labelled with a different identity number. To the laboratory staff, it would thus appear that urine samples had been received from two different patients, rather than the same patient. In the absence of measurement error, near identical values would be obtained from measurement of these hidden duplicate urine samples. Comparison of the discrepancy in measurements between duplicate samples therefore provided an objective assessment of the degree of assay / laboratory variation. For one of the main study end-points, log urinary albumin concentration, the agreement between measurement of duplicate urine samples is illustrated in Figure 1. The level of agreement between duplicate measurements was also estimated by the reliability coefficient (R) as follows:
\[ R = 1 - \left( \frac{s^2_w}{s^2_b} \right) \]

where

\( s^2_w \) is the within person variance of the difference between duplicate measurements and \( s^2_b \) is the between person variance of a single measurement.

The mean difference between 356 pairs of duplicate measurements was 0.46 mg/l, and the standard deviation 51.1; the mean albumin concentration in the whole sample (n=3054) was 92.2 mg/l and the standard deviation 302.5. Thus,

\[ R = 1 - \left( \frac{51.1^2}{302.5^2} \right) \]
\[ = 0.97 \]

The coefficient of reliability gives a measure of the relative magnitude of measurement error to between person variability and takes values from 0, indicating no reliability, to 1, indicating complete reliability. Because the difference in repeated measurements within individuals was small relative to the wide range of values found in different individuals, the reliability of measurement of urinary albumin concentration in this study was high.
Fig 1. Hidden duplicate measures of urinary albumin concentration
Focus, clarity and field definition were excellent, good or adequate for grading in 91% of all retinal fields received. Grading was complete (i.e. four fields graded) in 2138 patients, and grading of one eye was complete in a further 91 patients. All patients with at least one graded field (2479) are included in this report because exclusion of patients with incomplete grading would have led to underestimation of patients with more severe retinopathy.

In twenty-five patients, the more rigorous protocol (Diabetic Retinopathy Study Research Group: Report 7, 1981b) of the Wisconsin Epidemiology Study of Diabetic Retinopathy (WESDR) which requires stereoscopic photographs of seven retinal fields was followed in addition to the EURODIAB protocol. Both methods rely on comparison of photographs with sets of standard photographs, but the WESDR grading is more detailed and specifies 10 levels for each eye. The WESDR retinopathy levels can be summarised as follows:

<table>
<thead>
<tr>
<th>Level</th>
<th>Summary definition (Klein et al. 1989a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>No retinopathy</td>
</tr>
<tr>
<td>21-31</td>
<td>Minimal to mild background retinopathy</td>
</tr>
<tr>
<td>41-51</td>
<td>Moderate to severe background retinopathy</td>
</tr>
<tr>
<td>60</td>
<td>Fibrous proliferations only</td>
</tr>
<tr>
<td>65</td>
<td>Early proliferative changes</td>
</tr>
<tr>
<td>70-80</td>
<td>Proliferative retinopathy with high risk of visual loss</td>
</tr>
</tbody>
</table>
Table 2 shows the gradings for each of the twenty-five patients in whom both protocols were followed. For ease of comparison, levels of retinopathy are also categorised as background or proliferative. Comparison indicates close agreement in the detection of any retinopathy, but some disagreement in the level of retinopathy, with grading by the WESDR protocol tending to be more severe than the EURODIAB protocol.
Table 2. **Comparison of grading by two retinal photography protocols**

<table>
<thead>
<tr>
<th>EUROI DIAB protocol</th>
<th>WESDR protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grading Category of Retinopathy*</td>
<td>Grading Category of Retinopathy*</td>
</tr>
<tr>
<td>RE / LE</td>
<td>RE / LE</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>0 / 0</td>
<td>No retinopathy</td>
</tr>
<tr>
<td>0 / 0</td>
<td>No retinopathy</td>
</tr>
<tr>
<td>0 / 0</td>
<td>No retinopathy</td>
</tr>
<tr>
<td>2 / 1</td>
<td>Background</td>
</tr>
<tr>
<td>4 / 4</td>
<td>Background</td>
</tr>
<tr>
<td>2 / 3</td>
<td>Background</td>
</tr>
<tr>
<td>4 / 4</td>
<td>Background</td>
</tr>
<tr>
<td>1 / 1</td>
<td>Background</td>
</tr>
<tr>
<td>3 / 4</td>
<td>Background</td>
</tr>
<tr>
<td>2 / 2</td>
<td>Background</td>
</tr>
<tr>
<td>4 / 4</td>
<td>Background</td>
</tr>
<tr>
<td>4 / 4</td>
<td>Background</td>
</tr>
<tr>
<td>4 / 4</td>
<td>Background</td>
</tr>
<tr>
<td>1 / 3</td>
<td>Background</td>
</tr>
<tr>
<td>1 / 2</td>
<td>Background</td>
</tr>
<tr>
<td>1 / 3</td>
<td>Background</td>
</tr>
<tr>
<td>4 / 4</td>
<td>Background</td>
</tr>
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<td>3 / 4</td>
<td>Background</td>
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<td>Background</td>
</tr>
<tr>
<td>4 / 3</td>
<td>Background</td>
</tr>
<tr>
<td>5 / 1</td>
<td>Proliferative</td>
</tr>
<tr>
<td>5 / 5</td>
<td>Proliferative</td>
</tr>
<tr>
<td>1 / 5</td>
<td>Proliferative</td>
</tr>
<tr>
<td>5 / PH</td>
<td>Proliferative</td>
</tr>
<tr>
<td>5 / 5</td>
<td>Proliferative</td>
</tr>
</tbody>
</table>

* Grading of worse eye. PH - pre-retinal haemorrhage. RE/LE - Left eye / Right Eye
2.7 Statistical Analysis

The statistical power to detect relations between key variables within centres was estimated on the assumption that most centres would be able to recruit and investigate 140 patients. This would allow a difference of 14% in the frequency of macroalbuminuria between patients in the upper and lower halves of blood pressure distribution to be detected, with over 80% power at 5% significance, assuming the prevalence of macroalbuminuria in the lower half to be 3%.

All statistical analyses were performed using SAS software (SAS Institute Inc., 1988). Median systolic and diastolic blood pressure were calculated after assigning patients on antihypertensive treatment to the upper quartiles of the distributions (White et al. 1994). AER was analysed as a categorical variable (normo, micro and macroalbuminuria) and as a continuous variable after logarithmic transformation. The relation between blood pressure and log AER was examined in the whole sample, within centres, and by retinopathy status, level of glycaemic control, sex and parental history of hypertension. Blood pressure was treated as a series of discrete (dummy) variables i.e. each level of diastolic pressure (<65mmHg, 65-70mmHg etc) was treated as a separate variable and each individual assigned a score of 0 or 1 on each variable. At each level of blood pressure, mean log AER was adjusted for duration of diabetes and HbA1c using a least-squares regression model (SAS "proc glm", i.e. a statistical procedure which analyses data within the framework of general linear models (SAS Institute Inc., 1990)). The adjusted means are the values predicted in the model when the other variables are held at their mean values. The effect of
adjusting for other factors such as age, cholesterol and smoking status was also examined. Other terms were added to the model to see whether the relation of blood pressure to AER differed between groups of patients defined by retinopathy status, level of glycaemic control and history of parental hypertension (test for interaction). Statistically significant interaction terms indicated that the relation of blood pressure to AER differed significantly according to those factors. The relation between duration of diabetes in five year intervals and log AER was examined by the same statistical method; mean log AER was adjusted for diastolic blood pressure and HbA₁c using a least-squares regression model.

Within centres, the proportion of patients with raised albuminuria above and below the centre median diastolic pressure was compared separately in patients with and without retinopathy, using the Wilcoxon signed rank test (Kirkwood, 1988). Tests of significance for proportions (including prevalence of retinopathy) were otherwise based on the Mantel-Haenszel chi-square statistic (Mantel and Haenszel, 1959; SAS Institute Inc., 1988).

Prevalences of retinopathy and raised AER within HbA₁c groups were directly standardised to the distribution of duration in the whole sample. Prevalences of retinopathy within systolic and diastolic blood pressure groups were directly standardised to the distribution of HbA₁c and duration in the whole sample.
3. **Results**

3.1 **Prevalence of raised albuminuria by known risk factors**

The prevalence of raised albuminuria is shown in Table 3. Raised AER was more common in men (32.6%) than women (26.3%, \( p=0.001 \)).

<table>
<thead>
<tr>
<th>Albumin Excretion Rate - Men</th>
<th>n</th>
<th>% (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (&lt;20μg/min)</td>
<td>1052</td>
<td>67.4 (65.1 - 69.7)</td>
</tr>
<tr>
<td>Microalbuminuria (20-200μg/min)</td>
<td>357</td>
<td>22.9 (20.8 - 25.0)</td>
</tr>
<tr>
<td>Macroalbuminuria (&gt;200μg/min)</td>
<td>152</td>
<td>9.7 (8.3 - 9.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Albumin Excretion Rate - Women</th>
<th>n</th>
<th>% (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (&lt;20μg/min)</td>
<td>1101</td>
<td>73.7 (71.5 - 76.0)</td>
</tr>
<tr>
<td>Microalbuminuria (20-200μg/min)</td>
<td>274</td>
<td>18.4 (16.4 - 20.3)</td>
</tr>
<tr>
<td>Macroalbuminuria (&gt;200μg/min)</td>
<td>118</td>
<td>7.9 (6.5 - 9.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Albumin Excretion Rate - All</th>
<th>n</th>
<th>% (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (&lt;20μg/min)</td>
<td>2153</td>
<td>70.5 (68.9 - 72.1)</td>
</tr>
<tr>
<td>Microalbuminuria (20-200μg/min)</td>
<td>631</td>
<td>20.7 (19.3 - 22.1)</td>
</tr>
<tr>
<td>Macroalbuminuria (&gt;200μg/min)</td>
<td>270</td>
<td>8.8 (7.8 - 9.8)</td>
</tr>
</tbody>
</table>
The prevalence of micro- and macroalbuminuria by duration of diabetes is shown in Figures 2 to 4. In Figure 2, the top curve is the sum of the lower two. The prevalence of macroalbuminuria rose steeply between five and twenty years duration of diabetes and then declined, but the prevalence of microalbuminuria was relatively constant, ranging from 17% to 25% at five year intervals (Figure 2). The presence of microalbuminuria in a substantial proportion of patients with IDDM for less than five years was an unexpected finding, and was therefore examined in more detail (Figures 3 and 4). In patients with IDDM for less than six years (n=557), microalbuminuria was found consistently within each single year interval of duration (Figure 3). Seventeen percent of patients with diabetes for between one and two years had microalbuminuria. Figure 4 shows that raised AER in early IDDM was a consistent finding in all centres.

Sixteen percent of patients had HbA\(_{1c}\) values in the normal non diabetic range (2.9% to 4.8%). The distribution of HbA\(_{1c}\) and the relation between raised AER and HbA\(_{1c}\) is shown in Figure 5. The frequency of both microalbuminuria and macroalbuminuria increased steadily with increasing HbA\(_{1c}\).
Fig 2. Prevalence of raised AER by duration of diabetes

![Graph showing prevalence of raised AER by duration of diabetes. The graph plots the percentage of patients with macroalbuminuria, microalbuminuria, and raised AER against the duration of IDDM (years).]
Fig 3. Prevalence of raised AER in early IDDM

![Graph showing the prevalence of raised AER in early IDDM. The x-axis represents the duration of IDDM (years), ranging from 0-1 to 5-6 years. The y-axis represents the percentage (%). The bars indicate the prevalence of AER, with two categories: 20-200ug/min and >200ug/min. The duration 3-4 years shows the highest prevalence of raised AER, with AER >200ug/min highlighted.]

- AER 20-200ug/min
- AER >200ug/min
Fig 4. Prevalence of raised AER in early IDDM (duration 1-6 yrs) by centre

- AER 20-200ug/min
- AER >200ug/min
Fig 5. Prevalence of albuminuria by glycated haemoglobin

HbA1c (%)

- Microalbuminuria  - Macroalbuminuria

Prevalence adjusted for duration of diabetes
3.2 Prevalence of retinopathy by known risk factors

The frequency and severity of retinopathy is shown in Table 4. Background retinopathy was found in 35.9% (34.0, 37.8) of patients and proliferative retinopathy in 10.3% (9.1, 11.5). Prevalences were fairly similar in men (38.1% background, 9.6% proliferative) and women (33.6% and 11.0% respectively, \( p=0.06 \)).

Table 4. **Frequency and Severity of Retinopathy**

<table>
<thead>
<tr>
<th>Category</th>
<th>Level*</th>
<th>n</th>
<th>% (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Retinopathy</td>
<td>0</td>
<td>1335</td>
<td>53.9 (51.9 - 55.9)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>593</td>
<td>23.9 (22.2 - 25.6)</td>
</tr>
<tr>
<td>Background Retinopathy</td>
<td>2</td>
<td>53</td>
<td>2.1 (1.5 - 2.7)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>126</td>
<td>5.1 (4.2 - 6.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>117</td>
<td>4.7 (3.9 - 5.5)</td>
</tr>
<tr>
<td>Proliferative Retinopathy</td>
<td>5</td>
<td>255</td>
<td>10.3 (9.1 - 11.5)</td>
</tr>
</tbody>
</table>

* For definition of level see methods
Prevalence of retinopathy by duration of diabetes is shown in Figure 6. Background retinopathy increased most steeply between five and fifteen years to reach a plateau of 82% after 20 years. Proliferative retinopathy was rare before ten years, after which it increased steadily to reach a prevalence of 36% in patients with diabetes for more than 30 years.

Prevalence of retinopathy by HbA1c is shown in Figure 7. The relation of HbA1c to background retinopathy was quite different to that of proliferative retinopathy. Below an HbA1c concentration of 8%, the prevalence of background retinopathy increased steadily with HbA1c, while the frequency of proliferative retinopathy was fairly constant. Above an HbA1c level of 8%, the prevalence of proliferative retinopathy began to rise as the increase in frequency of background retinopathy reversed.
Fig 6. Prevalence of retinopathy by duration of diabetes

Duration of IDDM (yrs)

- Proliferative
- Nonproliferative
- Any retinopathy
Fig 7. Prevalence of retinopathy by glycated haemoglobin

Prevalence adjusted for duration of diabetes
3.3 Association between retinopathy and raised AER

The association between retinopathy and raised AER is shown in Table 5. The frequency of macroalbuminuria increased from 1.6% in patients with no retinopathy to 34% in those with proliferative retinopathy. Although macroalbuminuria without retinopathy was uncommon, the converse was not, as 67% of patients with background retinopathy and 40% of those with proliferative retinopathy had normal AER.

Table 5. Association between retinopathy and raised AER

<table>
<thead>
<tr>
<th>Frequency Row percent Column percent</th>
<th>Normal AER</th>
<th>Microalbuminuria</th>
<th>Macroalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>No retinopathy</td>
<td>1044</td>
<td>215</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>81.6%</td>
<td>16.8%</td>
<td>1.6%</td>
</tr>
<tr>
<td></td>
<td>60.9%</td>
<td>44.7%</td>
<td>11.5%</td>
</tr>
<tr>
<td>Background retinopathy</td>
<td>572</td>
<td>202</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>67.2%</td>
<td>23.7%</td>
<td>9.1%</td>
</tr>
<tr>
<td></td>
<td>33.4%</td>
<td>42.0%</td>
<td>42.0%</td>
</tr>
<tr>
<td>Proliferative retinopathy</td>
<td>99</td>
<td>64</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>40.1%</td>
<td>25.9%</td>
<td>34.0%</td>
</tr>
<tr>
<td></td>
<td>5.8%</td>
<td>13.3%</td>
<td>46.2%</td>
</tr>
</tbody>
</table>
3.4 Relation between blood pressure and AER

- in the whole sample

The relation between blood pressure and AER in all patients is shown in Figure 8. At diastolic pressure below 80mmHg, there was a shallow, linear increase in AER with blood pressure. Above 80mmHg, AER increased steeply with diastolic blood pressure. Ten percent of patients were on antihypertensive treatment. Blood pressure (mean ± sd) in these patients (systolic 141 ± 22 mmHg, diastolic 84 ± 12 mmHg) was considerably (p<0.001) higher than in untreated patients (systolic 119 ± 16 mmHg, diastolic 75 ± 11 mmHg). Assuming the pre-treatment levels of both blood pressure and AER were higher than the levels measured on treatment, exclusion of treated patients would probably lead to underestimation of the strength of the true relation between blood pressure and AER (Figure 9). To avoid such bias, treated patients were included in analyses of the relation between blood pressure and AER.

- by retinopathy

Figure 10a reveals a striking difference (F value 13.9, p<0.001) in the relation of AER to blood pressure between patients with and without retinopathy, after adjusting for duration of diabetes and HbA1c. In patients without retinopathy, there was virtually no rise in AER with blood pressure; the prevalence of macroalbuminuria was 1.4% when diastolic pressure was below 75mmHg, 1.3% at 75-85mmHg, 3.0% at 85-95mmHg and 2.8% above 95mmHg (p=0.3). By contrast, the prevalence of macroalbuminuria in patients with retinopathy was 7% below 75mmHg, 13% at 75-80mmHg, 25% at 85-95mmHg and 37% above 95mmHg (p<0.001).
Fig 8. Mean albumin excretion rate by diastolic blood pressure

Geometric mean AER adjusted for duration and HbA1c
Fig 9. Mean AER by diastolic BP and antihypertensive treatment

Geometric mean AER adjusted for duration and HbA1c
Fig 10a. Mean AER by diastolic blood pressure and retinopathy

Geometric mean AER and 95% CI adjusted for duration and HbA1c
Additional adjustment for cardiovascular risk factors (age, plasma cholesterol and smoking status) in addition to duration of diabetes and HbA1c had no appreciable effect on the relation between diastolic blood pressure and AER, stratified by retinopathy (Figure 10b).

Further analysis by severity of retinopathy (Figures 11 and 12) showed that the gradient of the relation between blood pressure and AER steepened with increasing severity of retinopathy. In figures 11 and 12, the outcome (AER) is expressed as the percentage of patients with raised AER. The increase in prevalence of raised AER with diastolic pressure was highly significant (p<0.001) for all groups except those with no retinopathy (p=0.64).

Analysis of the relation between blood pressure and albumin excretion by retinopathy status was repeated after excluding patients with normal AER (Figure 13). In the 236 patients (Table 5) who had raised AER without retinopathy there was still no discernable relation between blood pressure and albumin excretion. In patients with retinopathy (n=427), albumin excretion was three times higher in patients with diastolic pressure above 95mmHg than those with diastolic pressure below 65mmHg. The relation between diastolic blood pressure and AER in patients with raised AER therefore differed significantly (F value 2.8, p=0.03) by retinopathy status.
Fig 10b. Mean AER by diastolic blood pressure and retinopathy

Geometric mean AER and 95% CI adjusted for duration, HbA1c age, cholesterol and smoking status
Fig 11. Raised AER by diastolic blood pressure and retinopathy

%  
100  
80  
60  
40  
20  
0  
<70  70-  80-  90-  
Diastolic BP (mmHg)

- No retinopathy  - Background  - Proliferative
Fig 12. Raised AER by diastolic blood pressure and level of retinopathy

For definition of levels see methods
Fig 13. Mean AER by diastolic BP and retinopathy in patients with raised AER

Geometric mean AER and 95% CI adjusted for duration and HbA1c
A consistent pattern emerged from within centre analyses (Table 6): in patients with retinopathy, the frequency of raised albuminuria in patients with high (above centre median) diastolic pressure was greater than the frequency in patients with low (below centre median) diastolic pressure in all but three centres, and significantly higher in eleven centres; in patients without retinopathy, the frequency of raised albuminuria was higher (not significantly) in patients with high diastolic pressure in fourteen centres, lower (not significantly) in seven centres and the same in three centres, indicating no consistent relation ($p=0.8$) between blood pressure and AER in the absence of retinopathy.
Table 6. **Raised AER by Retinopathy, Blood Pressure and Centre**

<table>
<thead>
<tr>
<th>Centre</th>
<th>RETINOPATHY</th>
<th>NO RETINOPATHY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% with raised AER</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High BP</td>
</tr>
<tr>
<td>Athens</td>
<td>49</td>
<td>65</td>
</tr>
<tr>
<td>Bari</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>Budapest</td>
<td>60</td>
<td>47</td>
</tr>
<tr>
<td>Cagliari</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Cork</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Dusseldorf</td>
<td>36</td>
<td>61</td>
</tr>
<tr>
<td>England</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td>Gent</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Helsinki</td>
<td>71</td>
<td>69</td>
</tr>
<tr>
<td>Leiden</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>Lisbon</td>
<td>73</td>
<td>41</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>18</td>
<td>44</td>
</tr>
<tr>
<td>Milan</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td>Munich</td>
<td>68</td>
<td>38</td>
</tr>
<tr>
<td>N. France</td>
<td>31</td>
<td>41</td>
</tr>
<tr>
<td>Padua</td>
<td>52</td>
<td>24</td>
</tr>
<tr>
<td>Perugia</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>Pisa</td>
<td>40</td>
<td>56</td>
</tr>
<tr>
<td>Rome</td>
<td>62</td>
<td>54</td>
</tr>
<tr>
<td>Turin</td>
<td>21</td>
<td>44</td>
</tr>
<tr>
<td>Thessaloniki</td>
<td>26</td>
<td>83</td>
</tr>
<tr>
<td>Verona</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>Vienna</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>Zagreb</td>
<td>78</td>
<td>58</td>
</tr>
</tbody>
</table>

* Significant difference in frequency of raised AER between high and low diastolic blood pressure groups
Since duration and HbA\textsubscript{1c} varied little with blood pressure, adjustment for these risk factors had negligible effect on the relation between blood pressure and mean AER. HbA\textsubscript{1c} is, however, related univariately to AER as shown in Figure 5. The effect of glycaemic control on the relation between blood pressure and AER in patients with and without retinopathy was therefore examined by stratification (Figure 14). Patients were divided into four groups, according to retinopathy status and HbA\textsubscript{1c} concentration above (poor control) or below (good control) the median (6.5\%). In patients with no retinopathy, AER was generally low even when blood pressure was high and glycaemic control poor. In patients with retinopathy, the increase in AER at higher levels of blood pressure was more pronounced (F value 2.2, p=0.05) in patients with poor control than in those with good control.

- **by retinopathy and sex**

As noted above, the prevalence of raised AER was significantly higher in men than women. Median blood pressure was also significantly higher in men (123/77 versus 117/75 in women, p<0.001). Figure 15 suggests that differences in the frequency of raised AER between men and women can be partly explained by differences in blood pressure, since mean AER was similar in men and women with the same retinopathy status and level of blood pressure (F value 0.35, p=0.84).
Fig 14. Mean AER by diastolic BP retinopathy and glycaemic control

D)3

a : L U <

240
180
120
60
0

Diastolic BP (mmHg)

AER (ug/min)

<65 65- 75- 85- 95+

Retention, poor Retinopathy, good

No retention, poor No retention, good

Geometric mean AER and 95% CI adjusted for duration

92
Fig 15. Mean AER by diastolic blood pressure retinopathy and sex

Geometric mean AER and 95% CI adjusted for duration and HbA1c
3.5 Relation between duration of diabetes and AER by retinopathy

The relative constancy of the prevalence of microalbuminuria by duration of diabetes (Figure 2) and, in particular, the absence of a fall in prevalence of microalbuminuria after twenty years, suggested the existence of a substantial group of patients in whom microalbuminuria does not progress to macroalbuminuria. Since there was a striking lack of association between blood pressure and AER in patients without retinopathy, the hypothesis that microalbuminuria in patients without retinopathy is non progressive was investigated. The relation between duration and AER in 663 patients with raised albuminuria is therefore shown by retinopathy status in Figure 16. (Macroalbuminuric patients were included with microalbuminuric patients in this analysis to avoid restricting outcome (AER) to 20-200μg/min). In patients with raised albuminuria but no retinopathy, there was little relation (F value 2.3, p=0.05) between duration of diabetes and mean AER; a marked rise in AER with duration of diabetes was seen only in patients with retinopathy (F value 4.5, p=0.004).
Fig 16. Mean AER by duration of IDDM and retinopathy in patients with raised AER

Geometric mean AER and 95% CI adjusted for BP and HbA1c
3.6 Relation between parental history of hypertension and raised AER

Of the 2873 patients (88%) who answered questions about whether their parents had high blood pressure, 42.6% had a positive history of parental hypertension. Age-adjusted median blood pressure and the prevalence of raised albuminuria were significantly higher in patients with a positive parental history than in those with a negative parental history (Table 7).

Table 7. Blood pressure and raised AER by parental history of hypertension

<table>
<thead>
<tr>
<th>Parental history of hypertension</th>
<th>n</th>
<th>Blood pressure* (mmHg)</th>
<th>Frequency of raised AER (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Untreated</td>
<td>All Untreated</td>
<td>All Untreated</td>
</tr>
<tr>
<td>Positive</td>
<td>1224 979</td>
<td>123/77 120/75</td>
<td>33% 27% (30,46) (24,30)</td>
</tr>
<tr>
<td>Negative</td>
<td>1649 1430</td>
<td>119/75 118/74</td>
<td>27% 24% (25,29) (22,26)</td>
</tr>
</tbody>
</table>

* Age-adjusted median systolic and diastolic blood pressure

p values refer to differences between patients with a positive parental history and patients with a negative parental history of hypertension
The influence of parental hypertension on the relation between blood pressure and AER was therefore examined by stratification. Patients were divided into four groups according to retinopathy status and parental history of hypertension. Figure 17 shows that a parental history of hypertension makes little contribution (F value 2.4, p=0.12) to the relation already described between blood pressure and AER in patients with and without retinopathy (Figure 10). Since patients with known hypertension might be more aware of high blood pressure within the family than patients with normal blood pressure, the analyses were repeated after excluding patients on antihypertensive treatment. Median systolic pressure remained higher in untreated patients with a positive parental history than in untreated patients with a negative parental history, although the increased frequency of raised AER was no longer statistically significant (Table 7). Figure 18 shows that restricting the analysis to untreated patients had little effect on the findings in the whole sample.

The relation between blood pressure and AER was also examined using systolic blood pressure. The findings with systolic blood pressure were very similar to those described above for diastolic blood pressure (Figures 19 to 25).
Fig 17. Mean AER by diastolic BP, retinopathy and parental history of hypertension (PH)

Geometric mean AER and 95% CI adjusted for duration and Hba1c
Fig 18. Mean AER by diastolic BP, retinopathy and parental history of hypertension (PH) in untreated patients

Geometric mean and 95% CI adjusted for age, duration and HbA1c
Fig 19. Mean albumin excretion rate by systolic blood pressure

Geometric mean AER adjusted for duration and HbA1c
Fig 20. Mean AER by systolic blood pressure and retinopathy

Geometric mean AER adjusted for duration and HbA1c
Fig 21. Raised AER by systolic blood pressure and retinopathy

Systolic BP (mmHg)

- No retinopathy
- Background
- Proliferative
Fig 22. Raised AER by systolic blood pressure and level of retinopathy

Systolic BP (mmHg)

- Level 0
- Level 1
- Level 2-4
- Level 5

For definition of levels see methods
Fig 23. Mean AER by systolic BP and retinopathy in patients with raised AER

Systolic BP (mmHg)

- No retinopathy
- Retinopathy

Geometric mean AER and 95% CI adjusted for duration and HbA1c
Fig 24. Mean AER by systolic BP retinopathy and glycaemic control

Geometric mean AER and 95% CI adjusted for duration of diabetes
Fig 25. Mean AER by systolic BP, retinopathy and parental history of hypertension (PH)

Geometric mean AER and 95% CI adjusted for duration and HbA1c
3.7 Relation between blood pressure and retinopathy

- in the whole sample

The prevalence of any retinopathy tended to increase with diastolic (Figure 26) and systolic blood pressure (Figure 27), independently of HbA1c and duration of diabetes. Data for background and proliferative retinopathy are shown separately in Tables 8 and 9. Patients with blood pressure above 95mmHg diastolic or 145mmHg systolic had significantly more background retinopathy than patients with blood pressure below this level, after adjustment for duration of diabetes and HbA1c. However, the relation between blood pressure and background retinopathy was generally weak and over a third of patients in the lowest category of blood pressure had background retinopathy. The increase in proliferative retinopathy with blood pressure was more impressive, with a threefold increase in prevalence between the lowest and highest systolic blood pressure categories, independent of duration of diabetes and glycaemic control (Table 9).
Fig 26. Prevalence of any retinopathy by diastolic blood pressure

Prevalence adjusted for duration and HbA1c
Fig 27. Prevalence of any retinopathy by systolic blood pressure

Prevalence adjusted for duration and HbA1c
Table 8.  **Prevalence of retinopathy by diastolic blood pressure**

<table>
<thead>
<tr>
<th>Diastolic Blood Pressure</th>
<th>Background Retinopathy</th>
<th>Proliferative Retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude %  Adjusted* %</td>
<td>Crude %  Adjusted* %</td>
</tr>
<tr>
<td></td>
<td>(95% C.I.)</td>
<td>(95% C.I.)</td>
</tr>
<tr>
<td>&lt;65</td>
<td>31.5  34.2 (32.0, 36.6)</td>
<td>4.9  6.1 (3.7, 8.5)</td>
</tr>
<tr>
<td>65-</td>
<td>32.0  33.9 (32.3, 35.4)</td>
<td>8.2  9.2 (7.7, 10.6)</td>
</tr>
<tr>
<td>75-</td>
<td>37.5  36.9 (35.3, 38.5)</td>
<td>11.9 11.4 (9.9, 12.9)</td>
</tr>
<tr>
<td>85-</td>
<td>40.0  37.6 (35.4, 39.8)</td>
<td>15.3 13.0 (10.7, 15.1)</td>
</tr>
<tr>
<td>95+</td>
<td>47.4  44.1 (39.6, 48.1)</td>
<td>21.1 16.3 (12.0, 20.7)</td>
</tr>
</tbody>
</table>

* adjusted for duration and HbA1c

Table 9.  **Prevalence of retinopathy by systolic blood pressure**

<table>
<thead>
<tr>
<th>Systolic Blood Pressure</th>
<th>Background Retinopathy</th>
<th>Proliferative Retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude %  Adjusted* %</td>
<td>Crude %  Adjusted* %</td>
</tr>
<tr>
<td></td>
<td>(95% C.I.)</td>
<td>(95% C.I.)</td>
</tr>
<tr>
<td>&lt;105</td>
<td>31.6  35.5 (32.3, 38.9)</td>
<td>3.7  5.3 (2.8, 7.8)</td>
</tr>
<tr>
<td>105-</td>
<td>30.5  33.6 (31.8, 35.4)</td>
<td>8.4  10.1 (8.8, 11.4)</td>
</tr>
<tr>
<td>125-</td>
<td>40.7  37.6 (35.0, 40.2)</td>
<td>12.8 11.1 (9.3, 12.9)</td>
</tr>
<tr>
<td>145+</td>
<td>54.5  49.2 (45.1, 53.5)</td>
<td>27.0 16.7 (13.7, 19.7)</td>
</tr>
</tbody>
</table>

* adjusted for duration and HbA1c

110
The relation between blood pressure and retinopathy was also examined separately for background and proliferative retinopathy in patients with normal and raised AER, after adjustment for duration of diabetes and HbA\textsubscript{1c} (Figures 28-31). The relation between diastolic blood pressure and background retinopathy was weak, whether AER was normal (Mantel-Haenszel (M-H) statistic 2.63, \(p=0.11\), Figure 28) or raised (M-H statistic 0.88, \(p=0.35\)). Neither was there any discernable relation between diastolic blood pressure and proliferative retinopathy in patients with normal AER (M-H statistic 0.004, \(p=0.95\), Figure 29) but in those with raised AER there was a significant (M-H statistic 6.9, \(p=0.009\)) increase in prevalence of proliferative retinopathy with diastolic pressure above 65mmHg.

Relations with systolic blood pressure (Figures 30 and 31) were similar. Although the relation between systolic blood pressure and background retinopathy was statistically significant in patients with normal AER (M-H statistic 4.04, \(p=0.04\)) the magnitude of the relation was unimpressive (Figure 30), and in patients with raised AER the relation was not statistically significant (M-H statistic 2.66, \(p=0.10\)). Figure 31 shows no significant (M-H statistic 0.36, \(p=0.551\)) relation between systolic blood pressure and proliferative retinopathy in patients with normal AER, but a significant (M-H statistic 7.7, \(p=0.005\)) relation in those with raised AER.

The crude data, on which this thesis is based, (i.e. blood pressure, AER, HbA\textsubscript{1c} and retinopathy) are given, by sex and centre, in the appendix.
Fig. 28  Prevalence of background retinopathy by diastolic BP and AER

Prevalence adjusted for duration and HbA1c
Fig. 29 Prevalence of proliferative retinopathy by diastolic BP and AER

Prevalence adjusted for duration and HbA1c
Fig. 30  Prevalence of background retinopathy by systolic BP and AER

Prevalence adjusted for duration and HbA1c
Fig. 31 Prevalence of proliferative retinopathy by systolic BP and AER

Prevalence adjusted for duration and HbA1c
4. Discussion

4.1 Relation between blood pressure and albumin excretion

The major finding of this thesis is the striking difference in the relation of blood pressure to albumin excretion between patients with and without retinopathy. The rise in albumin excretion rate with blood pressure which is seen in the whole sample (Figure 8) is virtually confined to patients with retinopathy (Figures 10a and 10b).

This effect modification (or statistical interaction) by retinopathy is an original finding that has not been described before. It could not be explained in this study by differences in the distribution of established risk factors for nephropathy i.e. duration of diabetes and HbA1c, or putative risk factors such as age, plasma cholesterol and smoking status. Adjustment for duration of diabetes and HbA1c had no appreciable effect on the relation between blood pressure and albumin excretion because duration and HbA1c varied little with blood pressure. Age was closely related to blood pressure, but not albumin excretion rate, independently of duration, and therefore not an important confounder. The causal relation of other cardiovascular risk factors such as plasma cholesterol and smoking status to diabetic microangiopathy is uncertain. In this study, additional adjustment for cholesterol and/or smoking status had negligible effect on the relation between blood pressure and AER, stratified by retinopathy.

The stratified analysis by glycaemic control (Fig 14) illustrates more clearly that the
lack of association between blood pressure and albumin excretion in patients without retinopathy does not simply reflect good control in these patients. Even when blood pressure was high and control poor, patients without retinopathy seldom had high albumin excretion. In patients with retinopathy, albumin excretion was fairly low when blood pressure was low, but increased with blood pressure and severity of retinopathy. Together, these findings offer a plausible explanation for the well recognised observation that nephropathy without retinopathy is rare, but retinopathy without nephropathy is common. They also suggest that patients with retinopathy are particularly vulnerable to the effect of raised blood pressure on the kidney. Why or how this might be is not evident from this study, but the steepening of the relation between albumin excretion and blood pressure with severity of retinopathy (Figures 11 and 12) indicates a kind of dose response effect, rather than an all-or-nothing phenomenon. In addition to being highly correlated with retinopathy, the postulated vulnerability factor must be independent of blood pressure. The proportion of patients with both retinopathy and high (above median) blood pressure was just over 25%, which is not very unlike the proportion of insulin dependent patients who eventually develop nephropathy. Patients at high risk of developing nephropathy might therefore be distinguished at an early stage by the presence of both retinopathy and high blood pressure.

This interpretation assumes that patients develop retinopathy before nephropathy. Although the assumption cannot be examined rigorously in a cross-sectional study, the increase in prevalence of retinopathy with duration does begin earlier than the increase in prevalence of macroalbuminuria, and background retinopathy is generally
regarded as an earlier complication of diabetes than nephropathy.

The alternative explanation, in terms of blood pressure, for the association between retinopathy and nephropathy is that patients with nephropathy develop retinopathy regardless of blood pressure, while patients without nephropathy only develop retinopathy when blood pressure is high. This explanation clearly does not fit with the common occurrence of retinopathy in normoalbuminuric patients with low blood pressure (Figures 28 and 30), nor the weak association between blood pressure and retinopathy in these patients. It also assumes that retinopathy develops after nephropathy, which is less likely than the reverse.

In all analyses of the relation between blood pressure and albumin excretion, blood pressure has been the explanatory variable and albumin excretion the response variable. While it is agreed that raised blood pressure causes albumin excretion to rise once microalbuminuria has developed, the cause and effect relation between raised blood pressure and albumin excretion before this stage is highly controversial (Mogensen et al. 1992). The "chicken and egg" argument about whether a rise in blood pressure precedes or follows a rise in albumin excretion obviously cannot be resolved by cross-sectional analyses, but it was possible in this study to examine the relation between blood pressure and albumin excretion after excluding patients with normal albumin excretion. Even in patients with raised albumin excretion, there was no relation between blood pressure and albumin excretion in the absence of retinopathy. This was in marked contrast to the two to threefold increase in albumin excretion with blood pressure in patients with retinopathy (Figures 13 and 23).
4.2 Albumin excretion and duration of diabetes

The relation between macroalbuminuria and duration of diabetes (Figure 2) is in accordance with other studies which have shown a fall in the incidence of persistent proteinuria after fifteen to twenty years of IDDM (Andersen et al. 1983; Krolewski et al. 1985a). By contrast, the prevalence of micro-albuminuria varied little with duration and, in particular, did not fall after twenty years, suggesting the existence of a substantial group of patients in whom microalbuminuria does not progress to macroalbuminuria. Two other large cross-sectional studies (Parving et al. 1988; Orchard et al. 1990) have also found that the prevalence of microalbuminuria increases beyond twenty years of IDDM. More recently, a small prospective study has reported a low incidence (28%) of macroalbuminuria in microalbuminuric patients with IDDM of long duration (Forsblom et al. 1992). Evidence of non-progressive microalbuminuria in this study was therefore sought by examining the relation between duration of diabetes and raised albumin excretion in patients with and without retinopathy. The lack of association between duration and albumin excretion in patients without retinopathy suggests that microalbuminuria in patients without retinopathy does not progress, although prospective observation is needed to test this hypothesis. As discussed in the introduction, the high progression rates previously reported in patients with microalbuminuria are probably overestimates due to lack of adjustment for differences in duration of diabetes between micro- and normo-albuminuric patients. This study suggests that the proportion of patients with retinopathy would be another crucial factor in determining progression rates.
Another finding in this study that has not been reported before is microalbuminuria in early IDDM. Microalbuminuria is believed to be rare before five years of IDDM, but in this study the prevalence was 17% in patients with duration of diabetes between one and five years, and 15% in those with duration between one and two years. Part of the explanation lies in the method of measuring albumin excretion and definition of microalbuminuria. In the Microalbuminuria Collaborative Study (Microalbuminuria Collaborative Study Group, 1992) which is most often cited as evidence that microalbuminuria does not occur before five years, albumin excretion rate was measured from an overnight urine collection and the cutoff for microalbuminuria was 30μg/min. Since albumin excretion during the night is 25% lower than during the day, the equivalent cutoff in this study would be over 40μg/min. With this definition, the prevalence of microalbuminuria in patients with duration between one and five years in this study was 9% (95% confidence interval 6% to 12%). In the WHO Multinational Study (Jarrett et al. 1979), in which urinary protein was measured semi-quantitatively by the salicylsulphonic acid test, the prevalence of light proteinuria (which roughly corresponds to microalbuminuria (Watts et al. 1988)) in patients with IDDM for less than five years was 15% (95% confidence interval 4% to 26%). These two studies, conducted ten years apart in a total of 763 patients with early IDDM from thirty-six centres, are consistent in finding raised albuminuria before five years of IDDM, despite different methods of estimating microalbuminuria.

Another important feature of the Microalbuminuria Collaborative Study is the exclusion of patients with Albustix positive proteinuria before measurement of
albumin excretion rate. Although Albustix positivity is thought to equate with an excretion rate of around 200μg/min, excretion rates as low as 100μg/min have been measured in Albustix positive urine collections. Exclusion of Albustix positive patients might therefore eliminate up to one third of microalbuminuric patients (Martin Mattock, personal communication). Early prevalence studies could have failed to find microalbuminuria simply because they investigated few patients, while the larger, more recent studies (Orchard et al. 1990; Parving et al. 1988) have not apparently looked for microalbuminuria in very early IDDM, perhaps reflecting a self-perpetuating myth that microalbuminuria does not occur before five years of IDDM.

The significance of microalbuminuria in early IDDM is not clear from this study. Microalbuminuria can be found when glycaemic control is extremely poor, particularly when IDDM is first diagnosed, but none of the patients in this study had had diabetes for less than one year. In patients with diabetes for one to five years, HbA₁c and systolic blood pressure were higher in microalbuminuric than normoalbuminuric patients, but HbA₁c values in the microalbuminuric patients were not particularly high. Although poor control and high blood pressure were clearly related to early microalbuminuria in this study, they do not seem to explain its occurrence fully, as 15% (95% confidence interval 4% to 25%) of patients in the lowest quarter of both HbA₁c and diastolic blood pressure had microalbuminuria.
4.3 Albumin excretion and parental history of hypertension

The genetic contribution to essential hypertension in the general population is well documented (Williams et al. 1984). It appears from this study that insulin dependent diabetic patients are no exception, because age-adjusted median systolic blood pressure in patients who reported one or both parents being treated for hypertension was significantly higher than patients reporting antihypertensive treatment in neither parent. However, parents were not directly examined or questioned in this study. Questioning patients about their parents' blood pressure could introduce recall bias, because patients who are themselves on antihypertensive treatment are more likely to be aware of hypertension in the family than patients with no known hypertension. For this reason, age-adjusted median blood pressure by parental hypertension was recalculated after excluding patients on antihypertensive treatment. Systolic pressure remained higher in patients with a positive parental history of hypertension.

There are relatively few studies of the familial component of transmission of blood pressure in people with diabetes. One important study (Tarn et al. 1990) in which blood pressure was measured in 163 IDDM patients, 232 of their non-diabetic siblings and all available parents, found that parental blood pressure was a major determinant of blood pressure in both diabetic and non-diabetic sibling groups. Other significant correlates of blood pressure were age, sex, weight, and, in the diabetic offspring, urinary albumin concentration. The authors conclude from the similarity of the correlates of blood pressure between the two groups that the determinants of blood pressure in IDDM patients and in the general population are similar.
Interest in parental blood pressure has developed from the strong suspicion that genetic susceptibility to nephropathy is important, and the clear link between blood pressure and early diabetic nephropathy. The hypothesis is that susceptibility to diabetic nephropathy, which distinguishes the 35%-40% at risk of nephropathy from the remainder who are not at risk, is determined by a genetic predisposition to hypertension (Krolewski et al. 1988; Viberti et al. 1987). In this study, patients with a positive parental history of hypertension had higher albumin excretion than patients with a negative parental history. This does not, however, establish cause and effect. Given the relation between blood pressure and albumin excretion in the whole sample (Figure 8), one would expect albumin excretion to be relatively high in any group of patients distinguished by high mean blood pressure.

Analysis of the relation between blood pressure and albumin excretion by parental history of hypertension and retinopathy casts parental hypertension in a minor role. In this analysis, the distinction by retinopathy status (Figure 10) remains unchanged by the parental history of hypertension (Figures 17 and 18). This implies that the parental history of hypertension is merely a marker (and not a particularly good one in this study) of high blood pressure in offspring. It does not appear to distinguish a group of patients who have higher albumin excretion, independently of their level of blood pressure.

4.4 Retinopathy, glycaemic control and duration of diabetes

The relation between duration of diabetes and prevalence of retinopathy (Figure 6)
is similar to that described in population-based studies in Wisconsin (Klein et al. 1984) and Pittsburgh (Orchard et al. 1990), confirming a rapid rise in the prevalence of retinopathy between five and fifteen years and virtually no proliferative retinopathy before ten years. Absolute prevalence, however, was considerably lower in this study, with 82% of patients having retinopathy after twenty years, compared with almost 100% in the American studies. The prevalence of proliferative retinopathy after thirty years of IDDM was 36% in this study, but 60% (Klein et al. 1984) and 75% (Orchard et al. 1990) in the American studies. Few other studies have used centrally graded retinal photographs to measure retinopathy in large numbers of insulin dependent patients. Complete agreement on the presence or absence of retinopathy between the EURODIAB retinal photography protocol and the WESDR protocol used in the American studies suggests that retinopathy was unlikely to have gone undetected in this study (Table 2).

Alternative explanations for the lower prevalence of retinopathy are greater use of retinal photocoagulation and better glycaemic control. Differences in use of photocoagulation are difficult to compare between these studies, but fewer than 2% of patients in Wisconsin (Klein et al. 1984) compared with 6.4% of EURODIAB patients had signs of photocoagulation with no proliferative lesions, indicating successful treatment of proliferative retinopathy. Glycaemic control was also better in the EURODIAB patients: only 6.7% (Klein et al. 1988) and 1% (Trevor Orchard, personal communication) of patients in the American studies had glycated haemoglobin within the non diabetic range, compared with 16% in the present study.
The possibility of a threshold effect in the relation between retinopathy and glycaemic control has aroused much interest (Klein et al. 1988). It is difficult, in a cross-sectional study, to draw conclusions about an exposure like HbA₁₀ which is highly modifiable and an outcome like retinopathy which takes years to develop. Nonetheless, the decline in frequency of background retinopathy and coincident rise in proliferative retinopathy in the fifteen percent of patients with HbA₁₀ above 8% might indicate a threshold effect with important implications for avoidance of sight-threatening retinopathy. Whether or not this is correct, it is clear that the relation of glycaemic control to raised albuminuria differs qualitatively from its relation to retinopathy. Unlike retinopathy, the rise in frequency of macroalbuminuria with HbA₁₀ was not accompanied by any coincident fall in microalbuminuria. One interpretation of this data is that poor glycaemic control is a critical factor in the development of proliferative retinopathy, and a non-critical, but contributory factor in the development of clinical nephropathy. This interpretation fits with prospective data (Klein et al. 1988) showing a particularly strong relation between glycated haemoglobin and proliferative retinopathy (although that study did not report a glycaemic threshold) and the view that genetic factors determine susceptibility to diabetic nephropathy.

Observational studies have shown a strong and consistent relation between glycaemic control and retinopathy (Klein et al. 1988), and the DCCT has now shown a substantial reduction in retinopathy associated with improved control. Unfortunately, however, there is no doubt that improved glycaemic control increases the risk of severe hypoglycaemia (DCCT Research Group, 1991). Informed diabetic patients
should now be able to weigh the measured benefits against the risks of strict control. For those delivering diabetes care, one of the key issues will be the extent to which the benefits shown in the DCCT can be applied safely to the wider population of IDDM patients.

4.5 Relation between blood pressure and retinopathy

In this study, the prevalence of retinopathy tended to increase with blood pressure, independently of HbA\textsubscript{1c} and duration of diabetes, but the increase in background retinopathy was not clinically impressive. A prominent relation between blood pressure and retinopathy was found only for proliferative retinopathy in patients with raised albumin excretion (Figures 29 and 31).

Although a stronger relation between blood pressure and retinopathy might be anticipated from consideration of retinal pathophysiology and haemodynamics, the findings of this study are not dissimilar to other clinical studies. The marked increase in prevalence of proliferative retinopathy with diastolic blood pressure above 65mmHg (Figure 29 and Table 8) is similar to a report from the Joslin Clinic (Janka et al. 1989a) in which the risk of progression to severe retinopathy was much higher in patients with diastolic blood pressure above 70mmHg. Moreover, there was no relation in that study between blood pressure and the incidence of less severe forms of retinopathy. In the WESDR (Klein et al. 1989b), the four year relative risk of any retinopathy was estimated to be only 1.4 for each 10mmHg increase in systolic blood pressure. The few studies that have examined the relation between blood pressure
and retinopathy by proteinuria were cross-sectional and likewise reported no relation between blood pressure or hypertension and retinopathy in the absence of proteinuria (Krolewski et al. 1985b; Norgaard et al. 1991).

4.6 Methodological limitations

The limitations of using cross-sectional data to hypothesise about the temporal sequence of events linking blood pressure and AER have already been mentioned. A few other points warrant discussion. Most definitions of microalbuminuria are based on two out of three "positive" results from repeated urine collections. For practical and financial reasons, AER in this study was calculated from a single timed urine collection. There is therefore likely to be some misclassification of patients by categories of albuminuria; in particular, some patients classified here as microalbuminuric would probably have been classified as normoalbuminuric if urine collections had been repeated. However, the analytic approach taken here focuses on the relation of risk factors to AER as a continuous variable. This avoids "loss" of data through categorisation. Although a better estimate of an individual's usual AER could have been obtained from repeated measures, a single measure still provides useful information. This is analogous to the situation with blood pressure: two or three repeat measures may be required in order to classify an individual as hypertensive, but a single reading still gives a measure (albeit imprecise) of his/her level of cardiovascular risk.

Financial constraints also limited the frequency of transport of frozen urine samples
by courier post to the Co-ordinating Centre. The average time between collection of urine and measurement of urinary albumin concentration was therefore six months. Some studies have found that storage of frozen urine for more than a few weeks decreases the albumin content (Elving et al. 1989; Manley et al. 1992). Although the magnitude of this effect is fairly small (MacNeil et al. 1994), particularly when urinary albumin concentrations are well above normal (Manley et al. 1992), the effect of prolonged storage may have resulted in underestimation of the prevalence of microalbuminuria in this study. However, this effect is unlikely to have biased the main conclusions, concerning the relation between blood pressure and AER, because mean delay before measurement of urinary albumin did not vary importantly by level of diastolic blood pressure (ranging from a mean of 170 days in those with diastolic blood pressure below 65mmHg to 185 days in those with diastolic pressure above 95mmHg).

Use of the Hawksley random zero sphygmomanometer for measurement of blood pressure has recently been criticised (O’Brien et al. 1990; Conroy et al. 1993). The essential principle of this instrument is that it blinds the observer to the actual blood pressure until after the reading has been made. It does this by adding a random amount of mercury to the manometer as the reading is being taken. When the instrument falls back to zero the amount of extra mercury can be read and subtracted. Its design is simple and its purpose is to reduce operator bias in studies involving measurement of blood pressure. Its critics claim that blood pressure measured with the Hawksley random zero sphygmomanometer is a few millimetres of mercury lower than that measured with a conventional sphygmomanometer. The correspondence
(15th May 1993) which followed their paper in the British Medical Journal (Conroy et al. 1993) shows that underestimation of blood pressure with this instrument is controversial. Further, it is argued that the instrument succeeds in its aim of reducing operator bias in epidemiological studies, and that it was never intended to be used to measure blood pressure in individual patients for clinical purposes. Finally, in the absence of data showing differential underestimation at different levels of blood pressure, any genuine underestimation with the Hawksley random zero device is unlikely to bias the relations described here between blood pressure and AER or retinopathy.

4.7 Generalisation of findings beyond the study population

The EURODIAB study is the largest clinic-based study of its kind, combining standardised methods with central measurement of important outcomes. The study was clinic - rather than population - based, because population-based sampling frames from which IDDM patients can be identified and recruited for study are rare. What, then, is the likely impact of selection bias on these findings? Clinic-based estimates of the regional prevalence of complications will be biased if clinic-attenders have more or fewer complications than the population of IDDM patients from which the attenders are drawn. While some patients may be referred to clinics because of advanced complications, others’ motivation to attend may be associated with better control and fewer complications than in non-attenders (Hammersley et al. 1985). Individual clinics may therefore over- or underestimate the regional prevalence of complications, depending on the balance between these opposing biases.
Selection bias is less likely to affect associations between exposures and outcomes. For example, clinic-attenders may have longer (or shorter) duration of diabetes, and a higher (or lower) prevalence of retinopathy than non-attenders, but it is unlikely that the relation between duration of diabetes and retinopathy differs importantly between those who attend clinics and those who do not. Indeed the similarity of the relation between those two variables in both EURODIAB and population-based studies strengthens the view that complications in clinic-attenders do not develop in a way which is unlike that of the general population of IDDM patients. In general, although factors determining clinical referral and attendance are poorly defined and difficult to measure, they need not bias the association between risk factors and complications, providing the selective forces for exposure and outcome are independent of each other.

The strength of the EURODIAB Complications study therefore lies in elucidating the relation between specific outcomes and exposures. It is nonetheless important, in a clinic-based study, to consider referral or Berkson's bias (Berkson, 1946) when interpreting the relation between outcome and exposure. If the combination of exposure and disease carries a higher probability of clinic referral and attendance than either exposure or disease alone, a falsely high exposure rate will be found among cases (patients with the disease), leading to an exaggerated or even spurious association between exposure and disease. In the context of this study, the positive association between blood pressure and nephropathy would be biased if patients with either raised blood pressure or raised albumin excretion were less likely to attend diabetic clinics than patients with both characteristics. However, analysis of the
relation between blood pressure and albumin excretion by retinopathy status indicates that this potential source of bias was unimportant, because patients with high blood pressure but normal albumin excretion (identified by the absence of retinopathy) were well represented in the study sample.

4.8 Implications for future research

If susceptibility to diabetic nephropathy is genetically determined, the data presented in this thesis suggest that the genetic factor will be unrelated to blood pressure, as proposed by Danish researchers (Deckert et al. 1989; Mogensen et al. 1992). According to the Steno theory, genetic defects in regulation of heparan sulfate proteoglycan predispose IDDM patients to glomerulosclerosis and subsequent nephropathy. The epidemiological data presented here do not offer direct insight into the mechanism(s) by which such susceptibility to nephropathy is conferred. However, the data suggest that patients with this hypothetical genetic factor will have retinopathy and will be at high risk of nephropathy if they have high blood pressure, particularly if they also have poor glycaemic control (Derby et al. 1989). Patients may have high blood pressure because of familial hypertension, but that alone will be insufficient to determine susceptibility to nephropathy. One candidate genetic marker of susceptibility that is currently being explored is the angiotensin converting enzyme (ACE) gene. A large part of the variance in plasma and cellular levels of ACE is genetically determined by the ACE gene. Preliminary study (Marre et al. 1993) suggests that an insertion / deletion (I/D) polymorphism of the ACE gene may be a determinant of diabetic nephropathy, as both plasma ACE levels and the deletion
allele frequency were significantly higher in IDDM patients with nephropathy than in patients with no nephropathy or controls.

The predictions generated from this study clearly need to be tested by prospective observation. At its simplest, this would involve long-term follow-up of IDDM patients at risk of nephropathy, in whom the rate of progression of AER would be compared between patient groups with and without retinopathy at baseline. Identification of those at risk of nephropathy for recruitment could be based on initial level of AER (e.g. "high-normal" such as above 15μg/min), level of blood pressure (e.g. above the median pressure of 120/75mmHg in this study), or perhaps duration of disease (more than 5 years). In practice, entry criteria should not be so restrictive that recruitment of adequate numbers of patients becomes impractical. Baseline investigations would include degree of retinopathy, blood pressure, parental blood pressure where possible, glycated haemoglobin, duration of diabetes etc. Repeat measures of retinopathy, blood pressure and glycated haemoglobin would be recorded during follow-up, and the primary end-point would be some function of AER, such as annual percentage increase, or rate of change of AER. Sample size calculations, based on the expected rate of progression in patients without retinopathy, are an essential guide to recruitment of sufficient patients to enable the hypothesised effect to be detected. With a large study sample, the effect of different levels of blood pressure (and their relation to parental blood pressure) could be examined within retinopathy groups. Duration of diabetes and glycated haemoglobin would be treated as confounders, and controlled for in the analysis. A five year re-examination of all the EURODIAB study participants is currently being planned.
5. **Summary of Results**

- The relation between blood pressure and albumin excretion differed strikingly between patients with and without retinopathy. In patients without retinopathy, albumin excretion was generally low even when blood pressure was high and glycaemic control poor. In patients with retinopathy, albumin excretion increased with blood pressure above the median and, independently, with severity of retinopathy. Modification of the relation between blood pressure and albumin excretion by retinopathy provides a plausible explanation for the well-recognised observation that nephropathy is rare without retinopathy, while retinopathy without nephropathy is common.

- The association between blood pressure and background retinopathy was not strong. However, in patients with raised albumin excretion, proliferative retinopathy was two to three times more common in patients with blood pressure above 105/65mmHg than below this level.

- Both blood pressure and albumin excretion were higher in patients reporting a history of parental hypertension than in those reporting no parental hypertension. However, parental hypertension made no contribution to the relation between blood pressure and albumin excretion that was independent of blood pressure in the offspring.
The relation of glycaemic control to albuminuria differed qualitatively from its relation to retinopathy. These qualitative differences imply that hyperglycaemia makes a different aetiological contribution to the development of each of these complications.

Microalbuminuria was not a rare finding in patients with diabetes for less than five years. Eighteen percent of patients with duration of diabetes between one and five years of IDDM had microalbuminuria, and this was a consistent finding in all centres.

In patients with raised albumin excretion, there was no important relation between duration of diabetes and albumin excretion in the absence of retinopathy; in those with retinopathy, albumin excretion increased markedly with duration of diabetes.
6. **New hypotheses**

From the findings of this study, two hypotheses can be put forward:

- Patients at risk of developing nephropathy can be distinguished at an early stage by the presence of both retinopathy and raised blood pressure.

- The rate of progression of urinary albumin excretion rate differs importantly between patients with and without retinopathy. In patients without retinopathy, progression is minimal. In patients with retinopathy and blood pressure above 120/75, the progression rate is high (e.g. over 80% progression from microalbuminuria to macroalbuminuria within five years).

These hypotheses have been generated from analysis of cross-sectional data and clearly need to be tested by prospective observation. The EURODIAB study group is now conducting a randomised, double-blind, placebo-controlled trial of the long-term effect of the ACE-inhibitor lisinopril on renal function in IDDM patients with diastolic blood pressure between 75mmHg and 90mmHg. Target recruitment of 500 patients is nearly complete. The primary outcome measure is rate of change of AER. Analysis of outcome by retinopathy status in over 220 patients on placebo should provide a test of the hypotheses generated in this thesis.
7. **Statement of Authorship**

I carried out the work of this thesis between 1989 and 1993 while working as an Honourary Lecturer in Epidemiology and Public Health at University College London. I was the study co-ordinator for the EURODIAB IDDM Complications Study. My main intellectual contribution to the study was to re-define the study objectives by arguing that the strength of such a clinic-based study lies in examining relations between specific exposures and outcomes within and across centres, rather than comparing the prevalence of complications between centres. My other contribution to this multi-centre collaborative study can be summarised as follows:

**May 1989 - January 1990: Planning and Preparation**

Supervision of translation, and back-translation into English, of questionnaires; writing much of, and editing, the detailed operational guide (Manual of Operations), designing diagrams to assist in preparation of laboratory samples and other data; introduction of hidden duplicate urine samples to the study protocol as a quality control measure; organisation of five regional training meetings for investigators; distribution of materials and equipment to local centres; supervision of "dry run" including detailed feedback to each centre on performance;

**February 1990 - February 1992: Data collection**

General responsibility for monitoring progress of the study, including regular communication with local centres, organising and participating in steering committee meetings; site visiting local centres throughout Europe, organisation of transport of
frozen urine samples from local centres to the central laboratory; regular meetings
with, and formal site visits to, the retinal grading centre and central laboratory;

**March 1992 - August 1992: Creation of EURODIAB dataset**

Supervision of data cleaning and double data entry (bespoke data entry programmes
were written by a computer systems manager and raw data was entered by two
research assistants); checking double entered data for discrepancies; assessing
reliability of laboratory data through analysis of hidden duplicate measurements;
writing programmes in SAS to read in the raw data, merge files into a permanent
SAS dataset and then create variables for analysis (e.g. a single grade of retinopathy
for each individual from 56 data fields); final "cleaning" of dataset by examination
of outliers, recovering missing data wherever possible and excluding protocol
violators.

**September 1992 onwards: Data analysis and writing up thesis**

(including text, figures and tables)

8. **Acknowledgements**

I thank all the investigators (listed in the appendix) and the patients who took part in
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References


Remuzzi, G. and Bertani, T. Editorial review: is glomerulosclerosis a consequence


Rostand, F.G. Diabetic renal disease in blacks - inevitable or preventable?


Sawicki, P.T., Muhlhauser, I., Baba, T. and Berger, M. Do angiotensin converting
enzyme inhibitors represent a progress in hypertension care in diabetes mellitus.


Shemesh, O., Golbetz, H., Kriss, J.P. and Myers, B.D. Limitations of creatinine as


Appendix 1.

Basic data by sex and centre
- Retinopathy
- Diastolic blood pressure
- Systolic blood pressure
- HbAlc
- AER

Centres abbreviated as follows:
Athe Athens
Buch Bucharest
Buda Budapest
Cagl Cagliari
Duss Dusseldorf
Engl England
Hels Helsinki
Krak Krakow
Leid Leiden
Lisb Lisbon
Luxe Luxembourg
Mila Milan
Muni Munich
NFra N. France
Padu Padua
Perg Perugia
Turi Turin
Thes Thessaloniki
Vero Verona
Vien Vienna
Zagr Zagreb

173
Frequency of retinopathy in men by centre.

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All 1269 38.1 (35.3, 40.9) 9.6 (8.0, 11.2)

* n = the number of men with gradable retinal photographs
Frequency of retinopathy in women by centre.

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All 1210 33.6 (30.8, 36.4) 11.0 (9.3, 12.7)

*n = the number of women with gradable retinal photographs
Distribution of Diastolic BP by sex and centre

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Diastolic Blood Pressure, mmHg

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Diastolic Blood Pressure, mmHg

Plots show 5, 25, 50, 75, 95 percentiles
Distribution of Systolic BP by sex and centre

Male

Female

Systolic Blood Pressure, mmHg

plots show 5, 25, 50, 75, 95 percentiles
Distribution of HbA1c by sex and centre

Male

Female

plots show 5, 25, 50, 75, 95 percentiles
Distribution of AER by sex and centre

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Log AER, ug/min

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Log AER, ug/min

Plots show 5, 25, 50, 75, 95 percentiles
Appendix 2

EURODIAB IDDM COMPLICATIONS STUDY

PROTOCOL

The EURODIAB IDDM Complications Study Group
SUMMARY

The EURODIAB IDDM Complications Study is a major component of a Concerted Action Programme on the Epidemiology and Prevention of Diabetes, funded by the European Community. It is a multi-centre cross-sectional study designed to measure the frequency and severity of microvascular and macrovascular complications, and the distribution of established and putative risk factors in representative samples of insulin dependent diabetic patients attending 31 clinics in 16 European countries, giving a total sample of over 3000 patients. The relation between specific complications and exposures, such as blood pressure and urinary albumin excretion rates, will be examined, within and across centres. Much emphasis is placed on achieving precise, standardised measurements throughout the study. Extensive quality control procedures are carried out by local investigators and co-ordinating centres.
Introduction

The European Community (EC) is funding a Concerted Action Programme on the Epidemiology and Prevention of Diabetes (EURODIAB). A major component of the EURODIAB programme is a study of the frequency and determinants of the complications of insulin dependent diabetes (IDDM) throughout Europe.

Background

Diabetes is a common, important and costly disease, in both human and economic terms. A rising incidence of insulin dependent diabetes (IDDM) has been reported in many European countries, with a rate equivalent to a doubling period of 20-30 years. Most of the mortality, morbidity and costs of diabetes are due to its chronic complications, which seriously disable a large minority. As highlighted by the recent Saint Vincent Declaration, much more precise and complete epidemiological data on diabetic complications - macrovascular disease, retinopathy, nephropathy and neuropathy - is needed for effective planning of treatment and preventive strategies in Europe.

Previous studies of mortality in diabetes have shown wide European variation in death rates. In the WHO Multinational Study of Vascular Disease in Diabetics, death rates in IDDM were at least twice as high in Warsaw and Berlin, compared to London and Switzerland. Studies in America have suggested that 45% of IDDM patients die from renal failure or coronary artery disease before the age of 55 years. Retrospective studies from Denmark and the USA found that the cumulative incidence of proteinuria
after 40 years of IDDM was around 40%. Similar information for the rest of Europe is not available, although a study in Leicestershire, UK, found a much lower prevalence of nephropathy in diabetics diagnosed before 17 years. Acceptance rates onto renal replacement programmes vary considerably across Europe, with the highest rates reported in Scandinavian countries, especially Sweden and Finland. The median age at acceptance in these countries is 38-42 years, compared with 50-58 years for central and southern European countries, which raises the possibility of differences in the natural history of diabetic nephropathy. In longterm survivors nephropathy is uncommon, indicating that only a subgroup of patients are at risk of diabetic nephropathy, even though the characteristic histology of glomerular sclerosis is found in over 90% of patients after 10 years of disease. Early identification of this high risk subgroup presents an important challenge. The close link between blood pressure and early nephropathy has been the focus of much research and the hypothesis that genetic predisposition to hypertension underlies risk of nephropathy in IDDM has emerged. Others believe that genetically determined control of metabolic factors associated with diabetes are the primary cause of nephropathy. The epidemiological approach seeks to measure the distribution of urinary albumin excretion and blood pressure in diabetic populations and to examine the relation between them. Further search for familial clustering of nephropathy in different populations would also advance understanding of the aetiology of diabetic nephropathy.

The observation that patients with diabetic nephropathy have high cardiovascular morbidity and mortality has now been complemented by longitudinal studies showing that raised albumin excretion is a strong predictor of both clinical nephropathy and cardiovascular mortality. But the explanation for the association
between renal and cardiovascular complications in IDDM is not clear. Several studies in patients with renal impairment have shown increased levels of vascular risk factors such as raised plasma lipoproteins and fibrinogen. These changes may, however, reflect a non-specific response to increased albumin loss. Similar findings (e.g. disturbances in lipoprotein and apolipoprotein concentrations, raised fibrinogen, von Willebrand factor and other haemostatic factors) in patients at much earlier stages of renal disease \(^{24-27}\) suggest that the increased risk of vascular disease in patients with clinical nephropathy may result from prolonged exposure to risk factors long before renal function fails. These interesting findings in small numbers of selected patients require confirmation in larger epidemiological studies.

Diabetic eye disease is the leading cause of blindness in the working population in industrialised countries. Background retinopathy is found in virtually all patients after 14 years of diabetes. After 10 - 15 years of diabetes, the incidence of proliferative retinopathy, increases rapidly and then remains constant, resulting in a cumulative risk of 62%-70% after 30 to 40 years.\(^{4,28}\) Numerous observational studies have shown clear correlations between poor glucose control and the development of retinopathy.\(^{29-31}\) But attempts to prevent deterioration of retinopathy by improving glyaemic control have been disappointing \(^{32-35}\) and some studies have reported transient worsening of retinopathy following intensified insulin treatment, compared to those remaining on conventional treatment.\(^{34,36,37}\) The hope that optimal control might succeed in preventing the development of retinopathy is currently being examined by the Diabetes Control and Complications Trial.\(^{38}\) The reasons why many IDDM patients are spared sight-threatening retinopathy are poorly understood. The search for genetic markers for development of retinopathy has produced conflicting
results. The role of blood pressure as a risk factor for retinopathy has been examined in several studies, some of which have methodological problems, including failure to allow for the confounding effect of nephropathy. The importance of cigarette smoking to the risk of nephropathy and retinopathy also requires further investigation.

Even less is known about the natural history of diabetic neuropathy and its risk factors, apart from recognition that prevalence increases with age, duration of disease, and, in many studies, degree of glycaemia. Until very recently, a particular problem in the study of neuropathy has been the lack of commonly accepted definitions and methods. Although measurable autonomic defects are common in IDDM, symptoms are not. The consequences of autonomic neuropathy include respiratory arrest following anaesthesia and cardiac arrhythmias. Cumulative survival after 10 years is 73% in symptomatic patients, compared to 90% in those without symptoms. The assertion that autonomic neuropathy increases the risk of severe hypoglycaemia, through loss of warning symptoms or inadequate hormonal responses, has recently been questioned amidst much concern and controversy about the effect of insulin species on hypoglycaemic risk.

Although the importance of diabetic complications is well recognised, their underlying aetiologies are still largely unexplained and the scope for prevention very limited. Better understanding of the risk factors for complications and the ability to predict the development of serious complications in individual patients is required to advance the prospect of effective prevention.
OBJECTIVES

To measure the frequency of renal, retinal, neuropathic and macrovascular complications in representative samples of IDDM patients attending 32 clinics in 16 European countries, using standardised, validated methods.

To measure the distribution of established and putative risk factors (exposures) for these complications, including, for example, blood pressure, plasma lipids, urinary albumin excretion, fibrinogen, quality of glycaemic control, genetic markers and dietary factors.

To examine the relation between complications and exposures, within and across centres.

Examples of specific relations to be examined are:

1) the relation between blood pressure and urinary albumin excretion
2) the relation between dietary protein intake and urinary albumin excretion
3) the relation between blood pressure and retinopathy
4) the relation between severe hypoglycaemia, and autonomic neuropathy
5) the relation between dietary fat intake, serum lipids and cardiovascular disease
6) the relation between smoking and microvascular complications
7) familial clustering of diabetic complications
STUDY DESIGN

At each centre, a stratified random sample of up to 140 insulin dependent patients is recruited into this cross-sectional study and the level of diabetic complications and risk factors is measured using standardised methods. Extensive quality control procedures are carried out centrally and locally, including training and monitoring of investigators and independent validation of centralised measurements. Local ethical committee approval for the study must be obtained.

Sampling and recruitment

The target population, to which the findings of the study may be applied, is all people with IDDM in that part of Europe from which the centres are drawn. Within each centre, the study population consists of all eligible (see below) IDDM patients living in a defined catchment area who have attended the centre at least once during the preceding 12 months. The study population is stratified by age, sex and duration into 14 strata (table 1). The aim is to investigate up to 10 patients selected at random from each stratum, giving a sample of up to 140 patients in each centre and a total study sample of over 3000 patients. Stratified sampling will ensure that patients over the range of eligible age and duration are "fairly" represented and that samples from different centres are roughly comparable with respect to stratification variables. Stratification also allows sample characteristics to be estimated with greater statistical efficiency.
Eligibility criteria

Age range 15-59 years

Diagnosis of diabetes before age 31 years

Unbroken record of insulin treatment from diagnosis (allowing for short breaks and remission period up to 6 months in the first year)

Diagnosis of diabetes made at least 12 months before entry to the study.

Exclusion criteria

Pregnant women

Patients who are unrepresentative of regional ethnic groups

When numbers in the 14 age-gender-duration strata of clinic attenders are adequate, 12 potential recruits are selected randomly from each stratum, the additional 2 patients being selected as reserves. Patients are then invited to participate in the study after full explanation of purposes and procedures. Informed consent must be obtained from all patients. In cases of non-attendance, specified procedures are undertaken to determine the reason. A record of all patients in the study population is kept, including patients' responses to invitation to take part in the study.

METHODS OF EVALUATION

Complications

Nephropathy

Major outcome variables are the rate of urinary albumin excretion, plasma creatinine and treatment for renal failure (dialysis and transplant). Urinary albumin excretion
is measured from a 24 hour urine collection, after excluding urinary tract infection. After careful measurement of urine volume to the nearest 2 ml, seven aliquots are taken and stored at -20 C. Six frozen aliquots are sent to the Central Laboratory and one is retained locally.

A randomly pre-selected subgroup of 15% of patients in each centre is asked to repeat the 24 hour urine collection 2 to 3 weeks later, so that within person variability in urinary albumin excretion can be estimated.

**Retinopathy**

Visual acuity, corrected for distance, is recorded for each eye, using Snellen’s chart and coded as 1.0, 0.9, ....0.1. If visual acuity is <0.1, it is coded as CT (counting fingers), HM (hand movements) LP (light perception) or blind. The pupils are then dilated with tropicamide 1%. If pupils are < 5mm diameter, epinephrine 10% is added, except in patients with closed angle glaucoma. The presence and severity of retinopathy is assessed by retinal photography using a wide angle (45 degree) camera. Colour transparencies are taken of two fields per eye: disc-macula-temporal and disc-nasal, using Kodachrome 64 ASA diapositive film. Investigators are trained to produce transparencies of good focus and quality, with accurate field definition. Central grading of retinal transparencies is based on a system used in the Early Treatment of Diabetic Retinopathy Study (ETDRS) and modified subsequently for the Diabetes Control and Complications Trial (DCCT). The severity of the retinal lesions is judged by comparison with standard photographs.
Neuropathy

Diabetic neuropathy is assessed by validated questions designed to identify symptoms of muscle weakness and sensory dysfunction, by the presence or absence of knee and ankle reflexes and by measurement of vibration perception threshold (VPT) using the biothesiometer. The VPT is measured over the right medial malleolus and the tip of the right big toe and the mean of 3 readings at each site calculated. All biothesiometers are centrally calibrated before use in the study and observers are trained to ensure uniformity of method.

Autonomic neuropathy is assessed by clinical symptoms (of orthostatic hypotension, nocturnal diarrhoea, loss of bladder control and impotence), and by change in heart rate and blood pressure on moving from the lying to the standing position. The change in heart rate, recorded by ECG, is calculated as the ratio of the longest R-R interval between the 28th and 32nd complexes, to the shortest R-R interval between the 13th and 17th R-R interval. The fall in blood pressure 60 seconds after rising from the lying to standing position is measured using the random zero sphygmomanometer.

Macrovascular disease

The presence of macrovascular disease is assessed from a 12 lead resting ECG and a history of clinical disease i.e. myocardial infarction, stroke, coronary or peripheral artery bypass surgery, angioplasty, gangrene, or limb amputation. ECGs are coded by 2 observers according the Minnesota Code, and any discrepancies between the 2
observers are adjudicated by a third. Further assessment of peripheral vascular disease by palpation of femoral and foot pulses, and measurement of ankle/brachial pressure ratio is an optional investigation.

**Acute complications**

The frequency of hypoglycaemic attacks severe enough to require the help of another person and episodes of ketoacidosis requiring hospital admission over the last 12 months are recorded.

**Risk factors**

Major risk factors for diabetic complications are duration of diabetes and glycaemic control. Glycaemic control is estimated by current glycated haemoglobin concentration and up to 8 local measurements recorded over the previous 2 years. Method-related differences in glycated haemoglobin will be assessed using standard freeze-dried glycated haemoglobin samples. Cardiovascular risk factors to be measured are:

- total cholesterol, triglyceride, HDL cholesterol and fibrinogen
- blood pressure
- smoking history
- body mass index and waist/hip ratio
- physical activity
- dietary fat intake (cholesterol, saturated fatty acids etc.)
- parental history of hypertension or vascular disease
A single blood sample is taken and six aliquots of plasma separated and stored at -20°C. As with the urine samples, one aliquot of plasma is kept at the local centre as a backup, and the remaining samples are sent to the Central Laboratory for measurement of plasma lipids and fibrinogen. Samples of whole blood will be stored for analysis of genetic factors.

Sitting blood pressure is recorded in the right arm with a Hawksley random zero sphygmomanometer. The patient should rest for five minutes and avoid smoking and caffeine consumption for 30 minutes before blood pressure measurement. Diastolic pressure is recorded to the nearest 2 mm Hg at the disappearance of sound (phase V). The average of 2 consecutive blood pressure measurements is calculated. The use of antihypertensive medication, and current and past smoking habits are recorded.

Height and weight are measured using a stadiometer and calibrated beam balance where possible. Heavy outer garments and shoes are removed for measurement of weight. For measurement of waist and hip circumference, adequate exposure of the waist and hip area and standard positioning are important. Waist circumference is defined at the level midway between the costal margins and the iliac crests. Hip circumference is measured at the level of the greater trochanters - (if impalpable, the largest gluteal circumference is recorded).

The frequency of mild, moderate and vigorous physical activity is recorded.

Nutritional factors are assessed using 3-day (2 working days plus Sunday) dietary records (Nutri-diary) filled out by the patient and then checked by the local dietician.
The analysis makes use of food tables, with many additions to suit the range of national and local dietary practices throughout Europe. It uses household measurements, translated into grams by standardized methods, and has been used in other multinational European studies. The analytical variables include:

- total energy intake
- total carbohydrate
- protein - animal and vegetable
- fat - saturated, and polyunsaturated fatty acids, cholesterol
- fibre
- alcohol

In order to examine within person variability in dietary intake, the subgroup of patients who are asked to repeat a 24 hour urine collection are also asked to fill out another Nutri-diary.

Family history of diabetes is recorded to identify first degree relatives with IDDM who will be asked to participate in exactly the same manner so that familial clustering of complications can be sought. It is expected that about 5 first degree relatives in each centre will have IDDM. A detailed drug history is recorded including insulin dose and species (human or animal). Information on occupation, educational and marital status, and ethnic group is also recorded.
STANDARDISATION AND QUALITY CONTROL

The procedures described above are detailed in a Manual of Operations, which is the reference document for all investigators. All forms are centrally prepared and pre-coded. All labels used to identify urine and blood specimens, ECGs and retinal transparencies are prepared by the Co-ordinating Centre. The questionnaires (patient record form and Nutri-diary) have been translated into 12 different languages and then checked centrally by back-translation into English to ensure uniformity of meaning. They are administered by investigators who adhere to the precise wording of the questionnaires.

Training sessions are a very important method of standardisation and quality control. No centre may participate in the study until at least one representative has attended a training meeting. The meetings cover sampling method, allocation of identity numbers, blood pressure measurement, ECG recording, retinal photography, neurological examination, completion of questionnaires and forms, the nutritional assessment, the preparation, preservation and shipping of blood and urine specimens, and organisation of clinic visits.

All centres carry out a "dry run" on 4 patients which is assessed by the Co-ordinating Centre and discussed with each centre so that future errors can be avoided. No centre may participate without successfully completing a dry run. In addition to internal laboratory quality control methods, measurement error is assessed by creating hidden duplicate sets of urine and plasma aliquots from the 15% subgroup of patients in each centre.
STUDY ORGANISATION

The ultimate responsibility for completion of the EURODIAB IDDM Complications Study rests with the Concerted Action Project Leader (see appendix) who is responsible for the data co-ordination, quality control and statistical analysis of the study. A Steering Committee assists the Project Leader in overseeing the progress of the study. All local investigators are to be consulted on major policy decisions and operational matters arising during the study.

STATISTICAL ANALYSIS

The great majority of IDDM patients in Europe regularly attend clinics such as those taking part in this study; in many countries it is exceptional for IDDM patients to be cared for by family doctors. It is therefore reasonable to assume that the findings of the EURODIAB study will be applicable to its target population i.e. the population of insulin dependent diabetics in Europe. Since the study is not population based, estimates of specific regional prevalences of complications, however, are likely to be biased. The major objective is to examine exposure-complication relationships. These relations will be examined with adjustment for confounding at individual level, both within and across centres.

The sample size within each centre was based on a combination of statistical and practical assumptions. For example, in examining the relationship between blood pressure and urinary albumin excretion rate, 140 patients will allow a difference of 14% in the frequency of macroalbuminuria to be detected with at least 80% power.
at 5% significance, between patients in the upper and lower halves of blood pressure distribution, assuming the lower proportion to be 3%.

Acknowledgements

The European Community has been the main source of funding for the central coordination of this study. We are grateful to ICI (UK) Ltd, Miles/Ames (UK), Novo-Nordisk (Denmark) and Fidia (UK) Ltd for additional funding. We would also like to thank the INTERSALT Co-operative Research Group for advice on field methods.

This protocol was prepared on behalf of the EURODIAB IDDM Complications Study Steering Committee by Dr. Judith Stephenson.
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Dr. A-K. Sjolie (Aarhus)  
Dr. M. Toeller (Dusseldorf)  
Dr. J. Ward (Sheffield)

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**Table 1. Stratification of sample by age, sex and duration within each centre.**

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