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Glomerulopathy in normoalbuminuric adolescents with type 1 diabetes

**Relations between structure, function, metabolic
control and ambulatory blood pressure**

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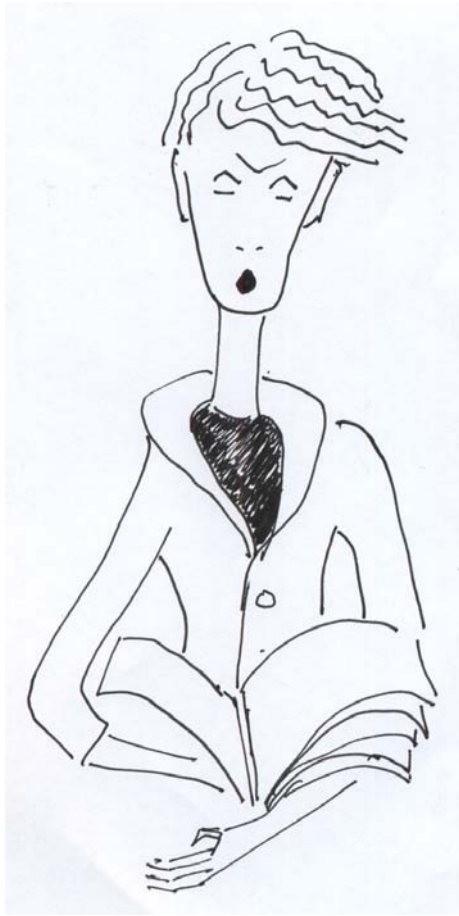
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To my family, for everything that really matters



“Even things that are true can be proved”.
Oscar Wilde

ABSTRACT

An increase in the incidence of type 1 diabetes in childhood has been reported in several countries, especially among the youngest children. Although clinically apparent diabetic vascular disease is rare in children and adolescents, they are at risk of developing nephropathy which leads to renal failure in about 30-40% during their lifetime. The determination of risk factors and screening for diabetic microvascular complications should be done even in childhood and adolescence to find those in the early stages who may respond to aggressive intervention strategies.

The main aims of this thesis were to improve the understanding of the very early events that initiate the process leading to albuminuria and renal injury and to find patients at risk of developing diabetic renal damage. Children and adolescents with type 1 diabetes in the Department of Paediatrics at Karolinska University Hospital Huddinge have been followed longitudinally with renal function tests since 1978 and with HbA_{1c}/HbA_{1c} since 1980. In 1992-1994 cross-sectional investigations were done on 46 patients over 12 years of age and who had had diabetes for more than five years. These included: 1. renal biopsies to evaluate the renal morphology on electron microscopy; 2. 24-h ambulatory blood pressure measurements to determine the day- and nighttime blood pressures (BP) and heart rate (HR); 3. renal clearances of inulin (glomerular filtration rate, GFR) and PAH (effective renal plasma flow, ERPF) to calculate the filtration fraction (FF=GFR/ERPF); and 4. the urinary albumin excretion rate (UAE). The mean age of the patients was 18 years and they had had a mean diabetes duration of 10 years. None of them had persistent microalbuminuria.

Basement membrane thickness (BMT), mesangial matrix volume fraction per glomerulus ($V_v(\text{matrix}/\text{glom})$) and foot process width were larger in the patients than healthy controls.

Spontaneous remission (period from the onset to the last visit when their insulin requirement was <0.5 U/kg/day and HbA_{1c} $<7.0\%$) occurred in 58% of the patients after a median duration of diabetes of 6.6 (range 2.6-16.2) months. Remitters had lower day- and nighttime diastolic BPs, thinner BMT and less $V_v(\text{matrix}/\text{glom})$ than those without a remission.

At least one third of the patients had systolic and diastolic nighttime BPs above the 90th percentile while their HR was within normal range. The nighttime mean arterial BP (MAP) correlated directly with the BMT, the $V_v(\text{matrix}/\text{glom})$ and the foot process width. In multiple regression analyses, 44% of the nighttime MAP and 57% of the BMT were explained by the long-term HbA_{1c}, nighttime HR and body height and 43 % of the $V_v(\text{matrix}/\text{glom})$ by long-term HbA_{1c}, nighttime HR and duration of diabetes. The effect of BP on BMT and $V_v(\text{matrix}/\text{glom})$ disappeared when the HR was included.

GFR and FF at the time of biopsy and the mean previous GFR and FF were higher in the patients with diabetes than in healthy controls. The mean previous FF correlated directly with the long-term HbA_{1c} and both variables correlated with the BMT and $V_v(\text{matrix}/\text{glom})$. UAE correlated directly with the mean previous FF and foot process width.

In conclusion, after about 10 years' duration of type 1 diabetes, we found typical diabetic glomerular changes, higher nighttime BP, increases in GFR and FF before and at the time of the biopsy in normoalbuminuric adolescents, as compared to controls. Poor metabolic control from the onset of diabetes was related to glomerulopathy and hypertension, and may in itself affect later metabolic control. Nighttime BP and especially nighttime HR seemed to be related to glomerulopathy changes which suggest that a disturbance in sympathovagal balance may have a pathogenic effect. An increase in FF may reflect intraglomerular hypertension and lead to glomerular changes with widening of the foot processes and to albuminuria.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Berg UB, **Torbjörnsdotter TB**, Jaremko GA, Thalme B.
Kidney morphological changes in relation to long-term renal function and metabolic control in adolescents with IDDM.
Diabetologia 41:1047-1056, 1998.
- II. **Torbjörnsdotter TB**, Jaremko GA, Berg UB.
Ambulatory blood pressure and heart rate in relation to kidney structure and metabolic control in adolescents with type 1 diabetes.
Diabetologia 44:865-873, 2001.
- III. **Torbjörnsdotter TB**, Jaremko GA, Berg UB.
Non-dipping and its relation to glomerulopathy and hyperfiltration in adolescents with type 1 diabetes.
Diabetes Care 27:510-516, 2004.
- IV. **Torbjörnsdotter TB**, Perrin NES, Jaremko GA, Berg UB.
Widening of foot processes in normoalbuminuric adolescents with type 1 diabetes.
Pediatric Nephrology, in press.
- V. **Torbjörnsdotter TB**, Perrin NES, Jaremko GA, Berg UB.
Metabolic control during the first years after onset in childhood type 1 diabetes in relation to glomerulopathy.
Manuscript.

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LIST OF ABBREVIATIONS

AMBP	ambulatory blood pressure
Ang II	angiotensin II
BM	basement membrane
BMI	body mass index
BMT	basement membrane thickness
BP	blood pressure
BSA	body surface area
CV	coefficient of variation
ERPF	effective renal plasma flow
FF	filtration fraction
GFR	glomerular filtration rate
HbA _{1c}	haemoglobin A _{1c}
HR	heart rate
JGA	juxtaglomerular apparatus
L _v (slit pore/glom)	length density of filtration slits
MAP	mean arterial blood pressure
RAS	renin-angiotensin II system
S _v (pcap/glom)	surface density of the peripheral capillary walls
UAE	urinary albumin excretion rate
V _v (cap/glom)	capillary volume fraction per glomerulus
V _v (matrix/glom)	mesangial matrix volume fraction per glomerulus
V _v (mes/glom)	mesangial volume fraction per glomerulus

1 BACKGROUND

1.1 INTRODUCTION

The research on complications in the kidneys in type 1 diabetes have mainly concerned the renal syndrome following diabetes; proteinuria, hypertension and progressive renal failure ¹ and the stage before that, i. e. the microalbuminuric stage ²⁻⁴. The last one to two decades, studies have been performed in a still earlier stage, when there are no clinical signs of complications - i. e., the normoalbuminuric stage. The importance of preventing these complications by optimizing blood glucose control ⁵, giving antihypertensive treatment ⁶ and educating the patients and their families ⁷ have been emphasized in the literature. However, it is now evident that these complications can be delayed, but not, always averted.

The main aims of the present studies were to improve the understanding of the very early events that initiate the process leading to albuminuria and renal injury and to find patients at risk of developing diabetic renal damage. We therefore investigated the early metabolic control, blood pressure and renal function and related them to the presumed “end-point” in our study; the morphological changes seen in the glomerulus. Moreover, known risk factors including duration of diabetes, metabolic control, hypertension and hyperfiltration are emphasized in the present studies, while others, like genetic predisposition ^{8,9}, hyperlipidemia ¹⁰, puberty ^{11,12} and smoking habits ¹³, are not.

To understand the present thesis, some background and basic facts about type 1 diabetes and the involvement of the kidneys are given.

1.2 BLOOD PRESSURE

The blood pressure (BP) is determined by cardiac output and systemic vascular resistance. The key determinant of the cardiac output is the circulatory blood volume and renal sodium handling ¹⁴, and while that of the systemic vascular resistance is vasoconstriction of the arterioles.

1.2.1 Investigation of ambulatory blood pressure

Measurements of ambulatory blood pressure (AMBP) give information, not only about the level of the daytime and nighttime BPs, but also about the diurnal variations in BP during regular activities. Moreover, AMBP enables identification of patients whose BP is elevated in the presence of health care workers but normal at home, the so called “white coat HT”^{15,16}.

An individual who has a normal nighttime decline of BP is referred to as a “dipper”, while a “non-dipper” has a reduced decline¹⁷. The definitions of dippers and non-dippers are based on arbitrarily chosen limits of the changes in the BP dip from day to night, and on the time intervals used to define day- and nighttime. There is no well-accepted definition. Many authors have used 10% as normal dipping at night¹⁸⁻²⁰. However, others who have studied younger subjects, have found greater diastolic than systolic dipping^{21,22}.

Reproducibility seems to be better for ambulatory than office BP over a three-month period^{23,24} and better for ambulatory BP than for the dipping values²⁴.

1.3 RENAL FUNCTION

One of the kidneys' functions is to filtrate the plasma volume, produce about 200 litres of primary urine and reabsorb all of it in the tubules apart from two litres that form the urine. The filtration, performed in the glomeruli, is due to a hydrostatic/colloid osmotic pressure gradient, permeability of the glomerular membranes and the surface area available for filtration²⁵. One method used to estimate the renal function, is to calculate the clearance of a substance – i. e., the volume of plasma from which that substance is completely cleared by the kidneys per unit time.

1.3.1 Investigations of renal function

The best way to measure the kidneys' function is to measure the glomerular filtration rate (GFR) – i. e., the volume that is filtered in the glomerulus per min (mL/min). Since the function is related to the body surface area (BSA), the value is normally about 120

mL/min per 1.73 m². The substances used to measure GFR are inulin, creatinine, Cr-EDTA and iohexol, which are supposed to be freely filtered by the glomeruli without tubular secretion or reabsorption.

Effective renal plasma flow (ERPF) is the volume of plasma that passes through the kidneys per min (mL/min), normally about 550 mL/min per 1.73 m². ERPF can be calculated by para-amino hippurate, which is completely removed after a single passage through the kidneys by glomerular filtration and tubular secretion²⁵: Therefore:

GFR or ERPF = the clearance of a substance = urine concentration of the substance X urine volume per unit time / plasma concentration of the substance.

The proportion of plasma filtered in the glomerulus is about 20 % - i. e., the filtration fraction (FF=GFR/ERPF). The clearance values of GFR and ERPF per body surface area are constant between two and 17 years of age²⁶, which was also found in the healthy control group up to 20 years of age, in the present studies (unpublished data). No significant difference in gender has been reported in children and adolescents²⁶ or adults²⁷.

1.4 ANATOMY AND NORMAL REGULATION OF BP AND GFR

The kidneys contain about one million functional units called *nephrons*, which consist of a glomerulus and its tubule that leads to the collecting duct^{14,25}. Urine is formed by filtration in the glomerulus which is then modified in the tubules by reabsorption and secretion of substances. Each kidney is supplied by a renal artery arising from the aorta, which eventually gives off the afferent arterioles that supply the glomerular capillary bed. The capillaries then join to form the efferent arterioles. Efferent arterioles from the cortical nephrons give rise to peritubular capillaries, which surround the tubules. The efferent arterioles from the juxtamedullary nephrons, descend into the medulla and give rise to the vasa recta in close proximity to the loops of Henle. Renal vascular resistance is produced mainly in the afferent and efferent arterioles. In each nephron, the distal tubule returns to the parent glomerulus thereby regulating its own GFR.

The *glomerulus* consists of an agglomeration of capillaries surrounded by Bowman's capsule into which the urine is filtered (Fig. 1A)^{14,25}. The glomerulus also contains

mesangial cells that support the capillary loops and have contractile properties; they can control the filtration surface area ²⁸. The *podocytes* surround the glomerular capillaries with long projections from which interdigitating foot processes originate. These podocytes are thought to maintain the filtration barrier ^{29 30} and to counteract the pressure-dependent elastic distension of the glomerular capillaries ³¹.

The filtration barrier has three layers: (1) the *endothelial cells* with numerous pores filled with negatively charged glycoproteins, (2) the *glomerular basement membrane*, which also contains negatively charged glycoproteins and (3) the filtration slits between the foot processes containing a highly organized network of various glycoproteins that form the slit pores ³². Filtration of a substance depends on both its molecular size ³³ and charge ^{30,34,35}.

The *juxtaglomerular apparatus (JGA)* is composed of afferent and efferent arterioles and a patch of distal tubular cells called the macula densa. Three types of cells are found in the JGA: the granular cells mainly in the afferent arteriole that secrete renin; the macula densa cells which respond to the tubular ion concentration and the extraglomerular mesangial cells.

Two mechanisms, namely the myogenic reflex and tubuloglomerular feedback, play an important role in the autoregulation of GFR during changes in BP. The *myogenic reflex* is induced by a rise in pressure that stretches the arterioles and causes reflex vasoconstriction, which reduces flow. The *tubuloglomerular feedback* refers to a regulation of the GFR of each nephron by the rate and composition of the distal tubular flow passing the macula densa. This signal is transmitted to the granular cells of the afferent arteriole, but also to the efferent arteriole, which together adjust the rate of filtration.

The *renin-angiotensin II- system (RAS)* is important in the regulation of the arterial BP. The release of renin from the granular cells of the arterioles promotes the production of angiotensin I, which turns to Angiotensin II (Ang II) by angiotensin-converting enzyme. Ang II acts via Angiotensin₁ receptors to constrict the afferent and efferent arterioles, and especially the efferent arterioles. The JGA releases renin in response to a reduction in afferent arteriolar pressure, a fall in the tubular flow rate, a rise in tubular

sodium concentration at macula densa, sympathetic nerve stimulation or a fall in Ang II levels. There is a local RAS in the kidney³⁶.

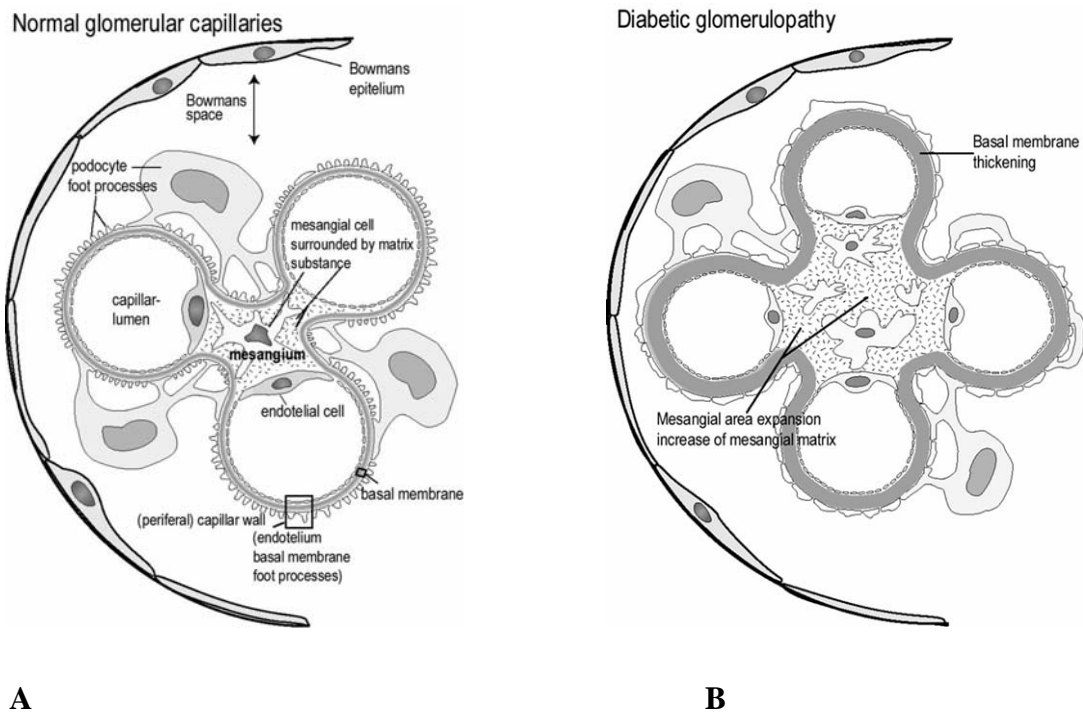


Figure 1. Schematic figure of a normal glomeruli (A) and of a glomeruli with diabetic changes (B)

A rich renal sympathetic innervation is distributed to the afferent and efferent arterioles and the JGA. Stimulation of these nerves releases noradrenaline causing constriction of the afferent and efferent arterioles. Aldosterone is mainly regulated by the RAS and stimulates increase in tubular sodium reabsorption. Many vasoconstrictors especially Ang II, ADH, endothelin, and noradrenalin stimulate the renal production of vasodilators such as prostaglandin, nitric oxid or bradykinin to protect the kidney from severe vasoconstriction.

1.5 TYPE 1 DIABETES IN CHILDREN

It has long been known that diabetes mellitus can present in two main age-related forms with different aetiologies, symptoms and treatment. Type 1 diabetes is characterized by insulin deficiency caused by autoimmune beta-cell damage with onset in childhood or

younger adult ages, while type 2 diabetes is associated with insulin resistance and non-autoimmune beta-cell failure with onset later in life. This thesis is about children and adolescents with type 1 diabetes.

The incidence of type 1 diabetes before the age of 15 years, varies widely in different parts of the world and is highest in Finland (45/100,000)³⁷, lowest in some parts of China (1/100,000)³⁸ and in Sweden it is 28/100,000³⁹. In several countries its incidence is increasing, particularly among the youngest children⁴⁰.

The main late complications of diabetes can be divided into two groups: macrovascular (i.e., cardiovascular) and microvascular complications (i.e., nephropathy, retinopathy and neuropathy). Moreover, despite modern insulin treatment, >50% of patients with childhood-onset type 1 diabetes developed retinopathy and/or microalbuminuria after about 12 years duration⁴¹. Although clinically apparent diabetic vascular disease is rare in children and adolescents they are at permanent risk of developing severe disease in one or two decades, early invalidity and having a shorter life expectancy⁷. The risk to develop diabetic nephropathy is 30-40% of these patients^{42,43}, but some studies suggest a lower incidence^{44,45}.

The determination of risk factors and screening for diabetic microvascular and macrovascular complications are needed even in childhood and adolescence⁴⁶, because it is important to find those in the early stages of their disease who may be helped by aggressive intervention strategies, such as strict glycaemic control and/or antihypertensive treatment.

1.6 BP IN DIABETES

While the clinic BP does not usually rise until after the onset of microalbuminuria⁴⁷, several studies of normoalbuminuric children, adolescent⁴⁸⁻⁵⁰ and adults⁵¹ with type 1 diabetes show elevated BP diagnosed by AMBP measurements. The most pronounced changes seem to be elevated BP at night^{48,49} and less nighttime dipping^{22,49}. In growing individuals the BP increases with the body height²¹.

The pathogenesis of the changes in BP in type 1 diabetes may be multifactorial. Factors that influence BP include autonomic neuropathy⁵², latent fluid retention⁵³, sodium retention⁵⁴ or elevated salt sensitivity⁵⁵ and activation of the RAS⁵⁶. In young adult patients with uncomplicated type 1 diabetes of short duration, mean arterial blood pressure (MAP), renal vascular resistance and FF are higher during hyperglycaemia, than during euglycaemia, and reduced by an Ang II blocker, suggesting that hyperglycaemia affects renal function by activating the RAS⁵⁶. The activation of the local RAS by hyperglukaemi has been found in the proximal tubular cells^{57,58}, mesangial cells⁵⁹ and podocytes⁶⁰. The mechanical strain on the podocytes has been shown to increase Ang II production resulting in podocyte apoptosis⁶⁰.

Nighttime hypertension is related to target-organ damage like left ventricular hypertrophy¹⁹ and function⁶¹, vascular compliance⁶², cerebrovascular lesions⁶³ and microalbuminuria in diabetes^{64,65}. The value of the non-dipping condition in diabetes is being increasingly recognised^{66,67}.

1.7 RENAL FUNCTION IN DIABETES

Increase in GFR and FF have been reported in type 1 diabetes from the onset of the disease⁶⁸⁻⁷⁴. This early glomerular hyperfiltration may be involved in the development of diabetic nephropathy⁷⁵⁻⁷⁸.

The causes of the hyperfiltration have been widely discussed⁷⁹ and are still disputed. Hyperglycaemia is thought to play a key role and might cause vasodilation⁸⁰, hypertonicity⁸¹, suppressed the tubuloglomerular feedback system⁸² or produce vasodilators^{83,84}. Hyperglycaemia increases total exchangeable body sodium and extracellular volume^{85,86} and thereby causing a compensatory increase in cardiac output, BP and filtration^{54,87}. Moreover, hyperglycaemia leads to an increased filtered load of glucose. This increase in reabsorbed glucose couples to sodium in the proximal tubules (shown by the enhanced expression of Na⁺/glucose co-transporter)⁸⁸⁻⁹⁰ lowers the concentration of Na⁺ at the macula densa^{88,91} and thereby increasing the GFR. Thus, the tubuloglomerular feedback system senses these lowered concentrations and induces glomerular hyperfiltration^{84,92}.

Diabetes mellitus is associated with a changed balance in the action of vasodilator and vasoconstrictor molecules⁹³. Hyperfiltration in early diabetic nephropathy may be due to an increase in the intrarenal production of nitric oxide⁹⁴. Growth hormone, insulin-like growth factor I⁹⁵ and glucagon are other possible mediators of diabetic hyperfiltration⁹⁶⁻⁹⁹.

1.8 NATURAL HISTORY OF DIABETES NEPHROPATHY

Diabetic *nephropathy* is a term used for the combination of proteinuria, an increase in BP and a decrease in GFR in diabetes, while the term diabetic *glomerulopathy* is reserved for the structural changes in the glomeruli in diabetes. The natural course of diabetic nephropathy can be divided clinically into five stages^{100,101} (Table 1) ranging from a normal renal appearance to end-stage renal failure, with renal dialysis or transplantation as treatment. Our knowledge about glomerular changes in diabetes mellitus began in 1936 when Kimmelstiel and Wilson¹⁰² described the typical light microscopic lesions in late diabetes, with nodular thickening of the mesangial regions.

Glomerular hypertrophy is present at the onset of type 1 diabetes¹⁰³ and is characterized by increases in the surface area of the capillaries and in GFR¹⁰⁴. After about 2 years, the basement membrane (BM) widens¹⁰⁵. Later, the picture is dominated by thickening of the peripheral BM and expansion of the mesangium¹⁰⁴.

In microalbuminuric patients, the fraction of the glomerulus occupied by the mesangium gradually increases and becomes marked after 6-15 years duration and an increase in the foot process width may be present¹⁰⁶ (Fig. 1B). In some patients, late glomerular hypertrophy with preservation of the capillary surface occurs as a compensatory phenomenon, which prolongs renal survival^{107,108}. In other patients the increase in mesangial volume cause a reduction in the capillary filtration surface area, which is closely associated with a reduce in GFR¹⁰⁹. At this stage, the number of totally occluded glomeruli (glomerular closure)¹¹⁰ increases, which eventually leads to end-stage renal failure.

Diabetic nephropathy consists not only of changes in the glomerulus, but also of hyalinosis of the afferent and efferent arterioles^{111,112}, interstitial fibrosis, tubular

Stage	Time course	UAE	Clinical BP	GFR	Structural changes	Effect of strict insulin treatment	Effect of antihypertensive treatment
1	Present at diagnosis	May be increased, but reversible	Normal	Increased (20-50%)	Increase in renal and glomerular size	GFR and UAE changes reversible	
2	0-5 years	Normoalbuminuria (< 20 µg/min)	Normal	Increased (20-50%)	Increase in BMT after 2 years	Hyperfiltration reduced	Filtration fraction and UAE may be reduced
3	6-15 years 35% of patients	Microalbuminuria (20 – 200 µg/min)	Incipient increase, (~ 3 mm Hg/year)	Decline from supra-normal values	Further increase in BMT and mesangium. Arteriolar hyalinosis, interstitium increased	Microalbuminuria and GFR stabilized. Structural damage slowed	Microalbuminuria reduced. Prevention of fall in GFR. Structural damage arrestable
4	15-25 years 35% of patients	Proteinuria, (> 200 µg/min)	High, increase (~ 5 mm Hg/year)	Decline (~10 ml/min/year)	Clear and pronounced abnormalities.	Greater fall in GFR with high HbA _{1c}	Progression slowed
5	≥ 25-30 years	Slight decline due to reduction in GFR	High	< 10 ml/min	Advanced glomerulopathy with glomerular closure	No effect	No effect

Table 1. Stages in the development of renal changes and lesions in type I diabetes ^{101,113}

atrophy and mononuclear cell infiltration ¹¹⁴ which are not present in early nephropathy.

Renal morphological changes are also seen in type 2 diabetes. About one third of the patients have near normal findings, one third have typical diabetic morphological changes and the other third have atypical patterns of renal injury ^{115,116}. The changes in the late stages of nephropathy in type 2 diabetes are well known, while those in the earlier stages have been studied less.

The main subject of this thesis is glomerulopathy due to type 1 diabetes in the normoalbuminuric range - i. e., stage 2 in Table 1.

1.9 REMISSION

In patients with type 1 diabetes, the clinical remission is a period characterized by residual beta-cell function, reduced insulin requirement and normalized HbA_{1c} levels. It occurs shortly after the clinical diagnosis is made after treatment with insulin has started. The remission is ascribed to a recovery of those beta-cells that were not destroyed at diagnosis and an improvement in sensitivity to insulin ^{117,118}. Factors affect the likelihood of partial or complete remission in children with type 1 diabetes, such as age, gender, pubertal status, metabolic findings at onset, HLA-types and presence of diabetes-associated autoantibodies ¹¹⁹⁻¹²¹. Several studies have been done to try to preserve the beta-cell function and promote or prolong the remission phase ^{120,122-125}. Indeed, it has been possible to increase the number of patients with remission by a strict initial control of diabetes ¹²⁶.

1.10 URINARY ALBUMIN EXCRETION RATE

Microalbuminuria has been regarded as the earliest sign of diabetic nephropathy ^{76,127}. During the past few years, a few studies have shown that over a three to 10-year period about one third of the microalbuminuric patients revert to normoalbuminuria, one third remain microalbuminuric and only one third develop proteinuria ¹²⁸⁻¹³⁰. In the latter group, the renal structural changes are well established ^{108,131,132}. However,

microalbuminuric ¹³³⁻¹³⁵ and normoalbuminuric patients ^{133,136,137} may also have well-established renal lesions. Therefore, the development of diabetic nephropathy can occur early without overt clinical signs or symptoms ¹³⁸ and it has been suggested that microalbuminuria may be a marker rather than a predictor of diabetic nephropathy ¹²⁹.

1.11 NEUROPATHY

Diabetic neuropathy is classified as polyneuropathy, focal neuropathy and autonomic neuropathy. The latter may raise the heart rate (HR) ^{139,140} and BP at night ⁵² and is associated with the most serious consequences, such as hypoglycaemia unawareness and cardiovascular dysfunction. Heart rate variability has been shown to detect early subclinical autonomic neuropathy in children and adolescents with type 1 diabetes ^{141,142}. The neuropathy in diabetes may reduce parasympathetic activity ^{141,143,144} or cause sympathetic dominance ^{64,144-146}.

A relation between nephropathy and autonomic neuropathy has been discussed by several authors ^{64,144,147,148}, and it may be due to the co-existence of two diabetic complications with a common background - i.e., poor metabolic control or genetic susceptibility. On the other hand, autonomic neuropathy may have a pathophysiological effect of its own and cause renal lesions by elevated BP, renal vascular dilation and elevated intraglomerular pressure ¹⁴⁸⁻¹⁵⁰.

1.12 THERAPEUTIC INTERVENTIONS

Treatments to reduce the risks of developing diabetic nephropathy include optimized metabolic control, administration of antihypertensives and protein or sodium reduced diets.

The importance of good metabolic control to lower the risk of microvascular complications in children ⁴⁴ and adults ¹⁵¹⁻¹⁵³ with type 1 diabetes has been reported by several studies. The Diabetes Control and Complications Trial (DCCT) established that normalized HbA_{1c} levels reduce the incidence of microalbuminuria, albuminuria and clinical nephropathy by 39%, 54% and 60%, respectively ¹⁵¹. An analysis of the findings in the cohort of teenagers who participated in the DCCT was presented

separately, but did not change the study outcome ¹⁵⁴. Moreover, the benefits of the treatment persisted 4 years after the conclusion of the DCCT ¹⁵⁵.

Some data have shown the importance of intensive control of BP in delaying the progression of the renal disease ^{156,157}. In particular, drugs that block the RAS (ACE-inhibitors, Ang II receptor blockers) have the advantage of reducing intraglomerular capillary pressure independently of changes in systemic BP ¹⁵⁸. This reduction can be induced by dilating the efferent arteriole lowering the sensitivity to Ang II ¹⁵⁹. These drugs also inhibit the formation of extracellular matrix and enhance matrix degradation ¹⁶⁰. ACE-inhibitors reduce the GFR ¹⁶¹ and FF in hyperfiltrating patients ⁴, diminish UAE in microalbuminuric patients ^{4,162} and also prevent further progression of glomerulopathy ¹⁶³. A combination with ACE-inhibitors and Ang II receptor blockers ¹⁶⁴ have been found to be more effective ^{165,166}.

A reduction in protein intake decreases GFR in young patients with type 1 diabetes ¹⁶⁷, but has no effect on microalbuminuria ^{168,169}, Lower sodium intake in type 2 diabetic patients during one week does reduce the GFR ¹⁷⁰, but long-term studies are lacking.

To conclude, apart from the best possible metabolic regulation, early treatment with antihypertensive drugs in microalbuminuric and/or hypertensive patients with type 1 diabetes ^{4,162} are needed. However, the relatively high transient microalbuminuria in paediatric patients ¹²⁸ with unknown prognostic relevance complicate the decision to start such treatment for a lifetime ¹⁷¹. Although our current tools are not perfect, the early detection of risk factors and appropriate intervention strategies are necessary to improve the long-term prognosis for children with diabetes.

2 AIMS

The general aim of the present thesis was to evaluate the relations between metabolic control, 24-h AMBP, renal function and renal morphology in order to determine the risk of developing glomerulopathy and nephropathy in an unselected group of normoalbuminuric and normotensive adolescents and young adults with type 1 diabetes.

The specific aims were:

- To investigate whether there are any differences between the renal morphology in these patients and healthy controls
- To study the effects of metabolic control on the development of renal morphological changes
- To determine whether hypertension can be diagnosed by 24-h AMBP measurements in the normoalbuminuric stage
- To study whether hypertension, detected by 24-h AMBP measurements, can be used to predict which patients run the risk of developing diabetic glomerulopathy
- To investigate whether renal function investigations can be used to predict which patients run the risk of developing diabetic glomerulopathy

3 MATERIAL AND METHODS

3.1 PATIENTS

All patients with type 1 diabetes above 12 years of age and with duration of more than five years at the Department of Paediatrics at Karolinska University Hospital at Huddinge were asked to participate in a kidney biopsy study at the time of routine renal function testing. Forty-six of 61 consecutive patients between 1992 and 1994 agreed to participate. None had persistent microalbuminuria but the patients were not selected on the basis of albumin excretion rate. Table 2 shows the patients who were included in the various studies and the reasons for their exclusions.

Table 2. The 46 patients participating in the studies (Papers I-V) and reasons of exclusion.

Paper	I	II	III	IV	V
Morphology and	Renal function	24h AMBP	"non dipping"	Early metabolic control	Renal volume
Included	36	41	40	46	39
Excluded total	10	5	6		7
< 3 Inulin PAH investigations	(10)				
No AMBP		(2)	(2)		
AMBP > 1 year after biopsy		(3)	(3)		
AMBP, no night time values			(1)		
Few HbA1c during the first 6 years					(7)

The publications about normoalbuminuric adolescents and young adults presented in this thesis are summarized in Table 2 and below:

Paper I. Renal morphology and function

This study included 36 patients investigated at 3-7 occasions with clearances of inulin and PAH in order to study the relation between long-term renal function, metabolic control and morphology.

Paper II. Renal morphology, ambulatory BP and HR

In this Paper, the 41 patients who had undergone a 24-h AMBP investigation within 10 months from the day of biopsy were included, in order to study the relations between renal morphology, function, metabolic control BP and HR.

Paper III. Renal morphology and non-dipping BP pattern

In this Paper, we included 40 patients in whom accurate BP and HR values during both day and night were available in order to study the non-dipping status in relation to renal morphology, function and metabolic control.

Paper IV. Renal morphology in controls and patients

Here we compared 46 patients with diabetes to 20 age- and gender-matched kidney donors kindly provided by Professor Michael Mauer, Minneapolis. The study concerned the relations with renal volume.

Paper V. Renal morphology, BP and early metabolic control

Data on the metabolic control during the first six years were available in 39 of the 46 patients. The influence of the duration of remission and early metabolic control on the development of glomerulopathy and hypertension was investigated.

The clinical data of the patients and some data concerning the controls are given in Table 3. The patients had a mean age of 17.7 years and duration of diabetes of 10.6 years at the time of biopsy. Fourteen per cent of the patients had an insulin pump; the remaining patients received one or two injections three to five times daily. The mean insulin dose was 0.95 (0.19) IU/kg per day. The median HbA_{1c} at biopsy was 7.7 % and median long-term HbA_{1c} 8.0 %. Eighty per cent were in Tanner stage 5 (adult sexual maturity: 95% of the girls, 67% of the boys). One patient had asymptomatic bacteriuria. Four patients had well-controlled hypothyroidism. Their office blood pressure was 121/74 mmHg. No patient was on antihypertensive treatment.

Table 3. Clinical data in normoalbuminuric adolescents with type 1 diabetes and comparisons with age-matched controls concerning clearances of inulin and para-amino hippurate. Mean (SD) or median (10th and 90th percentiles). (Papers I-V).

	Diabetes Mellitus	Controls		p-value
n	46	28	59	
Gender, female, n (%)	20 (43)	12 (43)	28 (47)	1.00, 0.7
Age at onset, years	7.1 (3.7)			
HbA _{1c} at onset, %	10.0 (7.7-12.9), n=28			
Age at biopsy, years	17.7 (2.8)	16.8 (2.7)	11.3 (4.4-19.5)	0.20, -
Duration at biopsy, years	10.6 (3.3)			
HbA _{1c} at biopsy, %	7.7 (6.8-10.3)			
Long-term HbA _{1c} , %	8.0 (7.4-9.1)			
UAE, µg/min	6 (3-25), n=38			
BMI, kg/m ²	22.3 (2.8)			
Blood pressure, mmHg	121 (12) /74 (10)			
Daytime blood pressure, mmHg	128(8) / 77(5), n=44			
Nighttime blood pressure, mmHg	113(9) / 60(7), n=43			
GFR, ml/min/1.73m ²	133 (22)	118 (10)		0.002
ERPF, ml/min/1.73m ²	610 (130)	641 (99)		0.28
Filtration fraction, %	22.3 (4.0)	18.8 (2.6)		<0.001
Mean previous GFR, ml/min/1.73m ²	136 (19), n=45		117 (10)	<0.001
Mean previous ERPF, ml/min/1.73m ²	629 (83), n=45		622 (97)	0.66
Mean previous FF, %	22.0 (2.5), n=45		19.2 (2.6)	<0.001

3.2 METHODS

3.2.1 Study design

Children and adolescents with type 1 diabetes have been followed longitudinally with renal function tests since 1978 and with HbA₁/HbA_{1c} since 1980. In 1992-1994, cross-sectional investigations were performed during a 3-day period:

- **Day 1.** Renal function test to determine glomerular filtration rate and renal plasma flow. Overnight urine to determine the albumin excretion rate.

- **Day 2.** Ultrasound to determine renal volume. Renal biopsy to determine renal morphological changes.
- **Day 3.** 24-h ambulatory blood pressure measurements.

3.2.2 Indices of metabolic control

Data regarding insulin treatment and metabolic control were obtained from the medical records of the patients. Initially, total HbA₁ with ion exchange chromatography (reference values for non-diabetic subjects 5.5-8.5%) was analysed. Between 1986-1988, HbA_{1c} was determined with fast protein liquid chromatography (FPLC, reference values 3.4-5.0%)¹⁷² and since 1988, with high-performance liquid chromatography (HPLC, reference values 3.4-5.0%). To compare the glycaemic control over time, HbA₁ data were converted to HbA_{1c}, using the equation $HbA_1 = 1.13 \times HbA_{1c} + 0.85$. The in-house correlation coefficient between the two methods was 0.957.

HbA₁ or HbA_{1c} was analysed three to four times a year. The “HbA_{1c} at onset” (available in 28 patients) was defined as the first value taken within 6 weeks at diagnosis of diabetes (Paper V). The mean of all HbA_{1c} determinations during three time intervals for each patient was calculated: 0-2, 2-4 and 4-6 years after diagnosis of diabetes (“HbA_{1c}⁰⁻²”, “HbA_{1c}²⁻⁴” and “HbA_{1c}⁴⁻⁶”, Paper V). “Long-term HbA_{1c}” was calculated as the mean of each year’s mean HbA_{1c} from the diagnosis to the biopsy (apart from the HbA_{1c} value at onset, Papers I-V).

Comments. Our HbA_{1c} values are 0.7-1.0% lower than those of the Diabetes Control and Complication Trial reference laboratory^{151,173} and 0.8% higher than the reference system developed by the IFCC Working group on HbA_{1c} standardization¹⁷³.

3.2.3 Definitions of onset and remission of diabetes

The onset of diabetes was defined as the day when the patients received their first injection of insulin. Spontaneous clinical remission was arbitrarily defined as an insulin dose < 0.5 U/kg/24h and an HbA_{1c} < 7.0%. The duration of remission was defined as the number of months from onset to the last visit when the need for insulin was < 0.5 U/kg/24h and the HbA_{1c} < 7.0 % (determined in 38 patients, Paper V).

3.2.4 Determination of albumin excretion rate

Timed overnight-collected urine was analysed for albumin by an automated immunonephelometric method (Behring Nephelometer Analyzer; Behringwerke AG, Marburg, Germany) and the urinary albumin excretion rate (UAE) determined. UAE was measured in 38 patients. Persistent microalbuminuria was defined as UAE > 15 µg/min in two of three samples during 6 months.

3.2.5 Determination of kidney volume

The ultrasound examinations were done with a 3.5 MHz vector scanner (Acuson, Mountain View, CA, USA). The patients were examined in the prone position. The volume of each kidney was calculated using the equation for an ellipsoid: $V \text{ (cm}^3\text{)} = 0.523 \times \text{length} \times \text{width} \times \text{depth}$ ¹⁷⁴, and the mean of the left and right kidney volumes calculated (Paper IV).

Control values. The calculated kidney volumes were compared to a material of normal kidney volumes, related to the body weight, in 325 children and adolescents using the same technique¹⁷⁴.

3.2.6 Determinations of blood pressure and heart rate

3.2.6.1 Office blood pressure

After 30 minutes' bed rest, the office blood pressure (BP, Papers II-IV) was measured in the right arm, with an Omron digital blood pressure monitor before the renal function test on Day 1 of the study (Model Hem-700C, Boehringer Mannheim, Scandinavia AB, Bromma, Sweden).

3.2.6.2 Ambulatory blood pressure

Ambulatory BP (AMBP, Papers II, III, V) was recorded for 24 h with portable automatic Space Labs 90 207 equipment. Daytime (08.00 a.m. to 20.00 p.m.) and nighttime (00.00 a.m. to 06.00 a.m.) BP and heart rate (HR) were used as described by Soergel et al.²¹. The monitor was programmed for cuff insufflations every 20 min during daytime and every 30 min during nighttime. In 24 patients, the recordings were started at about 12.00 a.m. on Day 3, when the patients were discharged from hospital.

In 17 of the patients, the 24-h AMBP measurement was performed later, but within 10 (median 7) months of the kidney biopsy. The patients were told to live as usual but to avoid sports. All patients had a median of 94 (range 71-100) % successful readings.

The mean arterial blood pressure (MAP) was calculated as the diastolic BP plus one third of the difference between the systolic and diastolic BP. The daytime and nighttime systolic, diastolic BP, MAP, and HR were calculated using ABP PC Direct/Base Station Interface 90 219.

The percentage of nighttime fall in BP (the “dipping”) was calculated as: (daytime BP – nighttime BP) x 100/ daytime BP^{21,148}. “Non-dippers” were defined as patients with a fall in BP of less than mean -1SD of controls²¹ - i.e., less than 7% for systolic BP and less than 14% for diastolic BP, while “dippers” were defined by a fall in BP during the night exceeding these values. “MAP non-dippers” was defined by a fall of less than 12%. The HR-dipping was similarly calculated. One patient interrupted the 24-h registration, and therefore, only daytime measurements could be recorded (Paper II).

Control values. For comparison, data based on 1141 healthy children and adolescents between five and 21 years of age were used (²¹ and personal communication). The mean (SD) values for systolic and diastolic dipping of BP in control subjects were 13 (6) % and 23 (9) %, respectively²¹.

Comments. The AMBP data were analysed using the same time intervals as those by Soergel et al.²¹. These intervals were chosen not only to allow comparison, but also because the bedtimes vary between children of different ages and some studies have shown that the BP changes considerably and rapidly from 20.00 to 24.00 p.m. and from 06.00 to 10.00 a.m.^{175,176}.

The definitions of systolic and diastolic dipping-non-dipping were not arbitrarily chosen, but based on the dipping data from healthy school children²¹. A similar approach was used by Lurbe et al.¹⁷⁷. Our MAP dipping-non-dipping division was similar to that of Staessen et al.¹⁷⁸.

The intraindividual repeatability of systolic and diastolic BP tends to be better for day- and nighttime BP than for systolic and diastolic dipping¹⁷⁸.

3.2.7 Renal function tests

Renal haemodynamics was evaluated every second or third year after the onset of diabetes. The glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by the clearances of inulin and para-amino hippurate during water diuresis with a standard clearance technique including continuous infusion after a prime dose and repeated during urine sampling^{68,136}. Before starting the renal function test in the morning the patients were given their ordinary insulin doses, followed by breakfast. The filtration fraction was calculated as GFR/ERPF.

The absolute GFR and ERPF (ml/min) were used for comparisons between renal function and body-size related parameters (e.g., renal volume, Paper IV). The relative GFR and ERPF values (ml/min/1.73m²) were used to compare patients of different sizes (Papers I- IV). We calculated the mean GFR, ERPF and FF of all previous renal function tests in each patient and these values are shown as “mean previous GFR, ERPF (ml/min/1.73m²) and FF (%)” (Papers I, III).

Controls. The renal function data in the patients were compared with data from healthy children and young adults investigated in the Paediatric Nephrology Unit. The relative GFR, ERPF and FF at biopsy were compared to those of 28-34 healthy children and young adults matched on a group level for age. The mean previous renal function estimates were compared to those of 59 healthy children and young adults 3.5 - 25.9 (median 11.3) years of age - i. e., the ages of the patients during the entire follow-up period.

Comments. The methods used to determine the GFR and ERPF with clearances of inulin and PAH are regarded as the “golden standard”¹⁷⁹⁻¹⁸¹.

3.2.8 Kidney biopsy

The biopsies were taken under local anaesthesia with ultrasound guidance (Acuson, Mountain View, CA, USA), using an automatic biopsy device (Biopty Bard Urological, Covington, GA USA) with a 16-gauge needle (Manan Medical Products, Northbrook, IL, USA)¹³⁶. The biopsy procedure is summarized here, but thoroughly described in Paper I. Samples for light and electron microscopy were immediately immersed in 4%

paraformaldehyde in phosphate buffer and for immunofluorescence quickly snap-frozen in liquid nitrogen ¹³⁶.

After the biopsy, the patients remained supine for 24 hours and the HR and BP were determined at regular intervals. Some patients developed microscopic haematuria and subcapsular haematomas, but no clinical complications occurred. A few patients had slight pain in the muscles overlying the biopsy site.

3.2.8.1 *Light microscopy*

The light microscopic examination was done on parts of fixed cortical tissue embedded in glycolmethacrylate (Technovit 7100, Kulzer, Germany).

The mean glomerular volume (Paper I) was estimated by Cavalieri's principle ¹⁸². The embedded tissue blocks were serially sectioned and stained with periodic acid-Schiff. The number of sampled levels per glomerulus ranged between 13 and 21. The area of the profiles was estimated by point counting using an ocular grid (magnification 410x) and the sum of the profile areas estimated. The mean glomerular volume was then calculated as: the sum of the profile areas x mean of the actual section thickness. At least five glomeruli were measured in each biopsy and the mean glomerular volume calculated. The coefficient of variation (CV) of the calculations was 21%.

Comments. The Cavalieri method is considered as the "golden standard" to measure the glomerular volume ¹⁸³.

3.2.8.2 *Electron microscopy*

Electron microscopy (Papers I-V) was performed on small tissue blocks postfixed and embedded in Polybed 812. Paper I describes the method used for sampling of the glomerular profiles taken for ultra-thin sectioning which were then stained with uranyl acetate and lead citrate. Between three and five glomeruli were analysed from each biopsy. Between 7 and 19 electron micrographs - i. e., approximately 40% of the area of each glomerular profile - were sampled in a systematic random manner. The micrographs were examined at a magnification of about x10 000 that was corrected by using a grating grid. The reference space, here called the "glomerulus", was defined as a minimal convex polygon enclosing the glomerular tuft ¹⁸⁴. All the estimated relative structural quantities were expressed in relation to this reference space.

Mesangial and mesangial matrix volume fractions per glomerulus ($V_v(\text{mes}/\text{glom})$, %, and $V_v(\text{matrix}/\text{glom})$, %) were estimated by point-counting, using a superimposed

lattice square grid. The total points hitting the mesangial and the mesangial matrix area were divided by the total points hitting the reference space. The mesangial matrix was defined as all extracellular material in the mesangial areas.

The same technique was used to estimate the *capillary volume fraction per glomerulus* ($V_v(\text{cap}/\text{glom})$, %) – i. e., the total points hitting the capillary areas were divided by the total points hitting the reference space.

Surface density of the peripheral capillary walls ($S_v(\text{pcap}/\text{glom})$, μm^{-1}) and *length density of filtration slits* ($L_v(\text{slit pore}/\text{glom})$, μm^{-2}) were estimated using the same square lattice grid as above. The intersections with the epithelial aspect of the basement membrane of the peripheral capillary walls and the number of their related filtration slits were counted, both divided respectively by the total points hitting the reference space.

The *mean foot process width* was estimated as the ratio of $S_v(\text{pcap}/\text{glom})$ and $L_v(\text{slit pore}/\text{glom})$ (nm).

The *basement membrane thickness* (BMT) (nm) was estimated by using the orthogonal intercept method of Jensen and co-workers¹⁸⁵.

Controls. Plastic embedded renal biopsy tissues blocks from normal control subjects (cadaver and living donors) were obtained from Professor Michael Mauer, Department of Pediatrics, University of Minnesota, MN, USA. These biopsy blocks and the biopsy tissues from our patients were treated in the same way (cutting and stereological measurements) by the same nephropathologist who had no knowledge of the subject's history. We compared our patients with the entire group of kidney donors (n=20) matched with our group of patients as regards age and gender, and with the living donors (n=8) who were older than our patients.

Comments. The mathematic method to measure three-dimensional structures by two-dimensional section planes, stereology, was described in the 1960s. The stereological symbols that are generally used are a double symbol of two letters. The first letter defines the structure you measure and the second, concerns the structure of the reference system, usually written as a subscript capital letter¹⁸⁶. In the present thesis, the volume, V, surface, S, or length, L are estimated and the reference system used are the total volume of the glomeruli, v .

The CV of electron microscopy measurements *in* each subject (variations between glomeruli) accorded with published data^{134,187,188} as did the CV *between* subjects

(Table 4) ^{106,189-191}. The greater variation *between* the patients than *in* each patient, especially in BMT, but also in $V_v(\text{matrix}/\text{glom})$, is important for the detection of very early morphological changes ¹⁹².

Table 4. Renal morphology in normoalbuminuric adolescents with type 1 diabetes and normal control subjects (all kidney donors or living donors alone). The coefficients of variation (CV) in three to five glomeruli in each subject¹ and between the subjects² are given. The significance of the differences between the groups is also shown after adjustment for age and gender: $p^3=0.040$, $p^4=0.025$ and $p^5=0.0004$, respectively (Paper IV). Mean (SD).

	Controls				Diabetes Mellitus (DM)			p-value	
	All donors (AD), n=20		Living donors (LD), n=8		n=46			DM - AD	DM - LD
		CV ¹ , %	CV ² , %			CV ¹ , %	CV ² , %		
Gender, female, n (%)	10 (50)			6 (75)	20 (43)			0.79	0.14
Age, years	19.1 (4.6)			22.5 (3.1)	17.7 (2.8)			0.12	<0.001
BMT, nm	320 (25)	5	8	323 (25)	505 (107)	7	21	<0.001	<0.001
$V_v(\text{matrix}/\text{glom})$, %	7.9 (1.0)	18	13	7.2 (0.8)	10.5 (2.1)	14	20	<0.001	<0.001
$V_v(\text{mes}/\text{glom})$, %	18.5 (1.6)	13	9	17.6 (1.7)	19.2 (3.0)	13	16	0.34	0.15 ³
$V_v(\text{cap}/\text{glom})$, %	39.3 (4.3)	10	11	36.5 (3.1)	46.7 (5.1)	9	11	<0.001	<0.001
$S_v(\text{pcap}/\text{glom})$, μm^{-1}	0.142 (0.012)	12	9	0.144 (0.014)	0.145 (0.019)	13	13	0.52	0.85
$L_v(\text{slit pore}/\text{glom})$, μm^{-2}	0.359 (0.055)	16	15	0.389 (0.036)	0.354 (0.058), n=45	12	16	0.74	0.11 ⁴
Foot process width, nm	398 (42), n=19	6	13	372 (12)	414 (36), n=45	5	9	0.13	<0.001 ⁵

3.2.8.3 Immunofluorescence

A subdivided part of fresh cortical tissue was quickly snap-frozen in liquid nitrogen and cut in a cryostat (Paper I). The sections were evaluated by a direct method, using fluorescein isothiocyanate-conjugated antibodies to human IgG, IgA, IgM, C3, C1q (Dakopatts AB, Sweden) and examined in a Zeiss epifluorescence microscope with appropriate filters (Carl Zeiss, Oberkochen, Germany)

3.3 STATISTICAL ANALYSES

The Shapiro-Wilk's W test for normality was used. For data not having a normal distribution, the medians with 10th and 90th percentiles are given, and for data of approximately normal distribution, mean values and standard deviations (SD) are given. UAE data were log 10 transformed for calculations because of a skewed distribution. The coefficients of variation ($CV = \text{standard deviation}/\text{mean}$) are given for the variations of morphological data within subjects and within groups.

The Student's t -test was used to compare two groups if the distribution was approximately normal and the variances equal. If the variances were not equal, the t -test was modified by the Welch-test. The Mann-Whitney U-test was used when the distribution was skewed and Fisher's exact test to compare categorical data.

In the correlation analyses, Pearson's (r) or Spearman's (r^s) correlation coefficient were given. Univariate and multiple regression analyses were done with the least square method. The residuals were checked for a normal distribution. Cook's D influence was done and if one observation had a strong effect on the regression line, this observation was excluded (Paper IV). R^2 or adjusted r^2 (to adjust for the number of X-factors used) were given in the multiple regression analyses.

A p -value <0.05 was considered significant. The statistical programme of JMP versions 3.1-4.0.5 were used.

Comments. During the period between the publications of these papers (1998-2005), the recommendations from the statisticians about how to present data have changed. Thus, the data are reported in various ways in different papers. To simplify reading this summary, only the mean (SD) or median (10th and 90th percentiles) values are given.

3.4 ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of Karolinska Institutet at Karolinska University Hospital, and performed after informed consent had been obtained from the patients and their parents.

4 RESULTS

The publications about normoalbuminuric adolescents and young adults presented in this thesis are summarized:

Paper I. Renal morphology and function

The relations between morphology, long-term renal function and metabolic control were studied.

Papers II and III. Renal morphology, ambulatory BP and HR

The relations between morphology, metabolic control, BP and HR and the BP non-dipping status were investigated.

Paper IV. Renal morphology in controls and patients

A comparison between renal morphology in patients and controls was performed. Relations with renal volume were investigated.

Paper V. Renal morphology, BP and early metabolic control

The influence of the duration of remission and early metabolic control on the development of glomerulopathy and hypertension was investigated.

4.1 REMISSION AND METABOLIC CONTROL

Remission occurred in 22 of 38 (58%) of the patients with a median duration of 6 (3-16) months. We found no difference between males and females. Remitters had lower HbA_{1c}^{0-2} and lower long-term metabolic control than those without a remission (7.3% vs. 8.7%, $p=0.004$ and 7.5% vs. 9.3%, $p=0.006$, respectively). Remitters also had significantly lower day- and nighttime diastolic BP (75 vs. 78 mm Hg, $p=0.045$ and 58 vs. 63 mm Hg, $p=0.032$), thinner BM (464 vs. 556 nm/1.73m², $p=0.006$) and less $V_V(\text{matrix}/\text{glom})$ (10.1% vs. 11.4%, $p=0.048$) than those without a remission.

The HbA_{1c} at onset correlated directly with the HbA_{1c}^{0-2} ($r^s=0.48$, $p=0.007$) and with long-term HbA_{1c} ($r^s=0.43$, $p=0.019$). The HbA_{1c} at onset correlated directly and the duration of remission inversely with the nighttime BP 10 years later (Table 5). Moreover, HbA_{1c} at onset tended to correlate with the duration of remission ($r^s=-0.35$, $p=0.062$).

Table 5. Correlations with blood pressure in normoalbuminuric adolescents with type 1 diabetes. Pearson's (r) or Spearman's (r^s) correlation coefficients are given. (Papers II, III, V). Prev., previous.

		n	Correlation coefficient	p-value
(II) Daytime diastolic BP, mmHg	Log UAE, Log $\mu\text{g}/\text{min}$	35	$r=0.39$	0.020
(II) Daytime MAP, mmHg	Long-term HbA _{1c} , %	41	$r=0.33$	0.038
(II) Nighttime systolic BP, mmHg	BMT, nm	40	$r=0.34$	0.029
(II)	Foot process width, nm	39	$r=0.41$	0.010
(V) Nighttime diastolic BP, mmHg	HbA _{1c} at onset, %	27	$r^s=0.47$	0.014
(II)	Long-term HbA _{1c} , %	40	$r=0.37$	0.017
(V)	Remission, months	36	$r^s=-0.45$	0.006
(II)	Log UAE, Log $\mu\text{g}/\text{min}$	34	$r=0.37$	0.033
(II)	Nighttime HR, beats/min	40	$r=0.53$	0.0004
(II)	BMT, nm	40	$r=0.34$	0.031
(V) Nighttime MAP, mmHg	HbA _{1c} at onset, %	27	$r^s=0.41$	0.031
(II)	Long-term HbA _{1c} , %	40	$r=0.40$	0.010
(V)	Remission, months	36	$r^s=-0.38$	0.021
(II)	Nighttime HR, beats/min	40	$r=0.45$	0.004
(II)	BMT, nm	40	$r=0.44$	0.004
(II)	Foot process width, nm	39	$r=0.38$	0.018
(II) Nighttime HR, beats/min	BMT, nm	40	$r=0.39$	0.012
(II)	V _v (matrix/glom), %	40	$r=0.37$	0.018
(III) HR dipping, %	MAP dipping, %	40	$r=0.36$	0.022
(III)	BMT, nm	40	$r=-0.48$	0.0016
(III)	V _v (matrix/glom), %	40	$r=-0.39$	0.012
(III)	V _v (mes/glom), %	40	$r=-0.34$	0.032
(III)	Mean prev. FF, %	40	$r=-0.32$	0.045

To be able to analyse the progression rate of the BMT and V_v(matrix/glom) changes, we reduced our morphological values with the mean value of the living controls and divided by the duration of diabetes (Table 6). The patients with the fastest progression of the BMT and increase in V_v(matrix/glom) had the highest HbA_{1c} during the first six years of diabetes after diagnosis (Table 6). In a multiple regression analysis, the progression of the BMT thickening was determined by the HbA_{1c} two to six years after

onset ($r^2=0.45$, $p<0.0001$), while the HbA_{1c} values at onset and during the first two years were of no significance.

Main conclusions. The metabolic control during the first years after the onset of type 1 diabetes seems to play an important role in the development of glomerulopathy and hypertension. Early metabolic control may in itself affect or predict later metabolic control.

Table 6. Early clinical data in normoalbuminuric adolescents with type 1 diabetes when divided into two groups according to the rate of increase in basement membrane thickening and mesangial matrix volume fraction/glomerulus about 10 years after onset. *mean value of living controls. Mean (SD) or median (10th and 90th percentiles) (Paper V).

	(BMT - 323*) / duration		(Vv(matrix/glom) - 7.2*) / duration	
	< 20 nm/year	≥ 20 nm/year	< 0.4%/year	≥ 0.4%/year
n	23	16	24	15
Gender, f (%)	13 (57)	5 (31)	11 (46)	7 (47)
p-value	0.19		1.00	
Age at onset, years	7.1 (3.3)	9.4 (2.6)	7.9 (3.4)	8.3 (3.0), n=11
p-value	0.024		0.72	
HbA _{1c} at onset, %	9.8 (6.2-13.3), n=15	10.1 (8.7-13.9), n=13	9.7 (6.7-13.1), n=17	10.8 (9.0-14.3)
p-value	0.32		0.074	
Remission, months	6 (0-16), n=22	0 (0-9)	6 (0-15), n=23	0 (0-10)
p-value	0.055		0.13	
Mean HbA _{1c} ⁰⁻² , %	7.7 (6.2-9.1), n=20	8.4 (6.7-10.2)	7.5 (6.2-9.5), n=21	8.7 (6.7-10.3)
p-value	0.050		0.030	
Mean HbA _{1c} ²⁻⁴ , %	7.5 (6.4-9.0)	8.9 (7.5-11.3)	7.7 (6.6-10.0)	8.8 (7.0-11.5)
p-value	0.001		0.029	
Mean HbA _{1c} ⁴⁻⁶ , %	7.7 (6.5-9.5)	9.7 (7.1-11.5)	7.8 (6.6-10.4)	9.4 (6.7-11.6)
p-value	0.002		0.067	

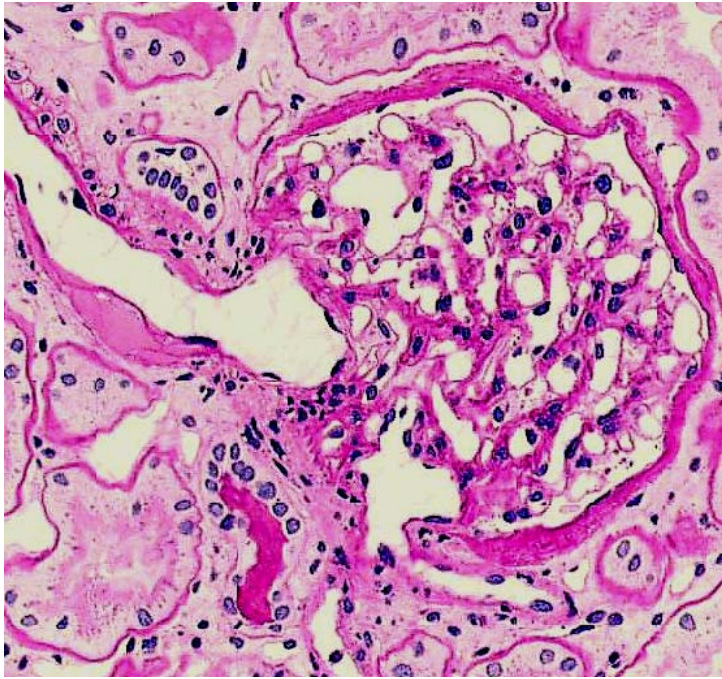


Figure 2. Light microscopy of a glomerulus from a patient with type 1 diabetes. (magnification 410X).

4.2 RENAL MORPHOLOGY

Light microscopy (Fig. 2) showed little or no thickening of the glomerular capillary walls and an increase in the mesangial areas. In some biopsies, mild focal hyaline arteriosclerosis close to the glomerular vascular poles was noted. No interstitial fibrosis, tubular atrophy or global glomerular sclerosis was seen. None of our patients had occluded glomeruli. A weak correlation was found between the mean glomerular volume and body surface area (BSA) and the mean glomerular volume was therefore related to 1.73 m^2 BSA. The mean glomerular volume was $2.7 (1.7-4.6) \text{ M}\mu^3 / 1.73 \text{ m}^2$.

On electron microscopy, the BMT, $V_V(\text{matrix}/\text{glom})$ and $V_V(\text{cap}/\text{glom})$ were significantly larger in the patients than in a group of gender- and age-matched kidney donors (Fig. 3 and Table 4). Foot processes were wider in the patients than in *living* donors and additional differences in $V_V(\text{mes}/\text{glom})$ were seen after adjustment for age and gender (Table 4). To assess the accuracy of the measurements of the individual morphological parameters, we determined the CV (Table 4). Girls with diabetes tended to have larger $V_V(\text{matrix}/\text{glom})$ and $V_V(\text{mes}/\text{glom})$ than boys with diabetes (11.2% vs. 10.0%, $p=0.052$ and 20.1% vs. 18.5%, $p=0.064$, respectively), which was not found in

the donor group (7.9% and 7.8%, $p=0.98$ and 18.5% and 18.5%, $p=0.94$, respectively). This did not reflect differences in metabolic control which was similar in girls and boys. The BMT correlated weakly with the BSA ($n= 46$, $r=0.30$, $p=0.048$) and directly with $V_v(\text{matrix}/\text{glom})$ (Table 7).

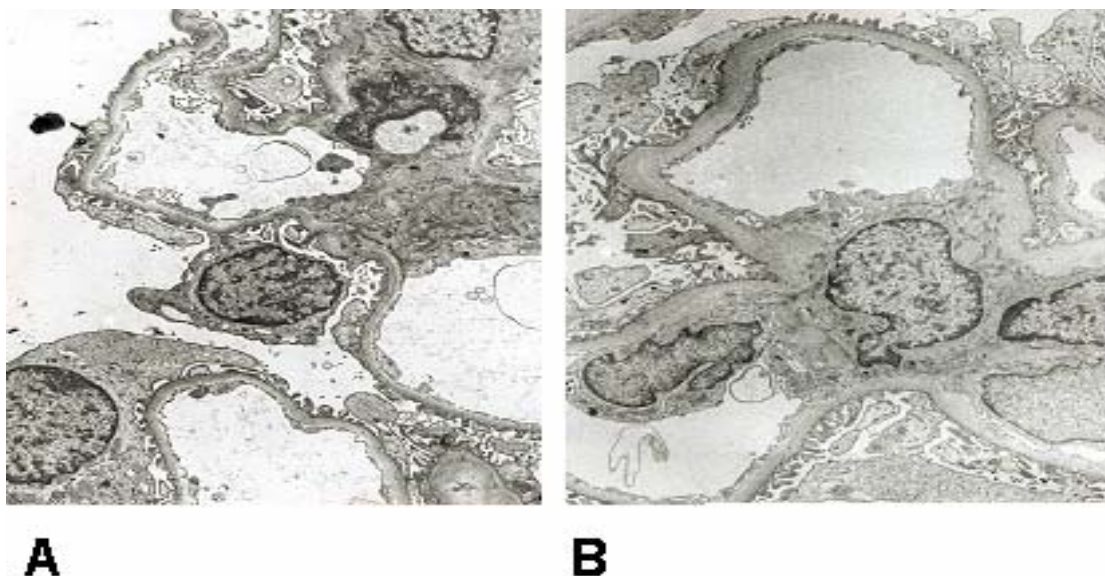


Figure 3. Electron microscopic pictures from a living control (A) and from a patient with diabetes (B)

Immunofluorescence microscopy revealed that four of 46 patients had diffuse positive staining for IgA and C3 in the mesangium. The only difference between patients with IgA deposits and those without was that the former had larger glomerular volumes than the latter ($p<0.01$).

Main conclusion. After type 1 diabetes for about 10 years, BMT, $V_v(\text{matrix}/\text{glom})$, $V_v(\text{cap}/\text{glom})$ and foot process width were larger in normoalbuminuric adolescents than in healthy controls.

4.3 KIDNEY VOLUME

We found no significant difference between the mean kidney volume in patients and healthy controls of similar body weight¹⁷⁴. The kidney volume was directly related to

Log UAE ($r=0.42$, $p=0.009$) and to the absolute GFR and ERPF ($r=0.73$ and 0.61 , respectively, $p=0.0001$). Fifty-two per cent of the variations in the log UAE were explained by foot process width, mean previous FF and renal volume (Table 8) (not published).

4.4 AMBULATORY BLOOD PRESSURE

At least one third of the patients had systolic and nighttime diastolic BPs above the 90th percentiles as compared to gender- and height-related controls ²¹ while the HR was within the normal range. However, none of these patients was hypertensive according to office BP. The boys had higher daytime systolic BP than the girls (131 ± 8 versus 125 ± 8 mmHg, $p=0.013$), while the girls had higher nighttime HR than the boys (69 ± 13 versus 62 ± 8 beats/min, $p=0.036$).

Table 7. Correlations with morphological parameters in normoalbuminuric adolescents with type 1 diabetes. Pearson's (r) correlation coefficients are given. ¹One patient excluded because of high Cook's D influence. (Papers I and IV).

		n	Correlation coefficient	p-value
(IV) BMT, nm (I)	Long-term HbA _{1c} , %	45 ¹	$r=0.62$	<0.001
(I, IV)	V _v (matrix/glom), %	45 ¹	$r=0.55$	0.0001
(IV)	V _v (mes/glom), %	45 ¹	$r=0.41$	0.0054
(IV)	Foot process width, nm	44 ¹	$r=0.41$	0.0059
(IV) V _v (matrix/glom), % (I)	Duration, years	45 ¹	$r=0.41$	0.0058
(I, IV)	Long-term HbA _{1c} , %	45 ¹	$r=0.29$	0.053
(IV)	V _v (mes/glom), %	45 ¹	$r=0.86$	0.0001
(IV) V _v (cap/glom), %	V _v (matrix/glom), %	45 ¹	$r=-0.48$	0.0009
(IV)	V _v (mes/glom), %	45 ¹	$r=-0.47$	0.0011
(IV) S _v (pcap/glom), μm^{-1}	Duration, years	45 ¹	$r=-0.39$	0.0078
(IV)	Log UAE, log $\mu\text{g}/\text{min}$	38	$r=-0.38$	0.020
(I, VI) Foot process width, nm	Log UAE, log $\mu\text{g}/\text{min}$	38	$r=0.47$	0.0027

Table 8. Multiple regression analyses used to estimate the effects of several variables on nighttime MAP, BMT, V_v(matrix/glom) and log UAE as the outcome variables. (Papers I-II).

Dependent variable	Independent variables	n	Estimate	Adjusted R ²	p-value
(II) Nighttime MAP, mmHg	Nighttime HR, beats/min		0.4		0.0002
	Body height, cm		0.4		0.0007
	Long-term HbA _{1c} , %		2.1		0.008
	Model	40		0.44	
(II) BMT, nm	Long-term HbA _{1c} , %		54		<0.0001
	Nighttime HR x long-term HbA _{1c}		3		0.004
	Nighttime HR, beats/min		3		0.037
	Body height, cm		5		0.006
	Model	40		0.57	<0.0001
(I) V _v (matrix/glom), %	Mean previous FF, %		0.6		0.002
	Duration, years		0.2		0.044
	Model	36		0.24	0.004
(II) V _v (matrix/glom), %	Long-term HbA _{1c} , %		0.7		0.003
	Nighttime HR x long-term HbA _{1c}		0.0		0.015
	Nighttime HR, beats/min		0.0		0.23
	Duration, years		0.2		0.040
	Model	40		0.43	<0.0001
Log UAE, log µg/min	Foot process width, nm		0.003		0.024
	Mean previous filtration fraction, %		0.062		0.0009
	Renal volume, cm ³		0.006		0.003
	Model	36		0.52	<0.0001

The highest single correlations were between the nighttime values of BP and HR and morphological findings (Table 5). No relations were found between the duration of diabetes and the HRs or BPs, or between the metabolic control and HR. In multiple regression analyses, 44% of the nighttime MAP and 57% of the BMT were explained by the long-term HbA_{1c}, nighttime HR and body height (Table 8). Forty-three per cent of the V_v(matrix/glom) was explained by long-term HbA_{1c}, nighttime HR and duration

(Table 8). The effect of the BP on BMT and V_v (matrix/glom) disappears when HR is included. Age, gender and log UAE had no effect in the multiple regression analyses.

The declines in systolic, diastolic BPs and MAP during the night were 12 (5)%, 22 (7)% and 16 (6)%, respectively, and in HR 23 (10)% - i. e., the same as in the controls²¹. Table 9 shows some data of the patients who were divided into dippers and non-dippers. Seventeen per cent of these patients were systolic, 12% diastolic and 27% MAP non-dippers. No differences were found between the groups in body height, gender, age at onset and at biopsy, duration of diabetes and UAE. The diastolic and

Table 9. Data on blood pressure, HR, mean previous kidney function and renal morphological changes of the patients when classified as dippers and non-dippers (Paper III). Prev., previous. Mean (SD) or median (10th and 90th percentiles).

	Diastolic blood pressure		Mean arterial blood pressure	
	Dippers ≥ 14%	Non-dippers < 14%	Dippers ≥ 12%	Non-dippers < 12%
n	35	5	29	11
Long-term HbA _{1c} , %	7.9 (6.8-10.9)	9.5 (7.7-10.8)	7.8 (6.8-10.9)	8.6 (7.3-10.8)
p-value	0.055		0.037	
Nighttime BP, mmHg	112 (9) / 59 (6)	123 (7) / 68 (7)	110 (8) / 57 (5)	122 (7) / 66 (5)
p-value	0.016/0.003		0.0001/0.0001	
Nighttime HR, beats/min	62 (49-87)	85 (66-94)	61 (49-87)	66 (56-94)
p-value	0.003		0.008	
Prev. GFR, ml/min/1.73 m ²	135 (18)	158 (13), n=4	132 (18)	151 (17), n=10
p-value	0.018		0.006	
Prev. ERPF, ml/min/1.73 m ²	622 (82)	677 (63), n=4	621 (86)	648 (65), n=10
p-value	0.20		0.37	
Prev. FF, %	22.0 (2.6)	23.5 (0.8), n=4	21.7 (2.4)	23.5 (2.5), n=10
p-value	0.27		0.054	
BMT, nm	492 (92)	636 (147)	485 (85)	574 (141)
p-value	0.004		0.020	
V_v (mes/glom), %	18.8 (2.6)	23.0 (3.8)	18.7 (2.7)	21.0 (3.4)
p-value	0.003		0.028	
V_v (matrix/glom), %	10.3 (1.7)	13.1 (3.0)	10.2 (1.8)	11.9 (2.5)
p-value	0.004		0.020	
Foot process width, nm	410 (37), n=34	441 (9)	412 (39), n=28	419 (30)
p-value	0.074		0.59	

MAP non- dippers had higher mean previous GFR, more renal morphological changes and worse metabolic control than the dippers. When adjusting for the long-term HbA_{1c}, the differences between the diastolic dippers and non-dippers as regards the BMT, V_v(mes/glom) or V_v(matrix/glom) remained significant ($p= 0.050$, $p=0.020$ and $p=0.039$ respectively).

Main conclusions. Nighttime BP and especially nighttime HR seem to be related to glomerulopathy changes in patients who have not yet developed persistent microalbuminuria. The effects of the HR on morphological findings suggest that a disturbance in sympathovagal balance may have a pathogenic effect.

MAP and diastolic non-dipper patients had thicker BM, larger V_v(matrix/glom), higher nighttime HR, long-term GFR and mean HbA_{1c} than dippers.

4.5 RENAL FUNCTION

The GFR and FF at the time of biopsy and the mean previous GFR and FF were higher (Paper I, page 1050, Fig. 2) in patients with diabetes than in healthy controls (Table 3, data in all 46 patients). The ERPF at biopsy and mean previous ERPF were about the same as in the controls.

Table 10. Correlations with renal function in normoalbuminuric adolescents with type 1 diabetes. Pearson's (r) correlation coefficients are given. (Papers I). Prev., previous.

		n	Correlation coefficient	p-value
Mean prev. FF, %	Long-term HbA _{1c} , %	36	r=0.48	<0.01
	BMT, nm	36	r=0.41	<0.02
	V _v (matrix/glom), %	36	r=0.43	<0.01
	Log UAE, log µg/min	30	r=0.42	<0.05

The mean previous FF correlated directly with the long-term HbA_{1c} and both variables correlated directly with the BMT and V_v(matrix/glom) (Table 10) In the multiple regression analysis, the mean previous FF and duration explained 24 % of the variation

in $V_v(\text{matrix}/\text{glom})$, while the age at biopsy and long-term HbA_{1c} were of no importance (Table 8).

Main conclusions. The patients still hyperfiltrate after having had type 1 diabetes for 10 years and a long-standing increase in FF plays a role in the increase in $V_v(\text{matrix}/\text{glom})$.

5 DISCUSSION

Since 1978, we have longitudinally followed all children having type 1 diabetes in our Unit with renal function tests, and since 1980, with HbA₁/HbA_{1c}. Cross-sectional renal biopsy and 24-h AMBP were performed in 46 unselected patients of about 18 years of age who had had the disease about 10 years. None had persistent microalbuminuria.

A few studies have suggested that the main factors predicting nephropathy in patients with type 1 diabetes are the degree of metabolic control ¹⁹³, microalbuminuria ¹⁹⁴, hypertension ¹⁹⁵ and hyperfiltration ^{78,196}.

5.1 REMISSION AND METABOLIC CONTROL

The relation between the duration of remission and HbA_{1c} at onset has also been reported by others ^{121,197,198}. An increase in HbA_{1c} at the onset would suggest a severer metabolic derangement two or three months before the diagnosis of diabetes ¹⁹⁷. Moreover, some data suggest that a longer remission may favour better metabolic control several years after the end of the remission ¹⁹⁸ with higher C-peptide levels (as a measure of residual insulin secretion) ¹⁹⁹. This accords with the present findings of the relation between the HbA_{1c} at onset and metabolic control during the following years. We found no difference in gender at onset between the patients with or without a remission period, which is consistent with the findings of Bonfanti et al. ²⁰⁰. Most studies, however, have shown that male gender is associated with a higher frequency of remission and/or a longer remission ¹⁹⁸.

5.2 RENAL MORPHOLOGY

5.2.1 Morphology

The morphological data of the control group of American children and young adults, who had been kidney donors, were similar to previously reported data from normal

children²⁰¹, young adults^{137,202} and adults^{106,132,191}. The mean BMT of the living controls, 323 nm, resembles those in teenagers and young adults reported by others^{137,201,202}. Small differences in BMT between various reports may be due to the variations in age of the controls¹⁹¹ and the sources of the kidney donors (living or cadaver)²⁰³. Some data show a gradual increase in BMT up to the fifth decade of life¹⁹¹. Caramori et al. found a thicker BM in cadaver than in living donors²⁰³. Moreover, since BMT and BSA correlated, we suggest that BMT values should be given per BSA, at least in children and adolescents. The mean $V_v(\text{matrix}/\text{glom})$ in our controls, 7.9%, is in agreement with some studies^{132,137,191}, but not with others^{201,204}. The difference in $V_v(\text{matrix}/\text{glom})$ may be related to difficulties in measuring mesangial structure. The mean of foot process width is similar to that of Bjorn et al.¹⁰⁶ who used the same technique.

The normoalbuminuric adolescents with type 1 diabetes in the present study had thicker BM, larger $V_v(\text{matrix}/\text{glom})$ and $V_v(\text{mes}/\text{glom})$ than controls, which accords with the findings of Drummond et al.¹³⁷ and Ellis et al.²⁰¹. Ellis et al., studying mainly normoalbuminuric teenagers, and Bangstad et al., studying microalbuminuric patients, also found a more marked difference in the matrix than in the mesangial volume fraction as compared with controls^{134,201}. The difference between the matrix and mesangial volumes suggests that the initial events in the mesangium are related to changes in the matrix rather than to proliferation of mesangial cells¹⁹². Only a few authors have measured $V_v(\text{cap}/\text{glom})$. We found larger $V_v(\text{cap}/\text{glom})$ in our normoalbuminuric patients than in controls unlike Ellis et al., who noted no difference in $V_v(\text{cap}/\text{glom})$ between younger, mostly normoalbuminuric patients and controls²⁰¹. Bangstad and Østerby, however, found a wider capillary diameter in microalbuminuric young adults than in controls^{134,189}. Our finding of wider foot processes in normoalbuminuric patients than in living donor subjects has not been reported before.

The correlation between BMT and $V_v(\text{matrix}/\text{glom})$, in the present study and that of Walker et al.¹³⁵ support the views of Østerby that changes in the peripheral BM and mesangial matrix develop together and both contribute to the early stage of glomerulopathy in patients with microalbuminuria¹⁹². The inverse correlation between $V_v(\text{cap}/\text{glom})$ and $V_v(\text{matrix}/\text{glom})$ or $V_v(\text{mes}/\text{glom})$ in our study may be caused by an increase in the amount of mesangium protruding into the capillaries, which reduces the filtering surface area of the glomerulus. This might lead to a decrease in filtration and

thereby lower the GFR¹³². The structural parameter most closely related to the loss of GFR is the mesangial volume fraction expansion, especially if >37%¹³². However, this occurs later during the development of diabetic nephropathy.

5.2.2 Morphology and gender differences

As noted by Drummond et al. in young patients with type 1 diabetes¹³⁷, the girls tended to have a larger mesangium than boys, but no such difference was found in the control group. This could be due to the age difference between the patients and controls, earlier puberty and maturation in girls (worse metabolic control and/or earlier rise in growth hormone during puberty) together with the longer duration and older age of the girls in our study. On a follow-up examination of our patients six years later, the difference in $V_v(\text{matrix}/\text{glom})$ and $V_v(\text{mes}/\text{glom})$ between females and males had disappeared²⁰⁵.

5.2.3 Morphology and duration

Our finding of a significant correlation between the duration of diabetes and $V_v(\text{matrix}/\text{glom})$ accords with that of Rudberg et al.²⁰⁶ who showed, in a multiple regression model, that this was the only factor that significantly affected $V_v(\text{matrix}/\text{glom})$. The inverse correlation that we found between $S_v(\text{pcap}/\text{glom})$ and duration is in line with the findings of Drummond et al., who reported that the surface density of the peripheral capillary walls increases early in the course of type 1 diabetes and falls with a longer duration of the disease¹³⁷.

5.2.4 Morphology and metabolic control

Only a few studies have evaluated the effect of metabolic control during the first years of diabetes on the development of microangiopathy^{41,207,208}. In a substudy of the DCCT, residual insulin secretion was associated with a lower HbA_{1c} and reduced risk of developing retinopathy and nephropathy^{207,208}. This is in agreement with our findings of a thinner BM and less mesangial matrix in patients with remission. The significance of early metabolic control is further supported by the observation that the level of the HbA_{1c} two to six years after onset affected the rate of progression of the BMT.

The higher correlation between the long-term HbA_{1c} and BMT than between metabolic control and V_v(matrix/glom) has also been found by others^{134,137,201,206}. However, our multiple regression models suggested that metabolic control plays one of the main roles in the increase of BMT, while the long-term FF seems to be an important factor in the increase of V_v(matrix/glom).

5.2.5 Morphology and UAE

The mechanisms and structural counterpart of the increase in permeability to albumin have not been identified. We found wider foot processes and a direct correlation between the foot process width and log UAE in normoalbuminuric patients. Foot processes are wider in microalbuminuric and proteinuric patients than in controls^{106,209} and foot process width and log UAE are directly correlated¹⁰⁶. In normal humans, podocytes do not increase in number from early childhood to late middle age²¹⁰. In diabetes mellitus, the number of podocytes declines probably within the first few years of the disease²¹⁰, possibly by apoptosis of podocytes activated by the local RAS⁶⁰. Since the podocytes have no real capacity to proliferate, the loss of podocytes widens the foot processes to cover the capillary surface²¹¹. This adaptive widening of the foot processes is always accompanied by their effacement and a decrease in permselectivity. For instance, Tenschert et al. found a strong inverse correlation between filtration slit length (i.e., increase in foot process width) and albuminuria after subtotal nephrectomy in rats²¹². The above-mentioned data together with our findings of a widening of foot processes, and the relation between the width of foot processes and log UAE, may partly explain the loss of albumin in urine in diabetes.

5.2.6 Immunofluorescence findings

Renal diseases other than diabetic nephropathy have been reported in patients with diabetes^{213,214}, such as immune complex glomerulonephritis and membranous nephropathy. IgA deposits have been reported in five of 47 (10.6%) patients with diabetes²¹⁵ as compared to 8.6% of 1,286 patients without diabetes²¹⁵. In a European study of 250 consecutive autopsy cases without renal disease, the frequency of such deposits was 4.8%²¹⁶. In Paper I, we found that four of 36 patients with diabetes - i.e., 11%, had IgA deposits. An analysis of all our patients shows that four of the 46 patients (9%) have IgA deposits.

Glycosylated IgG, IgA and IgM antibodies in serum are increased in both type 1 and type 2 diabetic patients with nephropathy as compared to diabetic patients without any complication ²¹⁷. The presence of antibodies and the corresponding antigens in the circulation may lead to the formation of circulating immune complexes deposited in the small blood vessels that cause microangiopathy ²¹⁸. In immunoglobulin A nephropathy recent data suggest that aberrant glycosylation of IgA in mesangial cells may modulate clinical progression ²¹⁹. Moreover, IgA nephropathy superimposed on diabetic nephropathy ²²⁰ has been found in a biopsy study of patients with diabetes and albuminuria which, however, had no effect on the clinical outcome ²²⁰.

5.3 KIDNEY VOLUME

We have previously reported an increase in renal volume in a small group of diabetic adolescents with very poor metabolic control ⁷³. In Paper IV, we could not confirm this, but these patients had better metabolic control. No relation has been found between the kidney volume and metabolic control in a group of patients having similar age, duration of diabetes and metabolic control ²²¹, but a relation has been shown in an older group of patients who had had diabetes for a longer period and a wider range of metabolic control ²²². Some authors have observed an increase in kidney size in early diabetes ^{223,224} with normalisation of the size after three months of insulin treatment ²²⁵. The correlation that we found between kidney size and renal function has also been reported by others ^{73,222,224,226,227}. Kleinman et al. proposed that early renal hypertrophy develops secondary to hyperfiltration and can be reversed by insulin therapy, but later renal hypertrophy is due to persistent production of growth factors, which is refractory to insulin therapy and does not respond to a reduction in GFR ²²⁸.

The correlations between renal volume and log UAE, in the present study accord with the findings of others ^{221,222}. Frazer et al. reported a larger kidney volume in children and adolescents with borderline and intermittent microalbuminuria than in those with normoalbuminuria, and suggested that nephromegaly is an early marker of incipient nephropathy ²²¹. Baumgartl et al. showed that adult normoalbuminuric patients with type 1 diabetes who had large kidneys at baseline ran a higher risk of developing severe renal disease ²²⁹.

In an analysis of our study of renal volume in a group of normoalbuminuric patients who had had diabetes for about 10 years and showed fairly good metabolic control, we believe that renal volume cannot be regarded as a risk marker of glomerulopathy.

5.4 AMBULATORY BLOOD PRESSURE

5.4.1 AMBP and heart rate measurements

The finding of a higher ambulatory systolic and diastolic BP as compared to healthy controls has also been reported in children with diabetes of shorter duration^{48,49} and in normoalbuminuric adults with longer duration of diabetes⁵¹. The higher systolic BP in boys than girls accords with the findings of other authors^{21,49} and may be due to the fact that the boys were taller than the girls.

We found a normal HR unlike other authors who have reported higher^{51,230,231} or lower²³² HR in normoalbuminuric adolescents and adults with varying durations of the disease. These differences in HR may be explained by the findings of Ewing et al.²³³, who noted a sequential increase in HR, which was thought to be due to cardiac parasympathetic damage, followed by a fall in HR because sympathetic damage had developed as well.

The direct correlation between the diastolic BP and HR in the present study has also been found by Moore et al.²³⁴ and confirmed by our multiple regression analyses, in which HR was shown to be an important determinant of BP. This relation may be secondary to autonomic neuropathy⁶⁴, sodium and fluid retention^{53,55,235} or to the physiological relation in which HR is a determinant of BP.

A few authors have observed less systolic and diastolic dipping in normoalbuminuric teenagers and young adults with type 1 diabetes than in controls^{22,49}. In our patients, the dipping at night in the entire group was within normal limits, which accords with some data^{177,234,236}.

5.4.2 AMBP and metabolic control

In our study, metabolic control seems to affect BP, especially diastolic BP and MAP. In a similar group of patients, a relation was found between worse metabolic control and higher 24-h diastolic BP²³⁴. In a mixed normo- and microalbuminuric group of adult patients, HbA_{1c} correlated with both the nighttime systolic and diastolic BPs²³¹, which accords with our finding of long-term HbA_{1c} as one of the determinants of nighttime BP. Recently, suggestions have been made that hyperglycaemia sensitizes endothelial cells to the barotraumas of BP²³⁷. The fact that HbA_{1c} at the onset of diabetes was related to the nighttime BP 10 years later and a higher diastolic BP in patients without remission, could indicate that early metabolic control affects the development of hypertension.

5.4.3 AMBP and UAE

Our patients had a higher BP, measured with an ambulatory blood pressure device, than controls, although none had persistent microalbuminuria. We therefore believe that the rise in BP precedes persistent microalbuminuria. This is in accord with the findings of others who measured the 24-h AMBP^{64,238-240}. Some studies of office BP have suggested that the development of microalbuminuria parallels the rise in BP¹⁹⁴.

The direct correlation between the diastolic BP and UAE in our group of patients is in agreement with a study by Moore et al.²³⁴. Some authors investigating mixed normo- and microalbuminuric patients have shown that systolic BP was related more to the albumin excretion rate than to the diastolic BP^{231,232}. This difference in the effects of the systolic and diastolic BPs suggests that the diastolic BP is higher in patients with diabetes of short duration who are normoalbuminuric and that the systolic BP increases in those with diabetes of longer duration who have microalbuminuria. MAP can be used to reflect both the systolic and diastolic BP.

5.4.4 AMBP and morphology

To our knowledge, no studies have been done on AMBP and HR in relation to renal morphological changes. We therefore compared our results with studies of office BP measurements. The relation between BMT and the nighttime diastolic BP in our

patients accords with the findings of Ellis et al., who studied a group of adolescents with diabetes of about the same duration as ours²⁰¹. They found a correlation between the mean of four office diastolic BP and a factor called “peripheral capillary decrease”, which included an increase in BMT. Other studies of microalbuminuric adolescents^{134,206} and adult patients^{135,241} have shown no correlation between morphological changes and office BP. Mauer et al.²⁴² studied 139 mostly adult patients with diabetes and overt diabetic nephropathy, who were being evaluated for pancreas transplantation. Their hypertensive patients had larger mesangial volumes than the normotensive ones. Our findings of a correlation between nighttime BP and BMT and foot process width reveal the value of the 24-h AMBP measurements in detecting relationships between BP and renal morphology.

In paper III, we have shown for the first time that nighttime BP dipping has a direct relation to target-organ damage - i.e., glomerulopathy changes. The relations between reduced dipping and various indirect signs of target-organ damage have been described²⁴³⁻²⁴⁵. Poulsen et al. studied 40 initially normotensive and normoalbuminuric type 1 diabetic patients during three years and observed that the patients who developed microalbuminuria had less initial diastolic dipping²⁴³. Moreover, diastolic dipping is positively correlated with diastolic cardiac dysfunction²⁴⁴. The absence of a decline in nighttime BP has been associated with an increase in the mortality rate of adult patients with diabetes and overt nephropathy²⁴⁵.

5.4.5 Nighttime hypertension

Type 1 diabetic patients with autonomic neuropathy have a diminished circadian BP rhythm with increases in nighttime BP^{148,149} and HR⁶⁴. In a stepwise regression analysis, an “autonomic score” was reported to be the variable of main importance for the day-night difference in BP in patients with type 1 diabetes⁶⁶. In addition, less variability in HR during deep breathing, due to autonomic dysfunction, seems to be related to HR dipping⁵¹. Therefore, these studies may indicate that autonomic neuropathy plays a role in the reduced dipping at night.

In the present study, autonomic function was not investigated, but the correlation between the nighttime HR and renal morphology may indicate that autonomic neuropathy contributes to renal morphological changes. Moreover, Spallone et al.

showed that patients with diabetes, who had been selected because they showed signs of autonomic neuropathy, had higher nighttime UAE than those without¹⁴⁸. This accords with the findings of Poulsen et al., who noted significant differences in sympathovagal balance between normoalbuminuric patients with UAE above or below the median⁶⁴.

5.4.6 Nighttime BP or dipping?

It is not known whether the reduction in the nighttime dip or the increase in BP at night induces damage to the target organ, or if it is the same thing. One study shows that the reduction in the nighttime dip precedes the increase in day- and nighttime BP⁴⁹. They found no increase in the ambulatory diastolic BP, although the decline in diastolic dipping was significant ($p < 0.03$) in 117 children and teenagers with type 1 diabetes, whom they followed for at least four years. In a multiple regression analysis of our patients, we found a closer correlation between the BMT and the nighttime MAP than with the MAP dipping. On the other hand, there was a stronger correlation between the mesangial volumes and MAP dipping than with the nighttime MAP. Although the dipping values may “contain” more information about a possible ongoing neuropathy they have poor repeatability. We therefore suggest that the BP values, and not the dipping values, should be used as a risk marker of glomerulopathy. Moreover, the present findings suggest that nighttime BP reflects end-organ damage better than daytime BP. Nighttime hypertension has been suggested to be associated more with end-organ damage because of the longer total period of an elevated BP (both day and night)²⁴⁶. One reason for not using the mean BP measured during the whole 24-hour period may be that it is affected by the number of hours the patient sleeps during the particular night when the measurements are made.

5.5 RENAL FUNCTION

5.5.1 Renal function

Increases in GFR and FF have been reported in type 1 diabetes even at the onset of the disease⁶⁸⁻⁷⁴. ERPF may be increased^{71,73,74,247}, normal^{68,223} or decreased²⁴⁸. In the

present study of patients who had had diabetes for about 10 years, we found a high GFR and FF.

5.5.2 Renal function and UAE

Some of our patients had had occasional microalbuminuria at biopsy, but none had persistent microalbuminuria. However, we found a relation between log UAE and the mean previous FF. Rudberg et al. reported that the only predictor of albuminuria was hyperfiltration after eight years' duration of diabetes⁷⁷. Chiarelli et al. followed two groups of children and adolescents having the same duration of diabetes during a 10-year period⁷⁸. Twenty-three had hyperfiltration (GFR >140 ml/min per 1.73 m²) and 23 age- and sex-matched patients had not. Of those in the former group, seven developed microalbuminuria, as compared to only one in the latter group. They concluded that persistent glomerular hyperfiltration is a risk factor for the development of microalbuminuria⁷⁸. Mogensen reported that patients with marked hyperfiltration about 13 years earlier had microalbuminuria and a reduced GFR at follow-up. He concluded that marked hyperfiltration may contribute to late glomerular damage⁷⁵. It has also been shown that the glomerular capillary barrier is very sensitive to a rise in hydrostatic pressure. Therefore minute increases in the intracapillary pressure greatly increase the permeability to albumin²⁴⁹. This observation accords with our finding of a relation between the mean previous FF and log UAE.

5.5.3 Renal function and AMBP

The absence of a correlation between the current GFR or FF and day- or nighttime BPs accords with studies by Wiegmann et al.²⁵⁰. However, we found long-term hyperfiltration in non-dippers. Glomerular hyperfiltration seems to be associated with a blunted reduction in diastolic BP at night and an expansion of extracellular fluid volume in normotensive and normoalbuminuric patients with type 1 diabetes²³⁵. They propose that a redistribution of extracellular volume in a recumbent position during the night can briefly increase the blood volume and explain the abnormalities in the diurnal pattern of the BP²³⁵.

5.5.4 Renal function and morphology

In most studies, no relation between renal function and structural changes has been found, probably because the investigation of renal function has been done only once and mostly at the same time as the biopsy^{134,192,251}. Moreover, many of these patients had had the disease for a longer period and were in a later stage of nephropathy²⁴². We correlated our findings with the *mean previous* GFR or FF, which reflects the renal function during the entire period from the onset of diabetes to the biopsy. We also performed the biopsy in a very early stage of the disease in an unselected group of patients of whom about 30% will develop nephropathy. We found correlations between BMT and $V_v(\text{matrix/glom})$ with the mean previous FF, which suggests that longstanding hyperfiltration (increase in GFR) without simultaneous hyperperfusion (increase in ERPF) - i.e., an increase in FF, contributes to the development of glomerular changes. It therefore seems that the previous FF is of greater importance than the previous GFR, which shows the value of determining both GFR and ERPF in patients with diabetes.

5.6 RENAL MORPHOLOGY, FUNCTION AND AMBP

A model for suggested interrelations between factors evaluated in the present studies is shown in Figure 4. An increase in the intraglomerular pressure may be a central mechanism in our findings. The metabolic control may have a direct effect on several factors.

In the present study, the metabolic control seemed to play an important role in the BM thickening, while an increase in the mean previous FF seemed to be essential for the increase in $V_v(\text{matrix/glom})$. This would suggest that the changes in BMT can vary with metabolic control, and perhaps in early stages in diabetes an improvement in metabolic control might reduce the thickening of the membranes. The effect of FF on $V_v(\text{matrix/glom})$ may indicate that hyperfiltration and therefore the increase in filtration pressure in the glomerulus can directly affect the mesangial matrix augmentation. Moreover, hyperglycaemia has been found to increase both FF and MAP by activating the RAS, and thereby promoting renal disease progression in diabetes⁵⁶.

↑ B-glucose → ↑ HbA_{1c}

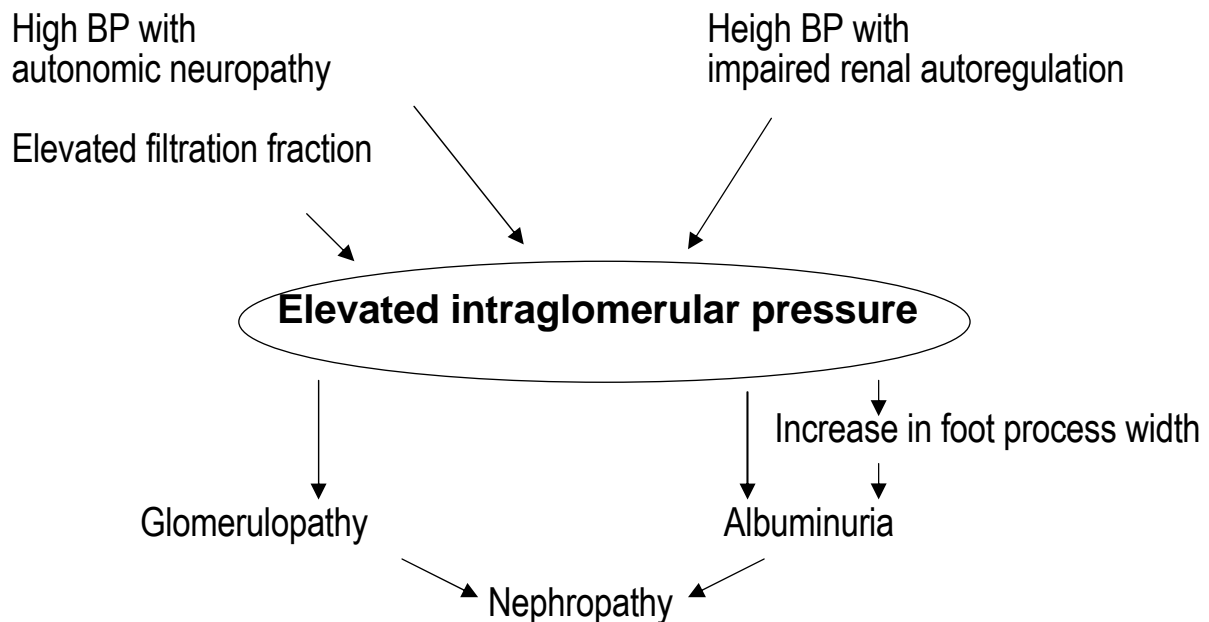


Figure 4. Dynamic model showing suggested interrelations between various findings in the present studies that may play a role in development of nephropathy.

Hashimoto et al. reported impaired renal autoregulation in rats with diabetes²⁵² - i. e., a finding indicating that an elevated systemic blood pressure leads to higher intraglomerular pressure. This is also thought to occur in patients with diabetes²⁵³. Autonomic neuropathy may be another reason for the increase in intraglomerular pressure^{149,150}. Therefore, the higher blood pressure that we found in our studies may raise the intraglomerular pressure and contribute to foot process widening, basal membrane thickening and an increase in the mesangial matrix - i. e., diabetic glomerulopathy. Moreover, since the podocytes cannot replicate, the finding of an increase in foot process width indirectly support the increase in intracapillary pressure (Fig. 4), a view which is also supported by the increase in long-term FF. Moreover, a high intracapillary pressure increases the permeability to albumin²⁴⁹, which is in agreement with our finding of a relation between the mean previous FF and UAE. These observations are also supported by the finding that the foot process width and mean previous FF explain part of the variations in log UAE.

Our findings concerning the relations between HR dipping and the mean previous FF, BMT and $V_V(\text{matrix}/\text{glom})$ accord with the view that autonomic neuropathy may play a role in the development of diabetic glomerulopathy. The relation between $V_V(\text{matrix}/\text{glom})$ and HR dipping, on the one hand, and to the mean previous FF, on the other, is of particular interest since mesangial volume is thought to be the most specific early change in diabetic glomerulopathy²⁵⁴. Thus, the persistent increase in intraglomerular pressure may damage the glomeruli²⁵⁵⁻²⁵⁷ and increase the BMT, widen the foot processes and enlarge the mesangial areas. Moreover, in our opinion, it seems possible that, in patients with type 1 diabetes, albuminuria, even within the normoalbuminuric range, is, among other aetiologies due to an increase in intracapillary glomerular pressure.

6 CONCLUSION

We found significant renal structural abnormalities, such as a thickened BM, enlarged $V_v(\text{matrix}/\text{glom})$ and widened foot processes in normoalbuminuric adolescents and young adults, of whom only about 30% will develop diabetic nephropathy. The metabolic control, from the onset of diabetes and onwards, seems to play a very important role in the development of hypertension and glomerulopathy. Urinary albumin excretion may be due partly to widening of the foot processes and an increase in FF. A higher HR and diastolic BP at night and the non-dipping status are related to a thicker BM and a larger mesangial matrix. However, in the multiple regression analyses, the nighttime HR seems to be more important as a cause of glomerulopathy than the BP, which would suggest that a disturbance in sympathovagal balance may have a pathogenic effect on the glomerulus itself. GFR and FF were increased before and at the time of the biopsy.

In view of the above data, we propose that in normoalbuminuric type 1 diabetes patients:

- Poor metabolic control from the onset of diabetes predicts the development of glomerulopathy and hypertension
- Poor metabolic control over a long period plays a considerable role in the increase in BMT
- A long-lasting increase in FF seems to be more important than persistent increased in GFR for the development of glomerulopathy
- An increase in FF may be a sign of intraglomerular hypertension
- Systemic hypertension may raise the intraglomerular pressure because of a disturbance in autoregulation and autonomic neuropathy
- An increase in intraglomerular pressure may widen the podocyte foot processes and contribute to albuminuria

In investigations of normoalbuminuric type 1 diabetes patients, we propose that:

- Hypertension, diagnosed with 24-h AMBP, precedes the development of microalbuminuria.

- The BP measured with 24-h AMBP is more sensitive than the office BP in finding patients at risk of developing nephropathy
- Diastolic hypertension at night may have the closest relation to glomerulopathy early in the development of nephropathy
- Renal function should include the determination of both GFR and ERPF to calculate the FF

Since renal biopsy cannot be done routinely, the early repeated 24-h AMBP, or perhaps the detection of autonomic neuropathy may be of value in finding the patients who may develop nephropathy when they are in the normoalbuminuric phase. Longitudinal determinations of the 24-h AMBP together with studies of the autonomic nervous system are therefore needed. In the meantime, we should give these patients the best possible care and try to keep the HbA_{1c} as close to normal as possible, from the onset.

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