

# Microalbuminuria

## Implications for micro- and macrovascular disease

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Microalbuminuria is diagnosed when the UAER is  $>20$  but  $<200$   $\mu\text{g}/\text{min}$ . The prevalence of microalbuminuria among diabetic patients is 15–20%. Persistent microalbuminuria in diabetic patients is a risk marker not only of renal disease, but also of proliferative retinopathy and cardiovascular morbidity and mortality. Even among nondiabetic individuals, those with microalbuminuria tend to have an increased cardiovascular morbidity. The established cardiovascular risk factors, such as smoking, elevated plasma cholesterol, fibrinogen, and hypertension, are seen more frequently in diabetic patients with persistent microalbuminuria than in normoalbuminuric diabetic patients of similar age, sex, and diabetes duration. However, these risk factors cannot by themselves explain the cardiovascular overmortality in these patients. In addition, insulin resistance or genetic disposition to hypertension or cardiovascular disease fails to be the missing link. Accumulating evidence suggests a common pathogenetic mechanism for microalbuminuria and premature atherosclerosis (i.e., qualitative alterations of the extracellular matrix, including decreased density and sulfation of HS-PG). Decreased density of HS in the glomeruli may lead to albuminuria and mesangial proliferation. In the intima of large vessel walls, decreased density and/or sulfation of HS may enhance several of the processes involved in premature atherosclerosis. Diabetes affects the composition and structure of the extracellular matrix in many ways and leads to decreased density and sulfation of HS-PG by several mechanisms. Genetic differences in the sulfation of HS and/or genetic defects in the coordinated biosynthesis of HS-PG might contribute to decreased concentration and sulfation of HS-PG in susceptible individuals. It is hoped that susceptibility genes can be identified soon, thereby making prevention of severe late diabetic complications more successful.

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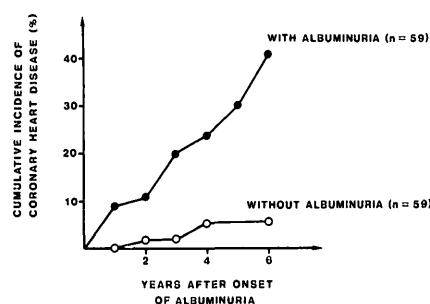
HS-PG, HEPARAN SULFATE-PROTEOGLYCAN; HS, HEPARAN SULFATE; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; ESRD, END-STAGE RENAL DISEASE; UAER, URINARY ALBUMIN EXCRETION RATE; NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; GFR, GLOMERULAR FILTRATION RATE; GBM, GLOMERULAR BASEMENT MEMBRANE; VLDL, VERY-LOW-DENSITY LIPOPROTEIN; TERA, TRANSCAPILLARY ESCAPE RATE OF ALBUMIN; LDL, LOW-DENSITY LIPOPROTEIN; CVD, CARDIOVASCULAR DISEASE; ACE, ANGIOTENSIN-CONVERTING ENZYME; IgG, IMMUNOGLOBULIN G; STZ, STREPTOZOCIN; CI, CONFIDENCE INTERVAL; ECG, ELECTROCARDIOGRAM.

Albuminuria in IDDM is associated with an extremely high risk of early death. The relative mortality (i.e., mortality in diabetic patients relative to mortality in the sex- and age-matched background population) in different age groups of patients with albuminuria is  $\sim 40$  times higher than in diabetic patients without albuminuria (1). Patients with persistently Albustix-positive urine (Boehringer Mannheim, Indianapolis, IN), in whom other nondiabetic renal diseases, urinary tract infection, and cardiac insufficiency have been excluded, exhibit increasing blood pressure and declining GFR (2). These patients suffer from clinical diabetic nephropathy. It is therefore tempting to believe that the difference in relative mortality between patients with and without albuminuria is attributable to death from ESRD. However, this is not the case.

### ALBUMINURIA: A NEW CARDIOVASCULAR RISK MARKER

— About 50% of the albuminuric patients will die from cardiovascular causes before progressing to ESRD. Thus, after 25 yr of diabetes duration at age 40–45 yr, the cardiovascular mortality (mostly from coronary heart disease or stroke) in albuminuric diabetics is 60 times higher than in the background population, whereas in patients without albuminuria, the relative cardiovascular mortality was only 2–3 times that of the background population (3). Most of these patients die from coronary heart disease. In a case control study of 59 IDDM patients who were followed from the onset of albuminuria, coronary heart disease developed 8 times more frequently than in a diabetic population of similar age, sex, and diabetes duration (4) (Fig. 1). Albuminuria in IDDM is, therefore, not only a marker of renal disease, but also a potent risk marker of cardiovascular disorder.

Albuminuria is normally diagnosed when strips such as Albustix are

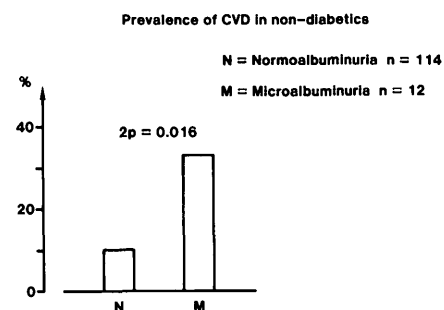


**Figure 1**—Cumulative incidence of coronary heart disease in IDDM patients with and without albuminuria. The two groups were carefully matched regarding age, sex, and diabetes duration. All patients had normal ECG at the onset of the study where UAER was 300 mg/24 h in the albuminuric group. From Jensen et al. (4). © by Diabetologia.

persistently positive. This corresponds to a UAER of  $\geq 300$  mg/24 h. However, in nondiabetic patients and in young patients with IDDM just after the onset of the disease, UAER is  $< 30$  mg/24 h (albumin/creatinin ratio in early morning urine  $< 2.5$  mg/mmol (5,6). Patients with clinical nephropathy, therefore, have passed a stage where UAER is  $< 300$  mg/24 h but  $> 30$  mg/24 h. In fact prospective studies using highly sensitive methods for the determination of UAER have demonstrated that patients who will later develop clinical nephropathy are characterized by an exponential increase of UAER (7,8). According to an international consensus conference, patients with UAER between 30 and 300 mg/24 h are, therefore, said to have microalbuminuria, and patients with persistent microalbuminuria are suffering from incipient nephropathy (9).

The prevalence of microalbuminuria among diabetic patients is 15–20% (10,11). Persistent microalbuminuria in IDDM patients is associated with many abnormalities (Table 1). Most of these abnormalities will not be discussed in this article in which we concentrate on the implications of microalbuminuria on macroangiopathy. The question here is:

Is microalbuminuria a risk marker not only for clinical nephropathy (12), but also for premature atherosclerosis? This is not known for IDDM patients of juvenile onset, because the low cardiovascular morbidity and mortality among these young patients require huge population studies that have not yet been conducted. However, among NIDDM patients, microalbuminuria is a potent cardiovascular risk marker, independent of hypertension and hyperlipidemia (13). In these patients, albuminuria is much more strongly associated with premature death from cardiovascular diseases than with ESRD. In a follow-up study of 175 NIDDM patients with microalbuminuria (urinary albumin concentration  $> 15$  mg/L), 120 (68%) patients died 10 yr after the onset of the study; but only 8 among these patients (7%) died from ESRD, whereas 58% died from acute myocardial infarction, cardiac failure, or stroke (13). Thus, microalbuminuria in NIDDM seems to be more relevant as a marker for CVD than for renal disease. Even in nondiabetic patients, microalbuminuria is a risk marker for premature atherosclerosis,



**Figure 2**—Six years cumulative incidence of CVD in 126 elderly nondiabetic patients with normal UAER ( $< 15$   $\mu$ g/min; N) or microalbuminuria (M). Calculated from Damsgaard et al. (15). © by the British Medical Journal.

as demonstrated by Yudkin et al. (14) and confirmed by other groups (15). Thus, in a population-based study of nondiabetic patients (normal fasting blood glucose), persons with microalbuminuria ( $> 15$   $\mu$ g/min) also had a several-fold increase in cardiovascular mortality during the following 6 yr compared with persons with normal UAER (15) (Fig. 2). Thus, persistent albuminuria is a

**Table 1**—Nonrenal changes in IDDM patients with persistent microalbuminuria in comparison with patients having normal UAER

CHARACTERISTIC	CHANGE
PROLIFERATIVE RETINOPATHY	MORE FREQUENT (76)
BLOOD PRESSURE	HIGHER (27)
CARDIAC OUTPUT	DECREASED (77)
END DIASTOLIC VOLUME	DECREASED (77)
WORKING CAPACITY	DECREASED (78)
TERA	INCREASED (49)
ALBUMIN CATABOLISM	INCREASED (79)
PLASMA FIBRINOGEN	INCREASED (26)
TER FIBRINOGEN	INCREASED (BENT-HANSEN, UNPUBLISHED OBSERVATIONS)
FIBRINOGEN SYNTHESIS	INCREASED (79)
FIBRINOGEN CATABOLISM	INCREASED (79)
TOTAL CHOLESTEROL	INCREASED (26)
VLDL	INCREASED (26)
LDL	INCREASED (26)
VON WILLEBRAND FACTOR	INCREASED (47)
TISSUE PLASMINOGEN RESPONSE TO EXERCISE	IMPAIRED (80)
ACE	INCREASED (81)

**Table 2—Clinical data, prevalence of death, cardiovascular risk factors and CVD in parents of type 1 (insulin-dependent) diabetic patients with nephropathy and in parents of type 1 diabetic patients with normoalbuminuria (17)**

	PARENTS OF DIABETIC PATIENTS	
	WITH NEPHROPATHY	WITH NORMOALBUMINURIA
SEX (F/M)	35/41	32/45
AGE (YR)	58 ± 8 (43–79)	58 ± 7 (43–71)
PREVALENCE OF DEATH (%)	10 (4–18)	8 (3–16)
PREVALENCE OF CVD (%)	12 (6–21)	13 (6–23)
UAER (μG/MIN)	4 (1–214)	5 (1–68)
PREVALENCE OF MICROALBUMINURIA (%)	5 (1–14)	11 (4–21)
SERUM CHOLESTEROL (MM)	5.7 ± 1.2	5.8 ± 1.0
PERCENTAGE OF SMOKERS (%)	40 (29–52)	34 (24–46)

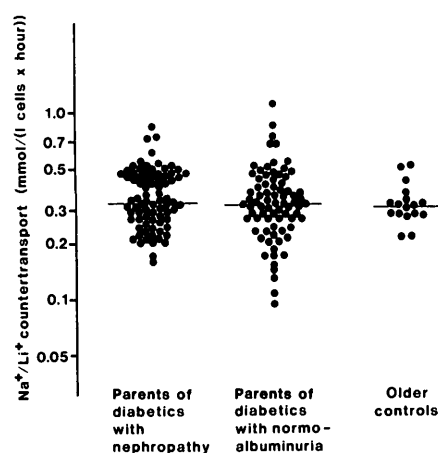
Values are means ± SD (range) or median (range) or prevalence (95%).

new potent cardiovascular risk marker not only in diabetics, but also in nondiabetic “healthy” subjects.

### GENETIC SUSCEPTIBILITY TO CARDIOVASCULAR DISEASE IN IDDM PATIENTS WITH ALBUMINURIA

Why is albuminuria a marker of cardiovascular disease and mortality? Some observations suggested that diabetic patients with albuminuria should have a genetic disposition for CVD (16). Such observations were not confirmed at our clinic (17). Thus, 153 nondiabetic parents of patients with IDDM were examined for cardiovascular morbidity and mortality. Of those, 77 were parents to patients with normal UAER, and 76 were parents to patients with nephropathy (Table 2). No significant differences in number of cardiovascular deaths, prevalence of cardiovascular morbidity, or frequency of cardiovascular risk factors were seen between these two groups (17). Other observations suggested that diabetic patients with albuminuria should have a genetic disposition to hypertension (18,19). However, our own and other more comprehensive studies found that blood pressure was not different in parents of diabetic patients with and without nephropathy (20,21). Further insight

into the predisposition to CVD in diabetic subjects with albuminuria comes from studies of cation transport systems, such as  $\text{Na}^+/\text{Li}^+$  countertransport in erythrocytes. Diabetic subjects with albuminuria usually have higher  $\text{Na}^+/\text{Li}^+$  countertransport activity in erythrocytes than diabetic patients without albuminuria (20,22). However, differences be-



**Figure 3— $\text{Na}^+/\text{Li}^+$  countertransport in nondiabetic parents of IDDM patients with and without clinical nephropathy. A group of control subjects of similar age but without diabetes in their families also is indicated. No difference was seen between the groups. From Jensen et al. (20). © by Diabetologia.**

tween parents of patients with and without albuminuria were not always observed (20,21) (Fig. 3), indicating that environmental factors are more likely to explain the differences in  $\text{Na}^+/\text{Li}^+$  countertransport between albuminuric and nonalbuminuric patients than genetic factors (23). Other abnormalities in cation transport systems have been reported in fibroblasts from diabetic patients with nephropathy kept in tissue culture for several weeks (24). These abnormalities might be genetic markers of cellular dysfunction, reflecting common pathogenetic mechanisms for renal and cardiovascular diseases in diabetic subjects. These results have to be confirmed, but they indicate that albuminuria and premature atherosclerosis may have some genetic susceptibility factors in common. The multifactorial pathogenesis of albuminuria and atherosclerosis, however, makes an identification of such factors highly complicated.

### ALBUMINURIA AND CARDIOVASCULAR RISK FACTORS

Other observations indicated the possibility that albuminuria is a marker of CVD because established cardiovascular risk factors (such as smoking, elevated plasma concentration of fibrinogen and cholesterol, and hypertension) are more prevalent among patients with nephropathy. Several authors (including ourselves [26]) have found a higher prevalence of heavy cigarette smokers (25), elevated fibrinogen, increased total cholesterol (26), and higher blood pressure in patients with albuminuria compared with patients with normal UAER (27). However, according to our calculations, a combination of elevated blood cholesterol (by 25%), fibrinogen (by 20%), blood pressure (by 15%), and heavy cigarette smoking can explain only a small part of the 10-fold higher cardiovascular mortality in such a group of patients. Other more important factors must contribute. Also in NIDDM, albuminuria is associated with increased blood pressure (11,28) and hyperlipidemia (29,30), and hemostatic parameters,

Table 3—Insulin resistance in IDDM patients without and with early nephropathy

	INSULIN DOSE (IU/KG, MEDIAN AND RANGE) N = 391	FASTING FREE PLASMA INSULIN (PM, MEANS ± SD) N = 74
NO NEPHROPATHY	0.72 (0.14–1.83)	50 ± 25
EARLY NEPHROPATHY	0.70 (0.11–0.69)	55 ± 24

n = number of patients.

such as von Willebrand factor, and fibrinogen might be elevated in patients with overt clinical nephropathy (31). However, as Schmitz and Ingerslev (31) pointed out on the basis of their comprehensive study, the increased mortality among NIDDM patients with microalbuminuria could not be explained by coexisting risk factors, such as hypertension, hyperlipidemia, or hemostatic disorders. Thus, from epidemiological studies, we must conclude that elevation of blood pressure, cholesterol, and fibrinogen contribute, but cannot by themselves, explain why albuminuria is such a potent cardiovascular risk marker. Recently, insulin resistance has been acknowledged as a potential cardiovascular risk factor. However, insulin resistance as reflected by the daily insulin requirement or the fasting free plasma insulin concentration seems not to be more pronounced in IDDM patients with early nephropathy than those without (26) (Table 3).

**POSSIBLY COMMON PATHOGENETIC MECHANISM FOR ALBUMINURIA AND PREMATURE ATHEROSCLEROSIS**

What is the missing link? We suggest a common pathogenetic mechanism of microalbuminuria and premature atherosclerosis, because of the close coincidence of albuminuria and atherosclerotic events and because of the similarity of structural and functional alterations of glomeruli and large vessel walls in patients with albuminuria. Mesangial and arterial myomedial cells are mesenchymal cells, with contractile properties equal to their phagocytotic properties. In patients with al-

buminuria, enhanced mesangial and myomedial proliferation takes place. Both these cells can synthesize collagen IV, fibronectin, laminin, and HS-PG. This also is reflected by the composition of the extracellular matrix, which is quite similar. In patients with albuminuria, not only is accumulation of extracellular matrix more pronounced than in patients without albuminuria (32), but changes in the quality of the extracellular matrix seems to be a characteristic feature (Table 4).

**DECREASED DENSITY OF HS-PG IN EXTRACELLULAR MATRIX OF DIABETIC ANIMALS AND HUMANS**— Shimomura and Spiro (33) have shown the density of HS-PG to be reduced ~50% in GBMs of patients with glomerulosclerosis (33), and similar changes have been demonstrated in extramural coronary vessels of diabetic patients (34). In diabetic animals, the synthesis, concentration, and sulfation of HS-PG are significantly decreased (35,36). But, is it possible that these qualitative changes of the extracellular matrix (i.e., decreased density of nega-

tively charged HS-PG) are the common cause of microalbuminuria and the enhancement of mesangial expansion and premature atherosclerosis? HS-PG is synthesized in the endothelial, mesangial, and myomedial cells. After sulfation has taken place in the Golgi apparatus (a process imitated by the enzyme *N*-deacetylase), HS-PG is incorporated into the extracellular matrix of glomeruli and large arteries, where it contributes to the structural integrity of the basal membrane and vessel walls (37).

**HS-PG AND ALBUMINURIA**— Decreased density of HS within the GBM leads to albuminuria (38,83). This also was seen in a recent study that demonstrated that injection of monoclonal antibodies against HS induces albuminuria within minutes (83). Furthermore, when researchers increased the density of HS-PG in glomeruli of diabetic rats by ACE inhibitors, the progression of albuminuria was arrested (39). Thus, in theory, it is not unlikely that the decreased density of anionic HS-PG demonstrated in glomeruli of diabetic patients might be the cause of the increased glomerular filtration of plasma albumin. HS-PG also contributes to keeping the mesangial (40,41) and myomedial (42) cells in a resting state. Decreased concentration of HS in tissue cultures of mesangial cells has been shown to lead to decreased inhibition and subsequently to proliferation of mesangial cells (40,41).

Table 4—Similarities of structural and functional alterations of glomeruli and large vessel walls in albuminuric patients with diabetes mellitus

ALTERATION	REFERENCE
ACCUMULATION OF EXTRACELLULAR MATRIX	(34, 82)
DECREASED DENSITY OF HS-PG	(33, 34)
PROLIFERATION OF MESANGIAL AND MYOMEDIAL CELLS	(40–42)
INCREASED POSTENDOTHELIAL MACROMOLECULAR PERMEABILITY ACROSS THE EXTRACELLULAR MATRIX	(49; BENT-HANSEN, UNPUBLISHED OBSERVATIONS)

**Table 5—Alteration in diabetic patients with early nephropathy, possibly related to decreased density of HS-PG within the extracellular matrix**

	NORMAL UAER	INCREASED UAER	REFERENCE
RENAL CLEARANCE RATIO OF NEUTRAL VS. ANIONIC MACROMOLECULES	2.5	1.0	(27)
TERA (% $\times$ h <sup>-1</sup> )	5.2	7.9	(27, 49)
P-TRIGLYCERIDES (mM)	0.98	1.28	(26)
P-VLDL (mM)	0.57	1.07	(26)
P-FIBRINOGEN ( $\mu$ M)	7.62	9.62	(26)
P-VON WILLEBRAND FACTOR (IU/mL)	0.86	1.20	(47)
IN VIVO PLATELET ADHESION (%)	36	46	(48)

All differences are statistically highly significant.

## HS-PG AND

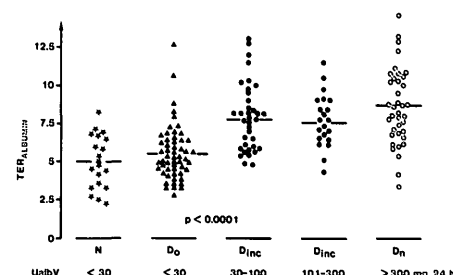
## ATHEROSCLEROSIS

— In large vessel walls, HS-PG has anti-atherogenic properties (43), and a negative correlation has been demonstrated between HS and the cholesterol content of human large vessel walls (44). HS-PG contributes to the structural integrity of the interendothelial clefts of large vessel walls. These interendothelial clefts contribute to the transendothelial pathway for lipoproteins and other macromolecules. HS-PG also helps to maintain the myo-medial cells in a resting state and, in large vessel walls, the anionic HS-PG contributes to binding of lipoprotein lipase and antithrombin III (reviewed [43]). A decreased density of anionic HS-PG in endothelial plasma membranes and extracellular matrices, as suggested in diabetic patients with microalbuminuria, is expected to lead to a decreased binding of lipoprotein lipase (45) and therefore to a decreased clearance of triglycerides and VLDLs. Increased plasma concentration of triglycerides and VLDLs is seen in IDDM patients with albuminuria (26). Decreased binding of antithrombin III would shift the hemostatic balance in a procoagulant direction, leading to deposition of fibrin on the endothelial cells' surface. Deposition of fibrin, however, leads to the release of von Willebrand factor (46). Increased

concentration of von Willebrand factor is seen in diabetic patients with albuminuria (47). Increased density of von Willebrand factor on endothelial cell surface will increase the in vivo platelet adhesion that also is seen in diabetic patients with albuminuria (48). Most signs and symptoms in patients with incipient nephropathy are compatible with decreased density of anionic HS-PG in extracellular matrices (27) (Table 5).

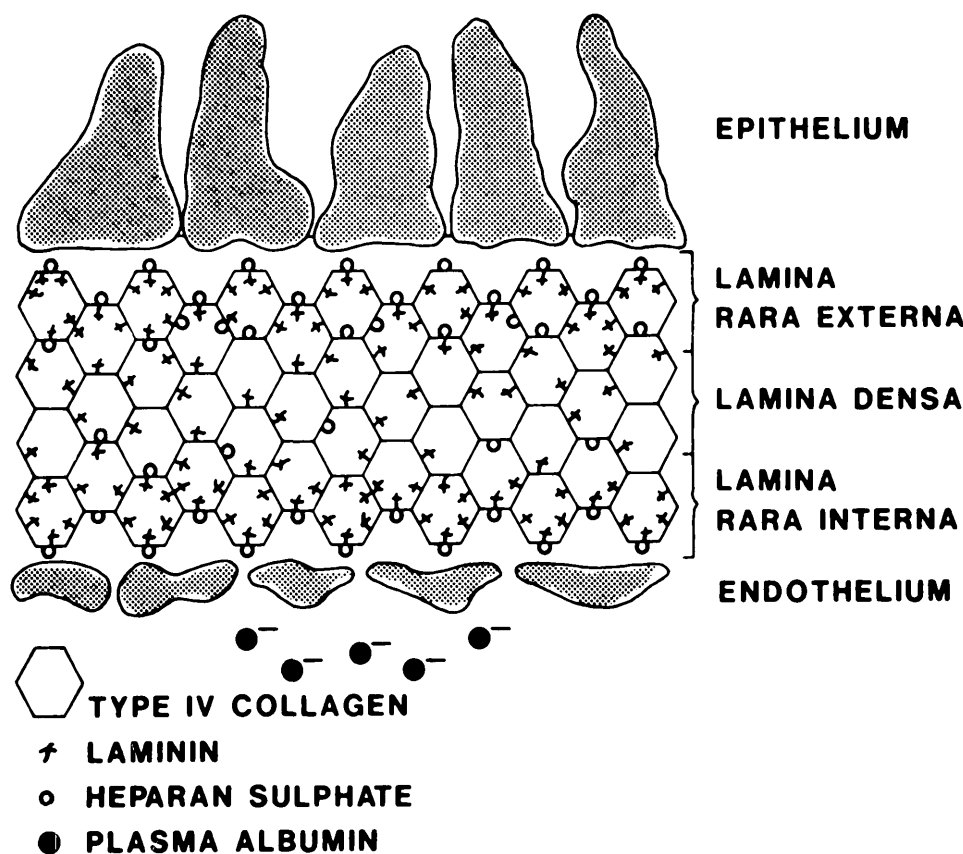
## DECREASED ANIONIC HS-PG: THE CAUSE OF ALBUMINURIA AND PREMATURE

**ATHEROSCLEROSIS?** — Is the decreased density of HS-PG in the GBM and large vessel walls actually the cause of microalbuminuria and premature atherosclerosis in diabetes? Accumulating evidence suggests that this might be the case. Most of this evidence comes from careful studies of the very first clinical signs of diabetic nephropathy, namely microalbuminuria and its concomitant increased TERA, a marker of postendothelial macromolecular permeability. The coincidence of microalbuminuria and TERA is rather striking (27) (Fig. 4). The transendothelial flux of albumin in patients with microalbuminuria is increased by ~50%, compared with long-term diabetic patients with normal UAER and similar metabolic control



**Figure 4**—TERA (percentage per hour) in control subjects (N) and IDDM patients with different levels of UAER. D<sub>0</sub>, long-term diabetics with normal UAER; D<sub>inc</sub>, incipient diabetic nephropathy; D<sub>n</sub>, clinical diabetic nephropathy. From Deckert et al. (27). © by Diabetologia.

(49). Is the increased UAER and the increased TERA of albumin caused by decreased density of anionic HS-PG? Increased TERA can be attributable to increased intravascular pressure, increased number and sizes of vascular macromolecular pathways, or compositional changes of the macromolecular pathway. Increased intravascular pressure seems not to be the cause because blood pressure (27), capillary pressure (E. Tooke, unpublished observations), oncotic pressure differences (50), and plasma flow were not different between patients with normal UAER and patients with persistently increased UAER (51,52). Also an increased number and size of macromolecular pathways have until now not been demonstrated in patients with microalbuminuria. Thus, vascular volume (50), capillary density (52), capillary surface (52), and even the number and width of interendothelial clefts, are not different between patients with and without microalbuminuria (T. Deckert and Egeberg, unpublished observations). These results might suggest that increased TERA in patients with microalbuminuria is caused by qualitative changes of the macromolecular pathway and the extracellular matrix. Structural-functional studies of the vascular extracellular matrix are difficult to perform in vivo. However, the extracellular matrix



**Figure 5**—Structure of GBM. ○, type IV collagen; ✕, laminin; ○, HS-PG (negatively charged); ●<sup>-</sup>, plasma albumin (negatively charged).

of the glomerular filtration barrier—the glomerular basement membrane (GBM)—can be studied more easily.

The composition of the GBM is well-known (Fig. 5). The GBM is negatively charged, and HS-PG contributes to this negative charge. The negative charge inhibits the filtration of negatively charged plasma proteins (i.e., exerts a charge selectivity). We have assessed renal charge selectivity in IDDM patients by measuring the fractional clearance of pairs of plasma proteins of identical size but different charges (27). We used nonglycosylated albumin and more negatively charged glycosylated albumin as such a pair (53), and neutral total IgG and the more negative subfraction IgG4 (27,54,55). With both pairs of plasma proteins, we found loss-of-charge selectivity in patients with microalbuminuria.

This means that most nephrons of patients with albuminuria are no longer able to differentiate between plasma proteins of different charges. Similar results were obtained by others using IgG and IgG4 (56) and pancreatic and salivary isoamylase (57) for the assessment of charge selectivity. It has been suggested that loss-of-charge selectivity might be attributable to an increase of the large pore area of the GBM. However, studies with dextran clearances indicate that the fractional dextran clearances in patients with microalbuminuria are identical to the fractional dextran clearance in nondiabetic and diabetic patients with normal UAER (58; T. Deckert, et al., unpublished observations). Thus, changes in size selectivity seem to be preceded by changes in charge selectivity. Alteration in tubular function also is sometimes

suggested to bias the estimation of renal charge selectivity. However, this is not very likely because the findings of loss of renal charge selectivity would suggest increased negative charge of the tubular membranes—even though no mechanism of such kind has been demonstrated. The excretion of  $\beta_2$ -microglobulin and retinol binding protein—two markers of tubular function with slightly different isoelectric points—are completely unchanged in diabetic patients with microalbuminuria compared with patients with normal UAER (T. Deckert et al., unpublished observations). Therefore, we suggest that loss-of-charge selectivity in patients with microalbuminuria is attributable to loss of negatively charged components within the glomeruli, probably HS-PG. A negative correlation is seen between the number of an-

**Table 6—Anionic charge of HS-PG in extracellular matrix of diabetic patients is reduced by certain factors**

DECREASED DENSITY OF HS-PG
INCREASED GENE EXPRESSION (60, 62) AND SECRETION (63) OF COLLAGEN IV AND FIBRONECTIN
DECREASED SYNTHESIS OF HS-PG (36)
DECREASED TURNOVER OF GLYCOSYLATED COLLAGEN IV (84)
DECREASED BINDING OF HS-PG TO GLYCOSYLATED COLLAGEN IV (69)
DECREASED SULFATION OF HS (65, 68)

ionic sites and albuminuria (59). A negative correlation has been seen between mRNA of HS-PG and albuminuria in diabetic mice (60). Furthermore, antibodies against HS will induce albuminuria (83), and ACE inhibitors given to diabetic rats has been shown to increase the content of HS within the glomeruli, and simultaneously arrest albuminuria (39). From these studies, we concluded that decreased density of anionic HS-PG might be the cause of microalbuminuria in diabetic patients. Because the anionic charge on the luminal surface of vascular walls has been shown to be decreased in diabetics patients (61) and because we have shown that charge selectivity of TERA also was found to disappear in patients with microalbuminuria (L. Bent-Hansen, et al., unpublished observations), we suggest that—in the extracellular matrix of vascular walls—a decrease of anionic HS-PG is occurring also, leading to an enhancement of the atherosclerotic processes.

### CAUSES OF DECREASED HS-PG IN DIABETES:

**HYPERGLYCEMIA**— How does decreased density of anionic HS-PG occur in diabetic patients? Hyperglycemia leads to decreased density of anionic HS-PG by several mechanisms (Table 6). The most important cause seems to be a defect in the coordinated genetic expression of components of the extracellular matrix. Cagliero et al. (62) have studied the biosynthesis of extracellular matrix components in tissue cultures of human endothelial cells. They found that, in most patients, hyperglycemia leads to in-

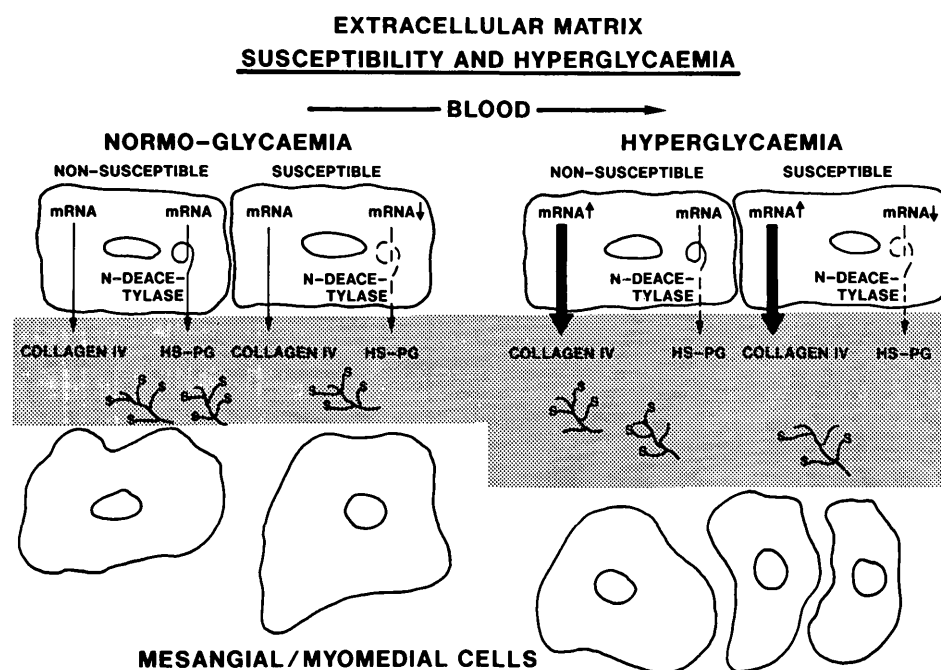
creased expression of collagen IV, laminin, and fibronectin. The activation of these extracellular matrix components seem to be coordinated. When collagen IV is activated by 300% the transcription of fibronectin also is increased by ~300%. Thus, hyperglycemia leads to a coordinated activation of the expression and secretion (63) of collagen IV, laminin, and fibronectin. This does not seem to be the case for HS-PG. Therefore, the ratio of mRNA for HS-PG/collagen IV is significantly reduced in diabetic KK mice (60). Increased gene expression and secretion of collagen IV without alteration of the expression of HS-PG would explain not only the thickening of basement membrane in diabetic patients, but also a relative loss of anionic HS-PG in the extracellular matrix.

Other mechanisms also affect the negative charge of the extracellular matrix in diabetic patients. Thus, it recently has been demonstrated that diabetes affects the activity of *N*-deacetylase, the key enzyme in the sulfation of HS-PG (Fig. 6) (64–66). In poorly regulated STZ-diabetic Sprague-Dawley rats, the activity of *N*-deacetylase in the liver and glomeruli is decreased by ~50%, a reduction that is correlated to mean blood glucose. The activity can be normalized by insulin treatment. In Sprague-Dawley rats, the reduction in *N*-deacetylase activity is correlated to albuminuria (66). Decreased activity of *N*-deacetylase leads to undersulfation of HS, which means loss of negative charges. Undersulfation of HS in diabetic animals has been demonstrated by several groups (35,68).

A third mechanism by which di-

abetes might affect the density of HS-PG in the extracellular matrix is nonenzymatic glycosylation. In poorly regulated diabetic patients, the degree of glycosylation of collagen IV and fibronectin is much higher than in control subjects (69). However, nonenzymatic glycosylation of collagen IV and fibronectin will decrease the affinity to HS-PG (69). This might lead to a greater turnover of HS-PG in poorly regulated diabetic patients (70) and reduced density of HS-PG in extracellular matrix. Because patients with albuminuria often have poor diabetes control and higher HbA<sub>1c</sub> than long-term diabetic patients with normal UAER, it is likely that these mechanisms also contribute to decreased density of HS-PG. Thus, diabetes affects the biosynthesis of the extracellular matrix in several ways and leads to reduced density of anionic HS-PG in the extracellular matrix. Besides poor diabetes control, genetic factors seem to influence the metabolism of extracellular matrix components.

**GENETIC FACTORS**— Epidemiological studies have demonstrated that only a minority of diabetic patients will develop clinical nephropathy and its associated cardiovascular complications (71). Thus, the majority of diabetic patients seem to be protected from the development of diabetic nephropathy. This protection cannot be explained exclusively by better metabolic control (72). Probably several susceptibility genes are involved. Recent studies have demonstrated a clustering of diabetic nephropathy within families that have several cases of diabetes among first-degree relatives (73,74). Other recent studies suggested a genetic defect in the coordinated secretion of glycosaminoglycans among patients with albuminuria. These studies demonstrated that fibroblasts from diabetic patients with nephropathy kept in tissue cultures for several months under identical condition secreted relatively less HS compared with hyaluronic acid than fibroblasts



**Figure 6**—Extracellular matrix in glomeruli and large vessel walls in normoglycemic (left) and hyperglycemic (right) individuals. *N*-deacetylase is the key enzyme in the sulfation of HS-PG (note branching symbols). For explanation, see text.

from control subjects, whereas fibroblasts from diabetic patients with normal UAER were similar to control subjects (75). Furthermore, experiments in diabetic rats of slightly different genetic background indicated differences in the regulation of the *N*-deacetylase as a susceptibility factor (65,66). Thus, besides poor diabetes control, genetic differences in the regulation of the biosynthesis of the extracellular matrix might contribute to individual differences in the compositional structure of the matrix and thereby to susceptibility to microalbuminuria and its associated complications. The hypothesis that emerges from these observations is as follows.

In nondiabetic patients not susceptible for albuminuria and premature atherosclerosis, collagen IV and HS-PG synthesis are normally regulated, resulting in a normal composition of the extracellular matrix. In susceptible normoglycemic individuals, however, defects in the regulation of HS-PG biosynthesis will result in a reduced density of HS-PG,

turning these individuals into subjects more vulnerable to albuminuria and atherosclerosis. In diabetic patients, the density of HS-PG is reduced mostly because of a relative increased synthesis of collagen IV. HS-PG is also undersulfated because of reduced *N*-deacetylase activity. The net alterations are, therefore, dependent mainly on the quality of metabolic control. In susceptible diabetic patients, however (i.e., patients with genetic defects in the regulation of HS-PG), extracellular matrix synthesis will result in a more pronounced reduction of the density and sulfation of HS-PG within plasma membranes and the extracellular matrix. In these patients, the biochemical alterations may lead to functional alterations, such as increased permeability and proliferation of mesangial and myomedial cells, and finally to the well-known structural alterations of glomerulosclerosis and premature atherosclerosis.

Because DNA probes of HS-PG and *N*-deacetylase are or will be available in the future, it is hoped that some of the

susceptibility genes will be identified soon. Then, identification of patients at risk might be possible. By concentrating our clinical efforts on these patients, it is hoped that prevention of some of the most severe complications of diabetes might, in the future, be more successful.

## References

1. Borch-Johnsen K, Kragh Andersen P, Deckert T: The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 28:590–96, 1985
2. Parving H-H, Smidt UM, Andersen AR, Svendsen PA: Early aggressive antihypertensive treatment reduces rate of decline in kidney function in diabetic nephropathy. *Lancet* 1:1175–79, 1983
3. Borch-Johnsen K, Kreiner S: Proteinuria: value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus. *Br Med J* 294:1651–54, 1987
4. Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T: Coronary heart disease in young type 1 (insulin-depen-

- dent) diabetic patients with and without diabetic nephropathy: incidence and risk factors. *Diabetologia* 30:144–48, 1987
5. Marshall SM: Screening for microalbuminuria: which measurement? *Diabetic Med* 8:706–11, 1991
  6. Lind B, Jensen T, Feldt-Rasmussen B, Deckert T: Normal urinary albumin excretion in recently diagnosed type 1 diabetic patients. *Diabetic Med* 6:682–84, 1989
  7. Christensen CK, Mogensen CE: The course of incipient diabetic nephropathy: studies of albumin excretion and blood pressure. *Diabetic Med* 2:97–102, 1985
  8. Mathiesen ER, Rønn B, Jensen T, Storm B, Deckert T: Relationship between blood pressure and urinary albumin excretion in development of microalbuminuria. *Diabetes* 39:245–49, 1990
  9. Mogensen CE, Chacati A, Christensen CK, Close CF, Deckert T, Hommel E, Kastrup J, Lefebvre P, Mathiesen ER, Feldt-Rasmussen B, Schmitz A, Viberti GC: Microalbuminuria: an early marker of renal involvement in diabetes. *Uremia Invest* 9:85–95, 1986
  10. Niazy S, Feldt-Rasmussen B, Deckert T: Microalbuminuria in insulin-dependent diabetes: prevalence and practical consequences. *J Diabet Complications* 1:76–80, 1987
  11. Gall M-A, Rossing P, Skøtt P, Damsbo P, Vaag A, Bech K, Dejgaard A, Lauritzen M, Lauritzen E, Hougaard P, Beck-Nielsen H, Parving HH: Prevalence of micro- and macroalbuminuria, arterial hypertension, retinopathy and large vessel disease in European type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 34:655–61, 1991
  12. Walker JD, Viberti GC: Aetiology and pathogenesis of diabetic nephropathy: clues from early functional abnormalities. In *Textbook of Diabetes*. Pickup J, Williams G, Eds. Oxford, UK, Blackwell Scientific Publications, 1991, p. 657–71
  13. Schmitz A, Vaeth M: Microalbuminuria: a major risk factor in non-insulin-dependent diabetes: a 10-year follow-up study of 503 patients. *Diabetic Med* 5:126–34, 1988
  14. Yudkin JS, Forrest RD, Jackson CA: Microalbuminuria as predictor of vascular disease in non-diabetic subjects. *Lancet* 1:530–33, 1988
  15. Damsgaard EM, Frøland A, Jørgensen OD, Mogensen CE: Microalbuminuria as predictor of increased mortality in elderly people. *Br Med J* 300:297–300, 1990
  16. Earle KA, Walker JD, Jones SL, Hill C, Viberti GC: Cardiovascular disease in the parents of type 1 (insulin-dependent) diabetics with nephropathy (Abstract). *Diabetologia* 33:A66, 1990
  17. Nørgaard K, Mathiesen ER, Hommel E, Jensen JS, Parving H-H: Lack of familial predisposition to cardiovascular disease in type 1 (insulin-dependent) diabetic patients with nephropathy. *Diabetologia* 34:370–72, 1991
  18. Krolewski AS, Canessa M, Warram JH, Laffel LMB, Christlieb AR, Knowler WC, Rand LI: Predisposition of hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *N Engl J Med* 318:140–45, 1988
  19. Viberti GC, Keen H, Wiseman MJ: Raised arterial pressure in parents of proteinuric insulin-dependent diabetics. *Br Med J* 295:515–17, 1987
  20. Jensen JS, Mathiesen ER, Nørgaard K, Hommel E, Borch-Johnsen K, Funder J, Brahm J, Parving H-H, Deckert T: Increased blood pressure and erythrocyte sodium/lithium countertransport activity are not inherited in diabetic nephropathy. *Diabetologia* 33:619–24, 1990
  21. Walker JD, Tariq T, Viberti GC: Sodium-lithium countertransport activity in red cells of patients with insulin dependent diabetes and nephropathy and their parents. *Br Med J* 301:635–38, 1990
  22. Mangili R, Bending JJ, Scott G, Li KL, Gupta A, Viberti GC: Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 318:1146–50, 1988
  23. Elving LD, Wetzels JFM, de Nobel E, Berder JHM: Erythrocyte sodium-lithium countertransport is not different in type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy. *Diabetologia* 34:126–28, 1991
  24. Davies JE, Ng LL, Li LK, Earl KL, Kofoed-Enevoldsen A, Trevisan R, Viberti GC: Intracellular pH and  $\text{Na}^+/\text{H}^+$  antiport activity of cultured fibroblasts in diabetic nephropathy. *Diabetic Med (BDA Abstracts)*:39A, 1991
  25. Chase HP, Garg SK, Marshall G, Berg CL, Harris S, Jackson WE, Hamman RE: Cigarette smoking increases the risk of albuminuria among subjects with type 1 diabetes. *JAMA* 265:614–17, 1991
  26. Jensen T, Stender S, Deckert T: Abnormalities in plasma concentrations of lipoproteins and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased urinary albumin excretion. *Diabetologia* 31:142–45, 1988
  27. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A: Albuminuria reflects widespread vascular damage: the Steno hypothesis. *Diabetologia* 32:219–26, 1989
  28. Nelson RG, Kunzelman CL, Pettitt DJ, Saad MF, Bennett PH, Knowler WC: Albuminuria in type 2 (non-insulin-dependent diabetes mellitus) and impaired glucose tolerance in Pima Indians. *Diabetologia* 32:870–76, 1989
  29. Mattock MB, Keen H, Viberti GC, El-Gohari MR, Murrells TJ, Scott GS, Wing JR, Jackson PG: Coronary heart disease and urinary albumin excretion rate in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 31:82–87, 1988
  30. Niskanen L, Uusitupa M, Sarlund H, Siitonen O, Voutilainen E, Penttilä I, Pyörälä K: Microalbuminuria predicts the development of serum lipoprotein abnormalities favoring atherogenesis in newly diagnosed type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 33:237–43, 1990
  31. Schmitz A, Ingerslev J: Haemostatic measures in type 2 diabetic patients with microalbuminuria. *Diabetic Med* 7:521–25, 1990
  32. Østerby R, Parving H-H, Hommel E, Jørgensen HE, Løkkegaard H: Glomerular structure and function in diabetic nephropathy: early to advanced stages. *Diabetes* 39:1057–63, 1990
  33. Shimomura H, Spiro RG: Studies on macromolecular components of human glomerular basement membrane and alterations in diabetes: decreased levels of heparan sulfate proteoglycan and lami-

- nin. *Diabetes* 36:374–81, 1987
34. Dybdahl H, Ledet T: Diabetic macroangiopathy. Quantitative histopathological studies of the extramural coronary arteries from type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 30:882–86, 1987
35. Kjellén L, Bielefeld D, Hook M: Reduced sulfation of liver heparan sulfate in experimentally diabetic rats. *Diabetes* 32:337–42, 1983
36. Wu V-Y, Wilson B, Cohen MP: Disturbances in glomerular basement membrane glycosaminoglycans in experimental diabetes. *Diabetes* 36:679–83, 1987
37. Gallagher JT, Lyon M, Steward WP: Structure and function of heparan sulphate proteoglycans. *Biochem J* 236:313–25, 1986
38. Rosenzweig LJ, Kanwar YS: Removal of sulfated (heparan sulfate) or nonsulfated (hyaluronic acid) glucosaminoglycans results in increased permeability of the glomerular basement membrane to <sup>125</sup>I-bovine serum albumin. *Lab Invest* 47:177–83, 1982
39. Reddi A, Ramamurthi R, Miller M, Dhuper S, Lasker N: Enalapril improves albuminuria by preventing glomerular loss of heparan sulfate in diabetic rats. *Biochemical Med Metab Biol* 45:119–31, 1991
40. Castellot JJ, Hoover RL, Harper PA, Karnovsky MJ: Heparin and glomerular epithelial cell-secreted heparin-like species inhibit mesangial-cell proliferation. *Am J Pathol* 120:427–35, 1985
41. Kitamura M, Mitarai T, Maruyama N, Nagasawa R, Yoshida H, Sakai O: Mesangial cell behavior in a three-dimensional extracellular matrix. *Kidney Int* 40:653–61, 1991
42. Pomerantz KB, Hajja DP: Eicosanoids in regulation of arterial smooth muscle cell phenotype, proliferative capacity and cholesterol metabolism. *Arteriosclerosis* 9:413–29, 1989
43. Deckert T, Jensen T, Feldt-Rasmussen B, Kofoed-Enevoldsen A, Borch-Johnsen K, Stender S: Albuminuria, a risk marker of atherosclerosis in insulin dependent diabetes mellitus. *Cardiovasc Risk Factors* 1:347–60, 1991
44. Hollmann J, Schmidt A, von Bassewitz DB, Buddecke E: Relationship of sulfated glycosaminoglycans and cholesterol content in normal and arteriosclerotic human aorta. *Arteriosclerosis* 9:154–58, 1989
45. Braun JEA, Severson DL: Diabetes reduces heparin- and phospholipase C-releasable lipoprotein lipase from cardiomyocytes. *Am J Physiol* 260:E447–85, 1991
46. Ribes JA, Ni F, Wagner DD, Francis CW: Mediation of fibrin-reduced release of von Willebrand factor from cultured endothelial cells by the fibrin  $\beta$  chain. *J Clin Invest* 84:4335–342, 1989
47. Jensen T: Increased plasma concentration of von Willebrand factor in insulin dependent diabetics with incipient nephropathy. *Br Med J* 298:27–28, 1989
48. Valdorf-Hansen F, Jensen T, Borch-Johnsen K, Deckert T: Cardiovascular risk factors in type 1 (insulin-dependent) diabetic patients with and without proteinuria. *Acta Med Scand* 222:439–44, 1987
49. Feldt-Rasmussen B: Increased transcapillary escape rate of albumin in type 1 (insulin-dependent) diabetic patients with microalbuminuria. *Diabetologia* 29:282–86, 1986
50. Hommel E, Mathiesen ER, Aukland K, Parving H-H: Pathophysiological aspects of edema formation in diabetic nephropathy. *Kidney Int* 38:1187–92, 1990
51. Feldt-Rasmussen B, Baker L, Deckert T: Exercise as a provocative test in early renal disease in type 1 (insulin-dependent) diabetes: albuminuric, systemic and renal haemodynamic responses. *Diabetologia* 28:389–96, 1985
52. Leinonen H, Matikainen E, Juntunen J: Permeability and morphology of skeletal muscle capillaries in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 22:158–62, 1982
53. Kverneland A, Feldt-Rasmussen B, Vidal P, Welinder B, Bent-Hansen L, Søgaard U, Deckert T: Evidence of changes in renal charge selectivity in patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 29:634–39, 1986
54. Deckert T, Feldt-Rasmussen B, Djurup R, Deckert M: Glomerular size and charge selectivity in type 1 (insulin-dependent) diabetes mellitus. *Kidney Int* 33:100–106, 1988
55. Deckert T, Feldt-Rasmussen B, Djurup R, Deckert M: Glomerular size and charge selectivity in type 1 (insulin-dependent) diabetes mellitus (Abstract). *Diabetologia* 30:513A, 1987
56. Pietravalle P, Morano S, Christina G, De Rossi MG, Mariani G, Cotroneo P, Ghirlanda G, Clementi A, Andreani D, Di Mario U: Charge selectivity of proteinuria in type 1 diabetes explored by immunoglobulin subclass clearance (Abstract). *Diabetes* 40 (Suppl. 1):552A, 1991
57. Fox JG, Quin JD, Paterson KR, O'Reilly DS, Boulton-Jones JM: Impaired glomerular charge selectivity in microalbuminuria and diabetic nephropathy (Abstract). *Diabetologia* 33 (Suppl.):A67, 1990
58. Nakamura Y, Myers BD: Charge selectivity of proteinuria in diabetic glomerulopathy. *Diabetes* 37:1202–11, 1988
59. Vernier RL, Steffes MW, Sisson-Ross S, Mauer SM: Heparan sulfate proteoglycan in the glomerular basement membrane in type 1 diabetes mellitus. *Kidney Int* 41:1070–80, 1992
60. Ledbetter S, Copeland EJ, Noonan D, Vogeli G, Hassell JR: Altered steady-state mRNA levels of basement membrane proteins in diabetic mouse kidneys and thromboxane synthase inhibition. *Diabetes* 39:196–203, 1990
61. Raz I, Havivi Y, Yarom R: Reduced negative surface on arterial endothelium of diabetic rats. *Diabetologia* 31:618–20, 1988
62. Cagliero E, Roth T, Roy S, Lorenzi M: Characteristics and mechanisms of high-glucose-induced overexpression of basement membrane components in cultured human endothelial cells. *Diabetes* 40:102–110, 1991
63. Haneda M, Kikkawa R, Horide N, Togawa M, Koya D, Kajiwarra N, Ooshima A, Shigeta Y: Glucose enhances type IV collagen production in cultured rat glomerular mesangial cells. *Diabetologia* 34:198–200, 1991
64. Unger E, Pettersson I, Eriksson UJ, Lindahl U, Kjellen L: Decreased activity of the heparan sulfate modifying enzyme glucosaminyl N-deacetylase in hepato-

- cytes from streptozotocin-diabetic rats. *J Biol Chem* 266:8671–74, 1991
65. Kofoed-Enevoldsen A, Eriksson UJ: Inhibition of N-acetyl-heparosan deacetylase in diabetic rats. *Diabetes* 40:1449–52, 1991
  66. Kofoed-Enevoldsen A: Inhibition of glomerular glucosaminyl N-deacetylase in diabetic rats. *Kidney Int.* 41:763–67, 1992
  68. Cohen MP, Klepser H, Wu V-Y: Under-sulfation of glomerular basement membrane heparan sulfate in experimental diabetes and lack of correction with aldose reductase inhibition. *Diabetes* 37:1324–27, 1988
  69. Tarsio JF, Reger LA, Furcht LT: Molecular mechanisms in basement membrane complications of diabetes: alterations in heparin, laminin, and type IV collagen association. *Diabetes* 37:532–39, 1988
  70. Klein DJ, Oegema TR, Jr., Brown DM: Release of glomerular heparan  $^{35}\text{SO}_4$  proteoglycan by heparin from glomeruli of streptozotocin-induced diabetic rats. *Diabetes* 38:130–39, 1989
  71. Kofoed-Enevoldsen A, Borch-Johnsen K, Kreiner S, Nerup J, Deckert T: Declining incidence of persistent proteinuria in type 1 (insulin-dependent) diabetic patients in Denmark. *Diabetes* 36:205–209, 1987
  72. Deckert T, Poulsen JE: Diabetic nephropathy: fault or destiny? *Diabetologia* 21:178–83, 1981
  73. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease. *N Engl J Med* 320:1161–65, 1989
  74. Borch-Johnsen K, Nørgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, Parving H-H: Diabetic nephropathy—an inherited complication? *Kidney Int.* 41:719–22, 1992
  75. Deckert T, Horowitz IM, Kofoed-Enevoldsen A, Kjellén L, Deckert M, Lykelund C, Burchard F: Possible genetic defects in regulation of glycosaminoglycans in patients with diabetic nephropathy. *Diabetes* 40:764–70, 1991
  76. Kofoed-Enevoldsen A, Jensen T, Borch-Johnsen K, Deckert T: Incidence of retinopathy in type 1 (insulin-dependent) diabetes: association with clinical nephropathy. *J Diabet Complications* 3:96–99, 1987
  77. Kelbæk H, Jensen T, Feldt-Rasmussen B, Christensen NJ, Richter EA, Deckert T: Impaired left-ventricular function in insulin-dependent diabetic patients with increased urinary albumin excretion. *Scand J Clin Invest* 51:467–73, 1991
  78. Jensen T, Richter EA, Feldt-Rasmussen B, Kelbæk H, Deckert T: Impaired aerobic work capacity in insulin dependent diabetics with increased urinary albumin excretion. *Br Med J* 196:1352–54, 1988
  79. Bent-Hansen L, Deckert T: Metabolism of albumin and fibrinogen in type 1 (insulin-dependent) diabetes mellitus. *Diabetes Res* 7:159–64, 1988
  80. Jensen T, Bjerre-Knudsen J, Feldt-Rasmussen B, Deckert T: Features of endothelial dysfunction in early diabetic nephropathy. *Lancet* 1:461–63, 1989
  81. Feldt-Rasmussen B, Mathiesen ER, Deckert T, Giese J, Christensen NJ, Bent-Hansen L, Nielsen MD: Central role for sodium in the pathogenesis of blood pressure changes independent of angiotensin, aldosterone and catecholamines in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 30:610–17, 1987
  82. Ellis EN, Steffes MW, Goetz FC, Sutherland DER, Mauer SM: Glomerular filtration surface in type 1 diabetes mellitus. *Kidney Int* 29:889–94, 1986
  83. Van Den Born J, Van den Heuvel LPWJ, Bakker MAH, Veerkamp JH, Assmann KJM, Berden JHM: A monoclonal antibody against GBM heparan sulfate induces an acute selective proteinuria in rats. *Kidney Int* 41:115–23, 1992
  84. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315–21, 1988