

American Diabetes Association 60th Scientific Sessions, 2000

Nephropathy

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This is the fifth in a continuing series of articles on the American Diabetes Association (ADA) 60th Scientific Sessions held in San Antonio, Texas, in June 2000. It covers topics related to nephropathy.

Hypertension and Nephropathy

A number of studies presented at the meeting gave insight into clinical and pathophysiological aspects of hypertension and nephropathy in patients with diabetes. Tarver-Carr et al. (abstract 783) reported 16-year follow-up analysis of 9,250 adults aged 30–74 in 1976–1980 who participated in the Second National Health and Nutrition Examination Survey (NHANES II). A total of 46 end-stage renal disease cases developed in the 521 diabetic adults, whereas 142 cases developed in those without type 2 diabetes at baseline, with cumulative incidence by age 75 of 7 vs. 3% among men and 8 vs. 0.8% among women with and without diabetes. For type 1 diabetes, Schultz and colleagues (abstracts 652 and 653) analyzed 511 patients developing diabetes before age 16 and followed for a median of 6 years. Of the patients, 63 developed microalbuminuria, showing both higher baseline albuminuria and a correlation of mean HbA_{1c} with the rate of increase of albuminuria, which also increased at a higher rate after onset of puberty. The risk of developing microalbuminuria was doubled for female patients, tripled after onset of puberty, and increased 36% for every 1% increase in HbA_{1c}. Tabak and

Orchard (abstract 658) reported greater baseline albuminuria, higher HbA_{1c}, longer diabetes duration, conventional treatment, younger age, male sex, and lower HDL cholesterol in females to be significant predictors of the development of microalbuminuria among 1,367 patients with type 1 diabetes in the Diabetes Control and Complications Trial (DCCT) who were initially normoalbuminuric. Thus, glycemia is an important explanation of diabetic nephropathy, but other factors must play a role in determining susceptibility.

Brown et al. (abstract 187) simulated effects of glycemia and blood pressure using algorithms derived from UKPDS (U.K. Prospective Diabetes Study), Framingham, and DCCT data. The benefits of control were greater among individuals with greater baseline risk levels, with the data suggesting that blood pressure treatment may be more efficient than glycemic treatment in preventing microvascular complications and increasing life expectancy. In an important reminder of the need for hypertension treatment of patients with diabetes, Geiss et al. (abstract 188) reported data from the 1988–1994 NHANES III, which included 1,507 adults with diagnosed diabetes; 71% had hypertension, with 71% of these aware of this and 57% treated. Only 12% had blood pressure <130/85 and 45% had blood pressure <140/90 mmHg.

Morioka et al. (abstract 73) followed 227 patients with diabetes on hemodialysis for up to 9 years, showing a 7% in-

crease in mortality for each 1% higher HbA_{1c} at initiation of dialysis. Janka et al. (abstract 74) treated 463 patients on stable antidiabetic therapy with the ACE inhibitor/calcium-channel blocker combination of verapamil with trandolapril versus a combination of atenolol and chlorthalidone for 6 months. Blood pressure fell similarly from 169/96 to 150/85 and from 168/95 to 145/83, and albuminuria decreased similarly 19 vs. 26 mg/day. However, HbA_{1c} was 7.9% before and after the former, but increased from 7.8 to 8.6% in the latter group, suggesting an adverse metabolic effect of β -blocker and/or diuretic treatment.

Czupryniak and Drzewoski (abstract 411) treated 19 normotensive, normoalbuminuric patients with type 2 diabetes who did not show nocturnal lowering of blood pressure with the ACE inhibitor trandolapril (2 mg daily for 12 weeks). Blood pressure fell from 126/89 to 118/76. Even 2–4 weeks after discontinuation of the drug, a nocturnal blood pressure fall was restored despite return of systolic and diastolic blood pressure toward pretreatment levels. Del Prato et al. (abstract 75) assessed glomerular hemodynamics in 8 micro- and 9 normoalbuminuric normotensive patients with type 1 diabetes. Mean blood pressure increased by 10 mmHg in the microalbuminuric patients who switched from a low- to a high-sodium diet. These patients had decreased insulin sensitivity and greater efferent arteriolar resistance and intraglomerular pressure, suggesting an effect of increased intraglomerular pressure in the pathogenesis of microalbuminuria, perhaps in turn related to insulin resistance. Strojek et al. (abstract 656) administered nonhypotensive doses of the sympathoplegic drug moxonidine to 15 normotensive microalbuminuric patients with type 1 diabetes, showing a 27% decrease in albumin excretion. This suggests that sympathetic tone, like angiotensin II, has direct effects on renal function in diabetes in addition to those mediated by blood pressure elevation.

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Abbreviations: ADA, American Diabetes Association; AGE, advanced glycation end product; CML, N^ε-carboxymethyl lysine; DCCT, Diabetes Control and Complications Trial; GFR, glomerular filtration rate; MMP, matrix metalloproteinase; NF_{κB}, nerve factor-κB; NHANES, National Health and Nutrition Examination Survey; RAGE, receptor for AGEs; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

A table elsewhere in this issue shows conventional Système International (SI) units and conversion factors for many substances.

Wilson et al. (abstract 1272) compared 159 Pima Indian adults with normal glucose tolerance whose parents both had diabetes, 78 with at least one parent with nephropathy and 81 with neither parent having nephropathy. The former group had fasting glucose 90 vs. 87 mg/dl and a 7% lower glucose disposal rate, suggesting a relationship between insulin resistance and nephropathy. Eddings et al. (abstract 784) compared 130 diabetic members of 58 African-American families with 181 diabetic members of 71 Caucasian families, with earlier onset of diabetes in the former group and with nephropathy affecting 31 vs. 11%, evidence for genetic susceptibility in the African-American group.

Sosenko et al. (abstract 655) followed 782 Native Americans with both normal glucose tolerance and normal albumin excretion at baseline, 52 whose urine albumin exceeded 30 mg/g creatinine and 105 who developed diabetes after 4 years. Subsequently, 37% of those who developed diabetes, but 12% of those who did not, developed microalbuminuria, with the extent of Native American heritage and baseline diastolic blood pressure also being risk factors. The degree of hyperglycemia after onset of diabetes was also associated with the microalbuminuria. Hyttinen et al. (abstract 642) analyzed a Finnish type 1 diabetic patient registry, showing a three- to fourfold increase in risk of nephropathy for diabetic siblings of a patient with diabetic nephropathy.

One potential basis of genetic differences in risk of renal complications may be differing susceptibility to the pathogenic effects of angiotensin in diabetes. De Cosmo et al. (abstract 79) reported a greater rate of decline in the glomerular filtration rate (GFR) among patients with type 1 diabetes and macroalbuminuria having both the K121Q polymorphism of the PC-1 gene and the D allele of the insertion/deletion polymorphism of the ACE gene. Their rate of GFR decline was 13-fold greater than in those lacking the two alleles, independent of blood pressure and degree of albuminuria. Solini et al. (abstract 80) reported lesser mesangial fractional volume and glomerular basement membrane width in 77 patients with type 2 diabetes having the insertion allele of the ACE gene than in those heterozygotic or homozygotic for the deletion allele. Kennon et al. (abstract 864) studied 12 patients with type 1 diabetes

homozygous for the D allele and 12 homozygous for the I allele of the insertion/deletion polymorphism of the ACE gene. Acute ACE inhibition with intravenous enalapril decreased the pressor response to angiotensin I to a greater extent in the II homozygotes, but after a 2-week course of enalapril (10 mg daily) both groups showed similar response. Whether the ACE polymorphism influences the local production of angiotensin II chronically in tissues with high ACE concentrations, such as the kidney, remains unknown.

In a study of the effect of dietary protein on nephropathy, Gross et al. (abstract 636) compared a normoproteic diet, with chicken as the only source of meat, with a low-protein diet and a usual diet. Among 15 microalbuminuric patients with type 2 diabetes, the urine albumin was 34, 52, and 64 g/min, and GFR was 102, 94, and 111 ml · min⁻¹ · 1.73 m⁻² on the three diets. LDL cholesterol was 104, 98, and 126 mg/dl, further suggesting benefit in patients with nephropathy. Gambaro et al. (late-breaking abstract 4) treated 240 patients with diabetic nephropathy with the oral glycosaminoglycan sulodexide at 0, 50, 100, or 200 mg daily, showing 31, 50, and 75% decreases in albuminuria after 4 months. Albuminuria remained 28 and 65% below baseline in the 100- and 200-mg groups after another 4 months without further treatment. They speculate the lasting drug effect may reflect replenishment of the glomerular membrane electrostatic charge barrier with the agent.

Pathogenesis of Nephropathy

Cohen et al. (abstract 628) reported an increase in urinary collagen IV in diabetic mice at 15 weeks, coinciding with the light microscopic appearance of glomerular matrix expansion. They pointed out that urinary collagen IV may be a useful marker of renal disease entering a phase of compromised filtration function and may help in evaluating potential therapies. Diamant et al. (abstract 631) measured serum and 24-h urine levels of the interstitial collagenases matrix metalloproteinase (MMP)-2 and -9, which are involved in extracellular proteolysis, and serum levels of vascular endothelial growth factor (VEGF), which stimulates expression of MMPs and endothelial proliferation after vascular injury in vitro, in patients with type 1 diabetes. Serum MMP and VEGF were not associated with albuminuria, but there were significant correla-

tions with urinary MMP excretion, suggesting this to be a marker of the degree of nephropathy.

Fuad N. Ziyadeh, Philadelphia, PA, discussed the role of the transforming growth factor (TGF)- β as the major common "downstream mediator" in the pathogenesis of diabetic nephropathy. A number of other growth factors are "upstream" of the TGF- β signal. Angiotensin II stimulates TGF- β , and ACE inhibitors and angiotensin receptor blockers lower TGF- β levels. Similarly, thromboxane and endothelin appear to increase TGF- β production. High glucose and advanced glycation end product (AGE)-modified proteins can modify collagen, also involving a TGF- β -mediated pathway. Hemodynamic stress cycles activate multiple pathways, including protein kinase C and mitogen-activated protein kinase, leading to upregulation of both TGF- β and its receptor. Glomerular TGF- β expression is also increased by administration of AGE-modified albumin. TGF- β is a prototypic fibrogenic cytokine, stimulating synthesis and inhibiting degradation of extracellular matrix, upregulating protease inhibitors and downregulating proteases, and upregulating the cell surface receptors for matrix, the integrins, thus potentially playing a role in matrix accumulation in nephropathy.

TGF- β may be important in both hypertrophic and fibrotic changes in the kidney and may play a role in tubular disease as well as in glomerular disease. TGF- β administration to normal animals stimulates glomerular fibrosis. Renal cells in culture, animal models of type 1 and type 2 diabetes, and human studies of renal vein TGF- β levels all suggest this effect. Ziyadeh also pointed out that clinical response to captopril is associated with decreasing levels of TGF- β . In streptozotocin-induced diabetic rats, anti-TGF- β antibody treatment decreased the response of type IV collagen to hyperglycemia.

Hyperglycemia can stimulate both bioactivity and immunoactivity of TGF- β . Mesangial cells grown in high-glucose conditions have a 72% increase in TGF- β production. Ziyadeh's group has been able to partially characterize a promoter of TGF- β ₁ transcription in mouse and human cells stimulated by hyperglycemia. The effect of high glucose to stimulate the TGF- β message is prevented by treatment with a specific inhibitor of an

enzyme in the mitogen-activated protein kinase pathway, showing the complexity of the signaling pathways involved in TGF- β action. Anti-TGF- β antibody administration to streptozotocin-induced diabetic mice decreases glomerular hypertrophy and matrix expression, although it is not clear that these several-week studies are sufficient to demonstrate the role of this factor in diabetic nephropathy. In an 8-week study beginning just after the onset of hyperglycemia, intraperitoneal administration of neutralizing anti-TGF- β antibody did not block the upregulation of glomerular TGF- β expression or of TGF- β receptor levels, but it did prevent mesangial matrix expansion. Plasma creatinine and creatinine clearance were improved by this treatment as well. Collagen type IV and fibronectin, which were upregulated in the cortex of diabetic controls, were present at normal levels in treated animals.

Ziyadeh concluded that TGF- β might be a critical mediator of diabetic nephropathy. Interrupting this system may hold promise for amelioration of diabetic nephropathy. Existing approaches to nephroprotection, including glycemic control, lowering of dietary protein, and administration of ACE inhibitors and angiotensin receptor blockers, may act, at least in part, via inhibition of TGF- β . Antisense oligonucleotides might have advantages over administration of neutralizing antibodies, and receptor blockers or intracellular signaling inhibitors are being sought.

In a study of the pathogenesis of nephropathy, Demaine et al. (abstract 76) analyzed the relationship between complications and the CA dinucleotide repeat polymorphism and the C(-106)T substitution in the promoter region of the aldose reductase gene, showing a specific haplotype present in 33% of those with retinopathy or nephropathy but in only 5% of those without complications. This suggests a role of this enzyme, the first and rate-limiting enzyme of the polyol pathway, in genetic susceptibility to complications. Hodgkinson et al. (abstract 641) reported the 1.1 polymorphism of GLUT1 to be present in 65% of patients with type 1 diabetes and nephropathy but in 42% of those without complications after 20 years. They speculated that functional differences in GLUT1 may modify the availability of glucose for aldose reductase. In a study offering an alternative

explanation, Schaan et al. (abstract 651) showed increased urinary albumin and TGF- β_1 excretion in streptozotocin-induced diabetes in association with an increase in renal cortical GLUT1. They suggested that increased glucose uptake by mesangial cells may potentiate the overexpression of TGF- β_1 .

Hiragushi et al. (abstract 640) reported elevations in adrenomedullin, a newly discovered vasodilator peptide, in streptozotocin-diabetic rats, with expression shown on renal vessels, glomeruli, and collecting tubules, suggesting a role in glomerular hyperfiltration in early diabetic nephropathy. Purohit and Chin (abstract 649) assessed intrarenal synthesis of fibronectin, a glycoprotein homodimer involved in cell adhesion present in glomerular and tubular extracellular matrix, showing that both a low-protein diet and ACE inhibitor treatment decreased fibronectin mRNA, suggesting a mechanism of their benefit in clinical treatment.

AGEs and Nephropathy

Helen Vlassara, New York, NY, discussed the interrelationships between AGEs, AGE receptors, and mesangial cells. AGE formation is a ubiquitous process occurring spontaneously in most body proteins and lipids and is accompanied by the production of reactive oxygen species. Serum AGE levels correlate inversely with renal function, and dietary sources of AGEs appear increasingly important, with levels particularly high after ingestion of AGE-rich foods in patients with renal disease at early as well as late stages. AGE levels correlate with the severity of microvascular complications, and these molecules may be directly pathogenic, showing a tendency to form irreversible protein cross-links and to increase oxidation, particularly of lipids containing free amines. In addition, AGEs have cellular effects, triggering signaling cascades of proinflammatory processes. A number of these effects are mediated through AGE-specific receptors, which play a role both in AGE clearance and in tissue remodeling, but may lead to pathological responses in diabetes and in aging. Vlassara pointed out that receptor-linked endocytic processes leading to the removal of AGEs are better described than the inflammatory events produced by AGEs. The NOD mouse shows increased AGE levels before the development of diabetes, in association with

a specific decrease in expression of AGE receptor-1. Clinical studies of patients with type 1 diabetes with and without complications suggest that the former group also show decreased levels of AGE receptor-1. Epstein-Barr virus-transformed lymphocytes derived from patients with severe complications, who have increased serum and tissue AGE levels, similarly show a decrease in expression of AGE receptor-1, suggesting that deficiency of this receptor, which is involved in AGE removal, represents a specific genetic component to the propensity to complications.

In another series of experiments, Vlassara pointed out that the protein lysozyme exhibits an 18-amino acid domain that shows AGE binding. Lysozyme-AGE complexes have marked enhancement of uptake and degradation, suggesting a mechanism of clearance. Indeed, AGE-mediated cytokine stimulation decreases in the presence of lysozyme. Studies of treatment with lysozyme in mouse models of type 1 and type 2 diabetes show increased urinary AGE excretion, with a reduction in albuminuria. Lysozyme-type peptides have specificity for a number of reactive AGEs and show high degrees of renal clearance, without evidence of toxicity, suggesting an important approach to therapy. Macrophages transfected to produce human lysozyme show a decrease in AGE-promoted TNF- α production, whereas a lysozyme construct that lacks the AGE binding domain has no effect. Finally, Vlassara reviewed a study of mouse models of type 1 and type 2 diabetes placed on low-AGE diets (eliminating heated products) for a 1-year period. The renal AGE accumulation and laminin- β_1 and TGF- β_1 overexpression typically seen in diabetes decreased, with prevention of albuminuria and evidence of advanced nephropathy, suggesting dietary AGEs to be "a major driving motor" of diabetic complications.

Several studies by Vlassara and co-workers gave further information pertaining to food-related AGEs. Koschinsky et al. (abstract 581) showed that AGEs derived from Coca-Cola, Nescafe, and Cacao Sarotti stimulated expression of platelet P-selectin and lysosomal integral membrane protein. Platelet activation may explain a tendency to thrombotic and ischemic events in the postprandial period. Zheng et al. (abstract 662) studied

diabetic mice placed on a diet low in glycotoxins, showing prevention of the increase in serum and renal AGE levels, of albuminuria, and of histological evidence of glomerular hypertrophy and sclerosis. Such a diet may play a role in patients with renal insufficiency. Chen et al. (abstract 220) transfected macrophages with lysozymes, AGE binding proteins that accelerate AGE clearance from circulation, enhancing cellular uptake and degradation, and showed suppression of AGE-dependent cell activation effects, such as expression of TNF- α and IGF-1A mRNA. Vlassara et al. (abstract 660) incubated mesangial cells with lysozymes, similarly showing suppression of AGE response. Further, she showed that AGE-bovine serum albumin induced mesangial cell lysozyme, a potential protective mechanism. Zheng et al. (abstract 663) treated diabetic mice with lysozyme, decreasing serum AGEs and increasing urinary AGE excretion and showing decrease in progression of albuminuria without adverse effect on renal function.

A number of additional presentations presented information pertaining to AGEs and nephropathy. Matsumura et al. (abstract 225) showed that overexpression of the enzyme glyoxalase I, which detoxifies the AGE precursors glyoxal and methylglyoxal, blocked the increase in angiotensin 2 seen in a retinal cell line with hyperglycemia. This angiogenesis-regulating factor may play a role in the pericyte loss and capillary regression of diabetes. Fu et al. (abstract 557) showed that glycated LDL increases expression of macrophage interleukin-8 via activation of protein kinase C and inhibition of nerve factor- κ B (NF- κ B). Koya et al. (abstract 644) reported a decrease in albuminuria in rat models of type 1 and type 2 diabetes with 4–6 months' oral administration of a protein kinase C β inhibitor. Yeh et al. (abstract 1414) showed that car-

boxymethyl lysine modified human serum albumin-induced NF- κ B target gene transactivation in monocytes via the receptor for AGEs (RAGE), increasing interleukin-1 β , TNF- α , and macrophage chemoattractant protein-1 secretion via mitogen-activated protein kinase activation and suggesting a link between AGEs and inflammation. Ihm et al. (abstract 572) showed that AGEs increase proliferation of aortic vascular smooth muscle, with evidence of activation of mitogen-activated protein kinase. Stitt et al. (abstract 197) showed that AGEs interact with endothelial receptors within caveolae, organelles involved with sequestration of membrane proteins, receptor binding, and vasopermeability responses.

Hudson et al. (abstract 824) reported that 56% of 106 type 2 diabetic patients with retinopathy, 73% of 101 without retinopathy, and 69% of 100 nondiabetic individuals showed the TT phenotype of the -429 polymorphism of RAGE, potentially altering RAGE expression and playing a role in microvascular disease. Beisswenger et al. (abstract 301) studied 20 patients with type 1 diabetes treated with regular or lispro insulin for 2-month periods. Postprandial glucose increments correlated with the increase in the toxic dicarbonyls methylglyoxal and 3-deoxyglucosone. Lispro insulin reduced postprandial glucose by 45 mg/dl and decreased the degree of formation of these AGE precursors. Johno et al. (abstract 575) showed that the AGEs imizazolone and N $^{\epsilon}$ -carboxymethyl lysine (CML) are formed from 3-deoxyglucose. Lin et al. (abstract 583) studied the effect of benfotiamine, a lipid-soluble thiamin derivative that decreases the intracellular formation of CML and methylglyoxal-derived AGEs. Six type 1 diabetic patients treated with benfotiamine (600 mg/day) for 28 days had a nonsignificant decrease in HbA $_{1c}$ from 7.2 to 6.9, but showed 40

and 70% falls in erythrocyte CML and methylglyoxal.

Treatment to decrease AGE formation may hold promise for prevention of complications of diabetes. Wolffbuttel et al. (abstract 618) incubated carotid arteries from rats with and without diabetes for 7 days in 5 and 25 mmol/l glucose, respectively, with the AGE breaker ALT-711 (4,5-dimethylthiazolium bromide), showing increase in carotid diameter at 80 mmHg perfusion pressure to control levels, suggesting reversal of the increased arterial stiffness caused by diabetes. Aminoguanidine inhibits AGE formation but causes vitamin B $_6$ deficiency by reacting with pyridoxal or pyridoxal phosphate. Miyoshi et al. (abstract 647) treated diabetic rats with aminoguanidine or an aminoguanidine-pyridoxal adduct that prevents loss of vitamin B $_6$, showing a decrease in albuminuria only with the latter and a greater decrease in glomerular volume and glomerular basement membrane thickness with the adduct than with aminoguanidine alone. Kern et al. (abstract 704) treated diabetic dogs with aminoguanidine for 5 years, preventing retinal microaneurisms and pericyte ghost formation and decreasing the fall in nerve conduction velocity by half, without amelioration or renal disease. A control group treated with aspirin showed inhibition of acellular capillaries in the retina and inhibited collagen-induced platelet aggregation, without effect on neural and renal abnormalities. Lin et al. (abstract 707) administered Tenilsetam [\pm -3-(2-thienyl)-2-piperazinone], an antidementia drug that lacks NOS-inhibitory and antioxidant actions but inhibits AGE formation, to streptozotocin-induced diabetic rats for 9 months, blocking formation of 3-deoxyglucose and methylglyoxal AGEs, with a 70% decrease in acellular capillaries to near control levels.