International Diabetes Federation Meeting, 1997

Nephropathy, retinopathy, and glycation

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This is the sixth and final report on the International Diabetes Federation (IDF) meeting held in Helsinki, Finland, in July 1997. It deals with a variety of topics related to neuropathy as well as with retinopathy and glycation.

Improving Prognosis of Nephropathy

At a symposium prior to the IDF meeting on improving the prognosis of type 1 diabetes, Per-Logstrup Poulsen, Aarhus, Denmark, discussed the characteristics and prognosis of patients with type 1 diabetes and normoalbuminuria. He noted that by the time a patient has microalbuminuria with urinary albumin excretion (UAE) >20 μg/min, there may be substantial renal pathology, and hypertension with loss of the normal nocturnal blood pressure fall is common. Thus, prevention of progression to microalbuminuria would be of great clinical benefit. In a group of 117 normoalbuminuric patients, those with UAE above the median value of 4.2 μg/min had mean HbA_{1c} 0.4% higher and systolic and diastolic blood pressure 5 and 3 mmHg higher than those below this level of albuminuria. There was also an association of increased night:day diastolic blood pressure ratio with increased albuminuria (1). Pointing out the association of hypertension, particularly nocturnal hypertension, with retinopathy in normoalbuminuric patients, Poulsen concluded that high normal albuminuria, particularly with poor metabolic control, is a risk factor for progression from normo- to microalbuminuria. Further, cigarette smoking is also a risk factor for progression, as it is for autonomic neuropathy with decreased heart rate variability. Poulsen showed data from the EURODIAB Controlled Trial of Lisinopril in IDDM (EUCLID) study demonstrating that in normotensive type 1 diabetic patients with UAE 20–70 μg/min treated with lisinopril or placebo, there was no threshold for benefit of treatment based on the degree of albuminuria (2). Thus, he suggested that angiotensin-converting enzyme inhibitors (ACEIs) should be investigated for use in normoalbuminuric patients to prevent progression to microalbuminuria.

Hans-Henrik Parving, Gentofte, Denmark, discussed improvement of prognosis in incipient and overt renal disease. Causes of renal disease include genetic factors, such as the insertion/deletion polymorphisms of the ACE gene; hemodynamic factors, such as increased glomerular pressure; local and circulating growth factors, including angiotensin II (A2); and the degree of glycemic control. Treatment options that have been explored include fish oil and lipid-lowering treatment, neither of which has been shown to have benefit, decreased dietary protein, and blood pressure treatment, in addition to the improvement of glycemia. In a meta-analysis, Wang and coworkers assessed a series of studies largely carried out in Scandinavia that showed that intensive glycemic control decreased the rate of development of nephropathy two- to threefold, presaging the findings of the Diabetes Control and Complications Trial (DCCT) (3). A recent analysis of the DCCT data suggests that intensive treatment leads to an average of 6 fewer years of end-stage renal disease (ESRD) per patient with type 1 diabetes—a critical achievement (4). Many studies have shown progression rates from microalbuminuria to macroalbuminuria of 20–40% without and of 5–10% with ACEI treatment. A meta-analysis of the Eurodiab data suggested a 2.5-fold greater rate of maintenance of normoalbuminuria with ACEI treatment (5). Finally, in patients with advanced nephropathy, low dietary protein may have a role in treatment (6), and ACEI treatment preserves the glomerular filtration rate (GFR) as well as decreasing albuminuria. Studies from Denmark, Finland, and Sweden suggest that the average survival for individuals with nephropathy has improved from about 7 to 14 years over the past one to two decades, further emphasizing the benefits of current therapeutic approaches.

In this context, the discussion by Eberhard Ritz, Heidelberg, Germany, of the improvement of prognosis in ESRD was relevant. The incidence of ESRD has increased in Germany, at a current cost of 1.3 billion DM/year. The mortality is mainly (57%) from cardiac complications, with sepsis from foot ulcers and cachexia being other prominent causes. Noting that among patients admitted to his center for dialysis, 80% had inadequately treated blood pressure, 95% were not receiving lipid-lowering treatment despite average cholesterol levels of 246 mg/dl and triglyceride levels of 296 mg/dl, and only 47% had had an ophthalmological examination within the previous year, he stressed physician education for "informing the medical community about the novel opportunities for preventing diabetic nephropathy."

At the IDF meeting, Per-Henrik Groop, Helsinki, Finland, spoke on the question of whether cholesterol lowering improves the prognosis of patients with nephropathy. The mortality of patients with proteinuria is 80-fold that of patients without proteinuria, and both micro- and macroalbuminuria increase cholesterol in the LDL, VLDL, and intermediate-density lipoprotein (IDL) groups. IDL and VLDL triglycerides increase

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Abbreviations: A2, angiotensin II; ACEI, angiotensin converting enzyme inhibitor; AG, aminoguanidine; AGE, advanced glycation end product; CCB, calcium channel blocker; CML, carboxymethyl lysine; CVD, cardiovascular disease; DCCT, Diabetes Control and Complications Trial; ESRD, end-stage renal disease; EUCLID, EURODIAB Controlled Trial of Lisinopril in IDDM; GFR, glomerular filtration rate; IDF, International Diabetes Federation; IDL, intermediate-density lipoprotein; LMW, low molecular weight; RAGE, receptor for AGE; UAE, urinary albumin excretion.

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

LDL particle size and decrease LDL particle mass. In several animal studies, lipid lowering decreases glomerulosclerosis. A study of patients with advanced nephropathy showed that the fall in GFR was most strongly correlated with the degree of hypercholesterolemia, but there is conflicting evidence as to whether treatment with statins is of benefit in decreasing albuminuria or in preserving the GFR. Thus, while the role of lipid-lowering treatment for prevention of CVD is unquestioned, that in nephroprotection is as yet uncertain.

Genetic and Other Factors in the Treatment of Nephropathy

In another presentation at the IDF meeting, Parving further discussed genetic and nongenetic factors in the treatment of diabetic nephropathy. Diabetes is the leading cause of ESRD and is responsible for 25% of cases in Europe and 35% in the U.S., with the number increasing progressively. Eight percent of the health care budget in Europe goes to diabetes, with 80–90% of this for costs of complications of diabetes. Microalbuminuria is associated with increased retinopathy, neuropathy, foot ulceration, and cardiovascular disease (CVD), as well as being the major marker of incipient nephropathy. Treatment involves improving metabolic control and the administration of ACEIs, with treatment decreasing risk of progression two- to fivefold.

Albuminuria is a risk marker for subsequent decline in GFR. In patients withdrawn from ACEI treatment after 8 years, albuminuria levels increase to those seen in patients who received placebo for the same period, but the GFR is preserved. Further, those who have the greatest fall in albuminuria have the least increase in GFR. There is similar evidence in patients with macroalbuminuria that the fall in GFR is correlated with the degree of albuminuria, as well as with HbA_{1c} and with mean blood pressure. Parving reviewed data from the Steno Diabetes Center suggesting that treatment directed at glycemic and blood pressure control led both to improvement in albuminuria and to decreases in the rapidity of fall in GFR.

An important part of understanding the role of ACEIs in prevention of nephropathy has been the demonstration of the adverse effects of the DD polymorphism in the ACE gene, which is associated with a doubling of circulating ACE levels. Patients with the DD or ID genotype have less fall in blood pressure and albuminuria when treated with ACEIs than do those with the II genotype. ("D" indicates "deletion" and "I" indicates "insertion" in the genome.) Furthermore, patients with the DD genotype have a greater fall in GFR than do those with the other genotypes. There are additive effects of having certain A2 receptor phenotypes in patients with DI or II ACE genotypes. A question is whether patients in the highest risk group based on ACE genotype and A2 receptor phenotype would benefit from additional blood pressure–lowering treatment beyond ACEIs alone. Current data suggest that below a mean blood pressure of 90 mmHg, ACEI and other blood pressure treatments have similar efficacy, with ACEI showing greater renoprotection only as the mean blood pressure increases above this level. The mean decline in GFR is 2 ml · min^{-1} · $year^{-1}$ at a mean blood pressure below 100 mmHg, 4 ml · min^{-1} · $year^{-1}$ at 105 mmHg, and 8 ml · min^{-1} · $year^{-1}$ at 110 mmHg, and at each blood pressure level patients with a lower HbA_{1c} have a lower fall in GFR, "suggesting an interaction between metabolism and hemodynamics." Parving concluded that "the remarkable thing about treating diabetic nephropathy is that you are saving money," and he mentioned a recent report analyzing results of these approaches and showing a direct benefit of $33,000 and an indirect benefit of $48,000 per patient treated (7).

Burt et al. (abstract 64) studied 58 patients with microalbuminuria for a mean follow-up period of 8 years. The mean rate of fall of GFR was 4.32 ml · min^{-1} · $year^{-1}$. Both mean HbA_1 and mean diastolic blood pressure were significantly and independently related to a faster rate of progression, while the II ACE genotype was independently associated with a slower rate of GFR fall. Tarnow et al. (abstract 66) found similar blood pressure levels with captopril treatment but a much greater fall in albuminuria in patients with the ID or II genotype than in those with the DD genotype of ACE. The rate of decline in GFR was lower in this group as well. Patients with the AA rather than the AC or CC genotype of the A2 type 1 receptor gene had a fall in GFR of 3.9 vs. 2.5 ml · min^{-1} · $year^{-1}$, with the lowest rate of progression in patients with both ID/II and AC/CC genotypes. However, Demidova et al. (abstract 1439) showed that hypertensive type 2 diabetic patients had similar serum ACE activity and response to the ACEI perindopril regardless of ACE gene insertion/deletion genotype. II genotype carriers did show a stronger correlation of ACE activity with the blood pressure response to treatment.

Ebbehoj et al. (abstract 37) presented data suggesting that ambulatory blood pressure monitoring is particularly useful in detecting the "white coat effect" in patients with diabetes whose clinic blood pressure is high but who are normoalbuminuric. Only 5 of 31 normoalbuminuric patients required initiation of antihypertensive treatment, with another 7 requiring intensification of ongoing treatment. Farmer et al. (abstract 62) analyzed ambulatory blood pressure in 26 patients with macroalbuminuria. Those patients whose mean sleeping blood pressure was >10% below the mean level while awake had a rate of decline of creatinine clearance of 2.9 ml · min^{-1} · $year^{-1}$, significantly less than "non-dippers," whose clearance fell by 7.9 ml · min^{-1} · $year^{-1}$. Nannipieri et al. (abstract 63) sought to find whether a mutation in the gene encoding atrial natriuretic peptide is related to susceptibility to diabetic nephropathy in 442 type 1 diabetic patients and 58 healthy controls. Of microalbuminuric patients, 23% had the mutation, in comparison to 10% of those with normoalbuminuria, 8% of those with macroalbuminuria, and 7% of controls.

How ACEIs Act and Whether A2 Blockers Are of Similar Efficacy

Mark Cooper, Heidelberg, Australia, discussed the questions of how the ACEIs act, whether A2 blockers are of similar efficacy, how ACEIs compare with calcium channel blockers (CCBs), when treatment should be started, and whether we have sufficient data about ACEI treatment of patients with type 2 diabetes. The classic concept of ACEI action is that with increased filtration per nephron, particularly with decreased renal mass, ACEIs decrease glomerular hyperfiltration. In a study of normotensive microalbuminuric patients treated with captopril (8), decreased progression to macroalbuminuria and borderline significant protection against decreased GFR were noted, but longer follow-up is necessary to definitively document these benefits. Biopsy studies suggest an effect on renal structure as well. There may also be nonhemodynamic factors. For example, one of the actions of A2 is to increase levels of transforming growth factor-α, which increases matrix protein production. Cooper cited animal studies showing similar benefits of ACEIs and A2 blockers and concluded that there is no evidence at this

point favoring either, unless the patient has a complication such as cough for which ACEI discontinuation would be advisable. He noted that albuminuria is a continuous variable and that in a given patient there can be a linear increase through the normoalbuminuric range to microalbuminuria and then to macroalbuminuria. A controlled study of enalapril showed that this slow rise in albuminuria can be prevented and that a fall in GFR can be blocked. At least in a subset of normoalbuminuric patients, then, enalapril treatment is useful.

The dihydropyridine CCB nifedipine appears to increase albuminuria, but other CCBs seem to have a benefit similar to that seen with ACEIs in decreasing albuminuria, particularly if blood pressure is controlled equally with both. In a multicenter European study comparing the A2 blocker losartan with the dihydropyridine CCB amlodipine, there was a lower fall in blood pressure but a greater fall in albuminuria with the former, although a study comparing amlodipine with the ACEI cilazapril showed a similar decrease in albuminuria and prevention of fall in GFR (9). Other studies suggest that combination treatment with CCBs and ACEIs may be more effective than treatment with either alone. Particularly in view of the strong association between albuminuria and CVD, it would be important to assess the effects of these drugs singly and in combination on CVD mortality and morbidity in individuals with diabetes. In research presented at the IDF meeting, the Lacidipine Multi-Center Study (abstract 2128) presented data pertaining to patients with type 2 diabetes treated for hypertension with this CCB for 12 weeks. (Abstract numbers are from Abstracts of the 16th International Diabetes Federation Congress, Helsinki, 20–25 July 1997, *Diabetologia* 40 [Suppl. 1]:A1–A722.) Fourteen microalbuminuric patients showed a decrease in albuminuria from 40 to 25 mg/l, but 87 normoalbuminuric patients had an increase in albuminuria from 3 to 13 mg/l, with 18% entering the microalbuminuric range, suggesting the possibility of an adverse effect. Crepaldi et al. (abstract 2145) compared the effects of lisinopril and nifedipine with placebo on the progression to overt proteinuria in 103 normotensive patients with type 1 diabetes and microalbuminuria who were followed for 3 years. The risk of progression to macroalbuminuria decreased 58% and 63% with the two treatments, although only lisinopril decreased the albumin excretion rate to within the microalbuminuric range, suggesting that microalbuminuria did not itself contribute to the progression of renal damage. Bojestig et al. (abstract 2139) showed that in type 1 diabetic patients with microalbuminuria and HbA_{1c} averaging 7.4%, ramapril in doses of 1.25 and 5 mg daily was associated with lack of progression of albuminuria, but that this also was seen with placebo. These authors suggested that microalbuminuric patients with good glycemic control may not need ACEI treatment.

Glycemic Control

An effect of glycemic control on the progression of nephropathy in type 2 diabetes was suggested by a report by Thorsby et al. (abstract 38). Of 54 individuals followed for a mean of 5 years, 26 treated with sulfonylureas had gradually worsening HbA_{1c}. Of these, 21 required insulin because of HbA_{1c} >10%. Those treated with insulin throughout the study had a decrease in HbA_{1c} from 8.7 to 7.8%. Of 23 patients with average HbA_{1c} <8.0%, none had progression of albuminuria from normo- to microalbuminuria or from micro- to macroalbuminuria, while 6 of 31 with average HbA_{1c} ≥8.0% had such a progression. Relimpio et al. (abstract 2080) studied 1,348 patients with type 2 diabetes, 461 of whom were microalbuminuric and 120 of whom were macroalbuminuric. In multivariate analysis, HbA_{1c}, creatinine, hypertension, male sex, age, diastolic blood pressure, coronary heart disease, and hypolipidemic therapy were independently associated with albuminuria category. Bangstad et al. (abstract 2085) performed renal biopsies in 18 patients with type 1 diabetes and microalbuminuria. Initial basement membrane thickness and mean HbA_{1c} during 6 years of follow-up were significantly correlated with the degree of progression of albuminuria. However, Molnar et al. (abstract 2078) found no correlation between albuminuria status in type 2 diabetic patients and glycemic control, cholesterol, or HDL cholesterol. They did find a positive correlation with triglycerides, uric acid, and serum creatinine, suggesting that the control of the insulin-resistant state may be of greater importance than that of glycemia in type 2 diabetes.

Dietary Protein and Progression of Diabetic Nephropathy

Siebenhofer et al. (abstract 2099) showed that not only does the GFR increase in patients with type 1 diabetes, but paradoxically it decreases after an oral protein load, which is opposite to the effect seen in individuals without diabetes. Hansen et al. (abstract 2134) showed that a 0.6 $g \cdot kg^{-1} \cdot day^{-1}$ protein diet reduced GFR in type 2 patients with nephropathy by 0.6 $ml \cdot min^{-1} \cdot (0.73\ m^2)^{-1}$ and decreased albuminuria by 28%, in comparison to a 1.0 $g \cdot kg^{-1} \cdot day^{-1}$ protein diet. Takahashi et al. (abstract 2136) restricted dietary protein to 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ in 22 patients with type 2 diabetes and nephropathy and observed decreased serum albumin and total protein, suggesting that nutritional status should be monitored closely during these diets. Addressing another component of diet, Mamos et al. (abstract 2113) studied 30 patients with type 1 diabetes and microalbuminuria and found that increasing dietary sodium from 83 to 302 $mmol \cdot l^{-1} \cdot day^{-1}$ increased albuminuria from 47 to 77 μg/min, as well as increasing mean blood pressure from 94 to 99 mmHg.

Heparin for Treatment of Diabetic Nephropathy

In a fascinating presentation, Allan Kofoed-Enevoldsen, Denmark reviewed the use of heparin for treatment of diabetic nephropathy. Heparin was discovered 80 years ago, and clinical use began 60 years ago. Heparin is a glycosaminoglycan polysaccharide composed of 20 to 120 linked saccharides, most of which are sulfated, with molecular weight of 5,000–30,000. Low molecular weight (LMW) heparin is composed of 16–32 saccharides and has molecular weight of 4,000–8,000. Unfractionated heparin is taken up by the endothelium and reticuloendothelial system, while LMW heparin is principally excreted in the urine in a more predictable fashion and with a longer half-life, allowing once-daily administration. Heparin may act in plasma, on extracellular matrix, or on the cell surface, where it interferes with the binding of various substances to certain receptors and activates other receptors, perhaps including specific heparin receptors. Heparin is also taken up by cells and may act intracellularly. In addition to its anticoagulant effect, heparin decreases angiogenesis, cellular proliferation, and matrix production. In vitro, heparin inhibits vascular smooth muscle growth at levels similar to those used for anticoagulation. This can be seen with small polysaccharides of four to six units and with heparan sulfate as well and may reflect inhibition of phosphatidyl inositol-4-phosphate kinase, which decreases protein kinase C

activation. Heparin decreases collagen accumulation after balloon injury of the carotid and decreases the inflammatory response, so it may be of direct benefit in decreasing CVD. Heparin also increases endothelial cell production of heparan sulfate proteoglycans, maintaining the charge of the glomerular capillary basement membrane, although extracellular heparan sulfate accumulation may decrease, so the overall story is complex. Heparin binds to matrix proteins, such as fibronectin, laminen, and collagen; to growth factors, such as fibroblast growth factor; to serine protease inhibitors, such as antithrombin III; and to other substrates, such as lipoprotein lipase and apoproteins A1 and B.

In examining the effects of heparin at the renal level, Purkeson et al. showed in a remnant kidney model that unfractionated heparin and an N-desulfated/acetylated heparin devoid of anticoagulant effect prevented the hypertension and fall in GFR seen with placebo (10). Both heparin preparations decreased glomerular sclerosis eightfold. Ganbaro et al. showed that streptozotocin-induced diabetic rats developed hyperfiltration with or without heparin treatment, but that albuminuria and increased glomerular basement membrane width were prevented by heparin treatment (11). Myrup treated 39 microalbuminuric patients with unfractionated heparin (5,000 U) or LMW heparin (2,000 U) twice daily for 3 months (12). There was no change in GFR, HbA_{1c}, or blood pressure, but albuminuria decreased with both heparin preparations and increased with placebo. Finally, Tamsma et al. reported that six patients with type 1 diabetes and nephropathy showed decreased albuminuria with heparin treatment (13). Potential problems are the anticoagulation effects of heparin, although this has potential benefits, the possibility of heparin-induced osteoporosis, and the need for injection (the heparin could not be mixed with insulin preparations containing protamine, which would reverse the heparin action). The mechanism of action still must be identified, and it may be possible to identify specific oligosaccharides that could be administered orally. Kofoed-Enevoldsen wondered why this topic has received so little attention, while ACEI and glycemic control benefits are much more widely recognized and have been so intensively studied. He commented that the area might represent "the future in diabetic nephropathy."

In related presentations, Shestakova et al. (abstract 2150) described studies in which they administered the LMW heparin glycosaminoglycan sulodexide for 3 weeks at a dose of 600 U/day i.m. to nine patients with microalbuminuria and nine with macroalbuminuria. Albuminuria decreased in both groups, and there was a lipid-lowering effect in macroalbuminuric patients, with cholesterol falling from 7.3 to 5.6 mmol/l. Poplawska et al. (abstract 2152) reported a similar effect in 15 type 1 diabetic patients, with microalbuminuria decreasing from 95 to 53 μg/min at 1 week and to 27 μg/min after 6 weeks, remaining at 36 μg/min during follow-up. Desenzani et al. (abstract 2146) and Desenzani et al. (abstract 2148) administered picotamide, a platelet thromboxane synthetase inhibitor and thromboxane antagonist, in a dose of 300 mg three times daily, or placebo, for 1 year to 30 patients with type 2 diabetes and microalbuminuria. Urinary thromboxane B2 excretion fell and albuminuria decreased from 60 to 30 μg/min with treatment without changes in blood pressure or glycemia, while albuminuria increased to 80 μg/min with placebo.

Other Issues in Nephropathy

Addressing another hematological problem, Yun et al. (abstract 2332) studied 192 anemic diabetic patients with creatinine clearance >30 ml · min^{-1} · m^{-2}. For 51% of these cases, there was no clear explanation for the anemia and serum erythropoeitin levels were 17 mU/ml, in comparison to the expected level of 145 mU/ml. The degree of anemia was correlated with albuminuria, suggesting that erythropoeitin deficiency plays a role in anemia in many diabetic patients without frank renal insufficiency.

Not all renal disease in individuals with diabetes represents diabetic nephropathy. Koselj et al. (abstract 2118) performed renal biopsies in 40 type 2 diabetic patients with rapidly progressive renal failure, unexplained renal failure, macrohematuria, unexpected nephrotic syndrome and acute nepritic syndrome, and normal or enlarged kidneys. Eight patients had only diabetic nephropathy, while glomerulonephritis was found in 27, acute interstitial nephritis in 3, fibrillary glomerulopathy in 1, and cholesterol microembolization in 1, suggesting that renal biopsy is an important procedure in patients with an unusual clinical course. Kibriya et al. (abstract 2130) performed renal biopsy in 29 proteinuric type 2 diabetic subjects either without diabetic retinopathy or with hematuria. Eight had diabetic nephropathy, and the remainder had glomerulonephritis. Addressing another factor potentially exacerbating diabetic renal disease, Silveiro et al. (abstract 2103) found that 20 type 2 diabetic patients who had undergone nephrectomy showed 45 and 25% prevalence of micro- and macroalbuminuria, in comparison with nondiabetic subjects, who had respective prevalences of 18 and 6%.

Retinopathy

At the symposium on improving the prognosis of type 1 diabetes, Eva Kohner, London, U.K., spoke on current approaches to improving prognosis for vision in diabetes. She stressed the DCCT data showing the effect of glycemic control on retinopathy, and in addition discussed the relationship between hypertension and retinopathy. The Wisconsin Epidemiologic Study of Diabetic Retinopathy showed an association of hypertension not only with the prevalence and incidence of retinopathy, but also with the rate of progression of background retinopathy to proliferative retinopathy. Her own data show that individuals whose retinopathy progressed had an average blood pressure of 156/84 mmHg, significantly higher than the level of 143/84 mmHg among those whose retinopathy did not progress (14). This may reflect the role of blood pressure treatment in restoring blood-retinal barrier permeability. In the EUCLID study, lisinopril administration to normotensive patients with no or mild microalbuminuria decreased the incidence and progression of retinopathy. Other risk factors for retinopathy include hypercholesterolemia, which increases maculopathy sevenfold and proliferative retinopathy fourfold, although Kohner did not show evidence that lipid-lowering treatment is of benefit in this regard.

Kohner also discussed the need for caution in performing cataract surgery in diabetic patients. She showed that in contrast to the >95% improvement among nondiabetic patients who undergo this procedure, there is only a 58% improvement among diabetic patients, along with a 20% deterioration in vision. Patients with maculopathy and proliferative retinopathy are particularly likely not to improve after cataract surgery. Those developing macular edema following cataract surgery require relatively urgent laser treatment.

In research presented at the IDF meeting, Kullberg et al. (abstract 1949) studied all patients in southeast Sweden who experienced onset of type 1 diabetes before the age of 36 and during the years 1983–1987.

Patients with mean HbA_{1c} levels from diabetes onset in the highest quartile (>8.6%) had a relative risk of retinopathy of 4.2 in comparison to patients in the lowest quartile (<6.6%). In addition, 61 of 166 patients with diabetes onset after 14 years of age had retinopathy, compared to 41 of 188 patients with younger onset (relative risk 1.7). Henricsson et al. (abstract 1972) studied 1,378 diabetic patients who were >40 years old at diagnosis. Of these, 333 were treated with insulin throughout the 3-year study, 871 were treated with diet or oral agents, and 174 had their treatment changed to insulin therapy during follow-up. Insulin treatment at baseline was not associated with an increased risk of retinopathy progression, but patients who changed treatment from oral agents or diet alone to insulin therapy had a relative risk of impaired vision caused by retinopathy progression of 2.7 compared with all other patients in the study. Poor glycemic control before the start of insulin therapy as well as the presence of retinopathy at baseline were significant risk factors for progression in the group with changed treatment. Nakagami et al. (abstract 1985) measured HbA_{1c} monthly for 10 years in 159 type 2 diabetic patients with background retinopathy at baseline. HbA_{1c}, diastolic blood pressure, and duration of diabetes were the risk factors for the progression to proliferative retinopathy. The prevalence of proliferative retinopathy was 8% (2/26) in the group with mean HbA_{1c} <7%, 5.3% (3/57) in the group with mean HbA_{1c} of 7.0–7.9%, 12.5% (5/40) in the group with mean HbA_{1c} of 8.0–8.9%, and 30.6% (11/36) when the mean HbA_{1c} exceeded 9%. Proliferative retinopathy was 2.5 times more frequent in patients with diabetes duration over 10 years, and the prevalence of proliferative retinopathy was 6 times higher in those whose diastolic blood pressure was ≥90 mmHg.

The EUCLID Study Group (abstract 1966) examined the effect of the ACEI lisinopril on the progression of retinopathy in a 2-year randomized controlled clinical trial of lisinopril versus placebo in 530 type 1 diabetic patients without macroalbuminuria. Of these patients, 336 had gradable photographs at 0 and 2 years, with a baseline prevalence of retinopathy of 66% in the placebo group and 60% with lisinopril. With placebo there was a 26% progression, while progression was 12% with lisinopril, for a relative risk of 0.41. Lachin et al. (abstract 2389) analyzed familial aggregation of retinopathy and nephropathy for 241 relatives of 217 DCCT subjects. The odds ratio for severe retinopathy among relatives of DCCT subjects with retinopathy was 3.1, and that for nephropathy was 5.4, with severity of retinopathy between parents and children also showing intrafamilial correlations. Stratton et al. (abstract 60) studied 788 patients in the U.K. Prospective Diabetes Study who had both eyes photographed and graded at years 0, 3, 6, and 9 and had direct ophthalmoscopy at year 0. Graded retinal photography showed greater sensitivity than ophthalmoscopy determination of severity in evaluating the risk of progression to photocoagulation at 9 years.

Danis et al. (abstract 59) reported that the protein kinase C inhibitor LY333531 decreases preretinal and optic nerve head neovascularization seen after branch retinal vein occlusion in pigs. There may be a role for this agent in the therapy of diabetic neovascular eye disease, which is also ischemia-mediated. Kern et al. (abstract 1932) studied alloxan-induced diabetic rats and nondiabetic rats fed 30% galactose that were randomly assigned to dietary supplementation with 0.1% α-tocopherol acetate and 1% ascorbic acid. The antioxidant diet significantly inhibited the development of acellular capillaries in retinas of diabetic rats and normalized the diabetes-induced increase in osmotic fragility of erythrocytes. Galactose-fed rats showed no defect in osmotic fragility, and antioxidants caused no significant inhibition of galactose-induced metabolic abnormalities or histopathology in the retina.

Obayashi et al. (abstract 1935) reported increased levels of vitreous advanced glycation end products (AGEs) and vascular endothelial growth factor in samples obtained during vitrectomy from 14 patients with proliferative diabetic retinopathy in comparison to samples from 14 patients without diabetes who underwent vitrectomy for retinal detachment. Grattagliano et al. (abstract 1940) reported increased subretinal fluid malondialdehyde and carbonyl proteins in samples obtained at vitrectomy in 8 individuals with diabetes, suggesting increased free radical production and lipid and protein oxidation.

AGEs

The IDF meeting included a symposium on AGEs, at which Michael Brownlee, New York, NY, discussed the increase in knowledge about the complications of diabetes over the past 20 years. From the earliest demonstration of the association of HbA_{1c} with glycosuria, through the DCCT, which demonstrated that reducing HbA_{1c} is associated with decreased incidence and progression of retinopathy, neuropathy, and nephropathy, we have progressed to an understanding of some of the mechanisms of tissue damage from hyperglycemia. Mechanisms include activation of the polyol pathway, the activation of diacylglycerol/protein kinase C, redox changes and the formation of reactive oxygen species, and nonenzymatic glycation of tissues and formation of AGEs. Brownlee reviewed the chemical sequence by which Amidori products are derived from glucose and form dicarbamyls, which react with amine groups on various biological molecules, causing protein crosslinking and the production of a wide variety of other products. Brownlee discussed the question of whether AGEs are associated with hyperglycemia, acting only as a marker of complications, or whether they are part of the pathogenic process that causes the complications of hyperglycemia. AGEs accumulate over 1–5 months in the renal cortex of diabetic animals, suggesting that they may be formed more rapidly than can be explained by the degree of hyperglycemia directly leading to glycation. There are three basic pathogenic effects of AGEs: intracellular glycation, matrix protein glycation, and interaction with specific receptors. Brownlee noted that in experimental animals, lens AGEs are not increased at 2 weeks although they increase sixfold over normal levels after 6 months of diabetes, while intracellular AGEs reach maximal levels after 2 weeks. He showed data suggesting that a specific protein, basic fibroblast growth factor, is the principal modified intracellular protein. This observation may be related to the relationship between hyperglycemia and decreased endothelial growth. Methylglyoxal, formed from degeneration of triose phosphate, a key glycolytic intermediate, either is metabolized by glyoxalase to D-lactate or reacts nonenzymatically with proteins to form AGEs. Administration of a glyoxalase inhibitor increases intracellular AGE levels. In a cell line overexpressing glyoxalase, the usual increase in methylglyoxal with hyperglycemia is blocked and D-lactate levels are increased. AGEs interfere with the collagen matrix and with matrix–cell interactions and block the antiproliferative effect of nitric oxide on smooth muscle. The integrin cell surface receptors identify specific cell types, and AGEs modify these mole-

cules, interfering with growth. Similarly, AGE-laminin modification of neurites decreases axon growth. AGEs also interact specifically with certain receptors, such as the macrophage AGE receptor, leading to increased cytokine production, and they have similar effects on endothelial cells. AGEs also have pro-oxidant effects, which can be blocked by antioxidants, and plasma AGEs are derived in part from intracellular production of highly reactive ketoaldehydes, which modify extracellular glycoproteins. Fructose and AGEs derived from foods may be additional sources of circulating AGEs, and dietary antioxidants may play a role in decreasing AGE formation. For example, carboxymethyl lysine (CML) formation decreases with vitamin E supplementation. All of these factors may contribute to the variability in AGE levels for a given level of glycemic control, perhaps explaining heterogeneity in the frequency of diabetic complications

Vincent Monnier, Cleveland, OH, discussed additional specific aspects of AGE chemistry. In combination with lysine residues on proteins, glucose produces fructose-lysine, the basis of glycated proteins, which can be oxidized, producing CML, or crosslinked, producing pentosidine. Antibodies to pentosidine demonstrate its presence in glomeruli affected by both early and late diabetic nephropathy and in subendothelial areas of the vascular wall, while anti-CML immune staining is most prominent in the renal tubules and the endothelium. Aminoguanidine prevents the changes in staining seen with diabetes. Using skin biopsy samples from patients in the DCCT, Monnier was able to address the relationship of glycemic control to AGEs. He used acid and pepsin solubility of collagen as a marker of crosslinking and determined pentosidine and CML as measures of AGEs. When the results were corrected for duration of diabetes and age, intensive treatment was shown to improve all parameters. Collagen glycation correlated most strongly with the most recent HbA_{1c} determination, while CML correlated best with the mean HbA_{1c} over the entire DCCT study, suggesting the influence of past as well as present glycemia. Skin collagen glycation, an HbA_{1c}-like measure, was most strongly associated with complications, while CML and pentosidine were associated only with neuropathy. However, an overall index of skin glycation and AGE measures did show a significant association with complications, even after correcting for HbA_{1c} levels. Monnier also discussed the new appreciation of the association of intracellular AGE formation with shorter-term elevations in glucose levels. Molecules such as 3-deoxy-glucosone are rapidly formed under conditions of hyperglycemia, and this can be prevented with aminoguanidine.

Helen Vlassara, New York, NY, discussed receptors for AGE. AGE formation is a dynamic process. Any given protein is likely to contain several classes of AGE, early and intermediate reactive glycotoxins, and advanced nonreactive AGEs. During catabolism of tissue components, each class is likely to provide reactive molecules, leading to secondary and tertiary end products, while nonreactive molecules are excreted in the urine. Serum AGE levels are strongly correlated with renal function. Tissue AGEs are important, with the kidney being not only a regulator but also a target of AGEs. Serum AGEs react with collagen and increase levels of certain cytokines and growth factors, affecting matrix protein, glomerular size, albuminuria, and the degree of glomerulosclerosis. It is noteworthy that food-derived and cigarette smoke–derived AGEs contribute to tissue damage. The body has devised mechanisms to deal with the continual onslaught of these toxic substances. AGE receptors were initially felt to be involved in removal of AGEs, but also have been found to act via cytokine and growth factor production to cause tissue remodeling, so they can be involved in cell proliferation, matrix accumulation, thrombosis, and ultimately chronic inflammatory and atherosclerotic changes. A number of cellular AGE receptors have been described. AGE-R1 is expressed on monocyte/macrophages, T-cells, endothelial cells, and renal mesangial and epithelial cells. These receptors are found in the cell as well as on the cell membrane. AGE-R2 is present on neurons. AGE-R3 is present intracellularly and intranuclearly and also is secreted into the circulation. The receptor for AGE (RAGE) is another receptor protein, which has also been found to bind β-amyloid peptide and to play a role in neural growth and in inflammation and oxidative stress. Finally, the scavenger receptor is a specific AGE receptor expressed on monocyte/macrophages that also recognizes modified LDL and is involved in endocytosis, adhesion, and foam cell formation. The three AGE-R receptors are all expressed on endothelium but are regulated differently, with AGE upregulating AGE-R1 and specific ligands phosphorylating and upregulating AGE-R2. Some of the genetic polymorphism in susceptibility to diabetic complications may relate to differing AGE uptake by receptors, so that nondiabetic as well as diabetic NOD mice have increased renal AGE deposition and decreased AGE-R1 and increased AGE-R2 and AGE-R3 expression. Similarly, in patients with diabetes, those without complications have increased AGE-R1 levels, while those with complications have decreased AGE-R1 and increased AGE-R2 and AGE-R3 expression, which may cause increased tissue damage. Soluble AGE-binding proteins may also be important. These include galectins, lysozyme, lactoferrin, and defensins. AGEs bind in a specific fashion to lysozyme, which leads to increased macrophage uptake and degradation, and some studies suggest that there is increased AGE clearance after lysozyme administration. There is also a specific circulating RAGE peptide, which has been used experimentally to increase clearance of circulating AGEs. Applications of these circulating factors may include infusion, topical use for wound healing, and incorporation into dialysis filters.

Hans-Peter Hammes, Diesen, Germany, spoke on trials of glycation inhibitors. He addressed several points that had been brought up by earlier speakers. First, the existence of two pathways to AGE formation, nonoxidative pathways such as those that produce 3-deoxy-glucosone and oxidative pathways such as those that generate CML, suggests that parallel approaches might be taken to the inhibition of AGE formation. Second, the slower occurrence of extracellular compared with intracellular glycation, particularly glycation of basic fibroblast growth factor, which loses its mitogenic activity with AGE modification, suggests another approach to prevention of the adverse effect of AGEs. Third, the AGE receptors should be of therapeutic consequence, with macrophage receptor-induced cellular and matrix proliferation and endothelial receptor-induced vasoconstriction being potential targets of treatment to prevent adverse AGE effects. Fourth, the ability of AGEs to crosslink high-molecular-weight proteins suggests that prevention of this might ameliorate AGE-related diabetic complications. Finally, AGEs modify cell surface proteins such as integrin and laminin, again an area that might allow therapeutic intervention. To date, only a few of these areas have been investigated. Hammes mentioned that metformin has been shown to decrease products of the methylglyoxal

pathway with increased conversion to D-lactate and that several thiamine-related B-complex vitamins may have therapeutic benefit. Preliminary studies have been described of proteins that break collagen crosslinking, one of which is the non–glucose lowering thiazolidinedione derivative OPB-9195, which has been shown to decrease albuminuria in a model of nephropathy. Tenilsetam, a drug developed for dementia, may act as an anti-AGE agent. Most interest to date has been in the compound aminoguanidine (AG). AG reacts with 3-deoxy-glucosone and other nonoxidatively formed compounds to prevent AGE formation in vitro and in a variety of animal models, which show decreased retinopathy, mesangial expansion, and albuminuria. In addition, AG has improved nerve conduction velocity in models of neuropathy. A 3-month Israeli study of 90 patients with renal insufficiency treated with 200–800 mg AG daily showed a modest fall in triglyceride and cholesterol and a 40% fall in albuminuria. Four other clinical trials are ongoing, but no clinical results are available. Hammes stated that one of the trials, in patients with type 1 diabetes with microalbuminuria, had been stopped in Europe because of recruitment and financial constraints but continued in the U.S. However, he did not believe that adverse reactions had led to discontinuation of any of the trials. In a study of treatment of AGE deposition, Nakamura et al. (abstract 2024) presented evidence that progression of nephropathy in spontaneously diabetic rats is prevented by OPB-9195, a new inhibitor of advanced glycation. Renal pathological examinations suggested decreased glomerular disease, with decreased AGE deposition detected by glomerular immunohistochemical staining. Beisswenger et al. (abstract 2314) reported increased levels of the reactive α-dicarbonyl methylglyoxal in 65 patients with type 1 and type 2 diabetes, and these levels were strongly correlated with blood glucose levels. Methylglyoxal has been postulated to produce diabetic complications as a direct toxin and as a precursor for AGEs.

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