

American Diabetes Association Annual Meeting, 1998

Nephropathy and retinopathy

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This is the last of seven reports on the American Diabetes Association (ADA) 1998 Annual Meeting and Scientific Sessions held in Chicago in June. It describes presentations on nephropathy and retinopathy.

Albuminuria and nephropathy

Among the symposia at the meeting was one on preclinical assessment of diabetes complications, at which Bo Feldt-Rasmussen, Copenhagen, Denmark, addressed the implications of microalbuminuria. Nephropathy develops in 30% of patients with type 1 diabetes, with peak incidence after 15 years duration. "We do not know," he stated, "whether albuminuria is a local renal phenomenon or a global phenomenon of vascular beds." Microalbuminuria can be defined as albumin excretion of 20–200 $\mu\text{g}/\text{min}$, 30–300 mg/day , or 30–300 mg/g creatinine. Initial studies of 130 patients with microalbuminuria at Steno and Aarhus in Denmark and at Guy's Hospital in the U.K. showed high rates of renal disease progression over 6–10 years. Predictors of progression from normoalbuminuria to microalbuminuria include high baseline albuminuria (within the normal range), hypertension (and high-normal blood pressure levels), cigarette smoking, and elevated HbA_{1c} . Signs of endothelial dysfunction, atherogenic changes in lipids, and increased evidence of cardiovascular disease, with doubling of cardiac mortality in some studies, are typically associated with microalbuminuria.

Scheen and Van Gaal, for the Diabetes Assistance and Nephropathy Awareness

Investigators, presented a study of 1,374 Belgian subjects with type 2 diabetes (abstract 513; abstract numbers refer to the Abstracts of the 58th Annual Meeting and Scientific Sessions of the ADA, *Diabetes* 47 [Suppl. 1]:1–A496). Microalbuminuria was associated with serum creatinine level, age, known duration of diabetes, body weight, smoking habits, male sex, plasma glucose level, HbA_{1c} level, waist-to-hip ratio, waist circumference, systolic and diastolic arterial pressure, and triglyceride level and inversely associated with HDL cholesterol level. Klein et al. (abstract 601) studied 891 patients with type 1 diabetes, whose 10-year cumulative incidence of renal failure or serum creatinine >2.0 mg/dl was 14.9%. For every 1% increase in HbA_{1c} , the risk of renal failure almost doubled, and hypertension almost tripled the risk. Brancati et al. (abstract 621) analyzed a nationally representative cohort of 984 adults aged 20–74 years with diabetes who participated in National Health and Nutrition Examination Survey II in 1976–1980. During 20 years of follow-up, 37 developed end-stage renal disease (ESRD), an incidence rate of 0.23/100 person-years. The incidence rates were 0.29 and 0.27 for patients with type 1 diabetes and diagnosed type 2 diabetes, but 0.08 in those whose diabetes was detected by a glucose tolerance test, similar to the nondiabetic population. The risk of ESRD was more than doubled by African-American race, by gout, and by hypertension.

Gomes et al. (abstract 492) showed similar, and high, mean intraindividual

coefficients of variation (CVs) of three specimens for determination of overnight urinary albumin excretion in 76 patients with type 1 diabetes (CV 61%) and in 66 with type 2 diabetes (CV 68%). Garg et al. (abstract 501) compared 24-h, timed overnight, and spot urine albumin measurements and albumin:creatinine ratio determinations in 40 patients with type 1 diabetes. Sensitivity was 97%, specificity 88%, and positive predictive value 97% for spot urine testing in comparison to 24-h urine collection for albumin determination.

A number of researchers have investigated the genetic contribution to nephropathy. Fogarty et al. (abstract 47) showed strong evidence of a genetic tendency to nephropathy, most likely with dominant inheritance. Their conclusions were based on a study of urinary albumin:creatinine measurements in 1,029 subjects, 416 with type 2 diabetes and the remainder without diabetes, from 77 multigenerational families. Wilson and Pratley (abstract 500) found 24-h urinary albumin excretion to correlate negatively with insulin sensitivity and to be a familial trait in nondiabetic Pima Indians. Zanchi et al. (abstract 202) compared 182 patients with type 1 diabetes and macroalbuminuria or ESRD to 229 without microalbuminuria. The ϵ -deletion allele in intron 4 of the endothelial nitric oxide synthase gene more than doubled the risk of renal disease. Maeda et al. (abstract 271) showed that macroalbuminuria was associated with increased erythrocyte aldose reductase content in 549 diabetic patients, but that there was no relationship to polymorphism of the aldose reductase gene. Araki et al. (abstract 295) used DNA analysis to determine apolipoprotein E genotype in 154 patients with type 1 diabetes with nephropathy, in 91 patients with type 1 diabetes without nephropathy, and from the parents. The apolipoprotein E ϵ 2 allele increased the risk of diabetic nephropathy, while the ϵ 3 allele seemed to be protective. Oue et al. (abstract 1423) found that 30 patients with type 2 diabetes whose microalbuminuria worsened during a 10-year follow up were older and had higher HbA_{1c} and a greater

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Abbreviations: ADA, American Diabetes Association; bFGF, basic fibroblast growth factor; CV, coefficient of variation; DAG, diacylglycerol; ESRD, end-stage renal disease; GH, growth hormone; MA, microaneurism; MAPK, mitogen-activated protein kinase; $\text{NF}\kappa\text{B}$, nuclear factor κB ; PKC, protein kinase C; VEGF, vascular endothelial growth factor.

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

frequency of the D/D genotype of angiotensin I converting enzyme than 37 whose microalbuminuria did not progress.

The effect of diet on nephropathy was also addressed. Holler et al. (abstract 311) studied the dietary intake of 37 patients with type 1 diabetes and microalbuminuria. Intake of sodium, saturated fatty acids, and mono- and disaccharides was associated with greater degrees of microalbuminuria. Protein intake was not. Among fatty acids, there were positive associations with myristic acid and arachidonic acid and a negative association with linoleic acid. Salt restriction and a restricted intake of saturated fats may have a role in the prevention and treatment of diabetic nephropathy. O'Hayon et al. (abstract 497) measured the glomerular filtration rate, albumin excretion rate, and dietary protein intake of 145 patients who had had type 1 diabetes for 5–10 years. Protein intake was significantly associated with the glomerular filtration rate and with male sex but did not have a relationship to albuminuria. These studies suggest it is incorrect to extrapolate the effects of protein restriction from studies in patients with ESRD not caused by diabetes to the treatment of patients with early diabetic nephropathy.

In another clinically important study, Tan and Tan (abstract 491) compared losartan at a dose of 50 mg daily with captopril at a dose of 25 mg twice daily in 12 normotensive diabetic patients. Albuminuria decreased from ~400 mg/day at baseline to ~300 mg/day at 2 months and to ~150 mg/day at 8 months, with the only difference between groups being development of cough necessitating withdrawal of captopril in two of the treated patients.

Tissue glycation mediated by toxic α -dicarbonyls, such as methylglyoxal, is a cause of glucose toxicity. Beisswenger et al. (abstract 312) measured methylglyoxal production and detoxification in seven type 1 diabetic patients who had developed overt nephropathy prior to having diabetes for 15 years and in seven who had no evidence of nephropathy after ≥ 25 years. Erythrocyte methylglyoxal production was increased in the former group, while its degradation was similar in the two groups. Clements et al. (abstract 199) reported that an orally effective glycation inhibitor, EXO-226, normalized glycated albumin in the *db/db* mouse despite persistent and severe hyperglycemia. The agent decreased urinary albumin excretion, prevented the usual decrease in creatinine clearance, and

diminished the abnormal accumulation of renal glomerular mesangial matrix material by 70%, suggesting a potential therapeutic role for EXO-226. Ceriello et al. (abstract 493) reported decreased cellular antioxidant defenses of fibroblasts obtained from nine patients with type 1 diabetes and nephropathy and cultured for 12–14 weeks under conditions of high glucose, in comparison with fibroblasts from six patients without nephropathy and six control subjects. Jenkins et al. (abstract 498) studied erythrocyte membranes from 30 patients with type 1 diabetes and 18 control subjects with normal serum urea nitrogen and creatinine. Fructose-lysine and glucitol-ethanolamine, markers of glycation, and carboxymethyl-lysine and other markers of glycooxidation/lipoxidation on proteins and on lipids were higher in the diabetic patients and correlated with HbA_{1c}. In addition, carboxymethyl-lysine, a marker of glycooxidation, was increased in patients with microalbuminuria. Tsuchida et al. (abstract 503) reported that OPB-9195, an inhibitor of advanced glycation, decreased transforming growth factor- β and type IV collagen production in a rat model of diabetic nephropathy.

Retinopathy

In a symposium on preclinical disorders in type 1 diabetes, Ronald Kline, Madison, WI, discussed the risk of progression to retinopathy in eyes with microaneurisms (MAs). The pathogenesis of retinopathy starts with hyperglycemia, which leads to increased levels of protein kinase C (PKC), nonenzymatic glycosylation, aldose reductase activity, vasoactive substances, growth factors, and free radicals. These cause both functional changes, such as increased vitreous fluometry and abnormal electrical conductance, and anatomic changes, which are those typically followed clinically. In the Wisconsin epidemiological study of >900 patients aged <30 at onset of diabetes, retinopathy affected 20% of patients at age 15, 60% at age 20, and 80% at age 30. Of the patients studied, ~20% had MAs only. For every 1% increase in HbA_{1c}, for every 10-mmHg increase in systolic blood pressure, and for every 3 years diabetes duration, the risk of retinopathy increased 50%. For patients with 1–5, 6–10, and 11–43 MAs in the worst eye, 10-year progression rates were 15%, 25%, and 35%. An increase in the number of MAs of 15 or more after 4 years was similarly a strong predictor of subsequent worsening,

independent of the level of glycemia and duration of disease. Karunakaran et al. (abstract 151) studied 631 subjects with fasting plasma glucose 99–139 mg/dl in the Early Diabetes Intervention Trial. Four-field color retinal photographs showed retinopathy in 21.4% of subjects, with 5.4% having hemorrhages and/or exudates in addition to MAs. In this study, 19% of subjects with glucose <110 mg/dl, 24% with glucose 110–125 mg/dl, and 22% of those with diabetes had retinopathy, and one patient with impaired glucose tolerance had proliferative retinopathy. This suggests that present World Health Organization and ADA diagnostic criteria for impaired glucose tolerance are above the glycemic threshold for developing diabetic retinopathy. Studying optimal therapeutic approaches, Chew and Ferris (abstract 553), for the Early Treatment Diabetic Retinopathy Research Group, reanalyzed early scatter photocoagulation treatment versus deferral of photocoagulation in 3,711 patients with retinopathy. Scatter photocoagulation was more effective in type 2 than in type 1 diabetes, particularly in patients with more severe early proliferative diabetic retinopathy.

The ADA meeting was remarkable for the new information from basic research that was presented to explain the mechanisms of development of retinopathy and potential approaches to treatment. George King, Boston, MA, characterized diabetic retinopathy as a disease of the microcirculation affecting pericytes and endothelial cells. Pericyte loss and abnormal basement membranes characterize the early stages, with endothelial dysfunction and abnormal blood flow leading to MA formation and macular edema mediated by growth factors such as angiostatin, endothelin, vascular endothelial growth factor (VEGF), and other vasculotrophic hormones. Subsequently, there is hemorrhage, exudate formation, capillary loss, and eventual proliferative retinopathy. Hyperglycemia clearly plays a role in the initial states. Potential mechanisms include advanced glycosylation end product formation, oxidative stress, including lipid glycation and oxidation/reduction alterations, increased sorbitol levels, and increased levels of diacylglycerol, which lead to PKC activation. There are interactions among all four pathways. Oxidants form via auto-oxidation and glycooxidation of glucose, subsequently causing PKC activation. Treatment approaches could include PKC inhibition with LY333531 and antioxidant treatment with vitamin E, noting that vitamin E also

can inhibit PKC action. There is evidence that diacylglycerol (DAG) and PKC cause a number of processes to occur. Increased levels of transforming growth factor- β , collagen, and fibronectin contribute to basement membrane thickening. Signal pathway activation with kinases and prostanoids leads to permeability changes. VEGF and other vascular growth factors are secreted, increasing angiogenesis. King hypothesized that vitamin E can inhibit both glucose oxidation and the metabolism of DAG via DAG kinase, both of which may play a role in glycemic complications. Vitamin E treatment can decrease oxidative stress levels in animal models of diabetes, and there is a dose-response relationship of its effect on DAG kinase activity and retinal DAG levels in animal models. Similarly, vitamin E can decrease glomerular PKC activity towards normal along with normalizing glomerular filtration rate, filtration fraction, and albuminuria in rats. Increased PKC activity in diabetic rat heart, aorta, and retina can also be normalized, and retinal blood flow can be restored with this treatment.

King discussed a clinical trial (reported at the meeting by Bursell et al. [abstract 391]), in which early retinopathy prevention in type 1 diabetes was investigated by looking at serum PKC and retinal blood flow. In the 8-month trial, 50 type 1 diabetic patients received vitamin E at a dose of 1,800 IU/day or placebo, with crossover at 4 months. HbA_{1c} levels were 7.9–8.3% and mean blood pressure levels were 86–90 mmHg without change during treatment. Retinal blood flow increased from 17% and remained normal during and after the 4-month vitamin E washout in the group receiving this agent initially. There was also normalization of renal hyperfiltration, without decreasing the glomerular filtration rate in patients with normal clearance at baseline. Plasminogen activator inhibitor 1 levels were increased at baseline and decreased with treatment. King noted that the recommended daily allowance for vitamin E is 15–45 IU, and supplements usually contain 100–600 IU, so the dose given was clearly pharmacological. Gastrointestinal side effects, increased prothrombin time, increased thyroid stimulating hormone levels, and worsening of retinitis pigmentosa are potential adverse consequences of this treatment, which should therefore be regarded as experimental. Nevertheless, a trial of retinopathy prevention in patients without clinical retinopathy appears justified. In a relevant

study presented at the meeting, Lyons et al. (abstract 1462) reported that the adverse effects of glycated and oxidized LDL particles on cultured retinal capillary cells in vitro could be attenuated by pretreatment of the LDL with vitamin E.

Martin Friedlander, La Jolla, CA, pointed out that retinopathy affects more than 300,000 people in the U.S. and that macular degeneration, another disease associated with proliferation of new vessels, affects more than 600,000 people. Angiogenesis inhibitors might also benefit patients with malignancy, rheumatoid arthritis, and psoriasis. Angiogenesis, the growth of new blood vessels from preexisting capillaries, can be initiated by vascular injury, tumors, local inflammation, and cytokines, with proliferation involving expression of proteolytic enzymes, cell migration, and growth of new cellular elements. The physiological ability to initiate angiogenesis is retained in development, and is displayed, for example, in wound healing and during ovulation and menstruation. Angiogenesis is induced by VEGF, platelet-derived growth factor, transforming growth factor, and angiotensin, and inhibitors include α -interferon, angiotensin, and endostatin.

Integrins are also involved in angiogenesis. The integrins are cell adhesion receptors, with α and β subunits recognizing extracellular matrix components. These are key factors in angiogenesis and are potential targets for therapeutic inhibition. In healing wounds in animals, integrins can be specifically localized, and monoclonal antibodies to these prevent cytokine- and tumor-stimulated angiogenesis. The $\alpha_v\beta_3$ integrin is selectively upregulated only on actively proliferating vascular endothelial cells. This can be demonstrated in animal models of neovascularization of the eye, where an antibody to $\alpha_v\beta_3$ integrin inhibits cytokine-induced proliferation. There are two cytokine-dependent angiogenesis pathways, with VEGF stimulating $\alpha_v\beta_5$ integrin and fibroblast growth factor stimulating $\alpha_v\beta_3$ integrin, and peptides have been discovered that inhibit both pathways. Upregulation of $\alpha_v\beta_3$ integrin is seen in a number of clinical states with neovascularization, and upregulation of $\alpha_v\beta_5$ integrin is seen in fibroblasts and other extracellular elements as well as in endothelium in diabetic retinopathy. Friedlander noted that metalloproteases are required to degrade the extracellular matrix to allow new vessel growth. $\alpha_v\beta_3$ integrin is

required for metalloprotease binding, suggesting an approach to inhibition of cell-associated collagenolytic activity. Friedlander envisioned therapeutic approaches to include “suicide” vascular precursor cells and implantable devices with antiangiogenic antibodies or peptides. He termed this a “less destructive and more effective approach” than laser photocoagulation. In a report at the meeting, Ruggiero et al. (abstract 557) discussed studies on the effects of glucose and insulin on expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in retinal microvascular cells. Insulin, alone or in association with high glucose concentrations, favored angiogenesis by increasing endothelial cell integrin expression while decreasing pericyte integrin expression, which could contribute to pericyte loss.

Lloyd Paul Aiello, Boston, MA, spoke on VEGF. The hypothesis that growth factors underlie proliferative retinopathy was initially suggested by Michaelson. These growth factors include basic fibroblast growth factor (bFGF) and VEGF, which are highly synergistic. This model suggests that blocking growth factors is important as a potential treatment. Because VEGF is produced in the eye and its level increases with hypoxia, it is a potential mediator of both proliferative and nonproliferative retinopathy. Physiological concentrations of VEGF increase retinal endothelial cell growth. In a study of 136 intraocular fluid samples from individuals with diabetes, those from patients with proliferative retinopathy showed high VEGF levels. Pan-retinal laser coagulation decreases VEGF levels. VEGF increases retinal permeability in animals. Studies of nonproliferative retinopathy show the presence of VEGF, and VEGF administration in nonhuman primate models causes decreased retinal blood flow and similar permeability changes. Once the retina is damaged, growth factors are released, and their binding to receptors generates a group of intracellular signals, so intervention could be made at several levels. Retinal hypoxia increases adenosine levels, increasing VEGF. Blocking this signal in animal models decreases VEGF levels. Chimeric receptor antagonists, neutralizing antibodies to VEGF, and antisense oligonucleotides can all prevent hypoxia-induced retinopathy. VEGF binds to a specific receptor, leading to phosphatidylinositol 3-kinase activation and, indirectly, to DAG and PKC activation. Administration of the PKC- β specific isoform inhibitor LY333531 can block the

VEGF-mediated increase in retinal endothelial cell growth and can block the increased permeability seen with VEGF administration.

Lois Smith, Boston, MA, discussed growth hormone (GH) as both a mediator and target in proliferative retinopathy. Potential approaches to clinical treatment of retinopathy include glycemic control, use of ACE inhibitors, aminoguanidine, and soluble receptor for advanced glycation end products, treatment with panretinal photocoagulation, and an approach now used infrequently, pituitary ablation. This approach was first discussed by Poulson (1) after noting resolution of retinopathy with postpartum pituitary infarction. Sharp et al. (2) presented a 15-year follow-up of ^{90}Y pituitary ablation of 117 patients, showing decreased retinopathy, and Alzaid et al. (3) showed that patients with diabetes and GH deficiency have a 12% incidence of proliferative retinopathy, as opposed to the 62.5% incidence among matched control subjects. Merimee et al. (4) described 31 GH-deficient diabetic patients without retinopathy, in comparison to a 41% incidence among control subjects. However, Ballintine et al. (5) showed no increase in frequency of proliferative retinopathy in a group of patients with acromegaly and diabetes, suggesting that GH is required for expression of retinopathy but is not itself a causal factor.

Animal studies have manipulated the GH receptor, IGF-I, and the IGF-I receptor. A model of severe proliferative retinopathy involves subjecting mice to 75% oxygen on the 7th day of life, which obliterates the central retinal vein. In transgenic mice with increased or decreased GH expression, dwarf mice with low IGF-I had a 35% decrease in retinopathy after 75% oxygen treatment, while those with increased GH levels tended to have increased retinopathy. The degree of proliferative retinopathy was strongly associated with the level of IGF-I. In a study in which somatostatin was used to suppress endogenous GH secretion, administration of either IGF-I, GH, or both in the same hyperoxia model showed that somatostatin profoundly decreased retinopathy and that IGF-I, but not GH, restored proliferative retinopathy to baseline levels. VEGF and VEGF receptor levels did not change in either treatment group. Clinical studies with somatostatin analogs have not shown improvement in retinopathy, however, although improvement in nephropathy was seen. Administration of an IGF-I receptor

antagonist showed a dose-response inhibition of proliferation, to a maximum of 60%, and Smith stated that the "take home message" was that VEGF inhibition and IGF-I inhibition might have additive effects. Somatostatin has potential adverse effects on growth, may cause cholelithiasis, and may increase hypoglycemia, and VEGF/integrin may decrease wound healing and adversely affect blood vessel "maintenance." The overall approach should be to control rather than to completely inhibit the factors that lead to neovascularization. Additional approaches to treatment include free radical suppression and the administration of vasoconstrictors, as well as the use of interferon and thalidomide.

Many studies presented at the ADA meeting addressed the role of cytokines and growth factors in diabetes. Grant et al. (abstract 148) showed that adenosine, which promotes endothelial cell proliferation and angiogenesis in various vascular beds, interacts with human retinal endothelial cells via a specific receptor and increases angiogenic growth factors such as VEGF, IGF-I, and bFGF. Aiello et al. (abstract 149) reported that bFGF increases expression of VEGF and its receptor in bovine retinal capillary endothelial cells. In this study, mitogen-activated protein kinase (MAPK) phosphorylation was increased >50-fold by bFGF PD 98059, an inhibitor of MAPK, inhibited VEGF receptor expression, as did the PKC inhibitor bisindolylmaleimide, suggesting that expression of VEGF and its receptor is upregulated by bFGF through a MAPK- and PKC-dependent pathway. This would explain the synergistic interaction of the two growth factors. Romeo et al. (abstract 150) found activated nuclear factor κB (NF κB) in the pericytes but not the endothelial cells of diabetic retinal vessels, suggesting that cytotoxic substances such as tumor necrosis factor- α may be induced by NF κB to affect viability of both pericytes and endothelial cells. Gidday et al. (abstract 152) studied factors leading to capillary occlusion by leukocytes of the retinal microcirculation in rats with streptozotocin-induced diabetes of 4 weeks duration. Leukocytes were present throughout the retina near or within small veins and capillaries, but did not adhere to the endothelium of larger arteries and veins. Leukocyte-mediated obstruction of capillary blood flow and free radical and protease release by activated leukocytes may affect vascular permeability and autoregulatory capacity.

A number of other studies explored relationships between eye disease in diabetes and oxidative stress. Kowluru et al. (abstract 554) and Kowluru et al. (abstract 555) reported that glutamate, the major excitotoxin in mammalian retina, was increased in retinal glial cells 2 months after onset of diabetes in streptozotocin-induced diabetic rats. An antioxidant-supplemented diet prevented this. In an in vitro model, incubation of retinas from normal rats in a high glucose solution for 6 h increased nitric oxide levels, PKC activity, and oxidative stress. Administration of the PKC inhibitor LY333531, an antioxidant (*N*-acetylcysteine), or an inhibitor of nitric oxide production [N(G)-nitro-L-arginine methylester] reversed the abnormalities. In this model, at normal glucose levels a glutamate agonist reproduced the elevation of retinal PKC activity, oxidative stress, and nitric oxide levels characteristic of hyperglycemia, suggesting that retinal glutamate excess may underlie the biochemical abnormalities of diabetic retinopathy. Nakamura et al. (abstract 1071) reported that advanced glycosylation end products induce aldose reductase, the first and rate-limiting enzyme of the polyol pathway, which catalyzes conversion of glucose to sorbitol. In an in vitro model, the antioxidant probucol prevented this. Madar et al. (abstract 1461) showed that the antioxidant α -lipoic acid decreased aldose reductase activity and cataract development in diabetic sand rats. Lyons et al. (abstract 1462) showed that glycated, minimally oxidized, and glycoxidated LDLs decreased retinal capillary endothelial cell and pericyte viability and that the antioxidant α -tocopherol normalized cell viability without changing the extent of LDL modification. In his concluding remarks, Aiello stated that human trials with a variety of agents are beginning and suggested that Robert Godard's statement that "the dream of yesterday is the hope of today and the reality of tomorrow" is a fitting conclusion to the exciting presentations on this area of research.

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