Diabetes Complications

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This is the second of three articles dealing with the International Diabetes Federation meeting, which was held in Paris, 24–29 August 2003.

C-peptide

John Wahren (Stockholm, Sweden) discussed the mechanism of action and biological role of C-peptide. He stated that C-peptide should be considered as an endogenous peptide hormone, playing an important role in the maintenance of vascular homeostasis and exerting physiological effects of importance for the prevention and treatment of type 1 diabetes. C-peptide is stored with insulin in β-cell granules, and the two are secreted in equimolecular amounts, although unlike insulin, C-peptide escapes first-pass hepatic clearance and is only cleared by the kidneys, so that circulating concentrations are ~10 times higher than those of insulin. Wahren addressed the question of whether there is evidence of a role of C-peptide deficiency in the microvascular diseases associated with diabetes, noting that persons with type 1 diabetes in the Diabetes Control and Complications Trial (DCCT) who maintained C-peptide in low nanomolar range, with saturation at 1 nmol/l in the physiologic range, expected no effect of exogenous C-peptide. Wahren commented that there is no effect of C-peptide in healthy persons but only in persons with type 1 diabetes who have C-peptide deficiency.

Wahren noted that it has been difficult to demonstrate binding of C-peptide with radioligand-binding techniques. Using fluorescence correlation spectroscopy with rhodamine-labeled C-peptide, it is possible to measure the diffusion time of C-peptide in tiny (0.2–2) volumes in incubation medium, as compared with such measures in similar volumes present just above cell membranes, renal tubular cells, and many other cell types, which show markedly delayed average diffusion time above the cell membrane, suggesting binding. This phenomenon shows competitive dislocation by unlabeled C-peptide, but not by insulin, proinsulin, IGF-I, IGF-II, or neuropeptide Y, confirming specificity. Using the technique, renal cells show C-peptide binding in the low nanomolar range, with saturation at ~1 nmol/l in the physiologic range, explaining the lack of effect of exogenous C-peptide in persons without type 1 diabetes who possess endogenous C-peptide. C-peptide increases sodium influx (2), further suggesting its biologic effect. C-peptide increases Na,K ATPase activity of renal tubular cells (3), an effect blocked by pertussis toxin. In vivo, streptozotocin (STZ)-induced diabetes is associated with decreased Na,K ATPase activity, which can be substantially restored with C-peptide administration (4). C-peptide also increases endothelial nitric oxide (NO) synthase (eNOS) activity and, over time, increases eNOS expression (5), suggesting a vasodilatory function. In a study reported at the meeting, Kobayashi et al. (abstract 140) showed that both insulin- and C-peptide–stimulated mitogen-activated protein kinase phosphorylation in cultured human aortic smooth muscle cells but that C-peptide decreased protein kinase B phosphorylation and increased translocation of Rho GTP-binding protein to the cell membrane, whereas insulin increased protein kinase B phosphorylation and had no effect on Rho.

Sciatic nerve blood flow is decreased with STZ-induced diabetes and partially restored by C-peptide administration (6). In persons with type 1 diabetes, with a stepped C-peptide infusion increasing C-peptide from 0 to 0.3 and to 1.1 nmol/l, forearm blood flow increases in a dose-dependent fashion. C-peptide may have insulinomimetic effects, acting on the insulin receptor tyrosine kinase (7). Wahren presented a model suggesting that C-peptide increases Ca2+ influx, increasing eNOS and Na,K ATPase, acting via a G-protein–coupled receptor that increases protein kinase C (PKC) activity, and stimulating the ERK 1/2 and p38 mitogen-activated protein kinase pathways.

Bo-Lennart Johansson (Stockholm, Sweden) discussed renal findings in humans with diabetes and in experimental diabetes. He reviewed the early findings of nephropathy in type 1 diabetes, with glomerular hyperfiltration, loss of renal functional reserve, renal enlargement, and glomerular hypertrophy with basal membrane thickening and microalbuminuria, as well as late findings of macroadmuminuria and hypertension and gradual loss of renal function. Glycemia and antihypertensive treatment have beneficial effect, but the underlying mechanisms are not fully understood. Lack of C-peptide may be one of the contributing factors, so that replacement might prevent or retard development of diabetic nephropathy in type 1 diabetes. Chibalin et
al. (abstract 1789) reported that C-peptide incubation with human renal tubular cells obtained from patients undergoing elective nephrectomy could be shown to have direct action in vitro, activating PKC isoforms d and e and increasing phosphorylation of extracellular signal-regulated kinases.

C-peptide administration appears to influence all of the renal pathogenic abnormalities of diabetes. C-peptide infusion lowers the hyperfiltration seen in type 1 diabetic animal models with both 1- and 2-h treatment courses (8). In a 1-month study of C-peptide administration to persons with type 1 diabetes, the glomerular filtration rate (GFR) decreased from 145 to 135 ml/min and there was reduction in albuminuria (9). In the early phase of type 1 diabetic animal models, renal functional reserve (the increase in GFR in response to glycerol infusion) is decreased, with restoration by C-peptide (10). In this model, diabetes was associated with an early and marked 45% increase in glomerular volume, with partial inhibition by C-peptide. At the same time, the increase in mesangial matrix volume seen with diabetes is almost completely blocked by C-peptide. In similar animal models, proteinuria increases with STZ-induced diabetes, with prevention over 2 weeks of administration by C-peptide. In a 6-month, double-blind, randomized, crossover study of 21 persons with type 1 diabetes and mean HbA1c of 7.6%, albuminuria decreased with C-peptide and returned to baseline with placebo administration (11).

Thus, C-peptide reduces glomerular hyperfiltration; preserves renal functional reserve; prevents renal enlargement, glomerular hypertrophy, and mesangial matrix expansion; and decreases albuminuria. The GFR is determined by the balance between the ultrafiltration coefficient (related to surface area and permeability) and the net driving force (related to the difference between glomerular capillary pressure and glomerular colloid osmotic pressure), suggesting that modulation of these factors may underlie the effects of the peptide.

Anders Sima (Detroit, MI) discussed the relationship between C-peptide and nerve function and morphology in diabetes. Diabetic polyneuropathy in type 2 diabetes affects 35% of patients at 25 years, with slow progression and no nodal changes, while affecting close to 100% of persons with type 1 diabetes by this time, progressing more rapidly, and with greater severity and progressive nodal/paranodal degeneration. Similar patterns can be seen in the BBZDB rat model (type 2 diabetes model) and BB/Wor rat (type 1 diabetes model) despite similar glucose levels, although with increased serum insulin and normal C-peptide in the former. Administration of C-peptide allowed normalization of levels in the type 1 diabetes model. In a study reported at the meeting, Sima et al. (abstract 909) showed that in an insulin-deficient diabetic rat model, there is evidence of deficiency of the cytoskeletal protein contactin in contact-associated protein (Caspr), a molecule in the paranodal apparatus related to the establishment of the Na current, and in the b1 sodium channel. This deficiency was not seen in similarly hyperglycemic-hyperinsulinemic animals and was prevented by administration of C-peptide.

Diabetic peripheral neuropathy includes both acute functional/metabolic effects mediated by Na,K ATPase, polyol, and decreased NO and chronic structural changes. In neuronal cell lines, there is synergism between insulin and C-peptide actions at lower insulin levels but inhibition of insulin effect at higher insulin levels. Nerve Na,K ATPase activity is increased in a dose-dependent fashion by C-peptide, and endothelial blood flow is similarly normalized in a NO-dependent process. NCV decreases to a much greater extent in the type 1 diabetes than in the type 2 diabetes model, despite similar glycemia, with C-peptide administration to the type 1 diabetic group increasing NCV to levels similar to that in the type 2 diabetic group. Axonal degeneration is present in the type 1 diabetes model at 8 months and, to a much lesser extent, in the type 2 diabetes model or the type 1 diabetes model with C-peptide administration from onset of diabetes. Fiber number is similarly lost only in the type 1 diabetes model and reversed by C-peptide administration.

Nerve regeneration is also increased by C-peptide administration. This process involves an early gene response with IGF-I-mediated nerve growth factor release, leading to cytokine activation and macrophage recruitment. In a type 1 diabetes model with a sciatic nerve crush injury, IGF-I mRNA does not show the same increase exhibited with C-peptide administration or in the type 2 diabetes model (12). β-Tubulin mRNA levels increase in nondiabetic rats and show little increase in the type 1 diabetes model, with an intermediate response seen with C-peptide administration. Morphometric analysis distal to the crush site after 20 days shows decreased nerve fiber caliber and growth distance with type 1 diabetes, with improvement following C-peptide administration. The progression of axoglial dysjunction on nerve biopsy is more marked in type 1 diabetes than in type 2 diabetes, with similar findings in animal models (13). Myelin forms an ion channel barrier in the normal nerve, with myelin degradation in type 1 diabetes leading to ion channels being dispersed from the nodes of Ranvier; therefore, the normal inward Na current initiating the depolarization is inhibited, leading to decreased NCV. C-peptide administration to the type 1 diabetes rat model normalizes axoglial dysjunction (14). The insulin receptor is localized to the nodal and paranodal region of the nerve, with reciprocal increase in insulin receptor in the insulopenic model normalized by C-peptide replacement. Caspr shows decrease in expression in the type 1 diabetes model with normalization by C-peptide administration, although full Caspr activation requires insulin as well as C-peptide. Another nerve protein, ankyrin, shows a similar pattern, requiring both C-peptide and insulin administration to restore normal in vitro function.

The consequences 8 months after onset of diabetes in the two animal models are of NCV decreasing mildly and more markedly with type 2 and with type 1 diabetes, respectively. NCV improves in the type 1 model with C-peptide administration either from time of onset or 5 months after onset of diabetes. In humans, C-peptide administration to patients with type 1 diabetes increases NCV and heart rate variability (15). Sima suggested that C-peptide improves the metabolic phase of diabetic peripheral neuropathy by effects on Na,K ATPase and NO activities and improves the structural phase by promoting nerve regeneration, counteracting axonal atrophy, preventing abnormalities in gene expression and posttranslational modification of key nodal and paranodal molecules, and preventing axoglial dysjunction, with demonstrated improvement of human autonomic and peripheral neuropathy.
Neuropathy

Andrew Boulton (Manchester, U.K.) gave the 18th Golgi lecture on the diabetic foot, recalling that T.D. Price, from Nottingham, first noted in 1887 that “diabetes itself may play an active part” in the development of foot ulcers. Paul Brand, pointed out that all one needs is “to remove the shoes and socks” to improve diabetic foot disease and, commenting on the prevention of neurotrophic ulcers, described pain as “God’s greatest gift to mankind,” although persons with diabetic neuropathy paradoxically do suffer from excruciating “burning” pain. It is not clear whether there has been improvement in the care of the diabetic foot. Over the past 5 years, amputation rates increased in the U.S. and U.K., with greater ulcer and amputation rates in ethnic minorities. Levels appear stable in Germany, however, and have markedly decreased in Sweden. Neuropathy was present at diagnosis in >10% of patients in the UKPDS, and, in a U.K. community study, was present in 42% of persons with diabetes, although it was asymptomatic in half. A prospective study measuring the vibration perception threshold showed that persons with definite neuropathy had a 7.99-fold increase in the risk of foot ulcers (16). In a community-based study of 9,710 diabetic persons measuring vibration, pin prick, and hot/cold sensation, 291 ulcers developed over 2 years, with an average annual incidence of 2.2%. Men had a 1.6-fold greater risk than women, and high neuropathy disability score was associated with a 2.3-fold increase in risk (17). In another study of 169 patients, reduced motor NCV was the best predictor of new foot ulcer and mortality (18). Neuropathy is the most important single cause of foot ulcers, with other factors leading to foot ulcer deformity, trauma, and ischemia, and >80% of ulcers are preventable (19).

High foot pressure is associated with both first and recurrent plantar neuro- pathic ulcers, Boulton noted, with foot pressure abnormality preceding neuropathy, foot pressure predicting ulcers, and plantar callus associated with high pressure as well as predicting ulcers. Limited joint mobility is an important factor in the genesis of abnormal pressures. With the use of ultrasound to measure subcutaneous tissue depth, there is an inverse relationship between plantar tissue thickness and dynamic foot pressure measurements (20). A footprint system can be used to assess abnormal pressure patterns (21). Approaches to reducing foot pressure include orthoses, padded stockings, removing callus, special footwear, surgery, and injection of liquid silicone. The latter was studied in a randomized controlled trial and was shown to be effective in forming a cushion under areas of abnormal pressure, but this needs to be repeated at 1- to 2-year intervals (22).

In a study of 194 patients with new foot ulcers, comparing the Wagner grade and the University of Texas grade and stage wound classification systems, the latter, which includes staging for ischemia and infection, is more predictive of outcome (23). The Charcot foot is common in neuropathic patients and should be suspected in the neuropathic patient with a warm and swollen foot. The pathogenesis involves both somatic and autonomic neuropathy, with increased blood flow and reduced bone density caused in part by repetitive minor trauma. In addition to reducing activity and use of casting, a potential pharmacologic approach is the use of bisphosphonates, which decrease skin temperature and alkaline phosphatases levels (24). In a randomized controlled trial of pamidronate, bone-specific alkaline phosphatases and markers of bone resorption decreased (25). Outcome studies have not been performed with these agents.

Wound healing follows a series of phases beginning with inflammation, followed by proliferation/regeneration, and finally remodeling. The normal orderly pattern is disrupted in chronic nonhealing wounds, which show decreased growth factors and increased protease activity. Wound healing is affected by serum albumin, tissue oxygenation, infection, hyperglycemia, cytokines, and proteases. A marker of nonhealing wounds may be the prolonged presence of extracellular matrix molecules in the dermis (26). Other markers and potential mediators include increased transforming growth factor (TGF)-β3 (27), proteolytic factors such as matrix metalloproteinases (28), and absence of IGF-1 (29).

Wound care includes a variety of approaches to enhance healing, with treatment of infection, vascular reconstruction, achieving adequate glycemic control, off-loading of pressure, and ongoing wound debridement being important. An adjunct to treatment may be the use of bioengineered tissues and growth factors, which are associated with significant improvement in healing, although not to as great an extent as would be ideal. The use of a total contact cast decreases hyperkeratosis, fibrosis, and inflammation and increases capillaries and granulating tissue. There is evidence that a total contact cast is better than a half shoe or aircast device (30), perhaps because patients do not wear the removable devices. A study that placed a pedometer in the cast found that patients wear removable casts only 28% of the time (31). An ongoing study is therefore comparing total contact casting with an “instant total contact cast” using a removable device that has been made “nonremovable.”

Boulton emphasized the importance of off-loading treatment for diabetic foot ulcers. Psychological stress is another important factor, with depression increased in persons with neuropathy and found to be associated with slow healing of acute wounds. He noted that wound healing is impaired in persons with diabetes and that we need to understand healing processes to develop new approaches, perhaps involving growth factor treatment or gene therapy approaches. Bone marrow-derived cells applied to chronic wounds may lead to dermal rebuilding, and interesting new studies suggest a protective effect of estrogens and adverse effect of androgens in wound healing (32).

A number of studies presented at the meeting discussed aspects of diabetic neuropathy. Manes et al. (abstract 1) studied the frequency of neuropathy among 1,491 persons attending Balkan Region diabetes clinics, reporting that 43% had decreased sensation, 36% abnormal tendon reflexes, and 8% foot ulcers. Abbott et al. (abstract 3), in Boulton’s group, screened 15,692 persons in U.K. diabetes clinics for neuropathy, reporting that 17 and 4% had plantar and dorsal insensitivity to the 10-g Semmes Weinstein monofilament. Edwall (abstract 2442) compared the Semmes Weinstein 4-g (4.56 log) and 10-g (5.07 log) monofilaments in screening for neuropathy, based on vibration sensation. Of 72 diabetic persons with and 270 without neuropathy, the 4-g monofilament missed 19 with neuropathy and incorrectly diagnosed 37 without, while the 10-g monofilament missed 31 and incorrectly diagnosed 15. Edwall suggested that the 4-g monofilament be
more widely used in clinical testing. Abbas et al. (abstract 929) studied 2,134 persons with diabetes admitted to a hospital in Tanzania from 1997 to 2002, of whom 350 had foot ulcers and 312 available outcome data, with 141 undergoing amputation and an additional 24 dying from sepsis before surgery, suggesting this to be a major cause of morbidity in developing countries. Pound et al. (abstract 930) noted that 46.5% of persons with healed foot ulcer in their practice in the U.K. had recurrence, with an additional 22.8% having an early death. Of 370 patients with diabetes admitted to a hospital in England, 35.4% never became ulcer free during 30 months' follow-up, while an additional 25% healed and then had a recurrent or new ulcer and 3% had a major amputation. Recurrence was less likely for patients ulcer free for >1 year after healing. van Schiel et al. (with Boulton) (abstract 922) measured peak plantar pressure using a prototype pressure-relieving dressing on the metatarsal head with highest pressure, showing 26–30% reduction in peak pressure over 3 days, suggesting applicability as an off-loading device.

In a study suggesting a nonperipheral nervous system effect of diabetes, Selvaraman et al. (abstract 902) measured the cross-sectional area of the cervical spine at disk space C2/C3 in 57 men with type 1 diabetes, showing evidence of volume reduction in persons with both early and more advanced symmetrical polyneuropathy, with particular reduction in width of the cord suggesting involvement of the spinothalamic (pain and temperature) tracts. Rafael et al. (abstract 2413) discussed the use of the anticholinergic agent dicyclomine (in the U.S., the generic term is dicylecymine) for treatment of diabetic diarrhea. By decreasing gastrointestinal motility, in 50 patients with evidence of autonomic neuropathy and three or more stools per day, the agent was reported to markedly decrease diarrhea in all, with few side effects, at a dosage of 10 mg three times daily. Parving et al. (abstract 906) used R-R interval analysis as a measure of cardiac autonomic neuropathy in 197 persons, with type 1 diabetes with and 191 without nephropathy showing severe and mild impairment of heart rate control in 39 and 38% of patients with and 10 and 24%, respectively, of those without nephropathy. Abnormality of the R-R interval was associated with 3.5- and 6.1-fold increase in mortality during 9.2 years of follow-up in the two groups.

Information pertaining to pharmacologic therapies potentially useful in diabetic neuropathy was also presented at the meeting. Bastyr et al. (abstract 913) and Skljarvski et al. (abstract 984) from Lilly Research Laboratories studied the effect of the PKC-β inhibitor Ruboxistaurin in 205 persons with diabetic peripheral neuropathy for 58 weeks, showing improvement in symptom score and in a composite of lower-extremity nerve electrophysiology score that was significant at a dosage of 64 but not at 32 mg daily. There was improvement in the Clinical Global Impression scale, which, on multivariate analysis, was most strongly correlated with patient complaints of pricking and burning sensations. Petrychka et al. (abstract 914) randomized 31 persons with type 2 diabetes and cardiac autonomic neuropathy to 600 mg α-lipoic acid daily or placebo for 6 months, showing improvement of heart rate variability and reduction in the QTc interval. Ziegler et al. (abstract 915) meta-analyzed four trials of 1,258 patients receiving 600 mg α-lipoic acid daily by intravenous infusion for 3 weeks, which showed a 24% improvement in neuropathy symptom score as 53% of treated persons vs. 37% receiving placebo showed improvement in total symptom score, with particular improvement in symptoms of pain, burning, and numbness as well as significant improvement in pinprick and light-touch sensation and ankle deep-tendon reflexes. Vinik et al. (abstract 918), noting that topiramate appears to prevent neuronal apoptosis and stimulate neuronal growth of diabetic seion- incubated neurons, showed that topiramate decreases advanced glycation end products and polyol, with each of these approaches for diabetic nephropathy. PKC-β inhibitors decrease albuminuria and mesangial expansion and attenuate the progression of nephropathy in rodent models of hypertension and diabetes. Other PKC isoforms may also be important. Forbes noted, with a mouse model not expressing PKC-α showing decreased glomerular vascular endothelial growth factor (VEGF) expression and albuminuria.

Oxidative stress may be an important mediator of diabetic nephropathy (34). Hyperglycemia increases mitochondrial superoxide formation, acting via a number of pathways, including PKC, and produces advanced glycation end products (AGEs) and polyl, with each of these distal pathways offering a potential treatment to prevent nephropathy. Mitochondrial antioxidant treatment can reduce both AGEs and albuminuria. Glucose interacts with protein, lipids, and nucleic acids to produce a range of AGEs that lead with type 2 diabetes >50 years of age with either micro- or macroalbuminuria treated for 3–6 years, showing no benefit in event-free survival and borderline decrease in albuminuria and blood pressure. Comparing this with the HOPE (Heart Outcomes Prevention Evaluation) study, in which there was marked reduction in cardiovascular disease mortality and overall end points, suggests the higher ramipril dose to be the critical factor. It is not, however, that there is no inhibition of the renin-angiotensin system, and Menard stated that blood pressure decreased similarly in the HOPE and DIAB-HYCAR.

The much higher frequency of coronary disease in the former study, and of albuminuria in the latter study, as well as the greater frequency of heart failure in the latter study, suggest important differences in study populations that may have contributed to the difference in outcome between the two. Furthermore, using N-acetyl-ery-seryl-aspartyl-proline, a hemo-regulatory peptide solely metabolized by ACE, as a measure of compliance, normal volunteers show a 8.8-fold increase in urinary levels, with urine samples in DIAB-HYCAR study participants 1.72 vs. 7.46 nmol/mmol creatinine in the placebo versus ramipril group, with distribution suggesting 24 vs. 63% were actually treated with an ACE inhibitor, weakening the study and perhaps explaining the lack of significant effect.

Mark Forbes (Melbourne, Australia) discussed potential new therapeutic approaches for diabetic nephropathy. PKC-β inhibitors decrease albuminuria and mesangial expansion and attenuate the progression of nephropathy in rodent models of hypertension and diabetes. Other PKC isoforms may also be important. Forbes noted, with a mouse model not expressing PKC-α showing decreased glomerular vascular endothelial growth factor (VEGF) expression and albuminuria.
to a variety of injuries, with two anti-AGE agents, aminoguanidine (AG) and ALT-946, as well as the soluble receptor for AGE (sRAGE) and lysozyme decreasing these adverse effects. In a study presented at the meeting, Allen et al. in Forbes’s group (abstract 141) administered aminoguanidine versus placebo to in a diabetic mouse model not expressing apolipoprotein E (APOE-KO), which showed a 43% reduction in atherosclerotic plaque area and a >50% decrease in gene expression of the profibrotic cytokine, connective tissue growth factor, although both were still three- to fivefold greater than in nondiabetic controls. ALT-946 and aminoguanidine decrease albuminuria in a rodent model (35), and similar benefits can be seen with the cross-link breaker ALT-711 (36), which decreases albuminuria with administration either early or late in the course of albuminuria, although with late administration, the degree of histologic glomerulosclerosis is not improved. There are interactions among these pathways, with PKC-β and -α expression both decreasing with ALT-711 and aminoguanidine treatment, and sRAGE decreasing albuminuria and glomerular basement membrane thickness.

Anti-angiotensin II drugs, either angiotensin receptor blockers (ARBs) or ACE inhibitors, also decrease AGE formation. Angiotensin II and AGEs activate a range of growth factors, including TGF-β and VEGF, with a variety of tyrosine kinase inhibitors and nonselective approaches potentially blocking these abnormalities. Administration of anti-TGF-β antibody to db/db mice reduced matrix expansion, although it did not decrease albuminuria (37). The effect of anti-TGF-β antibody is additive to that of ACE inhibitors (38). Anti-VEGF antibody decreases albuminuria, and a tyrosine kinase inhibitor decreases nephropathy in ApoE-KO mice. Connective tissue growth factor gene expression and collagen production are reduced with this treatment. In a study presented at the meeting by Lassila et al. (abstract 1125) from Forbes’s group, regarding the tyrosine kinase inhibitor imatinib (used clinically in treatment of chronic myeloid leukemia), STZ-induced diabetic ApoE-KO mice showed decreased expression of growth factors and cytokines in association with a 13% decrease in the atherosclerotic plaque area. In another study, Jerums et al. (abstract 995) reported that AGE peptides were increased in STZ-induced diabetes and decreased by treatment with insulin, aminoguanidine, or the ACE inhibitor ramipril. Bone morphogenic protein, which acts as an inhibitor to TGF-β, has as great a benefit as ACE inhibitors in decreasing nephrosclerosis in animal models. The ACE2 gene, which appears to be negatively related to circulating angiotensin II levels and to the development of hypertension, is downregulated in diabetes and can be upregulated with ACE inhibitor treatment. Glycation receptors include the ezrin, radixin, and moesin proteins, which may be new targets for treatment (39).

Microarray studies show additional genes that are downregulated in the diabetic kidney, suggesting new approaches to understanding and treatment. Combinations of ACE and AGE inhibitors have additive benefits, suggesting the need to combine hemodynamic and metabolic treatments. Another potential treatment is high-dose thiamine, which may have effects in retinopathy and nephropathy (40). Babaei-Jadidi et al. (abstract 1001), Thornerly et al. (abstract 1002), and Karachalias et al. (abstract 1182) studied STZ-induced diabetic rats on insulin maintenance therapy, showing reduction in the both elevated renal glomerular cytosolic and membrane PKC-β with high-dose thiamine and benfotiamine, as well as reduction in glomerular glycation and oxidation biomarkers and decrease in albuminuria and delay in development of hyperfiltration with the latter agent. Belmonto et al. (abstract 32) reported that incubation of human umbilical vein endothelial cells and bovine retinal pericytes with either thiamine or benfotiamine decreases the AGE production and increases the apoptosis seen with culture in 28 mmol/l glucose. In another presentation at the meeting, Nishikawa et al. (abstract 994) incubated human mesangial cells at 5.6 vs. 30 mmol/l glucose; the latter increased intracellular reactive oxygen species production, cyclooxygenase (COX)-2 gene expression, and prostaglandin E2 synthesis. The COX and prostaglandin effects were blocked by inactivating mutations of nuclear factor-κB, suggesting that hyperglycemia may in part cause glomerular hyperfiltration via oxidative stress–induced prostaglandin E2 synthesis. Saraheimo et al. (abstract 952) studied 67 persons with type 1 diabetes and normoalbuminuria, 64 with microalbuminuria, and 63 with macroalbuminuria, showing respective C-reactive protein levels of 2.0, 2.6, and 2.9 mg/l and interleukin-6 levels of 1.9, 2.9, and 3.6 ng/l, suggesting an association of inflammatory markers with nephropathy in type 1 diabetes.

Richard Gilbert (Melbourne, Australia) discussed commonalities of nephropathy and heart disease, pointing out that heart failure is a particularly important problem among persons with diabetes (41) and that albuminuria is a risk factor not only for renal disease but also for cardiovascular disease (42). There is a particular association between diabetic nephropathy and heart failure, with 20% of the 1,715 diabetic individuals with moderate renal insufficiency in the IDNT Nephropathy Trial (Irbesartan Diabetic) developing heart failure over a 2.5-year period (43). The increase in basement membrane matrix and fibrosis that leads to diabetic glomerulosclerosis occurs in all vascular beds. Endomyocardial biopsy of persons with diabetes shows increased fibrosis, particularly with concomitant hypertension (44). There is also evidence of disordering of extracellular matrix in diabetic cardiomyopathy. Functional concomitants suggestive of cardiac abnormality include early evidence of decreased left ventricular filling and systolic contraction (45). In the RALES (Randomized Aldactone Evaluation Study), increased procollagen type III NH₂-terminal peptide, a marker of cardiac fibrosis, was associated with response to spironolactone treatment (46). Levels of TGF-β are increased in diabetic cardiomyopathy and may be related to the increase in synthesis and decrease in degradation of matrix substances.

Gilbert noted that the ACE inhibitor perindopril decreases renal fibrosis and has been shown to decrease TGF-β (47). He suggested that hyperglycemia, AGEs, angiotensin II, cell stretch, free radicals, and other stressors may act via PKC to increase TGF-β, which may be an important common mediator of diabetes complications. Tranilast (N-3,4-dimethoxy-7-nitrophenyl) decreases keloid formation and has been shown to decrease TGF-β-stimulated fibroblast collagen formation. In a diabetic nephropathy model, tranilast did not affect blood glucose or blood pressure but decreased albuminuria (48). In persons with diabetic
nephropathy, tranilast similarly appears to decrease the degree of renal fibrosis, to reduce the rate of decline of renal function in association with decreasing urinary collagen levels (49). Animal models also have suggested an effect of tranilast in decreasing diabetic vascular pathology (50).

Hans Parving (Steno, Denmark) contrasted the concept that diabetic nephropathy is irreversible and that its progression accelerates in later stages with findings based on current therapeutic approaches, which suggests that regression and even remission of albuminuria may occur. Although improved glycemic control is important (51), Parving noted that 20% of the primary and 30% of the secondary intervention group in the DCCT developed worsening of renal disease and that glycemic control is only of modest benefit in improving nephropathy, although nephropathy reversal can be demonstrated a decade after pancreas transplantation (52). Persons with type 1 diabetes and microalbuminuria treated with ACE inhibitors for 2 years show halving of albumin excretion, with a 62% decrease in progression to macroalbuminuria (53). Treatment of 10 persons for 2 years prevented one progression from microalbuminuria to macroalbuminuria, suggesting this to be highly efficacious (54).

Parving concluded that the inexorable worsening of nephropathy seen several decades ago (55) is no longer necessary and that with multifactorial treatment, including a low-protein diet (56), intensive treatment of hypertension, and treatment directed at angiotensin II, it should be possible to further decrease the progression. He noted that dual blockade with both ACE inhibitors and ARBs is a promising future treatment, with a study of candesartan plus lisinopril showing that the combination leads to a greater fall in blood pressure and albuminuria than either agent alone (57). In a study of this approach presented at the meeting, Rossing et al. (abstract 980) treated 20 persons with type 2 diabetes and nephropathy with the ARB candesartan (16 mg daily) or placebo for 8 weeks, added to lisinopril or enalapril (40 mg daily) or captopril (150 mg daily), showing a 28% decrease in albuminuria from the baseline level averaging 706 mg/24 h, with a 3/2-mmHg decrease in 24-h ambulatory blood pressure, suggesting renoprotective benefit of combination A II blockade. Jacobsen et al. (abstract 982) studied persons with type 1 diabetes and nephropathy treated with enalapril 40 mg daily, finding a 25% decrease in albuminuria and 8/4 mmHg decrease in blood pressure with addition of irbesartan 300 mg daily for 8 weeks.

A number of presentations analyzed aspects of the natural history of diabetic nephropathy. Rossing et al. (abstract 83) reported 10-year follow-up of 593 persons with type 1 and 191 with type 2 diabetes who had urine albumin <30 mg/24 h at baseline. Risk factors for progression to microalbuminuria were any retinopathy at baseline, HbA1c, and having initial urine albumin >10 mg/24 h, suggesting that levels well below those we currently consider abnormal may have prognostic significance. Ebbelhoj et al. (abstract 1007) performed annual ambulatory blood pressure measurements in 131 nonalbuminuric persons with type 1 diabetes followed for 4 years, showing that although only 3 persons developed microalbuminuria, there was a progressive increase in blood pressure during the period, with 12 persons developing hypertension. Logstrup Poulsen et al. (abstract 1005) reported 23-year follow-up of 272 persons with type 1 diabetes, with 37% overall mortality. In multivariate analysis, microalbuminuria was associated with a 2.0-fold increase in mortality. Mortality was 1.6-fold greater for each 10-year greater duration of diabetes, macrovascular disease at baseline increased mortality 1.9-fold, smoking increased mortality 1.9-fold, and glycemic control and pulse pressure were also significant, with systolic, diastolic and mean blood pressure, sex, retinopathy, and serum cholesterol and creatinine not adding further to the model, suggesting the importance of modifiable risk factors in the prognosis of persons with type 1 diabetes. Hovind et al. (abstract 85) studied 126 persons with type 1 diabetes who had >2.5 g albuminuria daily, of whom 28 had sustained decrease to <0.6 g for at least 1 year (mean 3.6 years) and 21 were predominantly treated with ACE inhibitors. Over a mean 8.7-year follow-up, 2 of those with a sustained decrease developed end-stage renal disease (ESRD) and 4 died, as compared with 24 and 35, respectively, of those who did not attain remission of nephrotic range albuminuria, suggesting this to be a highly favorable response to treatment. Cooper et al. (abstract 959) presented analysis of the 1,513 type 2 diabetic patients with nephropathy participating in the RENAAL (Reduction of Endpoints in Type 2 Diabetics with the Angiotensin II Antagonist Losartan) study for a mean of 3.4 years. Thirty-five percent had diabetic retinopathy, which was associated with a 29% increase in mortality and a 46% increase in ESRD, both of which were significant in multivariate analyses. de Zeeuw et al. (abstract 981) presented additional data from RENAAL, showing that patients with albuminuria >3,000 mg/g creatinine had a 7.6-fold increased risk of progressing to ESRD and a 2.6-fold higher risk for hospitalization for heart failure compared those with <1,500 mg/g albuminuria. The 6-month reduction in proteinuria with losartan administration emerged as the strongest independent predictor of both renal and cardiovascular outcome, with a 50% reduction in proteinuria during this period halving the risks for both ESRD and heart failure during follow-up, suggesting that proteinuria should be considered both a cardiac and renal risk marker as well as a target for therapy. In a study of another aspect of renal disease among persons with diabetes, Boyko et al. (abstract 1413) compared 218 women with diabetes aged 55–75 years in a health maintenance organization with 799 nondiabetic women. Twelve versus 7% developed symptomatic urinary tract infection and 8 vs. 4% asymptomatic bacteriuria over a 2-year period, with a particular increase in risk among insulin-treated patients.

Andersen et al. (abstract 978) studied 133 patients with type 2 diabetes who had persistent microalbuminuria and had participated in the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study (IRMA-2), randomized to double-masked treatment with either placebo or irbesartan in doses of either 150 or 300 mg daily for 2 years, with conventional antihypertensive treatment as needed to reach a blood pressure target <135/85 mmHg. At baseline, respective mean arterial blood pressures were 112, 111, and 112 mmHg; albuminuria 46, 60, and 51 μg/min; and GFR 108, 117, and 113 ml/min–1.73 m2, while at study end mean blood pressures were 105, 103, and 102 and albuminuria was decreased by 8, 34, and 60%. Rates of decline in GFR were 1.3, 1.2, and 1.0 ml/min–1.73 m2–1 month–1 during the initial 3
months of the study and 0.3, 0.3, and 0.4 ml/min\(^{-1}\cdot m^2\) month\(^{-1}\) thereafter. One month after withdrawal of all antihypertensive medication, blood pressure was unchanged in the placebo group to 109 and 108 mmHg in the groups previously receiving irbesartan 150 and 300 mg. Albuminuria, however, had increased by 14% over baseline in the group previously receiving placebo and 11% in the irbesartan 150-mg group but was persistently reduced by 47% in the irbesartan 300-mg group, suggesting long-term renoprotective effects with higher-dose treatment independent of blood pressure effect, possibly reflecting reversal of renal structural and/or biochemical abnormalities.

There may be nephroprotective effects of statins and of thiazolidinediones. Papageorgiou et al. (abstract 88) analyzed 313 persons with diabetes participating in a randomized controlled trial of usual care or aggressive care with atorvastatin, with the latter group having lesser increases in serum creatinine levels, suggesting a statin benefit. Harry et al. (abstract 2294) presented effects of pioglitazone on the urinary albumin-to-creatinine ratio in comparative studies with other agents among 3,713 persons with type 2 diabetes. Urinary albumin-to-creatinine ratio decreased 19% with pioglitazone in comparison with a 1% increase with metformin monotherapy and decreased 20% with pioglitazone in comparison with a 17% decrease with glitazide monotherapy. In combination with sulfonylurea, the urinary albumin-to-creatinine ratio decreased 15% while increasing 2% with metformin, and the urinary albumin-to-creatinine ratio decreased 10% in combination pioglitazone/metformin treatment in comparison with metformin monotherapy while increasing 6% in combination glitazide/metformin treatment.

Retinopathy
Malone et al. (abstract 119) presented data from a study of 85 persons who had skin biopsy at the end of the DCCT. Compared with the 61 without a three-step worsening of retinopathy, the 24 with such worsening had higher cutaneous levels of the AGEs furosine and carboxymethyllysine, independent of the effects of HbA\(_1c\) and diabetes duration in multivariate analysis. Arun et al. (abstract 1150) studied 211 persons with type 2 diabetes for 3 years from time of initiation of insulin treatment, demonstrating clinically significant worsening of retinopathy in 15% with early and 67% with moderate nonproliferative retinopathy, showing no relationship to either HbA\(_1c\) at baseline or change in HbA\(_1c\), suggesting that this may represent an adverse effect of insulin treatment. Sailes et al. (abstract 1149) proposed that thyroxine-treated hypothyroidism offsets diabetic retinopathy, based on their finding that among 147 persons with coexisting type 2 diabetes and thyroxine-treated hypothyroidism versus 383 duration-of-diabetes-matched euthyroid control subjects, prevalence of diabetic retinopathy was 28% vs. 55%, with a mean time to development of retinopathy of 18 vs. 15 years, with similar mean HbA\(_1c\) and diabetes duration and with the thyroxine-treated group being 66 vs. 63 years old and having systolic blood pressure 148 vs. 144 mmHg. Aiello et al. (abstract 113) and Milton et al. (abstract 114) reported the effects of the PKC-\(\beta\) inhibitor ruboxistaurin (formerly LY333551) on macular edema and on diabetic retinopathy and showed no overall benefit, although subgroup analysis was suggestive that persons with intermediate elevations in HbA\(_1c\) were benefited.

References


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