The Epidemiology of Complications

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This is the seventh in a series of reports on the American Diabetes Association (ADA) 61st Scientific Sessions held in Philadelphia, PA, in June 2001. It covers topics related to the treatment of type 2 diabetes.

John Fuller, London, U.K., gave the Kelly West lecture on the epidemiology and prevention of diabetic complications. Data from the World Health Organization (WHO) multinational study of vascular disease in diabetes showed interesting discrepancies between national groups in rates of nephropathy and cardiovascular disease (CVD). Mortality rates increase with increasing cigarette use, blood pressure, and cholesterol; proteinuria, triglyceride, and fasting blood glucose are additional risk factors. Studies with electron beam computerized tomography (EBCT) show increased calcium levels in the coronary arteries of both men and women with diabetes, correlating with the duration of diabetes, systolic blood pressure, BMI, and total/HDL cholesterol ratio (1,2). Patients with diabetes have a 2.5- to 5-fold greater risk than those without diabetes of having an increased coronary artery calcification (CAC) score.

The EURODIAB study in 31 centers in 16 countries in Europe followed 3,250 patients with type 1 diabetes beginning in the late 1980s. Coronary heart disease (CHD) incidence rates of 10 and 12 per 1,000 patient-years were seen in men and in women, with age and albuminuria independent risk factors in both sexes, the waist-to-hip ratio (WHR) an additional risk factor in men, and systolic blood pressure an additional risk factor in women. Using baseline assessment in 1988–1991, of 1,134 patients with normoalbuminuria, 12.6 and 1.7% developed micro- and macroalbuminuria (3). Risk factors for worsening albuminuria are HbA_{1c}, baseline albuminuria level, triglyceride, and BMI. Among normo-, micro-, and macroalbuminuric patients, CHD event rates were 7, 12, and 22%, respectively. There was a 56% incidence of new retinopathy over 7.5 years for those without retinopathy initially; risk factors were the duration of diabetes, HbA_{1c}, albuminuria, triglyceride, WHR, fibrinogen, von Willenbrand factor (vWF), and γ-glutamyl transpeptidase (GGTP). Neuropathy, as assessed by symptoms, abnormal deep tendon reflexes, autonomic function, or vibration perception (measured with a bioesthesiometer) developed over 7.5 years in 24.6% of the patients, with independent risk factors of age, duration, HbA_{1c}, triglyceride, and BMI. For all complications, there appeared to be a log-linear relationship to glucose without evidence of a glycemic threshold.

Given current interest in markers of inflammation and endothelial dysfunction, a case-control analysis of patients participating in EURODIAB with and without complications was performed, measuring C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)-α, vascular cell adhesion molecule (VCAM), soluble E-selectin, homocysteine, and urine Amadori albumin. In multivariate analysis, TNF-α and VCAM showed very strong association with albuminuria, and there was a weaker but significant effect of IL-6 and homocysteine. TNF-α and IL-6 were associated with retinopathy, and TNF-α, Amadori albumin, and homocysteine were associated with neuropathy.

Fuller ended with a discussion of the Eurodiab Controlled Trial of Lisinopril in Insulin Dependent Diabetes Mellitus (EUCLID) of the effect of lisinopril treatment on nephropathy and retinopathy. There was a 50% decrease in progression of microalbuminuria to macroalbuminuria. After adjusting for diastolic blood pressure both at baseline and after initiation of ACE inhibitor, there was still a 46–53% fall in the degree of albuminuria with treatment, suggesting that the blood pressure effect of these drugs did not explain their benefit. Lisinopril also was associated with a >50% decrease in retinopathy progression and the appearance of proliferative retinopathy, which again was not explained by the decrease in blood pressure. Meta-analysis of studies of ACE inhibitors (ACEIs) has confirmed these findings, showing a threefold greater rate of regression to normoalbuminuria (4), suggesting a role of ACEI for all type 1 diabetic patients with microalbuminuria.

Protein kinase C

At a symposium sponsored by Medical Education Collaborative, Ronald Klein, Madison, WI, discussed the epidemiology and clinical consequences of diabetic complications. The disease currently affects 16 million individuals in the U.S., accounting for more than $44 billion in medical expenditures, almost $12 billion related to diabetic complications (5). Based on gross proteinuria measurement in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), 19 and 15% of patients with type 1 and type 2 diabetes have nephropathy. Klein showed decreased survival for patients with versus without nephropathy with both type 1 and type 2 diabetes. The WESDR also showed that 24 and 28% of those with type 1 and type 2 diabetes complained of

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Abbreviations: A2, angiotensin II; ACEI, ACE inhibitors; ACS, acute coronary syndrome; ALT, alanine amino-transferase; AST, aspartate amino-transferase; BNP, brain natriuretic peptide; CAC, coronary artery calcification; CHD, coronary heart disease; CHF, congestive heart failure; CRP, C-reactive protein; CVD, cardiovascular disease; DAG, diacylglycerol; DCCT, Diabetes Control and Complications Trial; EBCT, electron beam computerized tomography; EDIC, Epidemiology of Diabetes Intervention and Complications; ERK, extracellular-regulated kinase; ESRD, end-stage renal disease; ET, endothelin; FFA, free fatty acid; FMD, flow-mediated dilation; GGTP, γ-glutamyl transpeptidase; GPCR, G protein–coupled receptor; ICAM, intracellular adhesion molecule; IL, interleukin; IMT, intima-media thickness; KDR, kinase insert domain receptor; LBBB, left bundle branch block; LVH, left ventricular hypertrophy; MAP, mitogen-activated protein; MDA, malondialdehyde; NAFLD, nonalcoholic fatty liver disease; OHDG, oxo,2′-deoxyguanosine; PAI, plasminogen-activated inhibitor; PKC, protein kinase C; PPAR, peroxisome proliferator–activated receptor; QOL, quality of life; RGZ, rosiglitazone; SR-BI, scavenger receptor class B type I; TBARS, thiobarbituric reactive substances; TGF, transforming growth factor; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; vWF, von Willenbrand factor; WESDR, Wisconsin Epidemiologic Study of Diabetic Retinopathy; WHR, waist-to-hip ratio.

loss of tactile sensation and that 72 and 57% had some evidence of retinopathy.

In the WESDR, there was a highly significant relationship between glycosylated hemoglobin and both total and proliferative retinopathy, with evidence that individuals with levels short of proliferative retinopathy benefited from improved glycemia and without a glycemic threshold, below which further improvement failed to give additional benefit (6). For every 1% increase in glycosylated hemoglobin, retinopathy frequency almost doubled, with an increase of ~30% in frequency of visual loss, as well as of proteinuria and amputation (7).

Klein pointed out the similarities between these data and those in the Diabetes Control and Complications Trial (DCCT), as a 2% decrease in HbA_{1c} in the intervention group associated with a decrease in retinopathy risk of ~50%. Extrapolated to 1 million individuals with type 1 diabetes in the U.S., achievement of an HbA_{1c} of 7.2% would lead to a gain of close to 8 million person-years of sight, to close to 6 million person-years free of end-stage renal disease (ESRD), and of more than 5 million person-years of additional survival (8). Although the improvement in glycemia was less marked in the U.K. Prospective Diabetes Study (UKPDS) of patients with type 2 diabetes, at 7.0 vs. 7.9% over 12 years of follow-up, the decrease in retinopathy progression was qualitatively similar (9).

Additional benefit can be achieved with treatment of blood pressure. In WESDR, for each 10-mmHg increase in diastolic blood pressure, the rate of progression of retinopathy increased by ~50%, with individuals with diabetes and hypertension having almost a twofold increase in risk of progression to proliferative retinopathy. The EUCLID showed a 50% decrease in retinopathy progression in treated patients (10), and the blood pressure trial of the UKPDS showed that intensive blood pressure control decreased retinopathy progression from ~30 to 20% at 6 years and from 45 to 25% at 9 years (11). Klein also reviewed data from the Early Treatment Diabetic Retinopathy Study (ETDRS) showing that LDL cholesterol >160 mg/dl and triglyceride >400 mg/dl were associated with 50% increases in the development of retinal exudates (12). He concluded that glucose, blood pressure, and lipid control are of great importance in the management of patients with diabetes, but noted that >15% of patients treated with insulin and less than half of those not requiring insulin achieve excellent glycemic control, and that poorly controlled blood pressure and lipid levels remain distressingly common in these patients.

Bernard Zinman, Toronto, Canada, discussed additional aspects of diabetic complications, focusing on information from the Epidemiology of Diabetes Intervention and Complications (EDIC) follow-up study of patients who had been enrolled in the DCCT. The 7 vs. 9% differential in HbA_{1c} levels between the intervention and control groups of the DCCT was not maintained after these patients were returned to community care, with both groups showing HbA_{1c} of ~8% during the first 4 years of follow-up. Surprisingly, however, the cumulative incidence of further progression of retinopathy remained different: ~2 vs. 8% at 2 years and 6 vs. 18% at 4 years in the formerly intensive treatment and control groups, respectively (13). Similarly, microalbuminuria developed in 5 vs. 11%, and progression from micro- to macroalbuminuria was seen in 8 vs. 31%.

Zinman pointed out the similarity of the EDIC findings to the subgroup analysis of the DCCT, showing that 8% of those individuals in the intensive treatment group who nevertheless had HbA_{1c} of 9% developed retinopathy, while 20% of those in the control group with the same 9% HbA_{1c} showed retinopathy at 9 years. He suggested that there may be "something unique about intensive treatment independent of the HbA_{1c}." There are, however, difficulties with intensive treatment. It is costly (~$4,500 per patient per year) and associated with a marked increase in frequency of hypoglycemia, in both the DCCT and community studies (13).

A number of approaches have been studied for prevention of adverse effects of hyperglycemia, including aldose reductase inhibitors (14), AGE inhibitors (15), and measures to decrease oxidative stress. The development of inhibitors of protein kinase C (PKC) offers the ability to block a group of major direct and indirect (via vascular endothelial growth factor [VEGF]) consequences of hyperglycemia, and Zinman speculated that this may provide important benefit in the clinical prevention of complications.

Lloyd P Aiello, Boston, MA, discussed current treatment approaches for diabetic retinopathy, including glucose and blood pressure control and routine, lifelong ophthalmic evaluation, with laser photocoagulation when indicated. Pan-retinal laser photocoagulation decreases severe visual loss by 50% and by >90% in selected high-risk individuals. However, it destroys retinal tissue , commonly leading to loss of peripheral and night vision, changes in color vision, and the potential to cause a number of serious complications.

Aiello reviewed the biochemical pathway by which hyperglycemia leads to generation of diacylglycerol (DAG), which activates PKC, leading to effects on the vascular system, retina, kidney, heart, and nerve vasculature. A number of growth factors mediate retinopathy, with VEGF of greatest importance, showing marked elevation in human ocular fluids from patients with active proliferative diabetic retinopathy (16). VEGF interacts with a membrane-bound kinase insert domain receptor (KDR), leading to activation of phosphatidyl inositol 3 kinase and to phosphorylation of phospholipase C γ, which in turn increases DAG levels, activating PKC. PKC activation appears to be the final pathway mediating both an increase in retinal vascular permeability, causing macular edema, and an increase in retinal neovascularization, in the development of diabetic retinopathy (17). PKC activation also has effects in the vasculature, increasing basement matrix protein synthesis, activating leukocytes, causing endothelial cell activation and proliferation and smooth muscle contraction; activating cytokines such as transforming growth factor-β, VEGF, and endothelin; and promoting angiogenesis. The orally active agent LY333531 was first synthesized in 1994 (18). It has been shown to improve retinopathy and nephropathy in animal models, with promising early results in clinical trials (19). The agent decreases retinal blood flow toward the nondiabetic level in a dose-dependent fashion and may prove able to reduce progression of diabetic retinopathy, onset of neovascularization, and progression of macular edema, although actual efficacy data awaits the results of ongoing clinical trials.

In a conference addressing additional aspects of PKC, George L. King, Boston, MA, reviewed its relationship with diabetic vascular disease. Effects of hypergly-

cemia include oxidant stress, glycation, and increased PKC production via an increase in activity of the glucose sorbitol pathway and DAG. PKC can affect cytokine signaling, cell growth, contractility, immune regulation, and expression of ~10% of genes in the cell. PKC actually represents a family of 12 related phospholipid-dependent serine-threonine kinases, directly involved in the transmission of a wide variety of cellular processes, involved in proliferation and apoptosis. PKC plays a universal role in cellular function, with expression of individual PKC isotypes developmentally regulated. The individual isotypes serve distinct functions within the cell. There are three subgroups. The catalytic domains are similar in all, as they are in all serine/threonine kinases, but the regulatory domains differ among the classical PKCs, which have a DAG and phorbyl ester–binding domain; the novel PKCs, which do not require Ca for activation; and the atypical PKCs, which do not require calcium, phospholipid, DAG, or phorbyl ester for activation.

The classical PKC pathway mediates the effect of increased glucose in increasing DAG levels. This is seen in retinal endothelial cells and pericytes, in aortic endothelial and smooth muscle cells, and in the heart. There is a strong correlation of monocyte PKC activity with HbA_{1c} levels and with retinal and cardiac PKC activity. Monocyte PKC activity also correlates with the severity of retinopathy and with that of nephropathy. There is, however, a great deal of variation, potentially explaining genetic differences in susceptibility to complications. PKC-β and -Δ activation increases synthesis of transforming growth factor (TGF)-β, collagen, and fibronectin, leading to basement membrane thickening and to plasminogen-activated inhibitor (PAI)-1 and intracellular adhesion molecule (ICAM) production, which causes fibrinolysis and leukocyte adhesion. Signals such as mitogen-activated protein (MAP) kinases, prostaglandins, and VEGF are affected, causing changes in permeability and angiogenesis, and effects on endothelin (ET)-1 and endothelial nitric oxide synthase affect blood flow and contractility. All these processes affect retinal, renal, neural, and cardiac pathologies.

The agent LY333531 inhibits PKC-βI and -βII, preventing cardiac fibrosis in a transgenic mouse model of overexpression of PKC-βII. King showed evidence that LY333531 ameliorates diabetes-induced changes in renal hemodynamics in a dose-dependent fashion, that the increased expression of TGF-β1 in the diabetic kidney is blocked by the inhibitor, and that it can partially reverse established microalbuminuria and prevent the mesangial expansion seen in animal models of diabetes. In the retina, pericyte loss and abnormalities of endothelial flow lead to microaneurysms and macular edema, with subsequent hemorrhage, exudates, capillary loss, and hypoxia, causing neovascularizatrion and fibrosis. Mediators of microaneurysm formation include ET-1 and VEGF. Transgenic mice overexpressing PKC-βII show decreased retinal blood flow, with increased susceptibility to microaneurysms when they are made diabetic. Conversely, LY333531 normalizes the abnormalities seen in retinal blood flow in animal models and when administered to patients with diabetes. VEGF acts via the KDR receptor, leading to phospholipase Cγ phosphorylation, which increases DAG, then activating PKC. Ocular VEGF levels are increased in human ocular fluids with active retinopathy, suggesting it to be the major responsible growth factor. LY333531 and PKC-β knockout mouse models decrease the neovascularization seen in animal models of VEGF- or hypoxia-mediated proliferative retinopathy. In human phase I/B studies, LY333531 administered at doses of 8 mg twice daily, 16 mg daily, and 16 mg twice daily to seven, seven, and eight patients led to dose-related decrease in retinal blood flow. No adverse effects were seen in immune function or glycemic control, and results of a large clinical trial in patients with retinopathy are expected in the coming year.

Richard A. Walsh, Cleveland, OH, discussed the role of PKC modulators in preserving cardiac function. PKC plays a pivotal role in the development of congestive heart failure (CHF), with certain isoforms producing pathological hypertrophy, while PKCε and Δ contribute to ischemic preconditioning, the protective response to ischemia. There are 20 million persons in the U.S. with asymptomatic left ventricular dysfunction and 4.8 million persons with CHF, which has an incidence of 400,000–700,000 cases/year and mortality similar to that of most common malignancies. Walsh discussed the concept that hemodynamic and neurohormonal abnormalities lead to progressive worsening of CHF, so that we need "new therapies that focus on cellular abnormalities."

Increased cardiac pressures produce concentric hypertrophy, while increased volume leads to eccentric hypertrophy. Chamber remodeling occurs when there is abnormal balance between energy production and utilization, with cell death by necrosis and apoptosis, as well as pathologic growth and dysfunction, affecting myofilament and cytoskeletal proteins. Participating signal transduction pathways include cytokines, growth factors, angiotensin II (A2), and ET-1, acting via G protein–coupled receptors (GPCRs), cellular stresses, and mechanical deformation. The GPCR pathway acts on several PKC isoenzymes and shows cross-talk with the MAP kinase pathway. Walsh showed that activation of phospholipase C with inositol phosphate in models of chronic hypertrophy leads to increased translocation of PKC-α, -ε, and -γ. ACEI treatment improves left ventricular and myocyte function in association with decreased PKC translocation. Potential mechanisms for PKC activation in states of cardiac hypertrophy and CHF include mechanical deformation and GPCR activation. Hypoxia, ischemia, and A2 increase PKC-α, -βII, -ε, -γ, and -ζ. Contrasting exercise-induced physiologic hypertrophy with pathologic hypertrophy, Walsh showed that PKC-βII inhibition prevents the changes of the latter. PKC-βII overexpression in isolated myocytes decreases myocyte shortening, and inhibition improves these abnormalities. In the failing human heart, PKC-βI, -βII, and -α were increased in the membrane fraction of the cardiomyocyte, without change in PKC-ε. Using targeted cardiac transgenic overexpression of PKC-ε, concentric left ventricular hypertrophy (LVH) occurs, but without development of LV fibrosis. Myofilament calcium sensitivity decreases with PKC-βII overexpression while increasing with PKC-ε overexpression. Thus, the prevention of PKC-β activation by A2, ET-1, adrenergic stimuli, and prostaglandins, coupled with the administration of PKC-β inhibitors, may become an important treatment approach.

Catharine I. Whiteside, Toronto, Canada, analyzed the relationship between PKC activation and diabetic nephropathy, recalling the classic hallmark

findings of collagen IV deposition in the interstitium leading to obliteration of the glomerular capillary loops. The glomerular mesangial cell response to high glucose involves growth factor production, with consequent increased intraglomerular pressures and increased extracellular matrix protein deposition. TGF-β, A2, ET-1, and intraglomerular hypertension all lead to the sclerotic phenotype. PKC-β is involved in hyperglycemia-induced TGF-β1 synthesis and intraglomerular hypertension. PKC is also involved in mesangial cell expression of VEGF, presumably important in stimulating microalbuminuria. In diabetes PKC-βII expression is increased in glomerular mesangial cells. There is increased translocation to the nucleus and cell membrane of PKC-α, -βII, and -Δ when mesangial cells are cultured with high glucose concentration. High glucose increases DAG, activating PKC-β, with increased signaling in response to A2 and ET-1. The glucose effect also requires PKC-Δ, -ε, and -ζ. Reactive oxygen species may play an additional role in PKC activation. When mesangial cells are exposed to high glucose and ET-1, the MAP kinases extracellular-regulated kinase (ERK)-1/2 are increased, leading to increased transcription of Elk-1, which increases collagen IV synthesis. High glucose increases the response to ET-1, and with inhibitors of both PKC and of ERK1/2, the increase in collagen IV is blocked. Decreasing the expression of PKC-Δ, -ε, or -ζ reverses ET-1 stimulation of Elk-1 activity.

Normal glomerular pressure is regulated by afferent and efferent arteriolar tone. Hyperglycemia decreases afferent arteriolar tone, leading to glomerular hypertension. In addition, mesangial cells show decreased contractility when exposed to high glucose, potentially by DAG activation of PKC or by an ET-1 receptor–mediated effect. At 24 h after exposure to 30 mmol/l glucose with a PKC-ζ inhibitor, the loss of contractility is abrogated. Treatment with the aldose reductase tolrestat also prevents this, while other manipulations are ineffective. Both of these treatments restore the abnormalities of F- and G-actin seen with high glucose incubation. Whiteside suggested that prevention of diabetic nephropathy will require understanding of both normal and pathogenic PKC isozyme responses and development of targeted approaches to PKC isozyme inhibition, as well as addressing genetic predisposition to sclerosis. She noted that in animal models, PKC-β inhibition prevents the development of microalbuminuria.

Naruse et al. (295-PP) assessed mechanisms of adverse effects of incubation of vascular cells with high glucose concentrations (abstract numbers refer to the Abstracts of the 61st Annual Meeting of the American Diabetes Association, *Diabetes* 50 [Suppl. 2]:1–A649). In aortic tissue isolated from obese, highly insulin resistant Zucker diabetic rats, aortic DAG and membrane-associated PKC were increased, with resistance to insulin-induced phosphorylation of Akt and release of cGMP. These abnormalities were reversed with LY333531, suggesting activation of PKC-β to mediate endothelial dysfunction in states of insulin resistance.

Oxidative stress

Brian Hoogwerf, Oklahoma City, OK, introduced a conference on antioxidants and their influence in diabetes by reminding the audience that the concept of oxidized lipoproteins came to the forefront of thinking ~15 years ago, when evidence of the association between experimental diabetes and oxidation of LDL, HDL, and VLDL, which thus causes endothelial cytotoxicity and exacerbates many aspects of atherosclerosis, was shown. Individuals taking antioxidant vitamins have been shown in epidemiological studies to have decreased risk of CVD, and animal models have shown benefit, but intervention studies have been negative.

Arshag Mooradian, St. Louis, MI, discussed the increased oxidative stress in diabetes. There are two general categories of evidence: that of increased levels of byproducts of oxidation, measured as thiobarbituric reactive substances (TBARS), and that of decrease in levels of natural antioxidants. When hydroxy radicals are formed and interact with fatty acids, alkoxy radicals are generated as byproducts of lipid peroxidation, producing ethane, which can be measured in expired gases of experimental animals (or humans) breathing purified air. In diabetic rats, the rate of ethane exhalation is increased, and this improves, though does not normalize, with insulin treatment. Ethane exhalation also increases with an acute glucose load or with protein and fat administration. Conjugated dienes are additional lipid peroxidation products, with increased levels in individuals with diabetes, particularly in those subjects with complications. Lipid peroxidation byproducts can accumulate with either increased oxidation or increased availability of substrates, such as phospholipids or triglycerides, with most animal models suggesting the former to explain the increase in diabetes.

Malondialdehyde (MDA) is another lipid peroxidation byproduct, generated when glucose interacts with proteins, increasing with experimental diabetes, and improving with glycemic treatment. MDA may link with serum and tissue proteins, increasing their immunogenicity. Furthermore, protein glycation increases susceptibility to modification by MDA, and MDA itself increases protein glycation. Interestingly, glucose and other sugars may have either anti- or pro-oxidative activity, depending on the conditions of measurement. Antioxidant defenses in membranes isolated from diabetic rats are decreased, with correction by insulin treatment. Mooradian described a study of 30 individuals with diabetes assessed before and after institution of tight glycemic control, showing that both improved glycemia and, additively, vitamin E decreased TBARS (20).

Cheryl L. Rock, La Jolla, CA, defined oxidative stress as the imbalance between the generation of reactive oxygen species and the ability of the organism to dispose of them. There are a number of nutritional antioxidants, including tocopherols, carotenoids, vitamin C, and other phytochemicals, such as flavenoids in fruits and legumes. Because of the association between diabetes and increased oxidative damage, strategies to increase dietary antioxidants may be important, with either supplements or foods naturally rich in these substances. The epidemiologic data that have driven interest in the relationship between antioxidants and disease are, however, based on dietary data, so that lifestyle patterns not usually measured may play a role. Furthermore, "vegetables and fruits have a lot more going for them" in addition to their antioxidant activity, such as their high content of folate and of retinoids, which modulate cell growth and apoptosis. Many studies of effects of supplements on oxidative markers have shown ex vivo benefits on LDL, but randomized clinical trials that include in-

dividuals with diabetes have not shown evidence of benefit.

Rock asked two questions: "Is there a clinical benefit for persons with diabetes mellitus to optimize antioxidant status?" and "Does diet modification [with] more vegetables and fruits [change] biomarkers of oxidative stress?" The second question can be answered more readily. Biomarkers used in nutrition research include indicators to validate self-report of dietary intake (e.g., plasma carotenoids as a marker of fruit and vegetable intake), indicators of biological or cellular activity of the biological constituent (not necessarily related to the mechanism of benefit), and surrogate end point biomarkers, which are part of the effect of the dietary constituent related to the pathophysiologic process. Biomarkers used in human studies include MDA, TBARS, and other derivatives of lipid peroxidation, such as 8-isoprostane F2α, the oxidation product of PG-F2, and 8-oxo,2′-deoxyguanosine (OHDG), a marker of oxidative damage to nucleic acids.

Rock reviewed a study in which a period of avoidance of vegetable juice was associated with an increase in DNA strand breaks in peripheral blood lymphocytes and circulating levels of oxidized pyrimidine bases, both decreasing after supplementation with tomato juice and carrot juice. Another study in which vegetable and fruit intake were increased from 6 to 12 servings per day showed that women with the lowest baseline α-carotene had evidence of greater degrees of oxidative nucleic acid damage using 8OHDG measurement. Data from the Women's Healthy Eating and Living Study of a high vegetable and fruit diet for women who have had breast cancer, following initial chemotherapy and radiotherapy, showed that intensive telephone counseling increases vegetable and fruit consumption, verified by changes in plasma carotenoids. Preliminary analysis showed a decrease in 8OHDG, although many of these women supplemented with vitamins E and C. "The burning issue," Rock concluded, "is whether modifying diet can be linked to these markers" and whether this in turn leads to clinical benefit. Nishikawa et al. (121-OR) noted that OHDG is increased in urine from persons with diabetes. Urinary OHDG was 77.5 vs. 30.7 mg/g creatinine in patients with diabetes with carotid intima-media thickness (IMT) greater than versus ≤1.1 mm, and OHDG was also associated with increased HbA_{1c}, and with increased CVD risk scores, although not with neuropathy. Hirai et al. (255-OR) followed 396 persons with type 2 diabetes for 5 years, showing that those with higher baseline OHDG had increased progression of albuminuria.

Elisia Jenkins, Charleston, SC, discussed controversies in free radical–related diabetes complications. There are many theoretical reasons for oxidative stress in diabetes, including effects of hyperglycemia and of ischemia, as well as effects of diet. Oxidative stress is likely to vary between and within tissues and within cells. Currently, there is not a measure similar to the HbA_{1c} for assessing oxidative stress, and existing assays may not be sufficiently specific. Different measures in a given tissue may not be in agreement, and their relationship to the complications of diabetes is not straightforward. Oxidation of LDL is a major contributor to atherosclerosis, and may play a role in microvascular disease. LDL isolated by ultracentrifugation and then exposed to copper shows a lag phase during which lipid-soluble antioxidants are consumed, a propagation phase in which conjugated dienes are formed, and then a plateau phase. LDL assessed in this fashion has been shown more susceptible to oxidation in a number of studies of individuals with diabetes, although this may depend on the level of glycemic control of the patients studied. Glycated and nonglycated LDL show a similar degree of oxidation.

Glycation

AGEs, long known to form in foods during heating, are also orally absorbed (21). Lin et al. (33-OR) showed that streptozotocin-diabetic ApoE-deficient mice, at high risk of atherosclerosis, had a 50% decrease in atherosclerotic lesion area and a marked decrease in AGE on immunostaining at the aortic root when given a diet low in AGEs. Hofmann et al. (19-LB) reported 2.6-fold lower fasting insulin at 6 weeks and 4.8-fold lower insulin at 15 weeks, with a 1.6-fold greater fall in glucose in response to insulin in diabetic db/db (+/+) mice fed a diet low in AGEs or a regular chow diet with 3- to 4-fold higher carboxymethyl lysine content. Cai et al. (1537-P) showed that cellular injury by heat-promoted dietary AGEs involves saturable cellular uptake, presumably via AGE-receptor pathways, leading to depletion of intracellular antioxidant systems such as glutathione. The antioxidant *N*-acetylcysteine and the AGE-inhibitor aminoguanidine inhibited the effects of dietary AGE on endothelial cell glutathione.

Huang et al. (296-PP) reported decreased growth but increased type III collagen and sodium-hydrogen exchanger isoform 1 mRNA expression of cultured skin fibroblasts exposed in vitro to nonenzymatically glycated versus nonglycated serum proteins from 15 patients with type 1 diabetes . They speculate that variability of response to glycated proteins of fibroblasts from different patients may be related to risk of complications. Liu et al. (717-P) reported that methylglyoxal, a dicarbonyl reflecting glycation, induces apoptosis in rat mesangial cells, potentially contributing to the development of glomerulosclerosis. Beisswenger et al. (251-OR) reported that progression of mesangial expansion based on electron microscopy of renal biopsy specimens performed at baseline and at 5 years in 110 persons with type 1 diabetes correlated with HbA_{1c} and with 3-deoxyglucosone, an AGE intermediate.

HbA_{1c}

Little et al. (388-P) reported that the National Glycohemoglobin Standardization Program has shown that in year 2000, 1,949 of 2,146 surveyed laboratories reported GHB results as HbA_{1c} or equivalent, traceable to the DCCT and UKPDS methodology, compared with 422 of 840 laboratories in 1993, suggesting that the majority of glycohemoglobin assays in the U.S. are now standardized. Khera et al. (715-P) described studies showing that erythrocyte glucose influx and efflux are not equal, with lower intracellular than extracellular levels at steady state. They speculated that there may be interindividual variation in this glucose gradient that could contribute to variation in hemoglobin glycation for a given level of glycemia. Cohen et al. (252-OR) noted discordance between measured HbA_{1c} and that predicted based on serum fructosamine in 160 patients with diabetes. The glycosylation gap, defined as the residual when the measured HbA_{1c} is compared with the predicted HbA_{1c} based on the population regression between HbA_{1c}

and fructosamine, was less than −1% in 27 patients and more than +1% in 36 patients. In 29 patients studied twice over 15 weeks, the discordances were reproducible. The glycosylation gap was +0.5% in those with macroalbuminuria, but −0.7 and −0.4% in those without nephropathy and those with microalbuminuria, respectively, suggesting that there are sources of variance in diabetic complications beyond glycemic control per se.

Derr et al. (369-P) assessed the relationship between HbA_{1c} and home glucose results downloaded from meters of 124 individuals with diabetes testing at least 1.5 times daily, excluding tests performed within 1.5 h of a previous reading to eliminate postmeal or frequently repeated samples. Mean HbA_{1c} was 7.68% and mean glucose was 154.8 mg/dl, with significant correlation coefficient (r = 0.62). Although the standard deviation of each individual's blood glucose correlated with HbA_{1c}, with r equaling 0.34, correction for the mean glucose left this correlation not statistically significant, suggesting that HbA_{1c} reflects the mean rather than the variability of an individual's glucose levels. Levetan et al. (496-P) analyzed results of continuous glucose monitoring system glucose testing in 42 patients. HbA_{1c} correlated most strongly with mean glucose levels from midnight to 5:00 A.M., and with mean 90- and 120-min glucose levels following the evening meal, while not showing significant correlation with the fasting glucose, suggesting an important contribution of nocturnal and postdinner glycemia to HbA_{1c}, while morning glucose may have less effect.

Atherosclerosis mechanisms

Ohgami et al. (161-OR) found that AGE proteins, as ligands for scavenger receptor class B type I (SR-BI), inhibited in vitro both SR-BI–mediated selective uptake of HDL cholesterol ester and cholesterol efflux from peripheral cells to HDL, suggesting that AGE proteins might modulate SR-BI–mediated cholesterol metabolism in vivo. Du et al. (614-P) reported that incubation of endothelial cells with increasing levels of free fatty acids (FFAs) inhibited endothelial cell prostacyclin production and nitric oxide synthetase activity by inducing mitochondrial superoxide overproduction, suggesting effects similar to those of hyperglycemia, potentially contributing to atherogenesis. Unno et al. (165-OR) showed that peroxynitrite, which is generated from the reaction of nitric oxide with the superoxide anion, has actions similar to the hydroxyl radical in increasing levels of the AGE *N*ε-(carboxymethyl)lysine (CML) in vitro. Individuals with resistance to the glucose-lowering effects of insulin may be sensitive to its atherogenic effects. Pellegrini et al. (662-P) compared fibroblasts cultured from insulin-sensitive and insulin-resistant persons of similar age and BMI, showing 35 and 22% lower insulin-stimulated glucose uptake and glycogen synthesis, but similar insulin-stimulated PAI-1 release, suggesting a direct adverse effect of hyperinsulinemia in these patients. IL-6 increases endothelial permeability and mesangial cell growth, and its serum and urinary concentrations are increased with diabetes. Cahoon et al. (701-P) showed no effect of 30 mmol/l glucose, but doubling and quadrupling of in vitro endothelial production of IL-6 on incubation with physiologic levels of glycated human serum albumin and TNF, with synergistic 10-fold increase in IL-6 with both agents together and potentiation of the effect of both agents by insulin. An inhibitor of nuclear factor-κB transactivation decreased IL-6 production, suggesting the involvement of this transcription factor in IL-6 induction.

Benson et al. (323-PP) studied mice that did not express apoE and had a high rate of development of atherosclerosis. Given a high-fat and high-carbohydrate diet, hyperinsulinemia and hyperglycemia were reduced by treatment with rosiglitazone (RGZ) and aortic atherosclerotic lesion area showed significant decrease, confirming other reports of antiatheroscleotic effects of peroxisome proliferator–activated receptor (PPAR)-γ agonists. Collins et al. (292-PP) studied levels of atherosclerosis with A2 infusion in nondiabetic high-fat diet–fed mice lacking the LDL receptor. RGZ decreased lesion area 60.4%, and the nonthiazolidinedione PPAR-γ agonist Merck L805,645 decreased atherosclerosis 48.4% compared with control levels, without effect on glucose, insulin, blood pressure, triglyceride, or total cholesterol, but with a fall in FFA levels, suggesting these agents may protect patients with type 2 diabetes against atherosclerosis.

Lin et al. (311-PP) described a study with administration of the ET-1 receptor antagonist bosentan in a streptozotocin-induced diabetes rat model, which shows doubling of baseline ET-1 levels. The decrease in the rate of change of LV filling pressure with time, a measure of cardiac contractility, was attenuated with diabetes and returned to baseline with treatment, suggesting that the peptide, which is known to act as a potent vasoconstrictor, may mediate some of the adverse cardiac effects of diabetes.

Inflammation

Clark et al. (156-OR) used data from the Third National Health and Nutrition Examination Survey of 13,500 U.S. adults aged 18–74 years. Nonalcoholic fatty liver disease (NAFLD), also known as nonalcoholic hepatic steatosis (NASH)—based on aspartate amino-transferase (AST), alanine amino-transferase (ALT), or GGTP elevated above normal with negative hepatitis B and C serologies, transferrin saturation <50% and average daily alcohol intake <2 drinks for women and <3 drinks for men—was found in 23.5% of those surveyed. Type 2 diabetes occurred in 11.7% of adults with NAFLD but in 4.2% of those without NAFLD. Dean and Sellers (1581-P) found ALT and AST levels more than three times the upper level of normal in 22 and 16% of 49 individuals aged 8–18 years with type 2 diabetes, suggesting that NAFLD is frequent in youths with type 2 diabetes. Hockings et al. (1552-P) administered RGZ to Zucker fatty rats, showing prevention of the increase in liver fat content and size otherwise seen in this type 2 diabetes model. In treated animals, the fat-to-water ratio decreased from 7.8 to 2.3 and 3.7% at 4 and 13 weeks, respectively; in untreated animals, it increased from 10.6 to 13.4 and 13.8%, respectively. There was a 7% decrease in liver volume in the treated animals as compared with a 17% increase in controls. In an interesting analysis, Lu et al. (303-PP) reported that of 2,617 participants in the Strong Heart Study without prior diagnosis of diabetes, those who did not drink alcohol had a significant 1.97-fold increase in risk of diabetes adjusted for sex, age, BMI, blood pressure, cigarettes, physical activity, and family history. Those who formerly drank alcohol or drank small, moderate, or heavy amounts of alcohol showed no difference in diabetes risk.

Mayer-Davis et al. (157-OR) reported

that total vitamin E intake was not associated with the rate of development of diabetes among the 895 initially nondiabetic persons in the Insulin Resistance Atherosclerosis Study (IRAS). Among the 577 individuals who were not using vitamin E supplements, however, those in the lowest quintile of plasma α-tocopherol had a four- to fivefold lower diabetes risk than those in the two highest quintiles, suggesting a protective effect of vitamin E from food but not from high-dose supplementation. Lindsay et al. (154-OR) found that serum γ-globulin levels were higher with greater degrees of American Indian heritage and in the presence of a family history of type 2 diabetes among Pima Indians. γ-Globulin was a predictor of diabetes, controlling for sex, BMI, and the 2-h glucose. Bhally and Litvin (357-P) studied 73 patients with diabetes without evidence of infection or inflammatory disorder, vascular disease, or cigarette use. CRP was 6.1 mg/l in those with HbA_{1c} >7% and 2.7 mg/l in those with HbA_{1c} ≤7%, also showing correlation with BMI. Cressey and Streja (366-P) reported a correlation between CRP and fibrinogen in 200 patients with diabetes, with CRP showing an association with duration of diabetes, BMI, and statin and hormone replacement therapy controlling for fibrinogen, and with fibrinogen showing an association with microalbuminuria controlling for CRP. Festa et al. (617-P) showed a stronger relationship between CRP and the blood glucose 2 h after oral glucose than between CRP and fasting glucose, particularly adjusting for BMI, abdominal obesity, and insulin sensitivity, suggesting that postprandial glycemia may have impact on subclinical inflammation. Hayaishi et al. (624-P) reported a correlation between CRP and carotid IMT among 55 persons with type 1 diabetes with a mean duration of 14 years and a mean age 23 years. Weyer et al. (1307-P) found that among 32 nondiabetic Pima Indians, body fat showed a strong correlation with CRP, as well as with secretory phospholipase A2 and soluble ICAM-1, suggesting adipose tissue to be a determinant of humoral markers of inflammation.

Kim et al. (881-P) measured CRP levels in 484 persons without diabetes. Those with no components of the insulin resistance syndrome (triglyceride ≥200 mg/dl and/or HDL <35 mg/dl; BMI ≥25 kg/m^2; systolic and/or diastolic blood pressure ≥140/90 mmHg or antihypertensive treatment; impaired glucose tolerance by WHO criteria) had mean CRP 3.0 mg/l, and those with 1, 2, and 3 to 4 components had mean CRP levels of 3.7, 4.6, and 5.0 mg/l, respectively. An association between CRP and estradiol was also noted. Tominaga et al. (894-P) found that 1,706 persons with normal fasting and 2-h glucose concentrations had CRP levels of 0.8 mg/l, 46 persons with fasting glucose concentrations of 110–125 mg/dl but 2-h glucose values <140 mg/dl had CRP levels of 01.1, and 222 persons with normal fasting glucose but 2-h glucose concentrations >140 had CRP 2.6 mg/l, suggesting that high postload glucose is more strongly correlated with inflammation than high fasting glucose. Van de Ree et al. (684-P) studied 217 patients with type 2 diabetes without known coronary disease, with initial CRP levels of 2.8 mg/l, and found an increase of 0.2 mg/l after 30 weeks receiving placebo, a decrease of 0.2 mg/l with atorvastatin 10 mg daily, and a decrease of 1.5 mg/l with atorvastatin 80 mg daily, suggesting dose-related benefit of statin treatment.

Khan et al. (34-OR) studied 20 patients with type 2 diabetes and mean HbA_{1c} of 8.4%. Flow-mediated brachial artery dilation (FMD), a measure of endothelial function, decreased from 0.9 to 0.6% in nine patients after 1 week of placebo treatment, while but increased from 1.1 to 3.5% after 1 week of administration of vitamin C 500 mg twice daily. FMD was −0.5 and −0.9% at 4 and 8 h following a standard fatty meal at baseline, but was +2.5 and +3.1% after vitamin C 1 g daily for 7 days, with all differences significant, suggesting the potential to improve vascular risk. There was no change with placebo and no changes in endothelium-independent dilatation produced by sublingual nitrate. Khan et al. also (289-PP) studied 20 patients with type 2 diabetes treated with insulin lispro, showing a decrease in postprandial triglyceride and improvement in fasting and postprandial FMD.

CVD

Kanaya et al. (865-P) reported a meta-analysis of 24 studies assessing cardiovascular risks in type 2 diabetes. Compared with individuals without diabetes, coronary mortality was increased 2.2- and 2.8-fold in men and in women, nonfatal myocardial infarction was increased 1.6- and 1.7-fold, overall cardiovascular (including peripheral and cerebrovascular) mortality was increased 3.2- and 4.1-fold, and total mortality was increased 2.1- and 1.9-fold. Differences between men and women were not significant, and the greater absolute number of CHD deaths can be attributed to diabetes in men than in women. Meigs et al. (120-OR) reported that 109 of the 1,550 men and 1,820 women without clinical CVD in the Framingham Offspring Study had fasting glucose >125 mg/dl, and an additional 55 had 2-h glucose >200 mg/dl after an oral glucose tolerance test. During 12,242 person-years of follow-up, there were 118 CVD events. For each 20-mg/dl increase in 2-h glucose, there was a 20% increase in risk of CVD, with the postchallenge glucose significant even after adjustment for the fasting glucose. Hu et al. (153-OR) followed 117,653 female nurses aged 30–55 years who were free of diagnosed cardiovascular disease and diabetes at baseline in 1976 for 20 years. Among 5,897 who developed type 2 diabetes during follow-up, there were 3.7- and 4.7-fold increases in risk of myocardial infarction before and after the diagnosis of diabetes. The risk was 3.1- and 4.1-fold increased after adjustment for weight, cigarette use, and other CVD risk factors, suggesting the prediabetic state to be one intrinsically associated with increased atherosclerosis, with a 2.4-fold increase in risk even 15 years before the diagnosis of diabetes.

Bowden et al. (112-OR) measured familial aggregation of CAC in 122 patients with type 2 diabetes. Only 20% of the patients had no detectable calcification. In addition to its association with age, male sex, low HDL cholesterol, albuminuria, cigarette use, and history of angina, myocardial infarction, stroke, and vascular procedures, there was a significant heritable component. Snell-Bergeon et al. (674-P) measured CAC using EBCT twice over a mean 2.6-year interval in 97 patients with long-standing type 1 diabetes. Prior data show a 16% interperson reproducibility coefficient of CAC score; 24 patients showed an increase of at least 32%, representing progression, and 7 showed a similar decrease, suggesting regression. There was a 39% increase in risk of progression for each log score increment of baseline CAC and a 39% increase in risk for each 10-mg/dl increase in total choles-

terol. Risk of progression was 2.45-fold greater for each decade of age. Huskey et al. (630-P) reported that of 238 persons with childhood onset type 1 diabetes in the Pittsburgh Epidemiology of Diabetes Complications Study who had CAC measurement and were followed for a mean of ~2 years, 38 had CAC scores >400, 40 had scores between 100 and 399, 61 had scores between 1 and 99, and 165 had no calcification. The finding of CAC >400 had greater sensitivity, specificity, and positive predictive value for new CHD events than a history of a prior coronary event; history plus CAC scoring showed the highest correlation. Koshy et al. (635-P) compared coronary arterial intravascular ultrasound (IVUS) findings in 5 diabetic patients and 12 nondiabetic patients, showing similar vessel and plaque sizes and degree of remodeling, suggesting processes other than the differences in anatomy to underlie the worse outcome of coronary disease in diabetes.

Lazar et al. (490-P) treated 51 patients with diabetes undergoing coronary artery bypass surgery with parenteral GIK versus standard intravenous solutions beginning before anesthesia and continuing for 12 h postoperatively. Glucose levels were similar at 204 vs. 190 mg/dl in the two groups at baseline, but averaged 148 vs. 267 mg/dl with serum insulin 90 vs. 38 μU/ml for hours 0–12 and FFA levels 0.34 vs. 0.61 mEq/l at hour 6. There was improvement in cardiac index by 6 h postoperative that persisted through 18 h, with lower rates of atrial fibrillation, less ventilator time, and shorter length of stay in the intensive care unit and total hospitalization in the patients receiving GIK. Jurkovitz et al. (2-OR) compared an invasive strategy of early catheterization and revascularization for acute coronary syndromes (ACS) with a conservative strategy proceeding to revascularization only with recurrent ischemia or positive stress test in 2,220 patients (28% having diabetes), all of whom were treated with GPIIb/IIIa blockade, in the TACTICS-TIMI 18 trial. Death, myocardial infarction, or rehospitalization for ACS was seen in 14.2 vs. 16.4% of those without and in 20.1 vs. 27.7% of those with diabetes, and in-hospital cost was $13,724 vs. $12,669 for those without and $16,392 vs. $13,658 for those with diabetes at 6 months. The differences were significant only for the diabetic subgroup, suggesting benefit of an invasive management strategy for patients with diabetes, albeit at somewhat increased cost. However, the patients with diabetes had smaller improvements than nondiabetic subjects in physical functioning, angina stability, and angina frequency. Benjamin et al. (1623-P) analyzed the health-related quality of life (QOL) among 4,296 persons undergoing coronary angioplasty from 1995 to 1999, of whom 26% had diabetes, 33% were female, 93% were Caucasian, and 43% were age 65 years or older. Six-month follow-up studies, available for 925 patients, showed that physical function improved in 723 individuals without diabetes, while 202 diabetic patients, whose baseline physical function was lower, did not show improvement, leading the group with diabetes not to show overall improvement in QOL.

Bertoni et al. (836-P) used the 1995 Nationwide Inpatient Sample with demographic and diagnostic data on 6,500,000 discharges from over 900 representative hospitals in 19 states. Diabetes was present in 26.2% of 48,035 persons with primary cardiomyopathy but in 15.4% of 4,949,841 control subjects, with diabetes and cardiomyopathy showing a stronger association in Caucasians than in African-Americans on multivariate analysis. Saponieri et al. (402-P) compared 31 patients with diabetes alone, 22 with diabetes and left bundle branch block (LBBB), and 14 with LBBB alone. The cardiac ejection fraction was 49, 30, and 49%, left ventricular end diastolic volume was 147, 189, and 165 ml, and 24-h urine protein was 36, 81, and 12 mg, respectively, suggesting the combination to be a particular risk marker, for which ACEI and other treatment may be useful. Seyoum et al. (669-P) measured peak exercise oxygen consumption (V_{O_2}) at baseline in a cohort of 468 patients with type 2 diabetes, with 5-year follow-up showing that patients with versus without CVD events had peak exercise oxygen consumption at baseline 20.3 vs. 21.9 ml · kg^{-1} · min^{-1}, suggesting V_{O_2} as a potential risk factor. Epshteyn et al. (145-OR) measured plasma brain natriuretic peptide (BNP), secreted predominantly from the LV in response to both volume expansion and pressure overload, in 111 patients with diabetes undergoing echocardiography to assess possible LV dysfunction. BNP averaged 39 pg/ml in 38 patients with normal LV function, 474 pg/ml in 12 with systolic dysfunction, and 958 pg/ml in 22 with both systolic and diastolic dysfunction. A BNP of 64 pg/ml was 97% specific and 92% sensitive for detecting the presence or absence of LV dysfunction using echocardiography.

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