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Pathogenesis of type 2 diabetes, vascular disease, and neuropathy

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This report covers topics related to the pathogenesis of diabetes, vascular disease, and neuropathy from presentations at the 36th Annual Meeting of the European Association for the Study of Diabetes (EASD), which was held in Jerusalem in September 2000.

Pathogenesis of Type 2 Diabetes

The Aharon Cohen Memorial Lecture. Paul Zimmet of Melbourne, Australia, opened the meeting with the Aharon Cohen Memorial Lecture. He described the global epidemic of diabetes as a "clash between genes and the environment" that has caused one of the major public health issues of the 21st century. Given the location of the meeting in Israel, Zimmet recalled the work of Maimonedes in the 12th century, noting that diabetes was more frequent in certain geographic areas than others. Zimmet discussed the development of diabetes in ethnic groups with migration, such as the Yemenite Jews and Bedouins, who had little diabetes with traditional dietary patterns but more on exposure to modern high-calorie foods. In Australia, as in many other developed countries, the frequency of diabetes has tripled over the past several decades. A further doubling is predicted around the world over the coming decade, particularly in Asia, in association with increasing levels of obesity. Further, the age of onset of diabetes has lowered from the mid-50s to the mid-30s.

Zimmet suggested that the glucose tolerance test is still relevant to the diagnosis of diabetes. He noted that impaired glucose tolerance (IGT; a blood glucose level of 140–199 mg/dl 2 h after an oral glucose load) occurs with similar frequency in men and women, but that impaired fasting glucose (IFG; fasting blood glucose 110–125 mg/dl) is more common in women. Furthermore, cardiovascular disease (CVD) mortality is greater for individuals with IGT than for those with IFG. Indeed, studies Zimmet performed in Mauritius, which showed a 40% increase in rates of diabetes over the past decade in individuals of Asian and African ancestry under conditions of increasing obesity, also forecast a huge coming burden of CVD.

The original hypothesis of the thrifty gene was that hyperinsulinemia favors fat storage with selective insulin resistance in muscle, a survival factor in "hunter-gatherer" populations but one leading to the metabolic syndrome in modern humans. The Israeli sand rat (*Psammomys obesus*), originally studied by Cohen, develops insulin resistance, leptin resistance, and features of the metabolic syndrome when placed on a high-fat diet. A subset of these animals develop the diabetic phenotype with a progressive fall in insulin levels and progressive increase in blood glucose. Zimmet described studies performed in collaboration with G.R. Collier comparing lean and obese animals with differential display polymerase chain reaction from hypothalamic tissue of the two groups, allowing identification of genes expressed in the latter group.

A gene termed the Beacon gene (named after Collier's favorite surfboarding beach), which is also expressed in humans, was a prominent difference between animals in the two groups. The gene is composed of 2,194 bp and encodes a 73–amino acid protein that increases food intake and leads to obesity. It is expressed in the retrochiasmic area, an important site of leptin action. Leptin decreases hypothalamic Beacon expression, whereas intracerebroventricular (ICV) Beacon infusion increases body weight and food intake in association with increased hypothalamic neuropeptide Y (NPY) expression. When coinfused intracerebroventricularly with NPY, there is a synergistic increase in food intake and body weight. Zimmet stressed the importance of linking epidemiological, clinical, and basic science research in the development of new approaches to the treatment of diabetes, pointing out that Beacon may provide a target for pharmacological intervention in the maladaptive response to sociocultural change that is increasingly leading to type 2 diabetes.

Related studies. In studies from Collier's group, Sanigorski et al. (abstract 237) examined the expression of genes involved in lipid metabolism in the gastrocnemius muscle of lean, obese hyperinsulinemic, and obese diabetic Israeli sand rats. Muscle triglyceride levels did not increase, and there was no change in peroxisome proliferator–activated receptor-γ2 gene expression, suggesting that these mechanisms may not be primarily involved in this model of the insulin resistance syndrome. Morton et al. (abstract 662) showed that ICV Beacon had little effect on food intake in already obese or obese diabetic Israeli Sand rats, suggesting resistance to the appetite-stimulating effects of Beacon in these animals, paralleling the leptin resistance associated with obesity.

Diabetes epidemiology. Glümer et al. (abstract 435) reported the prevalence of type 2 diabetes and IGT among 1,332 men and 1,261 women in Copenhagen, Denmark. Diabetes was present in 1.8 and 1.5% of men and women at age 40

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Abbreviations: AGE, advanced glycation end product; CVD, cardiovascular disease; EASD, European Association for the Study of Diabetes; ECG, electrocardiogram; ET, endothelin 1; Gal-3, galectin-3; ICV, intracerebroventricular; IFG, impared fasting glucose; IGT, impaired glucose tolerance, IMT, intima-medial thickness, MTHFR, methylenetetrahydrofolate reductase; NO, nitric oxide; NPY, neuropeptide Y; PAI, plasminogen activator inhibitor; PKC, protein kinase C; RAGE, receptor for AGEs; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

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

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years, 9.3 and 3.2% at age 50 years, and 10.3 and 12.5% at age 60 years, whereas IGT was present in 9.1 and 10.4%, 14.1 and 18%, and 18.6 and 18.8% at the same ages. van Dam et al. (abstract 442) reported the relationship between glucose tolerance and physical activity among 424 male inhabitants of the Dutch town Zutphen, aged 69–89 years. The risk of developing IGT decreased 63% among men who cycled regularly and 67% among those who gardened for at least 20 minutes daily. Satman et al. (abstract 433) reported on the Turkey Diabetes Epidemiology study of 24,788 individuals aged at least 20 years from 540 regions, 63% urban and 37% rural. Diabetes was found in 7.2% and IGT in 6.7% of the population, increasing with obesity and hypertension and inversely related to income and education.

Obesity. In a study of low– versus high–glycemic index isocaloric diets given to 11 overweight men without diabetes for 5-week periods, Bouche et al. (abstract 14) reported that total fat mass decreased 0.5 kg with the low–glycemic index diet, mostly because of loss of abdominal fat. The loss of fat mass was associated with decreased subcutaneous abdominal tissue leptin gene expression and a fall in postprandial triglyceride levels. Will et al. (abstract 15) reported that among 101,285 men and 111,285 women enrolled in the American Cancer Society Cancer Prevention Study, men and women with intentional weight loss had 21 and 28% decreases in diabetes risk, the risk decreasing in proportion to the amount of weight lost. Addressing pharmacological obesity treatment, Segal et al. (abstract 170) studied 217 individuals treated with orlistat (120 mg) versus placebo three times daily. Weight loss of at least 3% at 3 months was achieved by 71% vs. 47% of patients and was associated with a fall in HbA_{1c} of ~1% and a weight loss of 7–8 kg at 13 months. Those failing to achieve 3% weight loss at 3 months, whether or not treated with orlistat, showed no significant change in HbA_{1c} and only a 2-kg weight loss at 13 months. Hawkins et al. (abstract 658) treated 243 obese patients with type 2 diabetes with BMI ≥027 kg/m^2 and HbA_{1c} ≥7.5% with orlistat (120 mg three times daily) vs. placebo. At 24 weeks, the two groups had 5.5 vs. 2.6% weight loss, 0.9 vs. 0.4% decrease in HbA_{1c}, and reductions in blood pressure and LDL cholesterol. Rissanen and Taskinen (abstract 657) treated 104 patients with diabetes and BMI >28 with sibutramine (15 mg daily) or placebo. Weight decreased 7.1 vs. 2.1 kg, and triglyceride decreased 12% vs. a 7% rise. Among patients with the small dense LDL phenotype, the fall in triglyceride was 25 vs. 1%. Pizzocri et al. (abstract 16) compared laparoscopic adjustable gastric banding with nonsurgical treatment for obesity among 126 and 115 patients with BMI >35. Both BMI and HbA_{1c} decreased only in the former group, whose visceral adipose tissue mass showed greater decrease than did subcutaneous adipose tissue.

Vascular Disease

The Camillo Golgi Lecture. Umberto Di Mario of Rome, Italy, gave the Camillo Golgi lecture on the relationship between hyperglycemia and the dysregulation of vascular remodeling in diabetes. Among patients who have had type 1 diabetes for 15 years, retinopathy occurs in ~50% and plateaus at this level, whereas CVD is seen in 25% but then increases progressively with time (1,2). This is one of many heterogeneous manifestations of diabetes in differing tissues. The dysregulation of vascular remodeling is uniquely caused by hyperglycemia in diabetes, as opposed to other diseases with vascular manifestations. Additional causative factors that "modify the impact of hyperglycemia" include insulin resistance and the many features of the insulin resistance syndrome, as well as underlying genetic and environmental predisposition. Thus, among individuals developing diabetes, it is those with insulin resistance who are most prone to CVD (3).

Important metabolic pathways for glucose include the polyol pathway, which leads to sorbitol accumulation in target tissues, and the hexosamine pathway (4), leading to protein kinase C (PKC) activation by diacyl glycerol. Both PKC-α and PKC-β play roles in vascular disease. Excessive glucose can also lead to nonenzymatic glycation, causing advanced glycation end product (AGE) formation, oxidative stress via superoxide and lipoperoxide formation, and lipid abnormalities. AGEs act via specific receptors, such as the receptor for AGEs (RAGE) and galectin-3 (Gal-3), and by glycation of glomerular and endothelial basement membranes, leading to increased molecular trapping. Soluble RAGE, which competes with membrane-bound RAGE, prevents activation of this receptor and adverse AGE effects (5), whereas Gal-3 appears to act in the disposal of AGEs, with a Gal-3 "knockout" model showing acceleration of vascular disease. All of these metabolic abnormalities are interrelated and may potentiate each others' actions in causing vascular disease. Thus, oxidative stress may increase both glycation and polyol formation, and administration of antioxidants may decrease PKC activation and AGE formation as well as lower superoxide levels.

PKC acts as another "crossroads" of pathways, activated by AGE, oxidative stress, and polyol accumulation. The PKC inhibitor LY333531 reverses renal and retinal abnormalities seen in animals with hyperglycemia (6). The metabolic abnormalities affect vascular cells, giving rise to release of mediators of cell-to-cell communication, including growth factors, such as transforming growth factor (TGF)-β, IGF-I, and vascular endothelial growth factor (VEGF), all of which are increased in mesangial cells cultured in high-glucose media (7,8). Vasoactive factors include nitric oxide (NO), endothelin (ET)-1, and prostaglandins. NO tends to increase acutely but to decrease with long-term exposure to high glucose levels, and vascular ET cultured in high-glucose media show decreased prostaglandin E2 levels. Hyperglycemia increases ET-1 levels (9) and also increases coagulation factors such as tissue plasminogen activator and plasminogen activator inhibitor (PAI)-1 (10) and the adhesion molecules E-selectin and vascular cell adhesion molecule. "All these," Di Mario stated, "form the basis of the dysregulation of vascular remodeling."

Normally there is ongoing cellular and matrix growth and removal, with changing vascular tone and permeability. The leukocyte-endothelium interaction occurs continuously, as does the equilibrium between fibrinolytic and thrombotic activities. In diabetes, there is increased matrix deposition and increased apoptosis of cellular elements. The former leads to increased basement membrane thickness, one of the best-studied abnormalities in diabetes. Vascular permeability is "one of the key lesions in diabetic nephropathy" (11) with altered endothelial function, as manifested by impaired forearm blood flow response to methacholine. Leukocyte adhesion to endothelium

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increases in response to hyperglycemia (12). There are increased levels of spontaneous platelet aggregation in type 2 diabetes (13) and in individuals with IGT or hyperinsulinemia. These factors lead to the small and large vessel occlusion characteristic of end-stage diabetic organ damage. Neovascularization then occurs as a manifestation of ischemia and hypoxia (14). These vessels may not be competent, leading to worsening tissue damage. An important question is the relationship of these abnormalities to the specific effects of diabetes in retina, kidney, eye, and large vessels.

In the diabetic retina, special features include the initial hemodynamic abnormalities causing increased vascular permeability, leading to apoptosis and high levels of cellular turnover. The acellular capillaries cause retinal hypoxia, increasing VEGF (15) and causing new vessel formation (16,17). This can be reduced by antagonists to VEGF. In the kidney, the earliest findings are selective proteinuria due to glomerular basement membrane changes. This leads to mesangial expansion with overexpression of fibronectin, laminin, and other constituents (18). TGF-β is increased (19) and is one of the growth factors sustaining the mesangial expansion, with an element of genetic predisposition.

In macroangiopathy, hyperglycemia causes dyslipidemia, hypertension, and other abnormalities, leading to endothelial dysfunction with subsequent atherosclerotic plaque and thrombus formation. ET-dependent vasodilation decreases, and clotting factors such as PAI-1 increase. Here, VEGF-induced neovascularization could be reparative, and both vascular wall VEGF levels and VEGF action are impaired in diabetes (20). Gene therapy to increase local VEGF expression and new vessel formation may be a potential therapeutic approach. Thus, to prevent diabetic complications "we have to understand better the interrelationships and hierarchies among the different mediators caused by hyperglycemia." In the future, targeted treatment specific for individual organ pathologies may then become a reality.

Related studies. In a study of CVD presented at the meeting, Hedblad et al. (abstract 2) followed 4,865 individuals from Malmö, Sweden, for 6 years, finding that fasting insulin levels were more frequently elevated among men, as were components of the insulin resistance syndrome, including hypertension, increased triglyceride levels, low HDL cholesterol, and central obesity. After adjustment for age, sex, history of myocardial infarction, HDL, triglycerides, and systolic blood pressure, the finding of fasting insulin above the 75th percentile was associated with 1.75- and 2.37-fold increases in cardiac events and in total mortality. Christiansen et al. (abstract 3) reported increased urinary orosomucoid to have been found earlier than microalbuminuria among 430 patients with type 2 diabetes and to have been associated with a 3.8-fold increase in mortality, as opposed to the 2.7-fold increase among patients with microalbuminuria. Mäkimattila et al. (abstract 5) reported that carotid artery intima-medial thickness (IMT) was 929, 549, and 187 μm in patients with type 1 diabetes in the highest, middle, and lowest tertiles of lifetime glycemia, respectively, based on HbA_{1c} measurement at ages 21 and 32 years in a cohort of 71 patients with type 1 diabetes of 22 years' duration. Chronic hyperglycemia was significant when controlling for age, blood pressure, apolipoprotein B, and cigarette use. Genuth et al. (abstract 6), however, reported data from the Diabetes Control and Complications Trial follow-up study of carotid IMT among 879 patients with type 1 diabetes, showing no significant difference between patients with diabetes and control subjects, although age, male sex, systolic blood pressure, and cigarette smoking were associated with increased IMT. Nichols and Brown (abstract 7) reported that age, duration of diabetes, weight, and obesity were risk factors for congestive heart failure among 9,591 patients with type 2 diabetes followed for 30 months. Paradoxically, those individuals whose HbA_{1c} or blood pressure improved during follow-up had an increase in risk. Gustafsson et al. (abstract 8) analyzed survival among 5,548 individuals hospitalized with congestive heart failure, 907 of whom had diabetes; 57% of those without and 75% of those with diabetes died during 6-year follow-up, with 1.4-fold increase in mortality among men and 1.7-fold increase in mortality among women with diabetes.

Silent ischemia. A number of different investigators addressed the complex issue of approaches to management of patients with diabetes and asymptomatic coronary ischemia. Naskret et al. (abstract 1095) performed single–photon emission computed tomography myocardial-perfusion measurement using technecium-labeled methoxy-isobutyl-isonitrile at rest and during ischemia induced pharmacologically with dipiridamol in 8 type 1 diabetic patients with and 12 without microalbuminuria, all with negative electrocardiographic exercise tests, mean age 26 years, and diabetes duration 15 and 13 years. Six of the former and two of the latter group had coronary blood flow abnormalities, suggesting the importance of albuminuria as an early indicator of coronary heart disease. Cerisier et al. (abstract 244) studied 383 asymptomatic patients with diabetes and abnormal electrocardiogram (ECG) or additional risk factors. Thallium stress imaging was positive in 124 patients; 60 subsequently treated medically showed a 33% cardiac event rate over 2.5-year follow-up, whereas the 64 undergoing immediate catheterization and revascularization when possible had an 8% event rate during the same period. This suggested the benefit of aggressive management. Janand-Delenne et al. (abstract 245) followed 73 patients with type 1 diabetes and 130 with type 2 diabetes with negative ECG and without cardiac symptoms. Exercise ECG or thallium stress testing was performed. Two-year cardiac mortality and major cardiac events occurred in 0.6 and 6.2% of the 171 patients with negative study, but in 6.4 and 16.1% of the 32 with positive evaluation. Coronary angiography in the latter group was positive in 19 patients, whose mortality and event rates were 10.5 and 26.3%, respectively. Valensi et al. (abstract 243) studied the predictive value of silent myocardial ischemia assessed by myocardial scintigraphy combined with a stress test, dipyridamole administration, or both, in 34.1% of 404 diabetic patients without cardiac history but with at least two additional CVD risk factors. Coronary angiography was performed in 63 of the 138 patients, showing significant coronary stenosis in 37. During a mean 37-month follow-up, myocardial infarction, severe arrhythmia, heart failure, unstable angina, sudden death, or coronary revascularization occurred in 53 patients, showing significant association with silent ischemia but not with coronary stenosis. In multivariate analysis of patients over age 60, silent ischemia was associated with a

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3.2-fold increase in risk of subsequent coronary events. Brulport-Cerisier et al. (abstract 1085) reported that one-third of 82 patients with positive stress Thallium scans, without history of myocardial infarction or revascularization, had negative findings on coronary arteriography, suggesting microcirculatory abnormality. The 39 patients without symptoms of angina had an average of three additional risk factors, showed anterior ischemia on scan in approximately one-quarter of patients, and had three-vessel disease in one-third of arteriograms, findings similar to those in the 43 patients with angina.

Mediators of CVD in diabetes. Stefanidis et al. (abstract 1089) and Melidonis et al. (abstract 1088) tested the impact of strict metabolic control by 72-h insulin infusion during unstable angina or non-Q myocardial infarction in 48 type 2 diabetic patients on combined systolic and diastolic myocardial performance. Systolic function and overall myocardial performance both showed 13% improvement. Total duration of ischemia was 10 vs. 40 min during the first 48 h of treatment of the groups with versus without insulin infusion.

Erkens et al. (abstract 1075) reported that 4,864 patients receiving oral antidiabetic therapy were 3.5 times more likely than nondiabetic control subjects to require treatment with CVD drugs at the time diabetes treatment was started, and that the increased requirement for CVD treatment became significant 7 years before diagnosis of diabetes. A number of potential factors other than hyperglycemia may therefore play roles acutely and chronically in the effects of diabetes on the heart. Dyntar et al. (abstract 148) reported that palmitic acid (0.5 mmol/l) but not glucose (33.3 mmol/l) caused apoptosis of cardiomyocytes in long-term culture, although both showed destructive effect on the cytoskeleton and on the myofibrillar apparatus. Kaye et al. (abstract 296) studied the relationship between plasma homocysteine, serum and red cell folate, pyridoxine (B_6), and methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and the presence of clinical coronary artery disease in 355 patients with type 1 and 392 with type 2 diabetes. A low folate level was associated with increased homocysteine, particularly with the MTHFR gene mutation, but the mutation decreased the response of homocysteine to folate supplementation. B_6 supplementation was only of benefit for those deficient in this vitamin. Leinonen et al. (abstract 1065) found a negative correlation between IGF-I and carotid artery IMT, in addition to the expected positive correlation with albuminuria and apolipoprotein B, suggesting a new risk association that may explain features of atherosclerosis. Dekker et al. (abstract 1081) described benefits of moderate alcohol use in the Hoorn Study of 2,407 men and women aged 50–75 years at baseline in 1989. There were 270 deaths over the subsequent decade. For those with normal and abnormal glucose tolerance, mortality was 48 and 65% lower with up to 10 g alcohol per day. With >30 g/day alcohol in those with normal glucose tolerance and >10 g/day alcohol among those with abnormal glucose tolerance, the apparent benefit was lost.

Dyslipidemia is another factor contributing to cardiac disease. Bos et al. (abstract 1107) found, in the Hoorn study, that individuals without diabetes had a 1.6- to 1.8-fold increase CVD mortality with triglycerides >177, HDL <39, or both, whereas individuals with diabetes had a 1.5-fold increase in risk with low HDL alone but a 3-fold increase in risk with high triglyceride, either with or without low HDL. Sattar et al. (abstract 286) analyzed factors predicting development of hyperglycemia in 192 of the 5,940 participants in the West of Scotland Coronary Prevention Study who did not have evidence of this at study entry. In multivariate analysis, BMI, leukocyte count, triglyceride level, and pravastatin treatment were significant. Patients randomized to pravastatin had a 26% decrease in the risk of developing hyperglycemia.

Diabetic Neuropathy

A number of studies addressed issues of diabetic neuropathy. In a report suggesting that diabetic neuropathy is associated with disease of the spinal cord, Eaton et al. (abstract 951) studied 19 diabetic patients with neuropathy (9 with chronic pain and 10 without), 10 with diabetes without neuropathy, and 10 normal healthy control subjects. Spinal cord cross-sectional areas using T2-weighted axial magnetic resonance images were 86.9 vs. 99.9 and 51.9 vs. 57.3 mm^2 at C4/5 and T3/4 in patients with neuropathy versus the two control groups. Atrophy, defined as area <2 SD below the mean of control subjects, was present in 9 of the 19 patients with neuropathy. Agrawal et al. (abstract 196) treated 60 patients with type 2 diabetes and painful neuropathy with sodium valproate (1,200 mg) or placebo for 1 month, showing significant improvement in pain score without change in electrophysiological parameters. One patient had elevations in hepatic transaminase levels.

Foot Ulcers

Slater et al. (abstract 941) reported that cultures of superficial swabs, outer necrotic tissue, tissue taken from the deepest removed debrided material, and a deep swab after the completion of debridement of 25 infected diabetic foot wounds were all positive, with identical organisms in 19 of the 25 wounds. The superficial swab identified all pathogens recovered from the deep-tissue specimen in 22 of the wounds, suggesting this to be a useful site for cultures. Armstrong and Nguyen (abstract 60) presented a double-blind, randomized controlled trial of intermittent foot compression with a functioning versus placebo (nonfunctioning) foot compression device following debridement of infected diabetic foot wounds in 115 patients; 75 vs. 51%, healed, suggesting that edema reduction may play a role in wound healing following debridement. Markevich et al. (abstract 59) reported a 30-month randomized, multicenter, double-blind controlled clinical trial of maggot therapy for diabetic neuropathic foot wounds as compared with conventional modern treatment in 140 diabetic patients. Sterile maggots (larvae) of the green-bottle fly (*Lucilia sericata*) were applied to the wound (6–10/cm^2) for 72 h. At 10 days, granulation tissue covered >50% of the wound in 60 vs. 34%, and wound area had decreased by >50% in 51 vs. 27%. This may be a useful method for debridement of necrotic tissue from diabetic foot wounds, with particular benefits in stimulating tissue growth and improving the rate of healing.

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