Inflammation and Insulin Resistance

ZACHARY T. BLOOMGARDEN, MD

This is the second of two articles describing a symposium on the relationship between inflammation and insulin resistance that was held in Niagara Falls, NY, 20–21 September 2002.

Studies in man
Antonio Ceriello (Udine, Italy) discussed the role of glucose intake and postprandial hyperglycemia in the development of diabetes complications, as well as the relationship of hyperglycemia to oxidative stress. The DECODE (Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe) study showed high 2-h postload glucose to be associated with increased mortality independent of fasting glucose (1), and the Pacific and Indian Ocean Study showed isolated 2-h hyperglycemia to double the risk of mortality (2). The Funagata Diabetes Study showed that impaired glucose tolerance (IGT) but not impaired fasting glucose was a risk factor for cardiovascular disease (CVD) (3). There is evidence that lowering postprandial glucose improves outcome. Post hoc analysis of the STOP-type 2 diabetes study showed that myocardial infarction and hypertension decrease with use of the prandial glucose-lowering agent acarbose (4). In the Kumamoto study, postprandial hyperglycemia strongly predicted retinopathy and nephropathy (5).

Endothelial dysfunction (ED) is a potential mediator of the effect of prandial glyemia, with altered vasodilation and procoagulant abnormalities. ED can be induced by hyperglycemia following a 75-g oral glucose load in persons with normal or IGT or with diabetes, with reduction of flow-mediated brachial artery dilation proportional to the degree of hyperglycemia (6). In a study of 225 persons with hypertension followed for 32 months, forearm ED was a marker of future CVD events (7), with a 4.5-year follow-up of 281 persons showing both ED and measures of oxidative stress to predict CVD events (8). Acute hyperglycemia may suppress vasodilation, which may involve oxidant stress, as it is reversed with antioxidant or L-arginine treatment (9).

Glucose increases endothelial cell free radical production leading to activation of nuclear factor (NF)-κB and protein kinase C, as well as enhancing intracellular advanced glycation end product formation and sorbitol accumulation (10). Glucose increases superoxide anion production and activation of NAD(P)H oxidase, with peroxynitrite leading to single-strand breaks in DNA. Glutathione reverses systemic hemodynamic changes induced by acute hyperglycemia (11). In endothelial cells, hyperglycemia leads to considerably greater increase in superoxide than nitric oxide, leading to peroxynitrite and nitrotyrosine production. Nitrotyrosine is not only a marker of oxidative stress but may itself lead to cardiomyocyte apoptosis and to vascular DNA damage. Nitrotyrosine is proportional to hyperglycemia in persons with diabetes (12), and nitrotyrosine levels are increased in persons with ED (13). Following glucose loads a variety of measures show increase in oxidant stress. Postprandial hyperglycemia, increase in nitrotyrosine, and ED in persons with diabetes can be prevented following a standardized meal with insulin administration. Persons with or without diabetes show ED following fat or glucose ingestion, particularly with the two in combination.

Ishwarlal Jialal (Sacramento, CA) discussed the major biological antioxidants, enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, antioxidant scavengers, and antioxidant nutrients such as the d-α-tocopherol form of vitamin E. In epidemiological and case control studies, plasma vitamin E levels are associated with CVD. There is evidence of increased oxidative stress in diabetes, perhaps potentiated by protein glycation, with low plasma and monocyte ascorbate and increased production of reactive oxygen species. Persons with diabetes have increased levels of oxidized LDL (ox-LDL) autoantibodies and increased oxidative susceptibility of LDL. Urinary F2 isoprostanes, a novel direct measure for oxidative stress, are increased in persons with diabetes, and α-tocopherol supplementation significantly decreases these levels, as well as decreases levels of lipid peroxides. Monocyte superoxide production is increased with diabetes and can be decreased by administration of 1,200 units of α-tocopherol for 3 months. Levels of interleukin (IL)-1β, IL-6, C-reactive protein (CRP), intracellular adhesion molecule, vascular cell adhesion molecule, E- and P-selectin, and plasminogen activator inhibitor-1, as well as monocyte endothelial adhesion, lipid peroxidation, and platelet aggregation, are increased in persons with diabetes, and α-tocopherol supplementation reverses these abnormalities. In a study of 30 persons with diabetes and albuminuria, vitamin C, 1,250 mg, and d-α-tocopherol, 680 units, daily for 4 weeks, decreased albuminuria from 243 to 197 mg daily (14). Another study of 36 persons with type 1 diabetes treated with α-tocopherol, 1,800 units daily for 4 months, showed increased retinal blood flow and improved creatinine clearance (15).

There may be evidence of benefit of antioxidants in CVD prevention. The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study of 29,113 male smokers from Finland showed a 38% decrease in nonfatal myocardial infarction and a 9% decrease in angina, although subarachnoid hemorrhage mortality increased 181% (without significant overall effect on stroke) and there was an 18% increase in lung cancer, as well as increase in gastic and prostate cancer, with consequent studies confirming adverse effects of β-carotene (16). The Cambridge Heart Antioxidant Study (CHAOS) of 2,002 persons with CAD treated with α-tocopherol showed a 77% decrease in nonfatal myocardial infarction, although without

Abbreviations: CRP, C-reactive protein; CVD, cardiovascular disease; ED, endothelial dysfunction; ICU, intensive care unit; IFN-γ, γ-interferon; IGT, impaired glucose tolerance; IL, interleukin; iNOS, inducible NO synthase; NF, nuclear factor; ox-LDL, oxidized LDL; TNF, tumor necrosis factor.

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change in CVD mortality (17). The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study compared d-α-tocopherol 272 units, ascorbate 500 mg, both, or neither, showing a decrease in carotid IMT with both vitamins among men, although not among women (18). The Secondary Prevention with Antioxidants of Cardiovascular Disease in End-stage Renal disease (SPACE) study randomized 196 persons on hemodialysis with preexisting CVD to d-α-tocopherol 800 units daily versus placebo, showing a 46% reduction in CVD end points. The Primary Prevention Project antioxidant trial of 4,495 persons with hypertension, hypercholesterolemia, or other risk factors showed a 46% reduction in peripheral artery disease over 500 days, although without significant decrease in CVD events (19). In a study of 40 patients who had undergone cardiac transplant within 2 years, vitamin E 800 units and vitamin C 1 g daily for 1 year decreased plaque area measured by intravascular ultrasound (20). Jialal concluded that there may be some evidence of benefit with at least 800 units of natural d-α-tocopherol and suggested the need to use biomarkers of compliance to assure an adequate intervention, choosing persons with increased risk of oxidative stress and inflammation.

Several studies, however, do not demonstrate benefit of antioxidant vitamin treatment. Racemic α-tocopherol treatment of 26 persons showed no decrease in superoxide or CRP levels (21). In the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico (GISSI) study, an open label trial of 11,324 persons treated with N-3 polyunsaturated fatty acids 1 g, racemic α-tocopherol 300 mg, both, or neither, there was no effect of α-tocopherol, although N-3 polyunsaturated fatty acids decreased CVD mortality 20% (22). The HOPE study compared 400 units of vitamin E with placebo, showing no benefit, although the combination of tocopherols and tocotrienols from natural sources may not have effectively increased plasma α-tocopherol (23). The Heart Protection Study treated 20,536 patients, 3,982 of whom had diabetes, with 600 mg vitamin E, 250 mg vitamin C, and 20 mg β-carotene, or with placebo, without evidence of benefit and with a significant increase in LDL cholesterol of 3 mg/dl and in triglyceride of 19 mg/dl, as well as a fall in HDL cholesterol of 1 mg/dl (24).

Steven Haffner (San Antonio, TX) discussed diabetes and CVD risk. Two-thirds of deaths of persons with diabetes are from CVD. Comparing coronary mortality rates from 1971 to 1975 with those from 1982 to 1984, levels declined 40% in men and 20% in women without diabetes, while decreasing 17% in diabetic men and increasing 11% in diabetic women (25). Framingham data show that the relative increase in risk of events is greater among diabetic versus nondiabetic women than among men (26). During the first year following a myocardial infarction, mortality in diabetic men was 45 vs. 30% in nondiabetic men, and 38 vs. 20% in women, with out-of-hospital mortality 29 vs. 22% and 11 vs. 12%, respectively, hospitalization through 28-day respective mortality 11 vs. 8% and 20 vs. 8%, and 28 day through 1-year respective mortality 6 vs. 3% and 8 vs. 2% (27). The 7-year incidence of myocardial infarction in the Finnish East-West study was 4 vs. 19% in nondiabetic persons without and with history of prior myocardial infarction but 20 vs. 45% in diabetic persons, respectively (28). The Organization to Assess Strategies for Ischemic Syndromes (OASIS) study showed similar information with relative mortality risk of 1.71 in nondiabetic persons with CVD, of 1.99 in diabetic persons without CVD, and of 2.88 in diabetic persons with CVD (29). Thus, persons with diabetes without history of myocardial infarction have similar risk to nondiabetic persons with myocardial infarction history, with the highest rate among persons both with diabetes and history of myocardial infarction, suggesting that even more stringent goals may be appropriate for these persons. Furthermore, of 181 persons who had had myocardial infarction and were not known to have diabetes, 35 and 40% had IGT at discharge and 3 months later and 31 and 25% had new diabetes at these times; therefore, in total approximately two-thirds of persons with myocardial infarction had abnormal glycemia (30).

Approaches to treatment of macrovascular disease include improved glycemic control, which does not completely eliminate excess CVD risk, risk factor modification, and diabetes prevention. In the U.K. Prospective Diabetes Study (UKPDS), as mean blood pressure increased from 110 to 170 mmHg, rates of myocardial infarction increased from 18 to 35 per 1,000 person-years (31), and as mean HbA1c increased from 5 to 11%, myocardial infarction rates increased from 18 to 36 per 1,000 person-years (32). There is evidence that the prediabetic state is atherogenic (33). Those persons who develop diabetes have higher triglycerides, blood glucose, insulin, and blood pressure and lower HDL cholesterol. Insulin resistance is associated with increased risk factors (34). Among persons who develop diabetes, insulin resistance alone was present in 29%, insulin deficiency alone in 16%, both in 54%, and neither in 2% (35). In this study, insulin-resistant converters had greater levels of risk factors, including triglycerides, HDL, and hypertension, than those who were insulin deficient alone, with obesity not fully explaining the differences. Thus, CVD risk often is elevated before development of diabetes (36).

Andrew Selwyn (Boston, MA) gave “a cardiologist’s view” of inflammation, thrombosis, and stents, noting that intravascular ultrasound shows that >90% of “normal” arteries on angiography have significant plaque burden. Mean LDL in the population increases from ∼30 mg/dl at birth to 70 mg/dl at age 20 years and to 120 mg/dl after age 30 years, when atherosclerosis begins to be observed, with steady progression thereafter. Three potential areas of intervention in the cell biology of early atherosclerosis are ED, procoagulant abnormalities, and chronic low-grade inflammation (37). Worse coronary endothelial function is associated with greater upregulation of endothelial nitric oxide (NO) synthase, which appears to reflect partial compensation for increase in NO degradation (38). ED is related to the presence of risk factors, suggesting the importance of aggressive LDL lowering. Statin-treated persons showed decreased macrophages, T-cells, and apoptotic cells in atherosclerotic plaques, with a linear relationship between the degree of LDL lowering and the reduction in risk of CVD events that was shown recently in a report in which fluvastatin decreased death and myocardial infarction by 58%, with particular benefit in persons with diabetes (39). In another study, atorvastatin 80 mg daily without angioplasty decreased LDL cholesterol to 77 mg/dl, with 13% 18-month risk of ischemic events, in comparison to an ischemic event rate of 21% among persons undergoing angioplasty with mean LDL cholesterol 119 mg/dl (40). Selwyn noted,
Perspectives on the News

however, that too often physicians do not administer statins “with any vigor at all, despite evidence that those persons given such treatment have improved outcome.”

Hypercholesterolemia is associated with increased platelet deposition, which normalizes with pravastatin treatment (41), and with increased monocyte chemotaxis, which decreases rapidly with statin treatment, and can be reversed by administration of mevalonate (42). CRP levels decrease within 1 month with maximal fall after 12 months of statin treatment (43). Intracoronary infusion of the Rho-kinase inhibitor fasudil prevents acetylcholine-induced coronary artery constriction (44), and statins bind to integrins and interfere with the activation of Rho and other small protein triggers of inflammation. In the advanced lesion, ox-LDL particles are present and play a key role. In a study comparing 10 persons who had stable angina with 23 having unstable angina, atherectomy specimens showed increased ox-LDL–filled macrophages in the latter group (45). ox-LDL is involved in production of free radicals, which increase peroxynitrite production. ox-LDL also increases levels of the nuclear transcriptional activator, NF-κB (46).

CRP levels predict risk of restenosis, and among persons with diabetes, the risk of angiographic restenosis may exceed 40%. Use of stents decreases restenosis, with a 22% decrease in death and myocardial infarction in persons with unstable angina after stenting (47). In-stent restenosis involves leukocyte recruitment followed by inflammatory cell migration, and inhibition of vascular smooth muscle cell migration and proliferation appears to effectively prevent this, particularly with immunosuppressive or antiproliferative agents bound locally to the stent such as paclitaxel, the taxane derivative QP-2, rapamycin, actinomycin D, dexamethasone, tacrolimus, and everolimus. Sirolimus binds to an intracellular site and when used to coat a stent decreases restenosis from 26% to nil (48).

Greet van den Bergh (Leuven, Belgium) discussed the effects of maintaining normoglycemia with insulin for patients in intensive care units (ICUs). Hyperglycemia is common in ICU patients, caused by insulin resistance in liver and muscle, which may be seen as “adaptive” in providing glucose for brain, erythrocytes, and wound healing. In clinical practice, she stated, this is typically treated only when glucose levels exceed 215 mg/dl. However, hyperglycemia may predispose to complications. IGF binding protein 1, a marker of decreased insulin action, is a strong marker of poor outcome among ICU patients. She described a study of 1,548 patients admitted to the ICU on a ventilator, placed on a feeding regimen with glucose 9 g/h initially, with additional calories given over the subsequent several days, and randomized to intensive insulin to maintain glucose levels of 80–110 mg/dl or to conventional insulin administration for glucose levels exceeding 180–200 mg/dl (49). Mortality in ICU and in hospital, bacteremia, inflammatory markers, risk of renal failure, need for transfusion, hyperbilirubinemia, critical illness polyneuropathy, need for prolonged mechanical ventilation, and cost were assessed. The mean daily insulin dose was 71 vs. 33 units/day, leading patients to have blood glucose levels of 103 vs. 153 mg/dl, respectively. Of intensively treated patients, 10% required >20 units/h and only six patients were not controlled with doses up to 50 units/h on their protocol. Hypoglycemia was seen in 5 vs. 1%, occurring when feedings were reduced. ICU mortality was 4.6 vs. 8%, and among long-stay patients (>5 days) it was 10.6 vs. 20.2%. Multiple organ failure with sepsis was seen in 8 vs. 33%. Bacteremia decreased by 46%, prolonged antibiotic treatment by 35%, dialysis need by 41%, critical illness polyneuropathy by 44%, mechanical ventilation >14 days by 37%, and ICU stay >14 days by 27%. Among those in the ICU for >5 days, renal failure was seen in 15 vs. 24%. Overall, ICU stay was 3 days shorter with intensive insulin treatment. Cost decreased 23%, translating into a cost saving of >$2 million annually in their 56-bed ICU. CRP levels exceeding 150 mg/l were seen in 15 vs. 21%, and the decrease in CRP was an important predictor of decrease in mortality, with high CRP a risk marker in the control but not in the insulin-treated group. van den Bergh suggested that the glucose level itself is the important factor, as higher insulin dose was associated with higher risk of mortality, and for every 50 mg/dl higher glucose there was a 75% increase in mortality. Ongoing studies in medical ICUs, pediatric ICUs, and in general populations of hospitalized patients will be important. Potential mechanisms of the insulin effect may include changes in leukocyte function and fatty acid and triglyceride lowering.

Inflammation and the islet

John Corbett (St Louis, MO) discussed mechanisms of islet inflammation and macrophage activation following virus-induced β-cell damage. T-cells, both CD4 and -8, produce the cytokines IL-2, tumor necrosis factor (TNF)-α, and γ-interferon (IFN-γ), leading to B- and killer T-cell activation. IL-1 inhibits glucose-induced insulin secretion and results in fragmentation of islets, via signal transduction events leading to NF-κB production, with inducible NO synthase (iNOS) production leading to oxidative phosphorylation and ultimate β-cell death. Tissue macrophages resident in the normal islet are activated by TNF and IFN, producing IL-1 and leading to β-cell damage. Thus, rather than the β-cell, the target of environmental toxins or viral infections as trigger events may be these macrophages. Viruses induce diabetes in animal models, with viral double-stranded (ds)RNA stimulating iNOS expression and NO production, leading to cytokine expression and release, as well as the stimulation of PKR, which activates NF-κB. Using synthetic dsRNA and IFN-γ, insulin secretion can be inhibited with development of β-cell degeneration, with an IL-1 receptor antagonist blocking this effect. dsRNA-dependent protein kinase deficient mice still show the dsRNA and IFN-γ effect; therefore, other cellular mechanisms are involved in the regulation of proinflammatory cytokines and iNOS in response to viral infection. Phospholipase A2 has diverse physiological functions that are involved in glucose-induced insulin secretion from β-cells and in “housekeeping” in macrophages, and the activity of islet PLA2 is increased by dsRNA, suggesting another system active in islet inflammation.

Jonathan Lakey (Edmonton, Canada) discussed advances in clinical islet transplantation. Difficulties include procurement, preservation, enzymes used for dissociation, purification, graft characterization, and new regulations for islet transplantation. Islet isolation began in the 1970s, and current approaches take four to five technicians 8 h at a cost of ~$15–30,000 per processed pancreas. Factors predicting success of islet isolation are donor age, BMI, and pancreas procurement locally, while glucose level
and allowing temperature increase of the pancreas are among factors predicting failure. The use of a perfluorochemical to increase oxygenation may be helpful in increasing islet recovery. A purified enzyme blend developed with Roche may improve islet recovery, particularly when administered with an infusion pump. Product release criteria include sufficient islet mass (>5,000 islet equivalents/kg recipient weight), blood group matching, and negative results of gram stain and endotoxin testing. A total of 37 patients (16 men and 21 women) had been transplanted in Edmonton for type 1 diabetes, with 83% having hypoglycemic unawareness and 46% excessive glycemic lability. Their mean age was 42 years and mean duration of diabetes 26 years. On average, each person received 800,000 islets in two transplants, with 36 days between transplants. Two patients have required four donors. One person had portal vein thrombosis and did not achieve insulin independence. At 1 year, 89% were C-peptide positive and 85% insulin independent. HbA1c decreased from 8.1 to 5.8%. Complications of islet transplantation in 151 procedures performed worldwide include portal vein thrombosis in 4, liver bleeds in 3, gall bladder bleed in 1, and transient liver chemistry rise in approximately half of patients. Immunosuppressant treatment has led 51% of patients to require statin treatment and 78% to develop buccal ulcers, with infections and other typical immunosuppressant treatment adverse effects as well. Of persons who became C-peptide negative after initial response to islet transplantation, two appear to have had recurrence of autoimmune islet destruction. An ongoing nine-center trial has performed 20 transplants to date, of which led to insulin independence after single donors. Although there have been recent reports of administration of mixtures of porcine islets and Sertoli cells administered to 15 children in Mexico, Lakey expressed concern at the validity of that report and caution about the idea of islet xenotransplantation.

References

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