
Perspectives in Diabetes

Microvascular Complications of Impaired Glucose Tolerance

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Impaired glucose tolerance (IGT) serves as a marker for the state of insulin resistance and predicts both large- and small-vessel vascular complications, independent of a patient's progression to diabetes. Patients with IGT are at significantly increased risk for death and morbidity due to myocardial infarction, stroke, and large-vessel occlusive disease. IGT is more predictive of cardiovascular morbidity than impaired fasting glucose, probably because it is a better surrogate for the state of insulin resistance. IGT is also independently associated with traditional microvascular complications of diabetes, including retinopathy, renal disease, and polyneuropathy, which are the topics of this review. Inhibition of nitric oxide-mediated vasodilation, endothelial injury due to increased release of free fatty acids and adipocytokines from adipocytes, and direct metabolic injury of endothelial and end-organ cells contribute to vascular complications. Early detection of IGT allows intensive diet and exercise modification, which has proven significantly more effective than drug therapy in normalizing postprandial glucose and inhibiting progression to diabetes. To what degree intervention will limit recognized complications is not known. *Diabetes* 52:2867–2873, 2003

Ten percent of Americans will develop type 2 diabetes during their lifetime. However, >20% will develop a more modest defect in glucose metabolism, designated impaired glucose tolerance (IGT). IGT, defined as a serum glucose concentration between 140 and 199 mg/dl 2-h after a 75-g glucose load, reflects hepatic gluconeogenesis and slower uptake of glucose from blood into skeletal muscle and adipose tissue following a meal. Thus, an oral glucose tolerance test (OGTT) is a more sensitive measure of early abnormalities in glucose regulation than fasting plasma glucose or

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ADA, American Diabetes Association; DPP, Diabetes Prevention Project; eNOS, endothelial nitric oxide synthase; FFA, free fatty acid; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; OGTT, oral glucose tolerance test; ROS, reactive oxygen species.

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HbA_{1c}. A developing literature links IGT to complications that traditionally are attributed to diabetes. These complications include both macrovascular (myocardial infarction and stroke) and microvascular (retinopathy, and microalbuminuria, polyneuropathy) disease. Vascular endothelial and end-organ damage associated with IGT occurs due to a combination of transient hyperglycemia and lipotoxicity itself, as well as resulting from insulin resistance and compensatory hyperinsulinemia.

This review discusses IGT and its natural history and examines the clinical consequences and pathogenesis of metabolic and vascular damage associated with IGT. We searched MEDLINE for English-language articles published from 1966 through June 2003 about IGT, postprandial hyperglycemia, and early diabetes in relationship to health effects and complications. Reference lists from retrieved articles were examined for additional pertinent articles or sources. The authors reviewed references jointly and placed emphasis on prospective study data where available.

Compared with age-matched normoglycemic control subjects, patients with IGT are at greater risk for death from all causes (1,2) and have a two- to fivefold increased incidence of new-onset cardiovascular ischemia, fatal and total myocardial infarction, and stroke, independent of progression to diabetes (2,3). These macrovascular complications of IGT have been extensively reviewed elsewhere. This review will focus on the emerging recognition of IGT as a cause for microvascular complications: peripheral neuropathy, retinopathy, and microalbuminuria.

Recognition and natural history of IGT. Among routine tests of glucose metabolism, the 2-h OGTT most closely reflects postprandial glucose disposal. Patients fast overnight, then have blood drawn for a serum glucose immediately before and 2 h following an oral load of 75 g of dextrose. Under current American Diabetes Association (ADA) criteria (Table 1), IGT corresponds to a 2-h serum glucose level between 140 and 199 mg/dl, while diabetes is defined as a 2-h value ≥ 200 mg/dl (4). The 1997 ADA criteria also established a range of fasting plasma glucose (110–125 mg/dl) meant to correspond with IGT, designated impaired fasting glucose (IFG). However, numerous studies indicate that OGTT is more sensitive in detecting early glucose dysregulation than fasting plasma glucose. This disparity in sensitivity is particularly acute in younger patients: 23 of 112 obese adolescents (21%) had IGT, while only one had IFG (5).

A recent screening study in an unselected population

TABLE 1
1997 ADA criteria (4) for IFG, IGT, and diabetes

Condition	Fasting plasma glucose (mg/dl)	Test and values 2-h OGTT (mg/dl)	HbA _{1c} (%)
Normal	≤109	≤139	NA
IFG	110–125	NA	NA
IGT	NA	140–199	NA
Diabetes	≥126	≥200	Variable

IFG is preferred to IGT by the ADA because of its greater convenience, but IFG and IGT are regarded as equivalent. The ADA recommends against use of HbA_{1c} as a diagnostic test for diabetes. HbA_{1c} threshold for diabetes is variable, but typically between 6.1 and 7.0%. No HbA_{1c} values are recognized as equivalent to IGT or IFG.

found the prevalence of IGT to be 11.2% for ages 50–59 years, peaking at 14.2% for ages 60–75, and declining in individuals older than 75 (6). Investigations of the natural history of IGT reveal a dynamic and reversible state. In a study of 200 adults >70 years old in Finland, one-third progressed from normoglycemia to IGT. Of IGT patients in this cohort, one-third reverted to normal glucose tolerance and one-fifth progressed to diabetes. Almost one-fifth of those with baseline diabetes reverted to IGT (7). The Diabetes Prevention Program (DPP) randomized 3,244 patients with IGT to treatment with placebo, the biguanide antihyperglycemic agent metformin, or intensive diet and exercise counseling. Nearly 30% of 1,082 subjects receiving placebo progressed from IGT to diabetes >3 years, but during this same period 25% reverted to postprandial normoglycemia (8). Overall, most patients progress toward greater glycemic dysregulation, but this progress appears to be slow. In the DPP, mean fasting plasma glucose among placebo subjects rose from 107 to 112 mg/dl over the average 3-year follow-up (8). Unmonitored patients probably experience many years of occult insulin resistance and postprandial hyperglycemia before developing typical symptoms of diabetes.

IGT is a surrogate for, and largely a consequence of, two intertwined and self-reinforcing processes that are intimately associated with obesity and drive prediabetic hyperglycemia: insulin resistance and dyslipidemia (9). In obesity, as skeletal muscle loses sensitivity to insulin, more glucose is taken up by adipocytes, both stimulating production and release of free fatty acids (FFAs) and triglycerides and increasing adiposity. Elevated circulating FFAs in turn promote hyperglycemia by stimulating hepatic gluconeogenesis while inhibiting insulin-mediated glucose uptake and storage as glycogen.

Microvascular complications of IGT: neuropathy, retinopathy, and renal microproteinuria. Neuropathy is a common complication of diabetes occurring over time in more than half of patients with type 2 diabetes. Nerve conduction studies demonstrate that neuropathy is already present in 10–18% of patients at the time of diabetes diagnosis (10), suggesting that peripheral nerve injury occurs at early stages of disease and with milder glycemic dysregulation. The concept that neuropathy is an early clinical sign of diabetes was proposed >40 years ago, and most studies report an association between IGT and neuropathy. Prospective screening of patients with otherwise “idiopathic” neuropathy using 2-h OGTT shows that, using the 1997 ADA criteria (11,12), 30–50% of these

patients have IGT, a significantly higher prevalence than seen in the age-matched general population (6). Similarly, a cross-sectional study using a combination of physical examination, focused history, and vibration threshold measurements found neuropathy in 26% of 279 patients with diabetes, 11.2% of 89 patients with IGT, and only 3.9% of 577 age-matched normal control subjects (13).

Most patients with IGT and associated neuropathy have a symmetric, distal sensory polyneuropathy with prominent neuropathic pain (11,12). In the largest prospective series, 81% of neuropathy patients with IGT had exclusively sensory complaints, and 92% recognized neuropathic pain as a dominant symptom of their neuropathy (11). IGT neuropathy is phenotypically similar to early diabetic neuropathy, which also causes sensory symptoms, including pain, and autonomic dysfunction. In a survey of 669 patients with early diabetic neuropathy, sensory symptoms were present in >60%, impotence in nearly 40%, and other autonomic involvement in 33%, but evidence of motor involvement in only 12% (14). These clinical findings suggest prominent early involvement of the small unmyelinated nerve fibers that carry pain, temperature, and autonomic signals. Direct quantitation of unmyelinated intraepidermal nerve fibers from skin biopsies shows similar fiber loss and altered morphology in patients with neuropathy associated with IGT and early diabetes (15).

Autonomic dysfunction, particularly erectile dysfunction and altered cardiac vagal response, are common early features of neuropathic injury in diabetes (16). Work with IGT patients also suggests prevalent vagal dysautonomia: separate studies have found abnormal heart rate recovery following exercise, blunted R-R interval variability to deep breathing, and reduced expiration to inspiration ratio (all measures of vagal dysautonomia) in a greater fraction of IGT patients than age-matched normoglycemic control subjects (17).

There is accumulating evidence that IGT is also associated with both renal and retinal injury. As with neuropathy, a significant number of patients have nephropathy or retinopathy at the time of diabetes diagnosis (18,19), suggesting that these complications occur early in glucose dysregulation. Multiple studies document an increased prevalence of microalbuminuria in patients with IGT. Microalbuminuria, defined as a urine albumin-to-creatinine ratio of 30–299, is predictive of progression to frank proteinuria and is associated with cardiovascular mortality (20). Among >5,000 Maoria and European subjects, microalbuminuria was found in 21% of those with diabetes and 16% of those with IGT, but only 4% of normoglycemic individuals (21). A very large population-based study in China performed OGTT in 4,000 patients with elevated postprandial glucose selected from >100,000 subjects. The rate of albumin excretion was significantly higher among IGT subjects compared with the nondiabetic control group (7.2 ± 5.7 vs. 4.5 ± 2.8) (22). Similar data are available for several other ethnic groups (23).

Clinical studies on retinopathy by multiple investigators have documented reduced visual acuity and contrast sensitivity in subjects with IGT (24,25). Reduced acuity was observed in the Rancho Bernardo study despite a very low

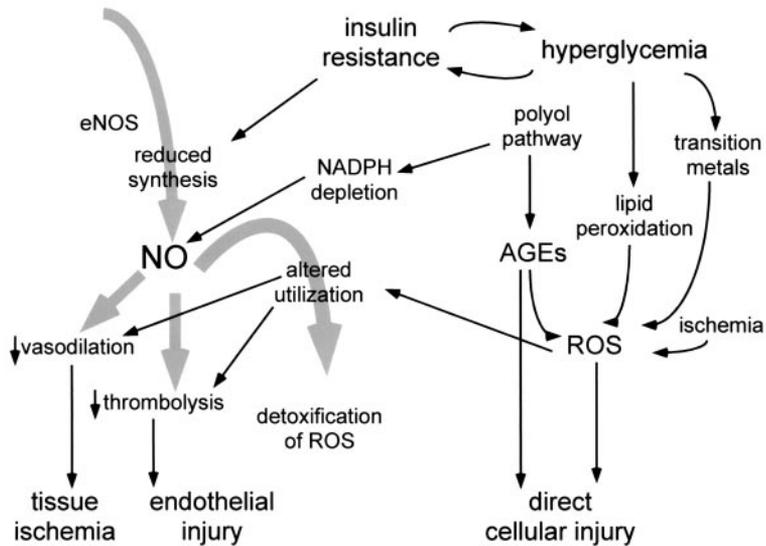


FIG. 1. Disruption of vascular NO signaling and direct metabolic injury contribute to IGT complications. NO synthesized by eNOS mediates large- and small-vessel vasodilation and promotes endothelial thrombolysis. Transient or sustained hyperglycemia depletes NADPH and increases formation of ROS. ROS directly damage endothelial and other target organ cells and alter utilization of NO from vasoregulatory tasks to detoxification of ROS. AGEs increase ROS formation and cause direct metabolic injury. These synergistic insults promote small-tissue ischemia, large-vessel atherogenesis and thrombogenesis, and contribute to complications in IGT.

incidence of frank retinopathy (24). Independent studies have noted an approximate fourfold increase in the prevalence of retinopathy among Pima Indians with IGT compared with age-matched control subjects (23). In the Western Samoa survey, 10% of IGT subjects had retinopathy as compared with 17% of those with newly diagnosed diabetes and 45% of known diabetic subjects. Collectively, the data suggest that the first stage of retinal injury occurs during IGT. Indeed, metaanalysis suggests that risk for retinopathy or nephropathy begins to increase with a 2-h glucose of 9.0 mmol/l, which is in the lower half of the IGT range (23).

Pathogenesis of IGT complications: general considerations. Direct metabolic injury, changes in vascular reactivity, and accumulating endothelial injury each play a role in the pathogenesis of complications observed in patients with IGT. However, the relative contributions of each of these mechanisms is probably different for each complication. For instance, direct tissue ischemia caused by a failure of reactive vasodilation is arguably more important in driving small vessel complications, while inflammatory atherogenesis and defects of thrombolysis allow clot formation and potentiate large-vessel complications, such as myocardial infarction and stroke. Insulin resistance, FFAs, and inflammatory cytokines released from adipocytes appear more important than hyperglycemia in causing chronic endothelial atherogenesis and thrombus formation. This may explain why insulin resistance is associated with complications, especially myocardial infarction, in patients who are still normoglycemic (26). It is less clear that insulin resistance is associated with microvascular complications in the absence of hyperglycemia, suggesting that elevated blood glucose plays a more important role in reducing small-vessel vasoreactivity. Finally, direct metabolic tissue injury caused by hyperglycemia may be more important to “microvascular” complications, especially neuropathy. Significant controversies remain regarding the relative contributions of direct hyperglycemic injury and effects of insulin resistance, hyperinsulinemia, and hyperlipidemia. Each of these factors is discussed in turn.

Hyperglycemia inhibits nitric oxide-mediated vasodilation. Vascular endothelial dysfunction is a pivotal feature of all IGT complications, and it develops in large part as a

consequence of acquired defects of nitric oxide (NO) signaling (Fig. 1). In endothelial smooth muscle cells, NO plays at least two critical and competing roles: vasodilatory signaling and detoxification of reactive oxygen species (ROS) that might otherwise cause oxidative tissue injury. NO is synthesized by NO synthase (NOS) in vascular endothelial cells in response to insulin and other stimuli, including acetylcholine, bradykinin, and mechanical shear stress. Once generated, NO diffuses locally to cause arterial vasodilation with a half-life of 3–20 s. Blunting of vasorelaxation and increased large-vessel blood flow in response to methacholine or hyperemia in patients with insulin resistance indicates an early partial failure of this NO-dependent system (27,28). This blunted vasoreactivity contributes to increased hypertension in patients with IGT (28). Local NO also inhibits endothelial atherosclerosis and platelet aggregation. However NO necessary to perform these vasoregulatory tasks is depleted by endothelial free radicals. NO reacts with and detoxifies endothelial ROS. This detoxification consumes NO, so large amounts of ROS induced by hyperglycemia serve as a sink for NO, making less NO available for vasodilation.

Hyperglycemia generates toxic ROS and drives a multifactorial depletion of NO. Hyperglycemia leads to activation of the enzyme aldose reductase, which converts excess glucose to sorbitol, which in turn is metabolized by sorbitol dehydrogenase to fructose (polyol pathway) (29). Glucose, and especially sorbitol and fructose, reacts non-enzymatically with proteins, lipids, and nucleic acids to produce advanced glycation end products (AGEs), which in turn induce ROS generation (30). Endothelial ROS formation is also increased in hyperglycemia by peroxidation of more abundant glucose and LDLs and by dysregulation of transition metals that serve as catalysts for auto-oxidation (31). Finally, ischemia itself accelerates ROS formation by decreasing mitochondrial efficiency and by reperfusion injury. The result is local endothelial damage by ROS and depletion of NO, impairing vasodilation.

These mechanisms appear particularly important for small arterioles, where failure of vasodilation may result in direct tissue ischemia. Drugs that decrease ROS, including ROS scavengers (α -lipoic acid, glutathione, vitamin E),

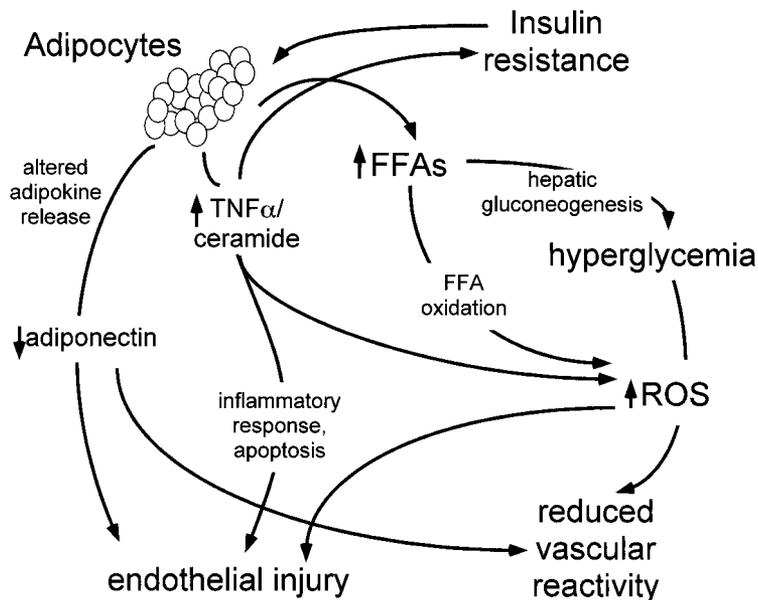


FIG. 2. Adipocyte lipolysis, release of FFAs, and changes in adipocytokines in response in states of insulin resistance and obesity contribute to both acute changes in vasoreactivity and chronic endothelial injury. Increased circulating FFAs reinforce insulin resistance and increase gluconeogenesis in the liver, accelerating hyperglycemia and feeding into the hyperglycemic effects described in Fig. 1. Fatty acid peroxidation directly increases ROS, and FFAs contribute to endothelial injury. Insulin resistance increases adipocyte release of tumor necrosis factor- α (TNF- α), which acts through the sphingolipid ceramide to damage endothelial cells. Adiponectin secretion is reduced, with inhibition of its vasoprotective effect.

lipid-lowering agents, and transition metal chelators (desferoxamine, α -lipoic acid) all slow or reverse peripheral nerve injury in animal models (31). However, there is good evidence for rapid vasoregulatory effects of transient hyperglycemia across a range of vessel calibers. IGT patients have reduced cutaneous vasodilation in response to acetylcholine (32). Moreover, Kawano et al. (33) found that oral glucose loading transiently reduced endothelial-dependent brachial artery vasodilation and that it was accompanied by a significant increase in circulating oxygen-derived free radicals. Significant transient vasodilatory blunting occurred in normal control subjects as well as those with IGT or diabetes. These results strengthen the concept of endothelial injury as a dynamic process, accumulating over years of intermittent hyperglycemic insult. **Insulin resistance.** Insulin resistance and compensatory hyperinsulinemia are intimately linked to the metabolic syndrome, a combination of abdominal obesity, hypertension, and dyslipidemia that is associated with increased vascular disease. Epidemiologic studies indicate that vascular complications are more frequent in insulin-resistant patients even before development of hyperglycemia (34). Similarly, in normoglycemic and normotensive subjects with a family history of diabetes, systemic insulin resistance correlates with blunted large-vessel NO-dependent vasoreactivity. This effect occurs independent of hyperglycemia (27,32).

Two recent studies suggest mechanisms whereby insulin resistance may disrupt insulin regulation of endothelial NOS (eNOS) activity. In a recent cross-sectional study of volunteers without diabetes (35), degree of insulin resistance was significantly correlated with increased plasma expression of asymmetric dimethylarginine, an endogenous NOS inhibitor, a risk factor for myocardial infarction. Treatment with rosiglitazone, an insulin-sensitizing agent, reduced asymmetric dimethylarginine levels, suggesting that insulin may directly regulate levels of this enzyme (35). Insulin binds the insulin receptor on the surface of endothelial cells to induce NO synthesis by eNOS. Activation by insulin of the serine/threonine protein kinase Akt increases phosphorylation of NOS in rat cardiac muscle,

increasing its activity (36). This stimulatory phosphorylation may be blocked by *O*-glycosylation of eNOS at the Akt-specific phosphorylation site as a consequence of hyperglycemia and increased hexosamine generation (37). However, the phosphorylation state of eNOS in the setting of insulin resistance has not been reported, and other mechanisms for insulin regulation of eNOS activity may also exist. Interestingly, disruption of vascular NO signaling in mice by inhibiting eNOS activity, or in eNOS knockout mice, leads to a state of insulin resistance (38). These findings point to a complicated regulatory relationship between insulin and NO in vascular tissue.

Endothelial proliferation is also a consequence of disproportionate mitogenic signaling associated with insulin resistance. In obese rats, insulin resistance selectively inhibits insulin signaling through the phosphatidylinositol 3-kinase Akt pathway, but signaling through the mitogen-activated protein kinase pathways remains permissive. As hyperinsulinemia develops, mitogen-activated protein kinase signaling increases, promoting vascular proliferation (39). Finally, important NO-independent mechanisms of vascular injury have been identified. Hyperglycemia increases signaling through the protein kinase C–diacylglycerol signal transduction pathway. In animal and human studies, protein kinase C alters blood flow and plays a role in damage of both small and large vessels, including basement membrane thickening and neovascularization (40).

FFAs and adipocytokines induce endothelial injury. As described above, obesity and insulin resistance promote production and release of FFAs from adipocytes, while FFAs increase insulin resistance and sustain hyperglycemia. Independent of these effects, accumulating evidence indicates that excessive FFAs induce both acute changes in vasoregulation and promote chronic endothelial injury (Fig. 2). FFAs potentially inhibit eNOS activation in cultured endothelial cells (41), and reduce acetylcholine-dependent vascular reactivity in human subjects, implicating NO-mediated signaling (42). Generation of ROS by increased lipid peroxidation has been implicated in these effects, and inhibitors of this process slow progression of

experimental hyperglycemic neuropathy. Insulin resistance and hyperlipidemia coexist in most IGT patients. Comparison of vascular reactivity in patients with hypertriglyceridemia stratified as either highly or only slightly insulin resistant suggest that insulin resistance is a more potent inhibitor of NO-dependent vasoreactivity than hyperlipidemia (43). Lipotoxicity probably also contributes to chronic endothelial inflammatory injury. In healthy volunteers, feeding with lipids (50 g/m²) resulted in a sustained postprandial increase in circulating neutrophils and the proinflammatory cytokine interleukin (IL)-8 (44).

Hormones secreted from adipocytes, termed "adipocytokines," play an important role in regulating food related behavior, metabolism, and vascular function. Altered secretion of two adipocytokines, tumor necrosis factor- α (TNF- α) and adiponectin, in obesity and IGT contributes to endothelial injury and promotes early vascular complications. TNF- α is a pro-inflammatory adipokine that acts through the sphingolipid second messenger ceramide. Ceramide has been shown to induce oxidative stress and apoptosis in endothelial cells and is associated with accelerated atherosclerosis in experimental models (45). TNF- α activation of ceramide also accelerates insulin resistance, probably by inhibiting insulin receptor coupling to IRS-1. There are mixed data regarding whether circulating TNF- α is increased in obesity and IGT. However, acute glucose infusion results in TNF- α release from adipocytes that is more profound and durable in IGT patients than in normoglycemic control subjects (46). Thus IGT patients may be at greater risk for TNF- α -mediated vascular injury.

Adiponectin is a novel adipocyte-derived peptide that has several salutary vascular effects: it inhibits vascular smooth muscle proliferation and appears to inhibit macrophage-mediated endothelial injury. Adiponectin secretion is reduced in obesity and hyperglycemic states. Reduced adiponectin is independently associated with reduced forearm blood flow, and may be permissive for arteriolar endothelial injury and atherosclerosis (47). Overall it is clear that increased adiposity and FFAs induce endothelial vascular dysfunction independent of their promotion of hyperglycemia in IGT.

Other metabolic effects of hyperglycemia. Although the above discussion has focused on vascular dysregulation and damage, it is clear that direct toxic and metabolic damage from hyperglycemia and hyperlipidemia play a role in development of IGT complications, especially neuropathy. Because of their long axons and high-energy requirements, peripheral neurons may be uniquely susceptible to the direct metabolic effects of hyperglycemia (48). Both ROS and AGEs are directly neurotoxic (29). AGEs promote inappropriate covalent cross-links between proteins. This process is accelerated in response to hyperglycemia and greater polyol pathway flux (30). AGEs cause neuronal-specific injury by inhibiting axonal transport, resulting in axonal degeneration that is proportional to the length of the axon (48). AGE-induced protein crosslinking is also implicated in sclerosis of renal glomeruli, capillary basement membrane thickening, and small-vessel atherosclerosis and occlusion (30). Defects in mitochondrial electron transport leading to increased superoxide generation has been suggested as a key contributor to metabolic injury mediated by the polyol, protein kinase C, AGE, and

hexosamine pathways (49). Mitochondrial dysfunction might account for persistent injury that occurs during periods of normoglycemia and contributes to genetic differences in susceptibility to hyperglycemic complications (49).

Treatment and areas for future study. Results from the DPP and similar large prospective studies in Finland and China make it clear that aggressive modification of diet and exercise can prevent or slow the progression from IGT to diabetes (8). In the DPP, diet and exercise modification was significantly more effective in diabetes risk reduction than pharmacological glucose reduction with metformin (58 vs. 31% reduction compared with placebo) (8). Diet and exercise was also more effective than metformin in returning IGT subjects to a normal glucose tolerance. DPP participants are being followed prospectively to examine changes in blood pressure, lipid profiles, atherosclerosis, and cardiovascular disease. In a separate study of IGT patients, 6 months of diet and exercise modification modeled on the DPP improved brachial artery reactivity, but not microvascular reactivity, suggesting that microvascular changes may be more difficult to reverse (50).

The results of the DPP and similar studies should transform the standard of care for hyperglycemic screening and treatment of patients with IGT. The 2-h glucose tolerance test has been criticized as less convenient and more variable than fasting plasma glucose. However, the epidemiological results highlighted in this review strongly argue that OGTT is a more sensitive marker of transient hyperglycemia and insulin resistance than fasting plasma glucose and is better correlated with risk for large-vessel atherogenic complications (5). Identification of IGT should prompt the clinician to screen for hypertension, dyslipidemia, and possibly cardiovascular disease. IGT patients should be referred to a nutritionist for coordinated counseling on modification of exercise and diet with the goal of losing 5–7% of body weight and increasing moderate aerobic exercise to 150 min weekly (the DPP goals) (8). Future studies are needed to more clearly define the natural history of progressive glucose intolerance; examine direct metabolic effects of hyperglycemia, insulin resistance, and hyperinsulinemia; and examine additive effects of lifestyle modification and pharmacotherapy on macrovascular and microvascular complications of IGT.

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