World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease

Part 1

ZACHARY T. BLOOMGARDEN, MD

This is the first of four reports on the 8th Annual World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease, held on 4–6 November 2010, in Los Angeles, California.

INSULIN RESISTANCE: NEW CONCEPTS
—Gerald Reaven (Stanford, CA) opened the meeting with a discussion of the implications of insulin resistance and of the interrelationships between hyperinsulinemia, hypertension, and cardiovascular disease (CVD). In his studies, insulin resistance is estimated from the steady-state plasma glucose (SSPG) during infusion of somatostatin, insulin, and glucose. In a study of 490 nondiabetic individuals, there was more than sixfold variability in insulin sensitivity with this measure. SSPG correlates with obesity, whether measured as BMI or waist circumference, but Reaven pointed out that there is an "enormous degree" of interindividual difference at any level of BMI or waist circumference, with obesity accounting for only approximately one-quarter of the variation in insulin sensitivity. Fitness, measured by the maximal oxygen consumption during exercise, was responsible for another quarter of the variation in his studies, with the remainder presumably caused by genetic factors. Hypertension is characteristically associated with glucose intolerance, with hyperinsulinemia, and with roughly a 50% reduction in insulin sensitivity, unaffected by antihypertensive treatment; Reaven reviewed a study of both treated and untreated hypertensive individuals, showing that 34 and 39%, respectively, had intermediate and that 52 and 47% had low insulin sensitivity, with the minority being insulin sensitive. Positive family history of hypertension is also

associated with insulin resistance, with elevated fasting insulin levels associated with development of essential hypertension (1). Similarly, hyperinsulinemia was associated with abnormalities of lipid and blood pressure levels in children and adolescents (2). In a study Reaven performed in an Italian population, insulin resistance was associated with higher levels of BMI, fasting glucose, insulin, and triglyceride levels and with lower HDL cholesterol levels, leading Reaven to suggest that those with insulin resistance "are the group who have all the cardiovascular risk factors." Among normotensive individuals, those with a positive family history of hypertension have similarly higher insulin and triglyceride and lower HDL cholesterol levels. Comparing hypertensive individuals with abnormal versus normal electrocardiograms, the former, Reaven said, had hyperinsulinemia and higher SSPG, leading to his suggestion that "insulin resistance predict[s] heart disease." Furthermore, in a study of normotensive and hypertensive individuals, those with low triglyceride and high HDL cholesterol had no increase in CVD with hypertension, while those with the insulin resistance lipid pattern of high triglyceride and low HDL cholesterol had increased CVD, which further worsened with hypertension (3). Hypertension and insulin resistance both are associated with increased mononuclear cell adhesion to the endothelium, and asymmetric dimethyl arginine, an inhibitor of nitric oxide (NO) synthase (NOS), is elevated with insulin resistance, with or without hypertension. Heart rate correlates with the insulin response to glucose challenge and with SSPG, suggesting a relationship between insulin resistance and enhanced sympathetic nervous system

activity, and sensitivity of blood pressure to a sodium load is more strongly predicted by hyperinsulinemia than by changes in renin, aldosterone, or atrial natriuretic peptide, with sodium sensitivity associated with weight gain and with lesser increase in NO to vasodilatory stimuli. Taken together, Reaven concluded, the insulin resistance and associated metabolic abnormalities present in hypertension, in those developing hypertension, and in relatives of hypertensive individuals both explain its pathogenesis and are related to its association with CVD.

In a second lecture at the meeting, Reaven suggested that "the concept of insulin resistance is not as simple as the words themselves." He termed compensatory hyperinsulinemia "the overlooked villain" and pointed out that insulin action varies as a function of organ system and insulin dose-response relationships. Adipose tissue is more sensitive to insulin-induced suppression of lipolysis than is muscle to stimulation of glucose uptake, he pointed out, an example of important differences in dose-response curves of different tissues, explaining the finding of elevated fasting but not postprandial free fatty acid (FFA)—as opposed to the much greater differences in postprandial than in fasting glucose in type 2 diabetes. Furthermore, neither the sympathetic nervous system nor the kidney is resistant to insulin action; insulin resistance is associated with increased sympathetic tone, and high dietary sodium induces elevated blood pressure in insulin-resistant states. Insulin resistance, hyperinsulinemia, and urate have a complex relationship, Reaven said, with insulin resistance reducing urate clearance and leading to higher urate concentrations. The liver should be considered separately in terms of glucose homeostasis and lipid metabolism. Hepatic glucose production is not elevated in type 2 diabetes at glucose levels below ~180 mg/dL and is modestly increased above this level, to a degree equal, he noted, to the rate of glycosuria, although it might be considered inappropriately high for the degree of hyperglycemia. Hepatic insulin resistance

should be differently understood in terms of lipid metabolism. Elevated fasting FFAs in turn increase VLDL secretion and, hence, plasma triglyceride levels, with no hepatic resistance to insulin inhibition of triglyceride secretion. Finally, the ovary appears to be more insulin sensitive to testosterone production in polycystic ovary syndrome (PCOS); perhaps ovarian hypersensitivity to insulin is required for the condition to appear.

Bart Staels (Lille, France) discussed the contemporary clinical role of the peroxisome proliferator–activated receptor (PPAR) system, focusing on new agonists. The PPARs are nuclear regulators of energy homeostasis, lipid and glucose metabolism, and inflammation, suggesting roles as modulators of the metabolic syndrome, type 2 diabetes, and its cardiovascular complications. PPARα, the target of the fibrates, plays a role in fatty acid oxidation, triglyceride lowering, and HDL raising, acting mainly in liver; PPARγ is insulin sensitizing and glucose and lipid lowering, acting mainly in adipose tissue; and PPARδ, "the still enigmatic PPAR," also plays roles in lipoprotein and energy metabolism, acting mainly in skeletal muscle.

Staels raised the question of whether fibrate PPARα agonists might reduce the "residual risk" seen after maximal dose statin treatment by further lowering LDL and raising HDL cholesterol or, perhaps, by other actions. Fibrates increase triglyceride and remnant lipoprotein metabolism, reducing apolipoprotein (apo)C-III production, increasing triglyceride clearance, and increasing hepatic mitochondrial fatty acid β-oxidation, which increases triglyceride degradation. PPARα agonists control HDL cholesterol production by increasing transcription of apoA1 and, also, by decreasing transcription of serum amyloid A, increasing the anti-inflammatory HDL cholesterol effect (4,5). PPARα may also play a role in intestinal cholesterol homeostasis, increasing apoA1 and *ABCA1* gene expression in human jejunal specimens.

The Veteran's Administration HDL Intervention Trial is the only study carried out in men with coronary heart disease whose primary lipid abnormality was a low HDL cholesterol level and did show that gemfibrozil reduced risk in diabetic patients (6). Reviewing the post hoc but consistent analysis finding of the Helsinki Heart Study, Bezafibrate Intervention Program, and Action to Control Cardiovascular Risk in Diabetes study (ACCORD) that the degree to which

fibrates decrease cardiovascular risk is greatest in those with lower HDL cholesterol and higher triglyceride levels, Staels analyzed this phenomenon in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study (7). Furthermore, Staels stated, "these compounds have potential not only to have effect in macrovascular complications," reviewing the finding from FIELD that fenofibrate decreased microvascular and cardiovascular complications independent of glycemia (8), with a similar finding in the ACCORD study of fenofibrate being associated with reduction in retinopathy; older literature also suggested that fibrates reduce retinopathy (9,10).

The action of PPARβ/δ involves multiple potential target tissues (11), appearing to increase HDL cholesterol levels and to reduce postprandial triglyceride levels by increasing their clearance (12), leading to potentially beneficial changes in fasting triglyceride, apoB, LDL cholesterol, insulin, liver fat, and urinary inflammatory markers (13). Staels further noted that there is cross-talk between PPARα and PPARδ in hepatic parenchymal cells and in macrophages (14).

PPARγ agonists, which Staels termed "the [glucose-lowering] compounds with the most durable action," have undesirable associations with edema, body weight gain, and skeletal fractures. Each PPAR compound, however, appears to have distinct pharmacological activity. When fenofibrate is compared with gemfibrozil, the latter markedly increases homocysteine; in a comprison of pioglitazone with rosiglitazone, the latter increases LDL cholesterol. There is need to develop compounds with improved clinical efficacy and fewer side effects, leading to the concept of selective PPAR modulators (SPPARMs) (15). Compounds with new PPAR effects include aleglitazar (16), and Staels reviewed his studies with the dual PPARα/δ modulator GFT505, which decreases apoB, apoC-III, apoE, triglyceride, and hepatic enzyme levels (suggesting benefit in nonalcoholic fatty liver disease); increases HDL cholesterol; and improves glucose homeostasis in individuals with impaired fasting glucose and impaired glucose tolerance. "There is future in this field of PPAR agonism," Staels concluded.

Philip Tsao (Stanford, CA) discussed the Apelin-APJ system in CVD and insulin resistance. APJ is a receptor that was first discovered in 1993, cloned by homology as an orphan G–coupled peptide receptor (GPCR) (17); A, P, and J are the initials of

the names of the discoverer's children. APJ has 40–50% homology with the angiotensin II (AII) receptor but does not bind AII. APJ is expressed in the cardiovascular system, central nervous system, skeletal muscle, adipose tissue, and liver. Apelin was identified in 1998 as the receptor's agonist. Its precursor is a 77 amino acid peptide, with 12, 13, and 36 amino acid isoforms. The expression of apelin in numerous tissues; in the endothelium; in neurons, including those of the hypothalamus; and in adipocytes suggests that it has both endocrine and paracrine effects. Apelin is the most potent inotrope known. All its isoforms decrease blood pressure in animal models, and human studies show endothelium-dependent reduction in blood pressure, an effect decreased by an NOS inhibitor (18). Apelin also may have a long-term protective effect against atherosclerosis, decreasing AII-induced atherosclerosis. Animals not expressing apelin have decreased cardiac contractility, and the atherosclerosis of mice not expressing apoE is worsened by apelin deficiency. Adipocyte apelin secretion is dependent on insulin in vitro (19) and its levels correlate with BMI in human studies (20), but intriguingly, apelin administration increases insulin sensitivity (21). Mice not expressing apelin have increased fasting insulin, decreased adiponectin, and insulin resistance. Insulin-induced insulin receptor substrate (IRS)-1 and Akt phosphorylation are reduced in these animals, and the insulin resistance is worsened with a high-fat diet, an effect reversed by infusion of apelin in vivo and in vitro. In *db/db* mice, apelin treatment improves insulin and glucose levels. Apelin, then, acts on skeletal muscle to increase insulin signaling, glucose uptake, and muscle blood flow. It also reduces FFA levels and lowers isoproterenol-induced adipocyte FFA release, with mice not expressing apelin having increased visceral fat and increased intramyocellular triglyceride. This effect appears to involve hormone-sensitive lipoprotein lipase and AMP kinase (AMPK) activation to increase intracellular cAMP levels.

Richard Johnson (Denver, CO) presented evidence of a relationship of fructose and uric acid to the pathogenesis of diabetes and obesity. He recalled the thrifty gene hypothesis (22) that evolutionary adaptations to lack of nutrient availability underlie many of our metabolic characteristics and noted that the increase in sugar intake over the past several centuries (23) leads to adaptations

with desirable effects under circumstances of famine to engender the development of illness. Sucrose is a disaccharide of glucose and fructose, and high fructose corn syrup is a mixture of 55% fructose and 45% glucose. Fructose itself appears naturally in foods such as honey, and fructose availability is increasing in our modern environment of low physical activity, high energy intake, and obesity. The specific mechanisms by which fructose causes adverse effects relate to its unique metabolism, with a specific transporter and with fructokinase, which catalyzes the phosphorylation of fructose to fructose-1-phosphate (F1P), not being product regulated, which potentially leads to transient ATP depletion (24).

Fructose has effects on the kidney (25), adipocytes, the vasculature, inflammation, and liver (26). It induces metabolic syndrome characteristics not seen with pair feeding of equicaloric diet given as starch (27), and even when calories are restricted but dietary sucrose levels are increased, Johnson showed animal models in which features of metabolic syndrome such as visceral obesity and fatty liver develop. When healthy men are given a diet adding 200 g fructose daily for 2 weeks, fasting triglyceride increased 55%, with increases in weight and blood pressure, reduction in HDL cholesterol, and reduction in insulin sensitivity (28). "Fructose," Johnson said, "correlates with the rise in metabolic syndrome throughout the world."

Soft drink consumption is increasing (29), correlating with increasing prevalence of gout, metabolic syndrome, and nonalcoholic fatty liver disease (30). Johnson reviewed studies of hypertension in association with gout dating from more than a century ago (31), with extensive subsequent corroborative evidence, although this correlation does not demonstrate a causal relationship of urate to hypertension. Stronger evidence comes from inhibition of uricase, the enzyme that degrades urate to allantoin, which functions in most mammals other than man. Administration of an inhibitor of this enzyme to rodents leads to a blood pressure increase, which can be prevented by allopurinol or probenecid administration (32). In animal models, urate activates the renal renin-angiotensin system, induces intracellular oxidative stress, inhibits NO, induces inflammation, and induces AII. Hyperuricemia induces preglomerular vascular disease, leading to sodium-sensitive hypertension (33),

which appears to be an early phase of hypertension, with low volume and renin dependency (34).

Serum urate is elevated in adolescents with newly diagnosed hypertension (35), and studies with allopurinol and with probenecid treatment in newly diagnosed hypertension in this age-group show blood pressure reduction, particularly in those whose urate level decreased below 5 mg/dL (36). Further evidence of urate-dependence of hypertension has been shown in a rodent model with fructose feeding (37), leading Johnson to wonder whether urate is "the catalyst that then leads to salt-sensitive hypertension." Allopurinol also improves dietary fructose-induced metabolic syndrome and prevents weight gain in an animal model (38). Urate induces a diabetic phenotype in adipocytes (39), and in the type 2 diabetes phenotype induced by sucrose, insulin deficiency, islet hyalinosis, and inflammation develop in association with upregulation of the islet urate transporter, suggesting that islet injury from fructose is in part mediated by urate. In preliminary studies, agents to block fructokinase lessen fructose-induced diabetes (40), while fructose increases fructokinase activity, a phenomenon also observed in nonalcoholic fatty liver disease models, with the fructokinase upregulation dependent on uric acid. Returning to the thrifty gene hypothesis, Johnson suggested that the diet of early hominids was high in fruits and hence in fructose but that periods of famine led to survival benefit of uricase deficiency because of the beneficial effect of insulin resistance in increasing fat storage. Today, of course, the same mechanisms increase risk of diabetes and obesity.

Grahame Hardie (Dundee, U.K.) discussed AMPK, stating with tongue in cheek, "If it's good for you, it must activate AMPK." AMPK levels are modulated by a number of drugs, including AICAR, biguanides, thiazolidinediones, resveratrol, epigallactechin gallate (in green tea), berberine (from Chinese Goldthread), and an agent in development by Abbott, A-769662. Cytokines acting on AMPK include leptin and adiponectin, the latter perhaps mediating effects of thiazolidinediones. French lilac or Goat's Rue, galaga officinalis, was used as an herbal remedy in medieval Europe. In 1923, its active component was identified as the guanidine derivative galegine, leading to the development of biguanide derivatives in the 1950s. Hardie reviewed the evidence that AMPK activation mediates the therapeutic

action of metformin. In a rodent model not expressing the serine/threonine kinase LKB1, AMPK is not activated by metformin and does not have hypoglycemic effect (41). In a study of 1,024 diabetic patients, the analysis of single nucleotide polymorphisms associated with the ability of metformin to reduce A1C, a locus on chromosome 11 linked to a different protein kinase, ATM (of the atypical phosphatidyl-inositol-3-kinase-like [PIKK] family) was found to be a marker (42). ATM mutation is the defect in human Ataxia telangiectasia. It is activated by double-strand DNA breaks, in turn activating AMPK, and is involved in DNA repair. ku55933 is an ATM inhibitor and inhibits activation of AMPK by metformin, blocking its glucose-lowering effect (43). Metformin's activation of AMPK requires intact cells. Hardie pointed out that both biguanides metformin and phenformin are inhibitors of complex I of the respiratory chain, suggesting that their effect is to increase the cellular AMP-to-ATP ratio and indirectly activate AMPK. The three-dimensional structure of AMP binding to AMPK has been elucidated (44). In a cellular model with AMP-insensitive AMPK, oligomycin fails to activate AMPK, while the direct activator A769662 remains effective, leading to the concept that metformin is "a metabolic poison" that has this beneficial effect.

INSULIN RESISTANCE AND CANCER—Hardie turned to new studies of AMPK activators and cancer. Breast and colorectal cancers are particularly associated with obesity and insulin resistance. AMPK activation causes cell cycle arrest in cancer cells, with the tumor suppressor lkb1 a key upstream kinase for AMPK and with evidence that AMPK turns off target of rapamycin (mTOR), which is hyperactivated in many tumors. In breast tumors, AMPK activation is reduced, a possible mechanism of cancer development suggesting the potential benefit of metformin. Diabetic individuals treated with metformin have lower cancer incidence, and metformin delays cancer development in a variety of animal models (45). Hypotheses are that AMPK has a direct cell cycle effect or, alternatively, that metformin activated hepatic AMPK, reducing glucose, insulin, and IGF1, which lead to the anticancer effect, although in non–insulin resistant animal models metformin has been shown to be cancer reducing. Thus, AMPK monitors the cellular AMP-to-ATP ratio and acts

as a sensor of cellular energy status, with AMPK activation switching cell function away from anabolism, growth, and proliferation toward catabolism and quiescence. AMPK is the target for metformin and other factors, and metformin acts by inhibiting mitochondrial function and increasing the AMP-to-ATP ratio. As AMPK activation is decreased in many cancers, the benefit of metformin in cancer treatment may be limited; another factor may be decreased levels in cancer cells of the organic cation transporters required for metformin cellular uptake.

Lorraine Lipscombe (Toronto, Canada) reviewed the evidence of an association between diabetes and cancer and the importance of insulin resistance (rev. in [46]). The association of diabetes and cancer has long been suspected, although the first prospective study, carried out by Elliot Joslin, failed to show an association, perhaps because of the high prevalence of type 1 diabetes in his clinic (47). Numerous large databases have subsequently revealed a cancer association for type 2 but not for type 1 diabetes. The initial reports were of association with pancreatic cancer, with the higher rates in individuals with longer duration of diabetes suggesting a real causal relationship. Hepatocellular cancer is associated with diabetes in the absence of risk factors such as hepatitis C and may be a consequence of nonalcoholic steatohepatitis cirrhosis. Endometrial cancer has long been recognized to be associated with obesity, likely from increased adipose tissue estrogen production, but an effect of diabetes independent of obesity has been demonstrated and there is evidence of effects of diabetes on colorectal cancer. Lipscombe reviewed her study comparing nearly 75,000 diabetic women with control subjects, showing 2.85 vs. 2.64 breast cancer cases per 1,000 person-years, an 8% increase, controlling for obesity and other risk factors (48), so that "diabetes or something about diabetes may be contributing to an increased risk of breast cancer." In a meta-analysis, diabetes was found to be particularly associated with carcinoma of the liver, pancreas, kidney, and endometrium; somewhat less strongly associated with colon and bladder cancers; and still less associated with non-Hodgkins lymphoma (49). Another study showed the strongest association to be between colorectal and pancreatic cancers (50). The same meta-analysis showed pancreas > colon > breast cancer associations with serum insulin and C-peptide and a

nonsignificant trend for endometrial cancer. It is interesting that prostate cancer risk is reduced among diabetic men, which Lipscombe suggested might be related to their higher rate of hypogonadism. Potential mechanisms of diabetes-induced malignancy include a direct effect of hyperglycemia or an indirect relationship caused by insulin resistance, inflammation, lipid and fatty acid abnormalities, adipokines, or IGF-1. The Warburg hypothesis that cancer cells predominantly use glycolysis for energy and have high glucose requirement (51) suggests that hyperglycemia might create a favorable environment for cancer. A relationship of intensive glycemic control to cancer and cancer mortality has not, however, been demonstrated, while the hyperinsulinemia hypothesis is supported by the 30% greater likelihood of malignancy among diabetic individuals treated with sulfonylureas and the 90% increase with insulin compared with metformin (52). Diabetes, Lipscombe commented, is also associated with worse prognosis among individuals with cancer, which may reflect an effect of hyperinsulinemia or may be due to less aggressive cancer treatment among patients with diabetes, lower likelihood of screening in this group, or reduced survival rates because of other diabetes complications.

Pamela Goodwin (Toronto, Canada) further discussed interrelationships of insulin with malignancy and the effects of metformin. Higher BMI is associated with greater risk of distant recurrence and mortality from breast cancer, with potential mediators including inflammation, adipokines, insulin, IGFs, and estrogens, all of which may interact. Higher insulin levels correlate with mortality among women with breast cancer (53) and men with prostate cancer (54). Cancers may express high levels of insulin, IGF-1, and hybrid receptors (55), and higher insulin receptor (insulin resistance) expression is associated with worse outcome (56). The α isoform expressed in breast cancer is the fetal receptor, which binds IGF1 and IGF2 and may switch insulin from metabolic to mitogenic and anabolic actions (49). Goodwin reviewed her study showing that metformin reduces cancer risk (57), agreeing with the other speakers' comments that it may act by lowering insulin levels and in an insulin-independent fashion, directly suppressing mammary tumor growth (58,59). Metformin activates AMPK and may have other cellular effects, upregulating

cell cycle–associated genes and possibly improving DNA damage recognition and repair. Organic cation transporters are required for cellular uptake of metformin, although the extent to which these transporters are expressed in human breast cancer is not known. Metformin has benefit across different subtypes of breast cancers (60) but may be most effective in "triple negative" breast cancer (61), with potential to abrogate resistance to human epidermal growth factor receptor-2–targeted therapies (62) and to increase aromatase inhibitor effects (63). Goodwin also reviewed rodent models in which metformin reduced tobacco-induced lung carcinogenesis (64) and had benefit in prevention of colon carcinoma (65).

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