Third Annual World Congress on the Insulin Resistance Syndrome

Mediators, antecedents, and measurement

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This is the first of three articles reviewing presentations at the 3rd Annual World Congress on the Insulin Resistance Syndrome, San Francisco, California, 17–19 November 2005.

Yehuda Handelsman (Tarzana, CA) introduced the 3rd Annual World Congress on the Insulin Resistance Syndrome and discussed the state of the insulin resistance syndrome, recalling its beginning in 1988 with Gerald Reaven's proposal that "Syndrome X" comprises a group of risk factors linked by insulin resistance, with the World Health Organization definition of metabolic syndrome in 1999, the modified definition proposed by the European Group on Insulin Resistance in 2001/20022, and the widely used Adult Treatment Panel (ATP)-III metabolic syndrome definition given by National Cholesterol Education Panel in 2001. Handelsman pointed out that the American Association of Clinical Endocrinologists (AACE) helped to gain the ICD-9 code of 277.7 for the constellation of conditions also termed the "metabolic" or the "dysmetabolic" syndrome, and he noted that the AACE definition of the syndrome adds the concept of differently assessing persons who belong to high-risk groups. The insulin resistance syndrome represents a continuum of risk, he stated, showing the use of the AACE-defined insulin resistance syndrome in predicting diabetes independently of glucose levels. Handelsman further reviewed what he termed the "war of the syndromes," which followed the International Diabetes Federation proposal for another definition in April 2005, placing central obesity as the defining characteristic of the syndrome and pointing out the need for ethnic group–specific criteria. The American Diabetes Association/European Association for the Study of Diabetes position statement in September 2005 said, in effect, that "there is no syndrome" and questioned whether the syndrome is actually useful in predicting cardiovascular disease (CVD). This was followed in the same month by the American Heart Association position statement insisting "yes, there is a syndrome," including central obesity as one potential but not required component. Perhaps, Handelsman concluded, we should realize that "the syndrome is not a disease" but instead represents a complex predisease state. Handelsman's introduction to the Congress was followed by two fascinating discussions of potential mechanisms of the insulin resistance syndrome.

Mediators of insulin resistance

Ira Goldfine (San Francisco, CA) discussed membrane glycoprotein placmacytoma-1 (PC-1) and insulin resistance. He reviewed the concept that type 2 diabetes starts with insulin resistance, either genetic or acquired, with β-cell activation and hyperinsulinemia leading to compensation with normal glucose tolerance and the insulin resistance syndrome, subsequently with impaired glucose tolerance (IGT) and then with overt type 2 diabetes. Focusing on genetic aspects of insulin resistance, he noted Reaven's findings that one-quarter of nonobese nondiabetic persons have the same degree of insulin resistance as persons with diabetes and suggested that the actual cause of this insulin resistance is to a large extent unknown.

The membrane protein PC-1 contains a phosphodiesterase site that is not related to insulin sensitivity and a somatomedin B domain that binds to the insulin receptor and inhibits insulin receptor signaling. In cells from insulin-resistant humans, Goldfine noted, there is evidence of an inhibitor of insulin receptor function that can be mimicked with PC-1 overexpression. Persons with insulin resistance, regardless of whether they have diabetes, have increased fibroblast PC-1. PC-1 content has inverse correlation with insulin sensitivity. Fibroblast and muscle PC-1 content show high correlation, suggesting this to be related to the pathophysiology of insulin resistance. Mechanistically, Goldfine showed evidence that PC-1 binds at a connecting domain in the insulin receptor α subunit, which transmits insulin-induced conformational change to the intracellularly located tyrosine kinase. PC-1 interferes with this process, reducing insulin receptor tyrosine kinase activity.

Transgenic mice overexpressing the PC-1 gene in liver and skeletal muscle have severe insulin resistance and moderately severe diabetes. Insulin-mediated 2-deoxy-D-glucose uptake is decreased in tissues from PC-1 mice, in muscle and also in brain. PC-1 is increased in muscle of nondiabetic obese persons and in obese primates and other animal models of obesity, where the increased PC-1 is associated with decreased insulin sensitivity.

The PC-1 Q allele is a lysine-to-glutamine polymorphism in exon 4 of the PC-1 gene that leads the protein to more potently inhibit the insulin receptor than the more common allelic variants. It does not have to be overexpressed to increase PC-1 activity, as it binds to the insulin receptor with stronger affinity than other variants. Q allele is found in 11.2 vs. 29.4 and 26.1% of normal versus nondiabetic insulin-resistant and diabetic persons. Insulin receptor activation is decreased in fibroblasts of persons with the Q allele, despite similar levels of the PC-1 protein. Persons overexpressing the Q allele have even greater insulin resistance. The Q allele is associated with a threefold increased risk of type 2 diabetes and with

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Abbreviations: AACE, American Association of Clinical Endocrinologists; ATP, Adult Treatment Panel; CVD, cardiovascular disease; FFA, free fatty acid; G6P, glucose-6-phosphate; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; IL, interleukin; NMR, nuclear magnetic resonance; PC-1, placmacytoma-1; PGC, peroxisome proliferator–activated receptor γ coactivator; PI3K, phosphatidylinositol-3-kinase; SSPG, steady-state plasma glucose; TNF, tumor necrosis factor; TZD, thiazolidinedione.

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increased risk of obesity (1), with genome scans of chromosome 6 confirming the association of the PC-1 gene with obesity and with insulin resistance. There are other "at-risk" PC-1 alleles that appear to be associated with PC-1 overexpression. PC-1 is expressed in adipose tissue, liver, and muscle, as well as in brain and pancreatic islets. The hypothalamic insulin receptor is important in appetite control, and the β-cell insulin receptor is important in insulin secretion, leading to the concept that increased PC-1 causes obesity via brain effects and decreased insulin secretion in the β-cell, as well as causing insulin resistance in fat, muscle, and liver, with Goldfine speculating that better understanding of PC-1 may allow discovery of new treatments for diabetes and the insulin resistance syndrome.

Gerald Shulman (New Haven, CT) discussed cellular mechanisms of insulin resistance in humans. Type 2 diabetes requires defects in the β-cell and the liver, but the earliest manifestations, by decades, appear to be abnormalities of skeletal muscle. Using nuclear magnetic resonance (NMR) spectroscopy (2), it is possible to noninvasively assess muscle glycogen content in vivo. Shulman showed evidence that persons with type 2 diabetes have a profound defect in insulin-stimulated muscle glycogen synthesis accounting for most of their insulin resistance. In studies to determine the rate-limiting step, glycogen synthase, hexokinase, and GLUT4 activities were assessed using phosphorus NMR to measure skeletal muscle glucose-6-phosphate (G6P) (3). When glucose is administered in the presence of insulin, in normal persons, intracellular G6P levels increase, while there is virtually no change in the muscle of the persons with type 2 diabetes, suggesting the abnormality to be at the level of GLUT4 or hexokinase. Offspring of two persons with type 2 diabetes have a 40% lifetime risk of developing the condition (4). Shulman's studies showed a defect in muscle G6P in these offspring similar to that seen in the type 2 diabetic persons. With a carbon-NMR method, he found that hexokinase is not a rate-controlling step (5). As an aside, Shulman noted, this implies that hexokinase and glycogen synthase will not be good targets for pharmacologic intervention.

Abnormalities in glucose transport via GLUT4 occur in diabetes. The GLUT4 transporter itself is, however, perfectly normal in persons with diabetes and their offspring. The best predictor of abnormality in diabetes is the plasma free fatty acid (FFA) level (6), and proton NMR studies to measure intramyocellular fat show this to be an even better predictor of insulin resistance (7). The question of how fat might cause insulin resistance in skeletal muscle was initially addressed by Randle, who suggested that increased intracellular FFA increased citrate, leading to greater pyruvate dehydrogenase activity, which would be expected to reduce phosphofructokinase, leading G6P levels to increase (8). In normal persons receiving infusions of heparin with a triglyceride emulsion to increase FFA levels, however, G6P levels decrease, suggesting that fatty acids block insulin activation of GLUT4 or hexokinase, rather than decrease pyruvate dehydrogenase activity (9). Furthermore, increasing plasma FFA levels leads to a reduction in intracellular glucose levels, suggesting that fatty acids block translocation and/or intracellular activation of GLUT4 (10). Potential mechanisms include actions of fatty acids to interfere with translocation of GLUT4 or with the insulin signaling cascade, via phosphatidylinositol-3-kinase (PI3K) activation, with insulin's ability to activate PI3K abolished by fatty acids (11). A fatty acid metabolite such as diacylglycerol is likely the mediator, increasing protein kinase C θ, which decreases insulin receptor substrate-1, in turn leading to decreased intracellular PI3K levels.

The absence of fat has deleterious effects as great as those seen from excess fat. A mouse model of lipodystrophy shows severe insulin resistance and hypertriglyceridemia (12). In the model, both muscle and liver are insulin resistant, with increased muscle and liver fatty acyl CoA. Transplantation of adipose tissue normalizes insulin action in this model, suggesting that it is the increased muscle and liver fat that is the culprit. Thus, a leading concept of the mechanism of thiazolidinedione (TZD) action is that the agents "keep fat in the fat cell . . . causing a redistribution of fat from muscle and liver." In humans with lipodystrophy, there are low circulating leptin levels, with leptin replacement therapy controlling the insulin-resistant diabetes, leading to normalization of glucose and reduction in liver and muscle fat (13). The mechanism of the leptin effect is as yet unknown, with leptin's role in decreasing energy intake unlikely to explain more than a small part of the phenomenon.

In a large study characterizing persons with the spectrum of insulin sensitivity that compared those with and without family history of type 2 diabetes, the former displayed mild glucose intolerance (14). There was no difference in tumor necrosis factor (TNF)α, resistin, interleukin (IL)-6, and adiponectin, but skeletal muscle glucose utilization was decreased, with increased intramyocellular fat. A potential explanation would be an increase in fatty acid delivery from fat cells, but no evidence can be found of this. Alternatively, there might be a decrease in muscle mitochondrial function. ^{31}P NMR flux through ATP synthase, a measure of mitochondrial function, shows a decrease of ~30%, and, similarly, mitochondrial tricarboxylic acid (TCA) cycle activity measured with ^{13}C NMR shows a 30% reduction in mitochondrial oxidation (15). There is a 38% decrease in mitochondrial density in muscle biopsies of insulin-resistant offspring (16). Mitochondrial content might be decreased by a defect in peroxisome proliferator–activated receptor γ coactivator (PGC)-1α, a transcription factor that turns on mitochondrial biogenesis, but this has not been demonstrated with direct assay of PGC-1. Protein kinase B (Akt) phosphorylation is decreased and serine phosphorylation of insulin receptor substrate-1 increased in insulin-resistant offspring, suggesting that insulin resistance in humans can be caused by increased calorie intake with consequent increase in adipocyte, muscle, and liver fat but that the syndrome may also be acquired and that inherited defects in mitochondrial metabolism may be factors.

After modest weight loss, persons with type 2 diabetes show decreased fasting glucose levels without change in intramyocellular fat or improvement in glucose utilization, but with markedly decreased liver fat (17), which Shulman termed "a very important target," associated with decreased hepatic glucose production and normalization of insulin-induced suppression of glucose production. He pointed out that exercise normalizes insulin-induced muscle glucose uptake, probably via AMP-activated protein kinase, the "fuel sensor for cells," which decreases malonyl CoA, increases fat oxidation via acetyl-CoA carboxylase, and increases PGC-1 and hence mitochondrial activity. Furthermore, exercise training decreases intramyocellular triglyceride, although this should probably be considered a marker rather than a mediator of insulin resistance, with long-chain fatty acyl CoA and diacylglycerol

most likely the actual explanatory factors. In this regard, it is fascinating to note that highly trained athletes, such as marathon runners, have increased intramyocellular fat but normal or increased insulin sensitivity.

Pediatric insulin resistance

Alan Sinaiko (Minneapolis, MN) reviewed the link between insulin resistance in childhood and adulthood, noting the association of puberty with insulin resistance, with boys having greater insulin sensitivity than girls around age 11 years but lower insulin sensitivity by age 19, despite the decrease in body fat in boys during adolescence. As a consequence, fasting insulin levels show little change in boys, while decreasing through adolescence in girls. Analysis of the National Health and Nutrition Examination Survey dataset suggests that the waist circumference and blood pressure criteria for the insulin resistance syndrome should be based on age-adjusted percentiles, but Sinaiko acknowledged that "we don't know exactly yet" how the cutoffs should be defined. He pointed out that there is a rather weak negative correlation between direct measures of insulin sensitivity and fasting insulin ($r = 0.35$), suggesting that the use of fasting insulin is even less appropriate in assessing insulin sensitivity in children than it is in adults.

As in adults, there has been increasing prevalence of obesity among children. The National Health and Nutrition Examination Surveys have shown that body weight above the age-matched 95th percentile was seen in ~4% of children in 1971–1974, in 6% in 1976–1980, and in 10% in 1988–1994 (18). The increase in rates of obesity has driven increases in hypertension, hypertriglyceridemia, low HDL, and high fasting insulin levels (19) and has been associated with increases in serum C-reactive protein and IL-6 and reductions in adiponectin (20). BMI and body fatness track from childhood into adulthood, with a correlation coefficient of 0.64 between childhood and adulthood BMI (21), so that "thin children become thin adults, fat children become fat adults" with similar tracking of blood pressure and lipid levels. Although the rate of weight increase is relatively constant through adolescence, the rate of increase in height slows after age 12 years, with the rate of weight increase rather than the starting weight at age 7 the major predictors of fasting insulin at age 24 years. A modifiable factor, weight gain, is the determinant of insulin resistance in adulthood. In a study of 29 persons, those in the third and fourth BMI quartiles at age 13 had tripling and a fivefold increase in risk of insulin resistance syndrome, with relatively thin 13-year-old subjects who subsequently develop insulin resistance syndrome having hypertension and low HDL as major factors, implying that there are multiple pathways to development of the syndrome. Those who are thin versus heavy who develop insulin resistance syndrome have similar degrees of insulin resistance, although Sinaiko pointed out again that fasting insulin is not a good marker, as levels are lower in the thinner persons. Using hyperinsulinemic clamp measurement of insulin sensitivity, insulin resistance and weight play roles in the development of insulin resistance syndrome, with adolescents who are both heavy and insulin resistant having particular elevations in fasting insulin and triglyceride and reductions in HDL cholesterol (19). Sinaiko concluded by saying, "To get on top of the [insulin resistance syndrome], to prevent it . . . we think there are some individuals who need therapy," suggesting that aggressive measures to prevent the development of obesity and insulin resistance are appropriate.

Francine R. Kaufman (Los Angeles, CA) addressed the question of whether the insulin resistance and type 2 diabetes seen in childhood differ from that in adults. She reviewed the marked increase that was seen from the late 1980s to the 1990s in the prevalence of pediatric type 2 diabetes among newly diagnosed children with diabetes (22). Of pediatric patients with new-onset diabetes, 8–43% have evidence of type 2 diabetes, with the SEARCH trial suggesting the emergence of a hybrid condition with both islet autoimmunity and insulin resistance (23), implying a pathophysiology differing somewhat from that in adults. Important contributions come from obesity, sedentary lifestyle, and genetic factors, with considerably stronger family history in families of children with type 2 than of type 1 diabetes, with predilection for girls, and with an association with polycystic ovary syndrome and ethnicity. One must ask, then, what effects β-cell function has on the background of insulin resistance. Important factors may include lipotoxicity, glucotoxicity, genetics, autoimmunity, and a link to the intrauterine environment, with intrauterine growth retardation and maternal gestational diabetes playing roles in subsequent abnormality of the β-cell. Multiple studies show a high prevalence of IGT but relatively low prevalence of undiagnosed type 2 diabetes during childhood and adolescence (24–26). Comparison of persons with IGT who progress to diabetes versus revert to normal glucose tolerance shows that important predictors are ethnicity, BMI, and the rate of increase in BMI (27). It is, however, unlikely that there is a long period of undiagnosed diabetes in children, as opposed to the situation in adults, with a feasibility study of oral glucose tolerance screening in ~1,800 children at age 13 years showing fasting glucose of 100–125 mg/dl in 40% but only 0.4% with fasting glucose ≥126 mg/dl and only 0.1% with 2-h glucose ≥200 mg/dl.

Kaufman showed that children appearing to have type 2 diabetes have a substantial (~30%) prevalence of islet autoimmunity (28). The "accelerator hypothesis" suggests that the β-cell stress of insulin resistance may make manifest an autoimmune process of β-cell destruction, with type 1 and type 2 diabetes perhaps being "the same disorder of insulin resistance, set against different genetic backgrounds," a concept proposed by Terry Wilkin (29). Thus, overweight in itself does not distinguish type 1 versus type 2 diabetes, the rapidity of development of type 1 and 2 diabetes may be similar, ketoacidosis may occur in both types (although to a greater degree in type 1 diabetes), and more than three-quarters of children with type 2 diabetes have a family history of type 2 diabetes, but up to 30% of children with type 1 diabetes also have family members with type 2 diabetes. C-peptide can be preserved at the time of diagnosis of type 1 diabetes and is normal or increased in type 2 diabetes. Antibodies are positive in 85% of type 1 and in 15% of type 2 diabetic children at diagnosis. Comorbidities include polycystic ovary syndrome in type 2 diabetes and autoimmune thyroid and adrenal disease, as well as vitiligo and celiac disease, in type 1 diabetes. Most type 1 diabetes is seen in Caucasians, while type 2 diabetes is seen, Kaufman stated, in "everybody else" (22).

The diagnosis of type 2 diabetes, Kaufman reiterated, "is made by symptoms, not by screening." She noted that treatment goals are similar to those in adults but that psychosocial factors may make it particularly difficult to achieve

this in adolescents. Approaches include self-monitoring of blood glucose and medical nutrition therapy, with family supervision producing better glycemic control outcomes (30). Preconception counseling, immunizations, dental care, and smoking and alcohol counseling are additional important components of treatment. Insulin treatment is probably needed initially for most pediatric type 2 diabetes, particularly for those with symptoms at diagnosis (31), although there has been interest in initial treatment with metformin (32). The STOPP-T2D (Studies to Treat Or Prevent Pediatric Type 2 Diabetes) have been initiated (750 patients at 13 centers), comparing metformin, metformin plus rosiglitazone, and metformin plus intensive lifestyle. The need for aggressive treatment of pediatric type 2 diabetes, Kaufman concluded, is suggested by data from Pima and indigenous Canadian diabetic adolescents showing a high rate of development of albuminuria; therefore, "this is a very serious disease."

Julia Steinberger (Minneapolis, MN) discussed the relationship between insulin resistance syndrome and dyslipidemia in children. Insulin resistance and increased nutrient availability lead to a state of increased FFA flux into the liver, with excess triglyceride leading to increased assembly and secretion of VLDL, enhanced by microsomal triglyceride transfer protein, a resident protein in the lumen of the endoplasmic reticulum. The hepatocyte endoplasmic reticulum produces apolipoprotein B, which either is degraded by both proteasomal and nonproteasomal pathways or is secreted. Insulin resistance enhances hepatic VLDL synthesis and causes resistance to the action of insulin on lipoprotein lipase in peripheral tissues and enhanced HDL cholesterol degradation. The consequence is the well-recognized pattern of increased triglyceride, with longer residence time in plasma leading to increased exchange of triglyceride (under the influence of cholesterol ester transfer protein) between these particles and LDL and HDL, with hepatic lipase leading to low HDL cholesterol levels and an increased proportion of small dense LDL particles (33), as well as postprandial hyperlipidemia. Childhood hyperlipidemia is associated with adult CVD, with fatty streaks beginning to appear during childhood and subsequently developing into advanced plaques. The extent of the early lesions is related to dyslipidemia. Children in the U.S. have higher intake of saturated fat and cholesterol than children in other countries (although other countries are catching up), and children with high LDL tend to have a family history of adult CVD, presumably with shared environmental as well as genetic factors. In the Muscatine, Iowa, study of children with cholesterol exceeding the 90th percentile, more than two-thirds had lipid levels that would qualify for intervention in adults, with the study showing association between childhood cholesterol and the carotid intimal-medial thickness measured in the same persons at age 33–42 years (34). In the Bogalusa Study, autopsies of 204 young persons 2–39 years of age showed effects of multiple risk factors on the extent of atherosclerosis in the aorta and coronary arteries in children and young adults; those with three or more risk factors had markedly increased prevalence of fatty streaks and fibrous plaques in the aorta and coronary arteries (35). Steinberger showed evidence that the serum triglyceride and HDL cholesterol correlate with insulin levels during oral glucose tolerance testing and with insulin sensitivity measured using glucose clamp methodologies. "The association of body fatness and abnormal lipid profile is present in early childhood," she stated, "progresses and becomes stronger in early adulthood, and is significantly associated with insulin resistance." In that lipid lowering in adults has resulted in great success in prevention and treatment of atherosclerotic CVD, and with lipid abnormality on the rise in children (related to the epidemic of obesity), the insulin resistance syndrome, and type 2 diabetes, she suggested that not only weight control but early lipid-lowering therapy may be necessary steps. It is not, however, currently accepted that lipid and blood pressure treatment are required in children, and we are far from defining the goals of treatment. Additional important questions remain, such as the relationship of rapidity of growth to subsequent insulin resistance and the adiposity rebound concept, which states that insulin resistance is an effect of intrauterine growth retardation with low birth weight and subsequent excessive weight gain.

Adipocytes and obesity

Tracy McLaughlin (Stamford, CA) discussed aspects of the relationships between obesity and insulin resistance, noting the importance of identifying the insulin-resistant subgroup of obese persons and of understanding factors leading some but not all obese persons to be insulin resistant. Steady-state plasma glucose (SSPG), a measure of insulin resistance obtained by infusing somatostatin, insulin, and glucose to observe the glycemic response to a standard glucose load at a fixed plasma insulin level, correlates with BMI ($r = 0.58$), suggesting that at any BMI, there is a great degree of scatter. Conversely, for persons in the most insulin-resistant tertile, ~10% have BMI <25, 40% have BMI 25–29.9, and half have BMI ≥30 kg/m^2, so being overweight is not synonymous with insulin resistance.

McLaughlin addressed the question of whether insulin itself causes weight gain, noting that diabetic patients receiving insulin tend to have weight gain, which is said to be caused by a greater frequency of hypoglycemia with attendant hunger/eating response rather than by a direct anabolic effect of insulin. Nondiabetic patients consuming a high-carbohydrate diet may have consequent hyperinsulinemia. The Zavaroni Study of 647 healthy nonobese persons followed for 14 years showed a sixfold variation in insulin response to a glycemic stimulus, with those in the lowest insulin quartile gaining 1.8 kg and those in the highest quartile gaining 2.3 kg (36). A study comparing 1,069 persons who gained weight with 424 who lost weight over an 8-year period, however, did not show an association between hyperinsulinemia and weight gain (37); other studies have shown greater degrees of weight gain in persons with greater insulin sensitivity (38). Furthermore, comparing persons with greater and lesser degrees of insulin sensitivity in a weight loss program, McLaughlin showed that both groups lost 11% of their initial body weight, suggesting that insulin resistance does not itself impair weight loss (39). Thus, neither hyperinsulinemia nor insulin resistance lead to weight gain.

On the other hand, obesity does worsen insulin resistance, as shown most clearly in studies of the effect of weight loss. Comparing the response to a 4-month hypocaloric diet of 11 insulin-resistant vs. 13 insulin-sensitive persons, despite the comparable weight loss in the two groups, the SSPG decreased dramatically in the insulin-resistant group, while although the SSPG in the insulin-sensitive group was below even the weight loss–induced level of the insulin-resistant group, it showed no change with weight

loss in these persons (40). McLaughlin further noted that some insulin-resistant persons have dramatic improvement in insulin sensitivity with weight loss, suggesting, "Not everybody does this, but mostly it is because not everybody loses enough weight." Those whose insulin sensitivity improves also show reductions in blood pressure and lipid levels. Clearly, persons with greater degrees of insulin resistance are at markedly greater risk of diabetes, hypertension, and dyslipidemia, as was observed in the Zavaroni 14-year follow-up study (41). In analysis of 147 healthy nonobese males followed for a mean of 4.7 years, 7 of 49 in the top tertile of SSPG had developed CVD, while this was only seen in 1 of 49 in the middle tertile and in none in the most insulin-sensitive tertile (42). In a 6-year follow-up of this cohort, 36, 17, and 0% developed clinical events including type 2 diabetes, CVD, hypertension, and cancer (43).

McLaughlin reviewed several additional studies, confirming the association between insulin resistance and hypertension, diabetes, cancer, and CVD and again showing that insulin-resistant versus -sensitive obese persons differ in 2-h glucose, total cholesterol, triglyceride, and HDL and LDL cholesterol, suggesting that it is possible to "be obese and be fairly metabolically healthy." Further comparison of BMI-matched insulin-resistant and -sensitive obese women before and after weight loss shows the naturally occurring inhibitor of nitric oxide synthesis asymmetric dimethylarginine to be higher at baseline in the insulin-resistant group and to decrease to the level seen in insulin-sensitive persons with weight loss. Similarly, C-reactive protein improves in the insulin-resistant obese group with weight loss, while levels begin lower and show no change in those who are already insulin sensitive at baseline.

McLaughlin addressed the question of determinants of insulin resistance versus insulin sensitivity among obese persons. She suggested that the widely held view that insulin sensitivity is determined by fat distribution (central versus peripheral, visceral versus subcutaneous) is debatable and suggested a more complex explanation involving variations in intrinsic metabolic activity of fat cells related to levels of adipokines, both the insulin sensitivity–inducing adiponectin and those associated with insulin resistance including leptin, resistin, TNFα, and IL-6. She reviewed the ectopic fat hypothesis of FFA overflow to nonadipose tissues (44) and the importance of inflammatory cells present in adipose tissue (45), with evidence that there is cross-talk between adipocytes and monocytes in coculture, with monocyte TNFα decreasing adipocyte adiponectin secretion, while increased adipocyte production of FFA and adipokines has effect on monocytes. A little explored question is whether morphologic and/or functional differences exist between adipocytes of insulin-sensitive and -resistant persons. Impaired fat oxidation, increased adipocyte size with altered production of adipokines, and differences between insulin-sensitive and -resistant obese persons in the expansion of adipocyte reserves may be additional factors.

Samuel Cushman (Bethesda, MD) reviewed the relationship between obesity and abnormalities of lipid and carbohydrate metabolism, discussing his studies of the role of adipogenesis in systemic insulin resistance and recalling the important degree of insulin resistance associated with lack of adipocytes in states of lipoatrophy. Adipocyte-specific GLUT4 knockout leads to systemic and muscle insulin resistance, suggesting effects of adipokines. Noting that TZDs cause fat cell differentiation in vitro while improving glucose metabolism in vitro, he continued McLaughlin's comments, pointing out that small adipocytes have long been shown to be associated with better glucose homeostasis, so that that recruitment of new adipocytes could explain TZD benefits. He showed studies measuring adipocyte size distribution in rosiglitazone-treated obese Zucker fatty rats, with reductions in insulin levels seen after 2 days, improvement in glucose levels at 6 days, and markedly increased skeletal muscle glucose uptake at 8 days. Frequency histograms of fat cell size distribution show a peak of small cells beginning at 4 days and continuing through 35 days of rosiglitazone treatment, while untreated Zucker fatty rats have larger cell size. Adipocyte DNA synthesis is not increased, which seems to contradict the apparent presence of new adipocytes. Thus, the early improvement in insulin sensitivity coincides with changes in adipose cell turnover, involving both differentiation and apoptosis rather than proliferation. In a proteomic study, analysis of proteins produced by adipocytes with and without TZD treatment shows changes in several hundred proteins, more than half of unknown function. Comparing adipose tissue needle biopsy of 26 insulin-resistant and -sensitive persons (from McLaughlin's clinical studies), all had a fairly large population of what appeared to be small cells and a great deal of scatter in the size of the larger cells, with electron microscopy showing that many of the adipocytes had attached macrophages. The insulin-resistant persons had a larger fraction of small cells, which Cushman suggested might be cells that are not fully capable of storing metabolic fuels as triglyceride, perhaps secreting factors leading to change in systemic glucose homeostasis, with the effect of TZDs potentially being to enhance differentiation of this population of cells in persons with insulin resistance.

Identifying persons with insulin resistance syndrome

Sun Kim (Stamford, CA) discussed surrogate metabolic markers of insulin resistance. She reviewed the determination of SSPG. Octreotide, insulin, and glucose are infused for 180 min, with the SSPG being the average plasma glucose at 150, 160, 170, and 180 min. The 1st decile of SSPG is 50 mg/dl, and the 10th decile is 300 mg/dl, suggesting that insulin resistance is a continuous variable. Therefore, there may be no absolute criterion for classifying a given person as insulin resistant or sensitive. The SSPG has relatively poor correlation with fasting plasma glucose, has best correlation with the insulin area under curve following an oral glucose load, and has intermediate correlation with fasting insulin and triglyceride levels, particularly in overweight and obese persons. Receiver operating characteristic curve analysis of the relationship between metabolic markers of insulin resistance and SSPG suggests that the best markers are the triglyceride-to-HDL ratio, the triglyceride level, and the fasting insulin, with the best cut points for distinguishing insulin sensitivity from insulin resistance as follows: triglyceride >130 mg/dl, triglyceride-to-HDL ratio >3, and plasma insulin >15 μU/ml. Kim suggested that in clinical practice, the triglyceride and triglyceride-to-HDL ratio are probably the best measures currently available for assessment of insulin resistance. The use of the ATP-III clinical criteria was no better than these simpler measures, having 52% sensitivity and 85% specificity, and fasting glucose measures had even lower performance, with glucose ≥100 mg/dl having 37% sensitivity and 88% specificity (46), confirming the relatively weak association between

the fasting glucose and SSPG. Kim noted that the commonly used fasting insulin–based measures, homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index, show almost perfect correlation with fasting insulin and are little better than insulin alone in the determination of persons with insulin resistance.

The subsequent presentations described two methodologies utilizing isotopically labeled glucose to estimate glucose disposition rates in more accurately assessing insulin sensitivity. The hyperinsulinemic-euglycemic clamp allows a measure of whole-body insulin sensitivity as the ratio of the glucose disposal rate to the insulin level. Approaches based on measurement of the insulin level alone, then, will by necessity be inaccurate, given the wide variability of glucose disposal rates. Marc Hellerstein (Berkeley, CA) presented information on a deuterated glucose disposal test. He reviewed studies showing that there is a relatively low correlation between clamp (or the equally accurate SSPG) and the insulin or triglyceride level, waist-to-hip ratio, presence or absence of metabolic syndrome, and other readily measured clinical variables. His company, Kinemed, has developed a test with administration of deuterated glucose and subsequent blood sampling to measure $^{2}H_2O$ production. This allows a kinetic measure of glucose flow through its metabolic pathway, with insulin sensitivity given as a ratio of $^{2}H_2O$ production to the fasting plasma insulin level. The test, then, measures insulin-mediated glucose utilization by tissues, as well as giving an assessment of β-cell compensation. In a study of 17 persons, with and without ATP-III metabolic syndrome, the $^{2}H_2O$ production measure had a correlation coefficient with euglycemic-hyperinsulinemic clamp insulin sensitivity of 0.9. Hellerstein suggested that this could become as important a cardiovascular risk indicator in assessing persons for metabolic syndrome as measuring cholesterol level.

Richard Lewanczuk (Alberta, Canada) described a similar dynamic test, the ^{13}C-glucose breath test, administering universally labeled 25 mg ^{13}C-glucose with 15 g dextrose after an overnight fast, with measurement of expired $^{13}CO_2$ before and 90 min later. The lower the amount of $^{13}CO_2$ in expired air, the greater the degree of insulin resistance. The test is available in Canada as a commercial product, Diatest. In a study of 54 persons with newly diagnosed type 2 diabetes and 50 normal control subjects, although the fasting glucose and the glucose tolerance test both had 100% specificity, their sensitivities were 52 and 62%, respectively. The fasting insulin–based HOMA had 70% specificity and 69% sensitivity for diabetes, while the ^{13}C-glucose breath test had 96% specificity and 78% sensitivity, suggesting performance characteristics superior than those for fasting insulin–based measures. Similarly, receiver operating curve analysis suggested the ^{13}C-glucose breath test to have superior performance to that of HOMA. Lewanczuk described acceptable test reproducibility, with coefficient of variation of ~8%. In studies in obese children, he showed evidence that the BMI and physical fitness were the major determinants of insulin sensitivity measured using the ^{13}C-glucose breath test. If these and related readily performed measures of insulin sensitivity become available, there is potential that the diagnosis of the insulin resistance syndrome may become much more widely available to clinicians and that it will be possible to directly assess responses of insulin resistance to therapy, allowing fuller delineation of the role of insulin-sensitizing treatment in the management of a wide variety of clinical conditions.

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