

# The 1st World Congress on the Insulin Resistance Syndrome

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This is the first of two articles covering the 1st World Congress on the Insulin Resistance Syndrome (IRS), which was held in Los Angeles, 21–22 November 2003.

Yehuda Handlesman (Tarzana, CA), the conference organizer, noted that it reflects an endeavor to bring an understanding of basic science into the clinical practice of medicine. Contributory causes of insulin resistance include obesity, sedentary lifestyle, and many other characteristics and conditions. Handlesman noted that the syndrome has been assigned International Classification of Disease (ICD)-9 code 277.7 as the dysmetabolic syndrome. Many aspects of the IRS are pharmacologically treatable when lifestyle modification is ineffective, and we need tools to determine which approaches are appropriate for particular individuals.

## Determinants of insulin sensitivity

Gerald Reaven (Stanford, CA) reviewed the six- to eightfold variation in steady-state plasma glucose (SSPG) with continuous octreotide, insulin, and glucose infusion among apparently healthy persons. He attributed the lack of glycemic abnormality of persons with insulin resistance to compensatory hyperinsulinemia, although noting that there is great variation in insulin levels for a given level of insulin sensitivity. Similarly, there is variation in the correlations between obesity and insulin resistance, as well as between maximal aerobic capacity, a measure of physical fitness, and insulin sensitivity. Reaven suggested that adiposity and physical fitness each account for ~25% of

the variability in insulin sensitivity, with genetic factors, which he illustrated with the differences in insulin sensitivity between persons of European and persons of South Asian or Mexican ancestry and with the similarity in insulin sensitivity of related persons in a family, responsible for an additional 50% of this variation.

Syndrome X, as Reaven originally termed it, included insulin resistance, hyperinsulinemia, dyslipidemia, hypertension, and increased risk of both diabetes and coronary heart disease. Other abnormalities associated with the IRS include glucose intolerance, small LDL particle size, postprandial accumulation of triglyceride-rich remnant lipoproteins, hypertriglyceridemia, and low HDL cholesterol. There is evidence of endothelial dysfunction, with increased circulating cell adhesion molecules, increased asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of endothelial nitric oxide (NO) synthase (eNOS), decreased endothelium-dependent vasodilation, increased plasminogen activator inhibitor-1, fibrinogen, and inflammatory markers, including C-reactive protein (CRP) and leukocyte count. The IRS is associated with decreased renal urate clearance, increased sympathetic nervous system activity and renal Na retention, increased ovarian testosterone secretion, and sleep-disordered breathing (1). Associated illnesses include cardiovascular disease (CVD), type 2 diabetes, hypertension, the polycystic ovarian syndrome, nonalcoholic fatty liver disease (NAFLD) (2), malignancies including breast cancer (3), and sleep apnea. Reaven noted that excess hepatic fat is more strongly associ-

ated with insulin resistance than visceral fat or hypertriglyceridemia. Reaven suggested that “most if not all of the manifestations of the IRS are related to the physiological effects of compensatory hyperinsulinemia associated with insulin resistance.”

Factors that increase the likelihood of the IRS include having CVD; hypertension; polycystic ovarian syndrome; acanthosis nigricans; a family history of type 2 diabetes, hypertension, or CVD; a history of gestational diabetes or glucose intolerance; non-Caucasian ethnicity; sedentary lifestyle; or obesity. Conversely, those persons in the upper third of insulin resistance in the population have increased risk of coronary heart disease and type 2 diabetes. Surrogate measures of insulin sensitivity (based on comparison with the SSPG) include fasting insulin or homeostasis model assessment of insulin resistance, related measures that explain 35–40% of the variance in insulin sensitivity. Glucose tolerance per se is associated with insulin sensitivity but again is only a partially effective marker. Overall, having impaired fasting glucose (IFG) has sensitivity 0.10 and specificity 0.97, impaired glucose tolerance (IGT) 0.26 and 0.95, fasting insulin in the highest tertile 0.66 and 0.83, and insulin 2 h after oral glucose 0.71 and 0.86, respectively, in ascertaining a person's insulin sensitivity status. Another useful measure is the triglyceride-to-HDL cholesterol ratio, which is as good a surrogate measure of insulin sensitivity as fasting insulin. Overweight is another strong predictor. Using receiver operating characteristic curve analysis, triglyceride level, triglyceride-to-HDL ratio, and insulin level are considerably stronger markers of insulin resistance than BMI, HDL cholesterol, and glucose levels. Reaven suggested that triglyceride >130 mg/dl, triglyceride-to-HDL ratio >3, and insulin >15  $\mu$ U/ml (Reaven pointed out that each laboratory must establish its own norms) are reasonable cutoff points and show greater predictive power than the use of the Adult Treatment Panel (ATP) III criteria for IRS (vide infra), which are specific but not sensitive.

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**Abbreviations:** ADMA, asymmetric dimethyl arginine; ALT, alanine transaminase; apo, apolipoprotein; ATP, Adult Treatment Panel; CRP, C-reactive protein; CVD, cardiovascular disease; DDAH, dimethylarginine dimethylaminohydrolase; eNOS, endothelial nitric oxide synthase; FFA, free fatty acid; IL, interleukin; IRS, insulin resistance syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NMR, nuclear magnetic resonance; ROS, reactive oxygen species; SSPG, steady-state plasma glucose; TNF, tumor necrosis factor; TZD, thiazolidinedione.

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In research presentations at the meeting, Helke Fenster (Stanford, CA) discussed the relationship between insulin resistance and clinical indexes of obesity, comparing waist circumference with BMI as measures, noting the difficulty in standardizing waist measurement. In a group of 208 persons without diabetes who underwent SSPG measurement, measuring waist circumference with a tape measure held horizontally at the superior iliac crest while standing, the BMI and waist circumference showed identical correlation to SSPG with  $R = 0.6$  for both measures. Using a BMI cutoff of  $25 \text{ kg/m}^2$ , 60% had insulin resistance, whereas with waist  $>88 \text{ cm}$  in women or  $102 \text{ cm}$  in men, 68% had insulin resistance. Sun Kim (Stanford, CA) discussed the impact of the degree of obesity on surrogate measures of insulin resistance. In analysis of data from 485 persons, 208 with  $\text{BMI} \leq 25 \text{ kg/m}^2$ , the fasting insulin, homeostasis model assessment of insulin resistance ( $\text{insulin } [\mu\text{U/ml}] \times [\text{glucose } \{\text{mmol/l}\}/22.5]$ ), and quantitative insulin sensitivity check index ( $1/[\log \text{insulin} + \log \text{glycemia in mg/dl}]$ ) showed extremely high intercorrelation because the latter two indexes are calculated based on fasting insulin. The insulin area under the curve following oral glucose administration facilitated distinction between insulin-sensitive and -resistant persons, particularly when correlations were separately calculated for normal, overweight, and obese persons.

### Mechanisms of insulin resistance

Gerald Shulman (New Haven, CT) discussed the mechanism of insulin resistance, using nuclear magnetic resonance (NMR) spectroscopy to assess muscle biochemistry (4). Carbon NMR studies allow measurement of muscle glycogen synthesis, with a profound defect in muscle glycogen synthesis in persons with type 2 diabetes "that accounts for virtually all of their insulin resistance." Phosphorus NMR studies comparing glycogen synthase, hexokinase, and GLUT4 as potential rate-limiting steps leading to this insulin resistance suggest transport defects at the level of hexokinase or GLUT4 to be primary (5). Similar studies in offspring of persons with type 2 diabetes suggest this abnormality to precede the onset of the disease (6). Further  $^{13}\text{C}$  NMR spectroscopy has suggested that the defect is at the level of GLUT4 (7). The ab-

normality in GLUT4 is strongly predicted by the free fatty acid (FFA) level and, even more strongly, by intramyocellular triglyceride levels. Lipid infusion to raise FFA levels in normal persons suggests this to be an important mechanism of muscle insulin resistance (8). A potential mechanism by which fatty acid metabolites inhibit glucose transport activity appears to involve the insulin signaling cascade, with decreased phosphatidylinositol 3-kinase caused by activation of a serine kinase cascade via protein kinase C- $\theta$  decreasing the translocation of GLUT4 to the cell membrane. (9)

Shulman reviewed the concept that peroxisome proliferator-activated receptor  $\gamma$  agonists act by increasing adipose tissue fat stores and preventing the increase in fatty acid metabolites in liver and muscle. In support of this hypothesis, studies of persons with diabetes caused by lipodystrophy show that leptin, which increases fat stores, reverses the glycemic abnormality, improving insulin action and reducing lipid deposition in muscle and liver (10). In a study of lean, healthy 70- vs. 20-year-old persons exploring the mechanism of age-related insulin resistance, increased liver and muscle fat is seen, with evidence of defects in skeletal muscle mitochondrial oxidative metabolism based on NMR measurement of  $^{13}\text{C}$ -labeled acetate and phosphate-labeled ATP (11). Mitochondrial biogenesis is regulated by peroxisome proliferator-activated receptor  $\gamma$  coactivator 1, AMP kinase, and other cellular intermediaries, suggesting potential therapeutic approaches.

### Endothelial dysfunction and insulin resistance

John Cooke (Stanford, CA) discussed the "tight coupling" between endothelial dysfunction and insulin resistance and focused on NO as an endothelial factor that maintains the balance between blood flow and vasoconstriction. "The endothelium takes on a different phenotype" in the insulin-resistant state, he stated, producing vasoconstrictors and growth factors. NO is the most potent endogenous vasodilator and inhibits vascular smooth muscle proliferation, leukocyte adhesion, and factors leading to oxidative stress, suggesting an antiatherosclerotic action. Flow (shear stress)-mediated vasodilation involves eNOS (12) and other vasodilatory factors, with superoxide dismutase

an important protective system that decreases superoxide anion levels that are also increased by flow-related factors. Exercise increases eNOS, improving vasodilation, whereas obesity and insulin resistance are associated with deficiency of NO leading to endothelial dysfunction (13).

Cholesterol-fed rats treated with L-arginine, an NO precursor, have decreased atherosclerosis (14). Insulin resistance is associated with elevations in circulating ADMA (15). ADMA acts as a competitive inhibitor of eNOS, and the enzyme dimethylarginine dimethylaminohydrolase (DDAH) increases ADMA metabolism, with insulin resistance decreasing DDAH levels and activity by increasing oxidative stress (16). ADMA is increased in a type 2 diabetes rat model in association with decreased DDAH activity (17). CVD risk factors that increase ADMA include increased cholesterol, increased glucose, hypertension, increased triglyceride, and increased homocysteine. ADMA is inversely related to the degree of flow-mediated vasodilation in persons with hypercholesterolemia, showing a stronger relationship than the LDL cholesterol per se (18). Of note, thiazolidinediones (TZDs), metformin, ACE inhibitors, angiotensin receptor blockers, statins, and antioxidants all have been shown to decrease plasma ADMA levels, representing potential approaches to improving vessel wall NO synthesis and decreasing superoxide anion levels. Thus, ADMA is an endogenous inhibitor of the NOS pathway, and reduction in ADMA appears to be a potential approach to improving endothelial function.

Cooke described a transgenic mouse model overexpressing DDAH, with levels of ADMA 50% lower than in controls, and NO generation doubled in association with decreased blood pressure and systemic vascular resistance. He concluded by showing the normal orderly pattern of alignment of endothelial cells with flow, which is disrupted in areas of "bends and branches," partially explaining the predilection of these sites for development of atheromas. Areas of atherosclerosis are associated with low blood flow, leading to increased particle residence time with potential for increased adherence of circulating inflammatory cells (19). In these areas, the endothelium loses its normal alignment and there is more rapid endothelial cell turnover due to what in effect

is a more rapid aging of these cells (20.) Aged endothelial cells produce less NO and show greater monocyte adhesion, while in a model with endothelial cells overexpressing telomerase, the cells show restored NO production and reduction in adhesion (21).

### Nonalcoholic fatty liver disease

Arun Sanyal (Richmond, VA) discussed NAFLD and nonalcoholic steatohepatitis (NASH), which he described as “the hepatic manifestation of the IRS” (22) and as “a disease of affluence,” associated with obesity, increased triglyceride, diabetes, and hypertension. Approximately 40% of persons with NASH have diabetes, and an additional 20% have impaired glucose tolerance, whereas ~50% of persons with diabetes have NAFLD, of whom 20% have NASH, with perhaps 20% of these persons ultimately developing cirrhosis.

Pathologically, in fatty liver, there may be replacement of the hepatocyte by small or large fat globules. Steatohepatitis also includes evidence of cytologic ballooning and pericellular fibrosis. The grade of steatohepatitis is related to the degree of inflammatory activity, while the stage refers to the degree of fibrosis. The current concept of pathogenesis is that increased fatty acid delivery to the liver, in a setting of increased fatty acid oxidation causing oxidative injury and de novo triglyceride synthesis, causes the development of fatty liver, which is associated with mitochondrial paracrystalline inclusions similar to those seen with mutations of mitochondrial DNA, which are known to be associated with oxidative stress. Indeed, both mitochondrial and peroxisomal fatty acid oxidation cause an increase in reactive oxygen species (ROS), one consequence of which is depletion of mitochondrial DNA. Tumor necrosis factor (TNF)- $\alpha$ , lipid peroxidation, microsomal oxidation, and iron overload are additional factors potentially causing mitochondrial dysfunction and increasing ROS.

Given the high prevalence of IRS (23), the frequency of NAFLD is predictable. Most patients with NAFLD are diagnosed incidentally. Sanyal pointed out that we “underplay clinical examination,” suggesting that hepatomegaly and persistent moderate elevations in liver chemistries (for example, alanine transaminase [ALT] as high as 250–300) are suggestive of the presence of NASH, although quite com-

monly the liver chemistries are normal, with indeed, “liver enzymes . . . highly overrated” as a screening test for NASH, and showing poor correlation with the degree of disease or the outcome. Ultrasound will show increased echogenicity but has poor specificity to rule out other liver disease and does not allow one to distinguish fatty liver alone from fatty liver plus fibrosis. Computed tomography and, particularly, MR scanning have greater sensitivity for fatty liver but are considerably more expensive than ultrasound and are not as useful as biopsy (24), which is required to determine the presence of fibrosis. The yield from biopsy is particularly great in older and more obese persons and in those with diabetes. There is, however, no evidence that screening for NASH is appropriate, as no specific treatment is available.

Treatment approaches address insulin resistance, particularly obesity, considering drugs and bariatric surgery as options, with insulin sensitizer treatment as a consideration, and recognition of the potential that insulin and insulin secretagogues might worsen the disease. Other agents that have been recommended, although with “very limited data,” include vitamin E, ursodeoxycholic acid, gemfibrozil, iron depletion agents, and betaine (25). Sanyal showed studies in which vitamin E treatment appeared to improve histology, with the combination of vitamin E and pioglitazone even more impressively decreasing liver fat and fibrosis, and multicenter trials of TZD treatment are being planned. Statins may decrease fat but could worsen inflammation, so they are not recommended as treatment for NAFLD per se.

Discussing the relationship between bariatric surgery and fatty liver disease, Sanyal noted that morbidly obese patients often have fatty liver and that the no-longer-recommended jejeunal-ileal bypass procedure was associated with development of liver dysfunction, sometimes progressing to hepatic necrosis. This may have been caused by bacterial overgrowth in the “blind loop” of excluded small bowel or by FFA toxicity because of the unrestrained lipolysis associated with rapid weight loss. He noted that liposuction in excess can cause liver failure, which is believed to be caused by such FFA toxicity. Proximal gastric bypass excludes the duodenum and part of the jejunum rather than the

jejunum and ileum and is not associated with development of liver dysfunction. Some patients are found to have underlying unsuspected cirrhosis at the time of obesity surgery, and there has been concern that the procedure may increase hepatic inflammation initially, although with follow-up, bariatric surgery has been found to be safe even with severe underlying NAFLD.

Sanyal made a number of interesting additional observations pertaining to the relationship between the IRS and liver disease. There may be a relationship between NAFLD and hepatitis C, with the genotype 3 virus, which is uncommon in North America but frequently found in Europe, producing fatty changes. Persons with hepatitis C who do have features of NASH have greater degree of fibrosis and are less likely to respond to interferon therapy, with some evidence that hyperinsulinemia interferes with interferon signaling, while leptin sensitizes the hepatocyte to interferon but ghrelin, which is increased in obesity, may cause leptin resistance. Discussing cirrhosis, he noted that with “the growing realization that cirrhosis is reversible,” one should be alert to clues for this, particularly thrombocytopenia, which is typically an early finding. Once cirrhosis is diagnosed, screening for hepatocellular carcinoma and esophageal varices is appropriate, and the growing interest in clinical trials for advanced NASH may be a rationale for liver biopsy. Finally, he criticized the current Food and Drug Administration recommendations requiring regular screening for TZD hepatotoxicity, noting that these agents cause rare, dose-independent, idiosyncratic reactions, so that screening of an asymptomatic person would only show abnormality in the unlikely event that toxicity happened to be developing at that particular moment.

In a research presentation at the meeting, Diego Ardigo (Parma, Italy) reported on the relationship between insulin resistance and liver fat content in 69 persons with normal serum transaminase levels, of whom 38% had normal liver sonogram and 62% evidence of some degree of abnormality. The plasma insulin was directly correlated with the degree of hepatic steatosis, as were BMI, waist, blood pressure, fasting glucose, HDL, triglyceride, and ALT (although not AST) concentration. In multivariate analysis, fasting insulin and BMI were the only in-



dependent predictors of the degree of steatosis, and fasting insulin was the only independent predictor of ALT, explaining 20% of the variance of this measure.

### Lifestyle approaches for the IRS

John Foreyt (Houston, TX) discussed lifestyle approaches for the IRS, calling attention to “the discrimination” that overweight persons experience. He presented evidence that body weight has increased ~15% over the past century, with BMI >25 and 30 kg/m<sup>2</sup> in 46 and 14%, respectively, of the population in 1980 and 65 and 31% in 2000 (26). One of the major approaches to management of the IRS is lifestyle change, with 7% weight loss and 150 min/week exercise in the Diabetes Prevention Program reducing diabetes by 58% (27). Realistic goals are a 5–10% weight loss, with focus on health, energy, fitness, well-being, and self-esteem, using food diaries to encourage adherence. However, we are subjected to a barrage of unhealthy food and advertisements for unhealthy food. The food industry produces ~3,800 cal · person<sup>-1</sup> · day<sup>-1</sup>, whereas the average requirement is 2,000 cal/day, with Foreyt terming “fat the real culprit” (although note Krauss’s recommendations below).

Foreyt recalled Mark Twain’s dictum, “Habit is habit, and not to be flung out of the window, but coaxed downstairs a step at a time” (28), suggesting an approach to changing behavior for lifestyle modification. Keeping a food diary is extremely important, although in treatment, one must recognize that typical patients underreport calories by one-third and overreport exercise by one-half. One must identify environmental cues associated with overeating and underexercising to develop approaches to “stimulus control,” such as trying to structure eating and exercise patterns (for example, putting out exercise clothes before going to bed). An approach to setting realistic expectations by starting with small changes is the “100/100” plan: eliminating 100 cal by diet and increasing activity by 100 cal, which should lead to a 20-lb annual weight loss (29). Stress management may be helpful and can include exercise, meditation, and “progressive relaxation.” Those persons able to modify lifestyle, participate longer in treatment, and increase physical activity to at least 1 h/day are most likely to succeed. A U.S. national weight control registry has shown that persons who have

been able to lose weight and maintain this follow a low-fat diet, exercise for 60–90 min per day, and eat regular meals, with eating breakfast every day found as an important predictor (30). Strength training is as good as aerobic exercise and may preserve lean body mass (31). Multiple short periods of exercise may be as good as one continuous bout of exercise (32), so it may be appropriate for a person to exercise for 10 min several times daily rather than for 1 h all at once.

Pharmacotherapy, very-low-calorie diet, residential diets (33), and “meal replacements” allow structured eating and are effective in producing sustained weight loss, Foreyt remarked, and should be considered appropriate for persons with BMI between 30 and 39 kg/m<sup>2</sup> who cannot adhere to lifestyle change. He discussed new approaches to behavioral counseling via internet (34), the Sibutramine Trial on Obesity Reduction and Maintenance (35), and the recently published XENDOS (XENical in the prevention of Diabetes in Obese Subjects) trial [Torgerson JS, Hauptman J, Boldrin MN, Sjöström L: XENical in the prevention of Diabetes in Obese Subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. *Diabetes Care* 27:155–161, 2004] with orlistat, with 4-year data showing new diabetes in persons with IGT decreased 37%. For those persons whose BMI exceeds 40 kg/m<sup>2</sup>, bariatric surgery should, Foreyt stated, be strongly considered. He concluded by suggesting that persons with the IRS must “Accuse not Nature: she hath done her part; do thou but thine” (36).

### Low-carbohydrate diet for atherogenic dyslipidemia

Ronald Krauss (Berkeley, CA) discussed dietary approaches to eliminating the atherogenic lipoprotein phenotype characterizing the IRS of increased triglyceride, low HDL cholesterol, and increased small dense LDL particles. There is a curvilinear relationship between peak LDL diameter and plasma triglyceride, although with moderate overlap in triglyceride between the two groups. In a group of 178 men with BMI 26–35 kg/m<sup>2</sup>, 30% with the IRS, and 47% with the pattern B of small dense LDL particles, 57% of those with pattern B had the IRS and 87% of those with the IRS had pattern B. The per-

sons with pattern B had triglyceride 197 vs. 109 mg/dl for those with pattern A, HDL was 40 vs. 51 mg/dl, LDL was similar at 134 vs. 133 mg/dl (although apolipoprotein B levels were 19% higher in the pattern-B group, reflecting their larger number of LDL particles), and the fasting insulin was 13.7 vs. 9.9 μU/ml, supporting the presence of lower insulin sensitivity in these persons. In an explanation of the mechanism, Krauss compared persons having a small hepatic triglyceride pool, leading to small VLDL particles and larger LDL, the less atherogenic pattern A. In contrast, with a large hepatic triglyceride pool, VLDL particles become larger, with remnants acted upon by lipoprotein lipase and hepatic lipase to produce small dense LDL particles and cholesterol ester transport protein interacting between remnants and small dense LDL to further reduce LDL size and enrich remnant particles in cholesterol, as well as leading to smaller and less atheroprotective HDL, the dyslipidemia pattern B (37). A number of gene variants predispose to pattern B.

With increasing dietary carbohydrate LDL pattern B increases, suggesting that reducing carbohydrate might improve the lipid pattern. Furthermore, as BMI decreases, there is a decrease in pattern B. Krauss therefore randomized the 178 men to one of four diets, an ATP III diet (30% fat/55% carbohydrate), a 40% carbohydrate, a 30% fat diet, and a 25% carbohydrate/45% fat diet, the latter with either high-saturated or -monounsaturated fat. After 3 weeks on the diet, calories were decreased by 1,000/day for 5 weeks, with a subsequent 4-week stabilization period.

From the baseline 40–50% pattern B frequency, the ATP III diet led to no change, while there was significant decrease in pattern B with the 40% carbohydrate diet and further decrease with 25% carbohydrate. During the weight loss phase of the diet, all groups had similar weight loss, despite differences in diet composition. Pattern B prevalence decreased on the ATP diet, but there was much more modest change in the low-carbohydrate diet groups. Apolipoprotein B decreased by 4, 10, 13, and 16 mg/dl on the initial diets, with weight loss similarly further lowering apolipoprotein B only in the 55% carbohydrate diet group. Krauss suggested that either weight loss or carbo-

hydrate restriction can be effective in improving the atherogenic lipid phenotype.

### Relationship between obesity and the IRS

Tracey McLaughlin (Stamford, CA) discussed the relationship between obesity and the IRS and potential approaches to treatment, reviewing the prevalence of obesity and noting that almost 5% of the population has BMI >40 kg/m<sup>2</sup>. She noted that even persons with BMI 23–25 kg/m<sup>2</sup> who are of Asian ethnicity, or of non-Asian ethnicity with insulin resistance, also usually require weight loss for optimal improvement. Insulin resistance is linearly associated with the BMI, although with wide scatter at any given level, so that 25–30% of the variance in insulin sensitivity is explained by BMI. Fewer than half of persons with BMI between 30 and 35 kg/m<sup>2</sup> have an SSPG in the highest tertile, with 11% of obese persons actually being in the lowest SSPG tertile, while 13% of persons with normal (<25 kg/m<sup>2</sup>) BMI are in the highest tertile of SSPG. She suggested that the clinical challenge is to focus on those persons with both obesity and low insulin sensitivity. Glycemic abnormality was not sensitive in studies performed at Stanford, as 2.5% of those in the highest insulin sensitivity tertile, 7% of those in the middle tertile, and 30% of those in the lowest insulin sensitivity tertile had IGT. Insulin sensitivity is, however, clinically important, as persons in the most insulin-resistant tertile had a 14% 5-year CVD event frequency compared with 2% of the middle tertile and none of the most insulin-sensitive group (38). In a study of 50 obese persons, 29 insulin resistant with mean SSPG 232 mg/dl and 21 insulin sensitive with mean SSPG 84 mg/dl, with similar age and BMI, the 2-h glucose was 144 vs. 112, triglyceride 199 vs. 125, and HDL 42 vs. 54 mg/dl, with a triglyceride-to-HDL ratio 5.4 vs. 2.5, suggesting that insulin resistance contributes to CVD risk independent of obesity.

In a study of weight loss with a 4-month hypocaloric diet and sibutramine in 13 insulin-resistant vs. 11 insulin-sensitive healthy obese women losing similar amounts of weight, the SSPG decreased 34% in the insulin-resistant group (from 210 to 140), whereas it did not change (at ~80 mg/dl) in the insulin-sensitive group, with the women whose insulin sensitivity improved having lower

blood glucose, insulin, triglyceride (39), and CRP (40). ADMA and adhesion molecules levels also decreased with weight loss in the insulin-resistant persons.

Using the Stanford database of ~500 persons whose SSPG had been measured, its correlation coefficients were 0.33 for fasting glucose, 0.56 for insulin, 0.42 for triglyceride, and 0.41 for the triglyceride-to-HDL ratio. The best cut point for the triglyceride-to-HDL ratio was 3.0, with sensitivity 72% and specificity 63% for insulin resistance, and the optimal triglyceride cut point of 131 mg/dl showed similar sensitivity and specificity. The ATP III criteria had sensitivity 55% and specificity 85%. McLaughlin noted that the fasting and, even more, the 2-h postglucose load insulin gave optimal discrimination between insulin sensitivity and insulin resistance.

### Adipocyte hormones and insulin action

Peter Havel (Davis, CA) discussed the role of adipocyte hormones in regulating insulin action, lipid metabolism, and energy homeostasis. Adipose tissue plays an important role in whole-body energy homeostasis, with a number of nonsecreted adipocyte proteins affecting energy balance and insulin action, including the glucose transporter GLUT4 and an enzyme involved in lipid synthesis, diacylglycerol acyltransferase-1, with animal models not expressing the latter gene being resistant to obesity. Adipocytes produce many cytokines, including leptin, with effects on food intake. The leptin-deficient *ob/ob* and leptin receptor-deficient *db/db* mouse are hyperphagic, with similar phenotype associated with leptin deficiency in humans (41) in association with severe hyperphagia (42), suggesting lack of satiety response. Partial leptin deficiency is also associated with increased body fat (43).

Both leptin and insulin act as long-term signals of body fat and energy intake, with feedback to the hypothalamus regulating food intake and energy homeostasis. During moderate energy restriction, leptin levels decrease within 1 week (44), correlating with and presumably contributing to the increase in hunger sensation. Infusion of insulin and glucose to maintain euglycemia in humans leads to an increase in circulating leptin levels after ~3 h (45). In streptozotocin-induced diabetic rats, leptin levels fall and can be re-

stored by insulin in a dose-dependent fashion, with prevention of the hyperphagia characteristic of uncontrolled diabetes. In adipocyte cell culture, insulin increases leptin production, an effect that can be blocked with 2-deoxyglucose in a dose-dependent fashion, suggesting that glucose uptake is required for the effect on leptin. If insulin-mediated glucose metabolism regulates leptin metabolism in fat, it is reasonable to assume that alterations in dietary macronutrients may change leptin and alter hunger during periods of dieting. Comparing high-carbohydrate and high-fat meals, the latter lead to lesser increases in insulin and to lower 24-h leptin patterns, with the greatest differences in leptin occurring ~4 h after meals. The lesser proportional increase in leptin may be more important than the absolute leptin level. In persons receiving a high-carbohydrate, low-fat diet, those with the largest fall in leptin have the greatest degree of weight loss (46). Comparing glucose with fructose, which is not metabolized to fructose 6-phosphate, which does not stimulate insulin secretion, and the metabolism of which favors lipid production, the postprandial glycemic and insulin increase is lower with fructose, in association with a 35% lower overall leptin production. Plasma ghrelin, which increases food intake and the sensation of hunger, decreases with glucose ingestion, while fat and fructose do not stimulate insulin and leptin. In a study of 11 overweight postmenopausal women consuming a high-fructose versus high-glucose diet for 10 weeks, triglyceride and apolipoprotein B levels increased with fructose.

Adiponectin is produced by adipocytes, but levels are inversely proportional to total adipocyte mass and therefore are lower in obesity. Adiponectin levels increase with weight loss following gastric bypass surgery (47) and with TZD administration (48). The cytokine has insulin-sensitizing actions, anti-inflammatory effects on vascular endothelium (49,50), and is associated with increased HDL cholesterol (51). In a lipotrophic mouse model, adiponectin levels are decreased and administration of either adipocyte cytokine leptin or adiponectin improved diabetes and insulin sensitivity (52). Two adiponectin receptors have been cloned, with evidence of activation of AMP kinase leading to fat oxidation. Low adiponectin is associated

with increased liver and muscle triglyceride content. Adipocytes increase adiponectin synthesis in vitro in response to insulin (53) with lesser insulin effect in adipocytes from obese animals, although in vivo insulin appears to decrease adiponectin levels (vide infra). Havel speculated that adiponectin, its receptors, and its subsequent metabolic steps may be targets for treatment of obesity and diabetes.

### Inflammation and the IRS

Peter Reaven (Phoenix, AZ) discussed the association of both obesity and insulin resistance with systemic inflammation, pointing out that atherosclerosis is a chronic inflammatory process, with non-vascular sources of inflammation including the adipocyte. Rudolf Virchow originally observed inflammation of the arterial wall in the 1800s. The initiating steps of atherosclerosis may involve inflammation due to lipoproteins, phospholipids, and other substances modified by oxidation or glycation, with subsequent expression by endothelial cells of chemoattractant molecules leading monocyte uptake into the arterial wall and activation into macrophages, which in turn produce proinflammatory molecules including TNF- $\alpha$  and interleukin (IL)-6. IL-6 acts as a messenger cytokine in the liver, leading to CRP and serum amyloid A production, further mediating atherosclerotic processes. CRP activates complement, stimulates cytokine secretion, increases endothelial cell adhesion molecule expression, decreases eNOS expression and bioactivity, increases plasminogen activator inhibitor-1 levels and activity, increases LDL uptake by macrophages, increases monocyte chemoattraction, increases expression of the angiotensin II type 1 receptor, and has many additional inflammatory effects. CRP is strongly associated with obesity and may be directly secreted by adipocytes, which also produce IL-6, stimulating further hepatic CRP secretion. Other inflammatory products of adipocytes such as TNF- $\alpha$  and interferon inhibit insulin receptor activation of insulin receptor substrate-1 and -2 via several intracellular pathways; therefore, increasing adipocyte mass increases proinflammatory factors and decreases the anti-inflammatory cytokine adiponectin, which inhibits endothelial nuclear factor- $\kappa$ B signaling, decreases endothelial adhesion molecule expression, inhibits

macrophage TNF- $\alpha$  secretion, and inhibits scavenger receptor expression and LDL uptake by macrophages. Adipocyte FFA secretion increases in insulin resistance, potentially enhancing endothelial cell ROS expression and IL-6 production, with enhancement of the effect by hyperglycemia and by LDL particles. Insulin increases plasma leptin (45) and decreases adiponectin in human clamp studies (54), further suggesting the complexity of effects of hyperinsulinemia and insulin resistance.

Reaven discussed the potential importance of CRP, although he noted that no studies of this marker have adjusted for insulin resistance status and that it is uncertain whether the measure should be considered a risk marker or a risk factor. He suggested that levels should be measured twice, 2 weeks apart, to avoid effects of intercurrent illness, particularly if baseline levels exceed 10 mg/l. CRP elevation predicts future events in studies of both high- and low-risk populations, in a fashion additive to information from traditional risk factors (55). In the Cholesterol and Recurrent Events study, persons with low CRP had less than half the risk of myocardial infarction of persons with higher levels (56). Studies of persons with unstable angina show that CRP may offer more information than the exercise electrocardiogram (57) and may improve risk assessment over that available from troponin measurement (58). Low-risk levels are <1 mg/l, average-risk levels are 1–3 mg/l, and high-risk levels are >3 mg/l and are associated with the doubling of CVD risk. CRP levels increase with age, are higher in women, and are associated with coronary disease and type 2 diabetes (59). Modifiable causes of CRP elevation include obesity, cigarette use (60), estrogen treatment, and chronic bronchial or periodontal inflammation. CRP decreases during treatment with statins, fibrates, antibiotics, metformin, and TZDs and with alcohol ingestion (61).

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