Third Annual World Congress on the Insulin Resistance Syndrome

Associated conditions

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

Relationships between insulin resistance and malignancy

Pamela Goodwin (Toronto, Canada) introduced the topic of insulin resistance and malignancy, pointing out that there is variation in cancer incidence around the world, with variations in diet and obesity as important determining factors, and perhaps with the insulin resistance syndrome (IRS) as the underlying state leading to both increased cancer risk and worse outcomes of cancers of breast, prostate, colon, and many other tissues. Mediators may include activation of tyrosine kinase signaling pathways of premalignant and malignant cells via the insulin, insulin-like growth factor (IGF)-1, and IGF-2 receptors. Insulin receptors are present on normal breast, colorectal, and other cells, and in cancer cell lines, binding of the insulin receptor activates the mitogen-activated protein kinase pathways, and insulin stimulates cell-cycle progression, with the potential to increase epithelial cell proliferation in colon and other tissues. Hormone receptor negative breast cancers, particularly those not expressing the progesterone receptor, may have increased signaling through the insulin receptor. There are two forms of the insulin receptor. The A form is mainly seen in the fetal state, and also in the adult central nervous system (CNS), with the B form seen in the adult. The A form has high-affinity IGF-2 binding, has mitogenic and antiapoptotic effects, and hybridizes with the IGF-1 receptor; therefore, a fruitful area of research in carcinogenesis may be the expression of this form of the insulin receptor.

Gerald Reaven (Stanford, CA) noted that differential tissue insulin sensitivity may be important in the relationship between the IRS and malignancy. The dose-response curve of adipose tissue to insulin shows a greater degree of insulin effect than that seen in skeletal muscle. Similarly, in the IRS not every tissue is insulin resistant. This differential tissue responsiveness may be related to the carcinogenic effects of insulin resistance. Furthermore, he commented on the need to accurately characterize the insulin sensitivity of persons with malignancy being studied to assess effects of interventions to improve this parameter, so that a study that fails to include a sufficient number of persons who are actually insulin resistant may show an apparently negative result.

Anne McTierman (Seattle, WA) discussed the IRS and cancer risk, commenting, "If your patient who has diabetes comes down with colon cancer, it's not just bad luck." Breast, colon, and endometrial cancer have been well demonstrated to be associated with obesity. The insulin receptor is expressed both on normal cells and on tumor cells, with insulin stimulating cell cycle progression in cancer cell lines, and with an association between overexpression of the insulin receptor and malignant transformation. Other potential mechanisms include increased estrogen and androgen bioavailability related to decreases in sex hormone–binding globulin (SHBG), as well as effects of decreased fertility, adipokines, leptin, diet, and lack of physical activity. There is a 50–60% increase in cancer mortality among persons with BMI exceeding 40 kg/m^2 (1). Excess weight and physical inactivity are believed to account for between one-quarter and one-third of breast, colon, and endometrial malignancies, with evidence that obesity is associated with total cancer mortality in men, particularly for malignancy of the pancreas (2) and liver, and in women, particularly for the kidney, uterus, cervix, and pancreas (3). More than 200 epidemiological studies have shown increased risk of postmenopausal breast cancer to be associated with obesity (4). In the Women's Health Initiative, analysis of 3-year follow-up with 1,014 new cases of breast cancer showed an association between BMI and risk only among those never using hormone replacement therapy, suggesting that the effect of obesity may involve an increase in circulating estrogen levels. In the study, waist circumference >86 cm (adjusted for BMI) also was associated with increased risk, suggesting a relationship to visceral obesity.

Endometrial cancer is most strongly related to obesity with estrogen excess and insulin resistance the most likely potential mechanisms. There is no clear relationship between ovarian cancer and obesity. The risk of colon cancer is increased by 20–50% risk in obese women and is doubled in obese men, with obesity also associated with an increased risk of colonic adenomas. Furthermore, persons with diabetes have a 20–40% increased risk of colorectal cancer, a 25% increased risk of breast cancer, a 20–60% increased risk of endometrial cancer, a 20–100% increased risk of pancreatic cancer, and a two- to fourfold increased risk of cancer

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Abbreviations: Aβ, Aβ42 isoform; ALT, alanine transaminase; CNS, central nervous system; CPAP, continuous positive airway pressure; CVD, cardiovascular disease; DCI, D-chiro inositol; IL, interleukin; IRS, insulin resistance syndrome; LFT, liver function test; LH, lutenizing hormone; LXR, liver X receptor; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OSA, obstructive sleep apnea; PCOS, polycystic ovary syndrome; PKC, protein kinase C; PSA, prostate-specific antigen; RAGE, receptor for advanced glycation end products; SDB, sleep disordered breathing; SHBG, sex hormone–binding globulin; SNP, single nucleotide polymorphism; SREBP, sterol regulatory element binding protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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of the liver. Among persons with diabetes, hyperinsulinemia and hyperglycemia may be risk factors for colorectal cancer (5). Increased insulin and C-peptide levels also may be associated with breast, colon, and endometrial cancer risks. Intriguingly, there is evidence of benefit of insulin sensitizers, with a suggestion that cancer risk is 15% lower in metformin-treated persons with diabetes (6). A strong relationship has been demonstrated between fasting glucose and both cancer incidence and cancer mortality in Korea, with increases in leukemia, stomach, bladder, pancreas, and esophageal cancer in men and in liver, pancreas, breast, and cervical cancer in women (7).

Martine Extermann (Tampa, FL) discussed the interactions between IRS and cancer, addressing the effects of the syndrome on malignancy outcome. Both cancer and IRS become more common with increasing age, and the strength of the association between obesity and cancer increases with age. Extermann reviewed a study of persons receiving adjuvant treatment for colon cancer, with worse prognosis for persons with diabetes, and with more frequent and earlier relapses. Comparing persons with versus without diabetes, there was a 48% vs. 59% 5-year disease-free survival rate and a 42% increased overall mortality rate (8). In a study of persons with stage II–III colon cancer undergoing adjuvant chemotherapy, mortality rates were 34% greater in obese than in nonobese women (9), with a 61% increased risk of recurrence in another study of obese men with rectal cancer (8).

Among men with prostate cancer, it is not as clear that obesity is associated with increased cancer initiation, but obesity is associated with more rapid progression of prostate-specific antigen (PSA) and with a more aggressive histology. In a study of 320 persons with stage T2–3 prostate cancer, hyperinsulinemia and low HDL cholesterol were associated with disease-specific mortality and with the PSA level (10). In a study of 512 women with stage T1–3 breast cancer, those in the highest fasting insulin quartile had a 2.1-fold greater rate of distant recurrence and a 3.3-fold greater mortality rate (11). Breast cancer relapses more frequently in obese women, with obesity accounting for 6–19% of attributable risk. Aromatase inhibitors may not be as effective in obese persons, Extermann commented, although she noted that this question has not been well explored.

Potential mechanisms of the association between insulin resistance and cancer progression include the IGF-1 pathway, or the inflammatory nuclear factor (NF)-κB cascade, with inhibitor of NF-κB kinase (IκB) β subunit (IKKB) thought to be an important mediator, and with interleukin (IL)-6 associated with prognosis of colon cancer. The IGF-1 receptor (IGF-1R) is overexpressed in colon cancer cells, and insulin may stimulate the IGF-1R. Although total IGF-1 decreases with age, Extermann noted that free IGF-1 may increase with age because of changes in IGF binding proteins. The receptor for advanced glycation end products (RAGE) is another potential mediator of the association of cancer with insulin resistance, binding AGEs, β-amyloid, S100/calgranulins, and amphoterin, an extracellular DNA-binding protein which plays a role in fibrinolysis and cell movement. RAGE blockade reduces tumor growth in some animal models, and there is histological evidence of increasing coexpression of RAGE and amphoterin being associated with worsening colorectal cancer stage, having effects on migration and invasiveness rather than on apoptosis. Yet another potential relationship is the negative association between insulin resistance and protein kinase C (PKC)-ζ, which like protein kinase B (PKB)/Akt is important in cancer. PKCζ acts as an antiapoptotic factor, increases glucose uptake and vascular endothelial growth factor (VEGF) production, protects the colonic mucosa against oxidative damage, and is decreased in muscle although not in liver of persons with the IRS. Both diabetes and the IRS are associated with decreased cellular immunity, and there may be local factors, including the expression of amphoterin, which decreases macrophage migration in areas of cancer. The association between cancer and VEGF may be complex, with VEGF stimulating tumor angiogenesis and with the anti-VEGF drug bevacizumab shown to prolong life for people with metastatic colorectal, breast, and lung cancer, suggesting the importance of studying this in persons with diabetes and malignancy.

A number of antidiabetic agents have been studied in animal models, with phenformin, buformin, and diabenol inhibiting colon carcinogenesis. PKCζ activity may be restored in muscle of persons with metabolic syndrome treated with metformin or with rosiglitazone. In a study of 106 men with prostate cancer and rising PSA, no effect was seen with rosiglitazone versus placebo (12), although there are animal models suggesting that peroxisome proliferator–activated receptor (PPAR)-γ activation inhibits prostate cancer growth. A number of other therapeutic agents may have an effect on malignancies. Statins are associated with reduced risk of malignancy in epidemiologic studies. Nonsteroidal antiinflammatory drugs (NSAIDs) could have effects via the COX2 pathway, or by suppressing the inhibitor of NF-κB (IκB), although high dosing would be needed, offering potential toxicity in view of recent evidence that COX2 inhibitors create cardiovascular side effects. Another potential treatment is orlistat, which blocks cell cycle progression and promotes apoptosis in mammary cancer models (13).

There has been a great deal of interest in the roles of diet and exercise in cancer. Calorie restriction in animals reduces G1-S transition, leading to decreased cell proliferation, and induces a pro-apoptotic pathway, possibly involving IGF-1 and IGF binding proteins, as well as via effects on insulin, corticosteroids, and leptin. Weight gain is often seen following chemotherapy, showing a pattern of loss of muscle mass with increase in fat (14), and appears to be associated with increased risk of recurrence. The Women's Intervention Nutrition Study in 290 women following surgery and systemic therapy for breast cancer compared low (15%) versus normal fat diets, with 33 vs. 51 g fat intake/day, resulting in a 1.5-kg weight loss vs. a 1.8-kg weight gain (15), with a 24% reduction in relapse, the greatest effect seen in estrogen receptor-negative women. In animal models, exercise reduces mammary carcinogenesis, although it has little effect on colon carcinoma. Among women with breast cancer, there is an association between self-reported physical activity and lower BMI, lesser degrees of weight gain, and lower risk of recurrence and death; both walking and vigorous activity lowered risk (16).

In a study of an exercise program in 173 50- to 75-year-old overweight postmenopausal women, there was an increase in SHBG and a decrease in E2 and estrone, suggesting potential benefit in prevention of breast, endometrium, colon, and other cancers. High level physical activity reduces cancer risk by approximately half, and although there is no data as to whether initiating a physical activity program will lower recurrence risk following cancer diagnosis and treatment, there is evidence of improved quality of life in people who have cancer and

engage in regular exercise (17). Programs promoting diet and physical activity in combination may have the greatest likelihood of success (18). It is important to note that antiandrogen treatment of men with prostate cancer and aromatase inhibitor/anti-estrogen treatment in women with breast cancer result in osteoporosis, so that weight-bearing exercise may be particularly beneficial for these patients. Certain cancer treatments may have adverse cardiac effects, including left chest irradiation and adriamicin, and the underlying insulin-resistant state may be associated with cardiovascular disease (CVD), so that cardiac evaluation may be useful. Exercise tolerance will be poor initially; therefore, it is necessary for patients to start slowly with an exercise program. Adverse effects of cancer treatment in children include loss of muscle and increase in adiposity with physical inactivity, and depression also is a factor, so that exercise may be particularly useful in this age group.

Polycystic ovary syndrome

Paulina Essah (Richmond, VA) discussed evidence that there is substantial overlap between the IRS and polycystic ovary syndrome (PCOS). The PCOS affects 6–10% of women of reproductive age, and is defined by hyperandrogenism, chronic anovulation and/or polycystic ovaries. Women with PCOS have a 43–47% incidence of the IRS (19–21), while among premenopausal women, those with IRS have higher androgen levels as well as lower insulin sensitivity levels (22). Adjusting both for both age and BMI, women with PCOS have at least doubling of IRS prevalence; low HDL cholesterol, high BMI, and hypertension are the most prevalent IRS components. In Essah's study, which compared women with PCOS with and without IRS, the former were more likely to have acanthosis and to have less frequent menses, higher serum-free testosterone, lower SHBG, higher blood pressure, lower HDL cholesterol, and higher fasting glucose; free testosterone and SHBG were important predictors of the presence of the IRS. Almost one-quarter of those with PCOS below age 20 had IRS in Essah's study; in a case-control series that analyzed 43 women age 18–22 with PCOS, 12% had IRS, and PCOS was associated with increased carotid intima-media thickness, suggesting potential association with CVD outcomes (23). Other studies substantiate the association of PCOS with IRS and with greater levels of coronary and aortic calcification (24). A number of CVD risk factors are associated with PCOS, including endothelial dysfunction, and elevated levels of plasminogen activator inhibitor (PAI)-1, endothelin-1, and C-reactive protein, as well as the typical abnormalities of HDL cholesterol and triglycerides associated with insulin resistance. In the Nurses Health Study of 82,439 women followed for 14 years, those with very irregular menses had a 50 and 90% increase in the likelihood of coronary heart disease and fatal myocardial infarction, respectively (25). Essah concluded that the high prevalence of IRS among women with PCOS, independent of the degree of obesity, with high levels of concomitant CVD risk factors, suggests the need to comprehensively assess women with PCOS for CVD risk factors.

Jean-Patrice Baillargeon (Sherbrooke, Canada) discussed the mechanisms of insulin resistance in PCOS, noting the complex potential interrelationship between hyperandrogenemia and insulin resistance. Both obese and lean women with PCOS are more insulin resistant than those without the syndrome, and may indeed have greater degrees of insulin resistance than women with diabetes (26), with evidence of decreased glucose oxidation rate (27). However, not all studies have shown insulin resistance among lean women with PCOS (28, 29). Gonadotropin-releasing hormone (GNRH) agonists normalize testosterone without improving insulin sensitivity, suggesting that androgen excess is not the mediator (30). Rather, an increasingly accepted concept is that insulin resistance (whether genetic or associated with obesity) causes androgen excess and decreased SHBG, leading to the classic features of PCOS (31).

An important question is whether the PCOS develops as a consequence of the hyperinsulinemia associated with insulin resistance or whether the PCOS is caused by a more specific defect in insulin action. Adipocytes from women with PCOS have decreased levels of the glucose transporter GLUT4 and decreased insulin-stimulated lipolysis (32). Abnormality of the insulin receptor has been demonstrated in cultured skin fibroblasts of women with PCOS (33), with increased phosphorylation of serine residues (34) and decreased insulin-stimulated tyrosine phosphorylation, as well as decreased phosphorylation of insulin receptor substrate (IRS)-1 and IRS-2. In addition, muscle biopsy studies confirm the cellular evidence of insulin resistance, with decreased insulin-mediated activation of IRS-1 leading to decreased glucose transport (35). D-chiro inositol (DCI) is an inositol isoform acting as a mediator of insulin action. Decreases in DCI may be caused by an increase in urinary clearance, by decreased conversion from myoinsitol, or by decreased transport, and may play a role in the development of insulin resistance. Baillargeon showed evidence that DCI clearance is sixfold greater in women with PCOS than in control subjects, suggesting that DCI deficiency may contribute to the insulin resistance of PCOS.

Another feature of PCOS is the relationship between insulin action and ovarian androgen production. There is greater androgen production by theca cells from women with PCOS (36,37), with evidence that both lutenizing hormone (LH) and insulin stimulate ovarian steroidogenesis. Women with PCOS have increased LH-stimulated androgen production (38,39), which improves with insulin sensitizing intervention (40); metformin, rosiglitazone, and the combination of both have similar effects in decreasing testosterone levels in PCOS (41). Comparing the effects of diazoxide-induced insulin-lowering and GNRH agonist-induced suppression of LH in eight nonobese normoinsulinemic women with PCOS, Baillargeon showed that insulin appeared to more strongly stimulate testosterone, suggesting the mediator of increased androgen to be insulin rather than LH, even in women with PCOS without insulin resistance, for whom the disease may be driven by increased ovarian androgen production in response to insulin.

Nonalcoholic fatty liver disease

Stephan Caldwell (Charlottesville, VA) discussed the clinical presentation and natural history, noting that patients typically present with abnormal liver function tests (LFTs) rather than with symptoms, with a number of studies suggesting that most persons with abnormality of liver function in U.S. and European populations have nonalcoholic steatohepatitis (NASH) (42,43). There is a strong correlation between LFTs and markers of the IRS, particularly obesity and dyslipidemia, with evidence that one-third of people with NASH have diabetes (44,45). Risk factors for development of fibrosis include age, the presence of diabetes, and an aspartate transaminase (AST)–alanine transaminase (ALT) ratio >1.0 (46) (al-

though LFTs may be normal despite actual development of cirrhosis). Other markers include increased ferritin, without evidence of hemochromatosis. Approximately 20% of individuals with NASH have a positive antinuclear antibody (47), with the possibility that the disease evokes an autoimmune response rather than indicating a different etiology. Gluten sensitivity may also be seen and may exacerbate NASH. Up to half of people with NASH have symptoms and physical examination abnormalities, including right upper quadrant pain, hepatomegaly, and acanthosis nigricans, and nonalcoholic fatty liver disease (NAFLD) is associated with an increased likelihood of gallstones. Familial clustering is common, particularly with more severe NASH, and may reflect either genetic or environmental/lifestyle influences. An unusual related abnormality is intermittent disconjugate gaze, with evidence of a relationship between NASH and the gaze palsies of mitochondrial diseases (48).

NASH is associated with a doubling of 10- to 15-year mortality (49), at least in part because of the development of cirrhosis. Findings suggesting cirrhosis include palmar erythema, thrombocytopenia, and varices of the esophagus, stomach, or rectum. Latent NASH-cirrhosis can present abruptly with rapid deterioration over weeks to months (50), and there is an inverse relationship between ALT and fibrosis, leading to the seeming paradox that LFT normalization may be a sign of disease progression. NASH appears to be the major cause of what was once termed "cryptogenic cirrhosis," typically presenting in women between 50 and 60 years of age and often with normal LFTs (51). The prognosis is related to histology, with fibrosis, ballooning hepatocyte degeneration, and inflammatory changes all associated with progression to cirrhosis. Caldwell summarized seven studies comprising 171 patients with NASH who were followed with serial biopsy for a mean of 5 years, of whom 35% worsened (including 11% who progressed to cirrhosis), 43% remained stable, and only 23% improved. Among persons with cirrhosis, over 10 years there is steady progression to ascites, encephalopathy, and variceal bleeding (52). Importantly, both NSAIDs and ACEI may worsen ascites and cause diuretic unresponsiveness, and must be used with great caution. Another important complication is the increased risk of hepatocellular carcinoma in persons with NASH, which more commonly occurs in persons with obesity (53) and with diabetes (54), usually with an interval stage of cirrhosis. Caldwell noted that pediatric NAFLD represents a somewhat different disease, with two types, a less common pericentral vein form and a more common type involving portal fibrosis, with advanced fibrosis or cirrhosis occurring in 8% (55).

Arun Sanyal (Richmond, VA) discussed advances in understanding of the pathogenesis of NAFLD, distinguishing hepatic fat accumulation alone from the conjunction of fat deposition with hepatocyte ballooning and injury, representing steatohepatitis. Hepatic fat contains a predominance of triglyceride, formed by esterification of fatty acids, either locally synthesized from acetyl-CoA or derived from circulating free fatty acids (FFAs), with evidence that the latter pathway is less important in NAFLD, suggesting that de novo lipogenesis is an important contributor to NAFLD pathogenesis (56). Lipogenesis is controlled by the transcription factor sterol regulatory element binding protein (SREBP)-1c, the nuclear isoform mediating insulin action on hepatic glucokinase and lipogenic gene expression, which is expressed to a greater extent in persons with than in those without NAFLD. SREBP-1c transcription is upregulated by insulin, by activation of the nuclear liver X receptor (LXR), and, negatively, by polyunsaturated fatty acids, with SREBP-1c precursor activation increased by insulin and by higher levels of saturated and lower levels of unsaturated fat, perhaps explaining the association of NAFLD with lower hepatic γ-linoleic and docosatetranoic acid content.

Sanyal characterized NAFLD as "very much a diet-driven disease," noting that high fructose corn syrup (HCFS) is preferentially metabolized into triglyceride with increasing dietary HCFS content considered an important driver of the epidemic of obesity in the U.S. (57). Persons with NAFLD have decreased leucine incorporation into apoB-100, suggesting an abnormality of triglyceride mobilization (58). Thus, fat accumulates in NAFLD because of a combination of increased de novo synthesis, increased FFA reesterification, decreased fat oxidation, or decreased triglyceride mobilization, the former and latter being the most important. Steatohepatitis involves not just the accumulation of fat, but hepatocyte injury, initially characterized by cytosolic ballooning, Mallory bodies, inflammation, and mild septal fibrosis, all to some extent mediated by oxidative stress (59). NASH may be associated with mitochondrial abnormality, with mitochondrial para-crystalline inclusions similar to those seen with diseases of mitochondrial DNA demonstrable on electron microscopy. NASH also is associated with decreased mitochondrial respiratory chain activity, indicating an uncoupling of oxidative phosphorylation (60), and with increased levels of cytochrome p450 2E1 (61), also contributing to oxidative stress. Mallory bodies are believed to contain contain heat-shock protein colocalized in proteasomes with the intracellular proteolytic protein ubiquitin (62), and there is increased hepatocyte apoptosis in NASH (63). A unifying hypothesis for liver injury in NASH (64), then, is of increased levels of lipids and glucose contributing to mitochondrial oxidative stress leading to the unfolded protein response, which activates the mitogen-activated kinase pathway and the NF-κB–mediated inflammatory cascade, potentially occurring in the adipocyte as well as in the liver. NF-κB also downregulates PPAR-α, decreasing its antioxidant effect. Given the association of these factors with insulin resistance, Sanyal suggested that rather than involving two separate processes—steatosis followed by cellular injury and inflammation—NAFLD may evolve from the single abnormality of increased adipocyte FFA release and hyperinsulinemia producing hepatic steatosis, while adipocyte-derived cytokines (65) cause inflammatory response of the hepatic Kupfer cells, with these macrophage-like cells releasing further cytokines, acting on the hepatocyte to cause injury. The inflammation further contributes to the development of cirrhosis by activating hepatic stellate cells, leading to fibrosis.

Nathan Bass (San Francisco, CA) discussed current approaches to NASH evaluation and management, reviewing the progression from NAFL, to NASH, to cirrhosis, and ultimately to hepatocellular carcinoma and to both liver-related and nonliver-related mortality. NAFLD usually presents with incidental findings, of elevation of aminotransferases, fatty liver on ultrasound, or hepatomegaly, but may present with complications of cirrhosis, or may be discovered during screening of high-risk clinical populations, such as persons with severe obesity who are candidates for bariatric surgery. Bass illustrated the difficulty of using clinical criteria to detect the disease by reviewing findings in a group of 65 morbidly obese persons undergoing liver biopsy during

gastric bypass, with BMI 48 kg/m^2. Liver chemistries were normal in 44, but only 34 had normal liver histology. On biopsy, 18 had NAFL, and 13 had NASH, with one having stage III hepatic fibrosis. Comparing persons with NASH of varying ethnicity, Bass showed evidence that Asians had lower BMI and were less likely to have diabetes, but were more likely than Caucasians and Hispanics to have hyperlipidemia. The exclusion of alcohol as a contributory factor is somewhat arbitrary, with "nonalcoholic" defined by ≤14 and ≤7 alcoholic beverages weekly in men and in women, respectively, recognizing that there is considerable variation in the amount of alcohol per "drink."

Bass observed that in the Third National Health and Nutrition Examination Survey (NHANES III), the finding of elevated ALT was associated with alcohol ingestion among overweight or obese individuals but not among people of normal weight. He also noted that these alcohol ingestion limits are not "permissible amounts" for people with NAFLD, who should be advised not to drink any alcohol. In addition to alcohol, drug-induced hepatitis, hepatitis B and C, iron overload states, and autoimmune hepatitis are particularly important conditions to be excluded. Asymptomatic persons lacking markers of these conditions with abnormal LFTs who meet ultrasound criteria for fatty liver have a 96% likelihood of having NASH (66). Computerized tomography is somewhat more specific in distinguishing fat from fibrosis, but the gold standard is histological diagnosis, allowing precise characterization of fat, inflammation, necrosis, Mallory's hyaline, ballooning, and fibrosis (67). Biopsy, however, is painful in 25%, with a 3% risk of bleeding and organ perforation and a 0.1% mortality.

Furthermore, although the grade of steatosis and the diagnosis of NASH can be made with confidence on biopsy, there is some risk of sampling error for ballooning, inflammation, and stage of fibrosis, so that Bass suggested that few patients truly require the procedure for clinical management, with noninvasive diagnosis using new ultrasound modalities offering promising new approaches. Approaches to management include serial LFT, platelet, and ultrasound assessment, emphasis on gradual weight loss, and careful treatment of diabetes and of dyslipidemia, although it is not entirely certain that statins are safe in these patients. Avoidance of hepatotoxins, particularly alcohol, is important, with limited evidence of benefit of metformin and thizaolidinediones (68–75) in nondiabetic persons with NASH, recognizing that the latter agents may cause weight gain, and that there may be potential for relapse with worsening upon discontinuation of an insulin sensitizer that cannot be assessed at this time. Ongoing studies will attempt to better characterize these approaches.

John Sninsky (Rockville, MD) discussed the use of genetics and genomics in understanding NASH-causing gene variants, reviewing work from his company, Celera Diagnostics, as well as by other researchers, suggesting that if studies are performed on sufficiently large patient groups with adequate replication of findings, it will be possible to induce "the genome . . . to yield up its secrets." This approach may be useful in determining which persons require biopsy and in stratifying patients for trials. Most characterized monogenic diseases, Sninsky stated, are caused by single nucleotide polymorphisms (SNPs) that alter the amount, function, or stability of a protein.

Applying this approach to common polygenic diseases, however, requires particular attention to adequate sample size and population stratification, addressing the multiple testing problem that occurs when tens or even hundreds of thousands of SNPs are examined, leading to very real risk that spurious associations will be found and that associations that are real but weak due to limited penetrance will be overlooked. Nevertheless, it is currently possible to screen ~40% of the genome with the 20,000–30,000 existing SNP markers, suggesting that we soon will be able to characterize genetic determinants of common complex diseases.

This approach has confirmed a number of accepted markers, such as HLA DR for rheumatoid arthritis on chromosome 6, HLA C for psoriasis on chromosome 6, factor V for thrombosis on chromosome 1, and ApoE for Alzheimer's disease on chromosome 19. New discoveries are the association of the PTPN22 phosphatase allele with rheumatoid arthritis (76) and a number of other autoimmune diseases, and of the association of two gene variants, the DDX5 Ser480Ala polymorphism of a RNA helicase (ATPase) involved in RNA unwinding and RNA binding (77), and the carnitine palmitoyltransferase 1a Ala275Thr polymorphism (78), with advanced hepatic fibrosis in hepatitis C, suggesting that it may be possible to characterize persons with NAFLD who are at 10-fold higher risk of progression and hence in need of treatment. These genes may lead to drug discovery and may allow optimal risk stratification for clinical trials.

Sleep disorders and insulin resistance

Daniel Einhorn (La Jolla, CA) reviewed the interrelationships between breathing/sleep disorders and insulin resistance, a relationship that has been termed "Syndrome Z" (79). There is now an emerging body of evidence suggesting that sleep disturbances are common and may contribute to insulin resistance, and that treatment of these conditions may improve insulin sensitivity. Einhorn observed, however, that most studies are small, and predominantly involve male Caucasians, with inadequate measures of insulin resistance or only with measures of glycemia. Abnormalities associated with obstructive sleep apnea (OSA), the most studied condition, may not be present with the myriad of other causes of sleep deprivation. Hypoxemia may complicate the interpretation of these studies.

Among studied persons with type 2 diabetes, approximately half of men and one-fifth of women have OSA, the prevalence increasing with age so that OSA affects two-thirds of diabetic men age 65 and over. Some degree of the sleep loss syndromes referred to as "sleep disordered breathing " (SDB) is present in 24% of adults (80), the prevalence increasing fourfold with each one SD increase in BMI (81). Snoring, well recognized to be a symptom of OSA, was associated with a doubling of the likelihood of subsequent development of diabetes in the Nurses' Health Study (82). In addition to sleep apnea, which may be obstructive or central, SDB includes chronic voluntary partial sleep deprivation, shift work syndrome, jet lag syndrome, restless leg syndrome, insomnia, and fragmented sleep.

The consequences of all forms of sleep loss are similar, leading to what is termed "sleep debt," manifesting in excessive daytime sleepiness. The average sleep time in the U.S. has decreased by >80 min since 1950 and continues to decrease rapidly, tracking with television-watching, with obesity, and with diabetes, as well as with hypertension, dyslipidemia, inflammatory cytokines, glucose intolerance, stimulation of the hy-

pothalamic-pituitary-adrenal axis, and sympathetic nervous system (SNS) stimulation (83). There is growing evidence that these sleep disturbances contribute to insulin resistance, perhaps with a vicious cycle of obesity and sleep disruption. A study of healthy young men who spent 18 nights in a sleep laboratory with six nights each of 8-, 4-, and 12-h sleep cycles showed that less sleep was associated with increased cortisol and with glucose intolerance in a dose-response fashion (84). Sleep-deprived individuals chose greater amounts of higher-energy density foods and chose to exercise less. In a study of 150 healthy middle-aged men, one-third had SDB, a finding associated with increased 2-h glucose and insulin levels (85). Greater degrees of sleep deprivation are associated with hyperinsulinemia, independent of age, sex, ethnicity, smoking status, BMI, waist circumference, and sleep duration. SDB also is associated with elevated fasting and 2-h post-challenge glucose levels (86). Levels of glucose, insulin, TNF-α, IL-6, and leptin are increased in OSA, which is associated with increased visceral fat when compared with obese control subjects (87). Furthermore, OSA predicts the components of the IRS, particularly low HDL cholesterol and elevated blood pressure, and hence the prevalence of IRS is more than twice as great in persons with than without OSA (88).

Given these relationships, it is encouraging that treatment of OSA appears to have metabolic benefits. The use of continuous positive airway pressure (CPAP) improves insulin sensitivity after 2 days, with evidence of continued benefit at 3 months, particularly in persons with BMI <32 kg/m^2 (89). In type 2 diabetic patients, euglycemic-hyperinsulinemic clamp studies similarly show evidence of improved insulin sensitivity with CPAP (90), with evidence as well of improvement in postprandial glycemia in those using CPAP >4 h/night, with progressive improvement in A1C in these persons over a 5-month period of treatment (91) and evidence of improved glycemic patterns with continuous glucose monitoring studies. The mechanisms of adverse effect of OSA appear to involve sympathetic activation, with increased muscle sympathetic activity in persons with OSA with and without hypertension and with increased circulating norepinephrine levels (92), with sympathetic nerve activity and blood pressure increased during periods of nocturnal wakefulness, responding to CPAP treatment (93). Experimental sleep deprivation is associated with increased serum cortisol levels on the following evening (83), another potential mechanism of adverse metabolic effect, with CPAP reversing the hypercortisolemia (94). Given these findings, Einhorn speculated that "there is a lot going on in cardiac patients," and that there may be a number of beneficial cardiovascular as well as metabolic effects of sleep disorder treatment.

Brain function and insulin resistance

Suzanne Craft (Seattle, WA) discussed the "very critical relationship" between insulin resistance and aspects of central nervous system (CNS) function, noting that insulin plays a role in normal brain function, and that insulin resistance is associated with increased risk of cognitive impairment and Alzheimer's disease (AD). Potential mechanisms include inflammation, increased β-amyloid, and decreased cerebral glucose metabolism, with intriguing evidence emerging that there may be effects of thiazolidinedione treatment on cognition. Insulin receptors are distributed in the medial temporal cortices and surrounding areas. Although insulin does not increase glucose transport into the brain, it promotes glucose utilization in specific brain regions (particularly the hippocampus [95]), affects levels of neurotransmitters (96), and modulates membrane potentials, membrane expression of *N*-methyl-D-aspartate (NMDA) receptors, and neuronal firing rates (97).

There is a close linkage between peripheral and CNS insulin, with insulin crossing the blood-brain barrier via saturable receptor-mediated transcytosis (98) and increasing peripheral insulin associated with increased insulin binding in the hippocampus (99). Thus, although there is debate as to whether insulin synthesis occurs in the CNS, there is clear evidence that peripheral insulin acts on brain function. Insulin enhances memory when given intravenously with euglycemia maintained (100), and this effect may also be demonstrated with intranasal insulin administration (101); Craft speculates that memory-encoding events surrounding feeding may have particular importance to the organism. Chronic effects of insulin may, however, reduce the effect of insulin on glucose and neurotransmitters, leading to decreased brain insulin uptake (102), which increases insulin's inflammatory, mitogenic, and oncogenic effects, and is associated with the phenomenon of reduced memory with insulin resistance in older persons. Craft noted that insulin exhibits both pro- and antiinflammatory effects. High chronic levels increase CRP, cytokines, and F2-isoprostane, leading to the question of whether insulin may regulate brain inflammation. In a study of 16 normal persons age 55–81 years who were receiving saline versus insulin infusion, hyperinsulinemia (with euglycemia) increased cerebrospinal spinal fluid (CSF) IL-1α, IL-1β, IL-6, and TNF-α, with increased CSF levels of F2-isoprostane, an eicosanoid biomarker of free radical–mediated arachidonic acid oxidation derived exclusively from brain, suggesting a direct CNS rather than peripheral proinflammatory effect.

Insulin resistance and inflammation are associated with increased risk of Alzheimer's disease (103,104), and insulin regulates memory and pathophysiological features of Alzheimer's disease (105). β-amyloid is a peptide produced by many cell types, with aggregation of the Aβ42 isoform (Aβ) thought to have neurotoxic effects in Alzheimer's disease. Insulin promotes release of intracellular Aβ (106) and inhibits degradation of Aβ by a metalloproteinase, insulin degrading enzyme (IDE) (107), the main enzyme clearing Aβ. As IDE preferentially degrades insulin, increased CNS insulin may increase levels of Aβ. In the study of the effect of insulin administration, there was no change in CSF Aβ in those younger than 70 years, but a 20% increase was seen in those age 70 or older, correlating in this subgroup with CNS F2-isoprostane, suggesting a mechanism through which insulin resistance might increase Alzheimer's risk. Two studies have addressed potential benefits on Alzheimer's disease of insulin sensitizer treatment. In a comparison of pioglitazone 30 mg daily versus nateglinide 120 mg three times daily versus placebo in 71 persons with a mean age of 74 years with impaired glucose tolerance or type 2 diabetes, a measure of memory impairment showed some evidence of improvement with pioglitazone, with the change in 2-h glucose correlating with the degree of improvement. In a second study, 30 persons with early Alzheimer's disease were randomized to rosiglitazone 4 mg daily versus placebo, with evidence of improved memory (108).

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