

# Third Annual World Congress on the Insulin Resistance Syndrome

## Associated conditions

ZACHARY T. BLOOMGARDEN, MD

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 pre-malignant 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 nor-

mal 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<sup>2</sup> (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

Zachary T. Bloomgarden, MD, is a practicing endocrinologist in New York, New York, and is affiliated with the Division of Endocrinology, Mount Sinai School of Medicine, New York, New York.

**Abbreviations:** A $\beta$ , A $\beta$ 42 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, luteinizing 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.

DOI: 10.2337/dc06-zb09

© 2006 by the American Diabetes Association.

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)- $\kappa$ B cascade, with inhibitor of NF- $\kappa$ B kinase (I $\kappa$ B)  $\beta$  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,  $\beta$ -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)- $\zeta$ , which like protein kinase B (PKB)/Akt is important in cancer. PKC $\zeta$  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. PKC $\zeta$  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)- $\gamma$  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- $\kappa$ B (I $\kappa$ B), 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 adriamycin, 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 myoinositol, 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 luteinizing 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  $\gamma$ -linoleic and docosatetraenoic 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 mitochon-

drial 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 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- $\kappa$ B-mediated inflammatory cascade, potentially occurring in the adipocyte as well as in the liver. NF- $\kappa$ B also downregulates PPAR- $\alpha$ , 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<sup>2</sup>. 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  $\leq 14$  and  $\leq 7$  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 im-

portant, with limited evidence of benefit of metformin and thiazolidinediones (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  $\sim 40\%$  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 char-

acterize 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- $\alpha$ , 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<sup>2</sup> (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 in-

creased 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  $\beta$ -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 inflam-

matory, 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 fluid (CSF) IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , 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).  $\beta$ -amyloid is a peptide produced by many cell types, with aggregation of the A $\beta$ 42 isoform (A $\beta$ ) thought to have neurotoxic effects in Alzheimer’s disease. Insulin promotes release of intracellular A $\beta$  (106) and inhibits degradation of A $\beta$  by a metalloproteinase, insulin degrading enzyme (IDE) (107), the main enzyme clearing A $\beta$ . As IDE preferentially degrades insulin, increased CNS insulin may increase levels of A $\beta$ . In the study of the effect of insulin administration, there was no change in CSF A $\beta$  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).

## References

- Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ: Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348:1625–1638, 2003
- Stolzenberg-Solomon RZ, Graubard BI, Chari S, Limburg P, Taylor PR, Virtamo J, Albanes D: Insulin, glucose, insulin resistance, and pancreatic cancer in male smokers. *JAMA* 294:2872–2878, 2005
- Calle EE, Thun MJ: Obesity and cancer. *Oncogene* 23:6365–6378, 2004
- McTiernan A: Associations between energy balance and body mass index and risk of breast carcinoma in women from diverse racial and ethnic backgrounds in the U.S. *Cancer* 88 (Suppl. 5):1248–1255, 2000
- Chang CK, Ulrich CM: Hyperinsulinaemia and hyperglycaemia: possible risk factors of colorectal cancer among diabetic patients. *Diabetologia* 46:595–607, 2003
- Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD: Metformin and reduced risk of cancer in diabetic patients. *BMJ* 330:1304–1305, 2005
- Jee SH, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM: Fasting serum glucose level and cancer risk in Korean men and women. *JAMA* 293:194–202, 2005
- Meyerhardt JA, Catalano PJ, Haller DG, Mayer RJ, Macdonald JS, Benson AB 3rd, Fuchs CS: Impact of diabetes mellitus on outcomes in patients with colon cancer. *J Clin Oncol* 21:433–440, 2003
- Meyerhardt JA, Catalano PJ, Haller DG, Mayer RJ, Benson AB 3rd, Macdonald JS, Fuchs CS: Influence of body mass index on outcomes and treatment-related toxicity in patients with colon carcinoma. *Cancer* 98:484–495, 2003
- Hammarsten J, Hogstedt B: Hyperinsulinaemia: a prospective risk factor for lethal clinical prostate cancer. *Eur J Cancer* 41:2887–2895, 2005
- Goodwin PJ, Ennis M, Pritchard KI, Trudeau ME, Koo J, Madamas Y, Hartwick W, Hoffman B, Hood N: Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J Clin Oncol* 20:42–51, 2002
- Smith MR, Manola J, Kaufman DS, George D, Oh WK, Mueller E, Slovin S, Spiegelman B, Small E, Kantoff PW: Rosiglitazone versus placebo for men with prostate carcinoma and a rising serum prostate-specific antigen level after radical prostatectomy and/or radiation therapy. *Cancer* 101:1569–1574, 2004
- Menendez JA, Vellon L, Lupu R: Antitumoral actions of the anti-obesity drug orlistat (Xenical) in breast cancer cells: blockade of cell cycle progression, promotion of apoptotic cell death and PEA3-mediated transcriptional repression of Her2/neu (erbB-2) oncogene. *Ann Oncol* 16:1253–1267, 2005
- Demark-Wahnefried W, Peterson BL, Winer EP, Marks L, Aziz N, Marcom PK, Blackwell K, Rimer BK: Changes in weight, body composition, and factors influencing energy balance among premenopausal breast cancer patients receiving adjuvant chemotherapy. *J Clin Oncol* 19:2381–2389, 2001
- Chlebowski RT, Blackburn GL, Buzzard IM, Rose DP, Martino S, Khandekar JD, York RM, Jeffery RW, Elashoff RM, Wynder EL: Adherence to a dietary fat intake reduction program in postmenopausal women receiving therapy for early breast cancer. The Women's Intervention Nutrition Study. *J Clin Oncol* 11:2072–2080, 1993
- Holmes MD, Chen WY, Feskanich D, Kroenke CH, Colditz GA: Physical activity and survival after breast cancer diagnosis. *JAMA* 293:2479–2486, 2005
- Courneya KS: Exercise in cancer survivors: an overview of research. *Med Sci Sports Exerc* 35:1846–1852, 2003
- Goodwin P, Esplen MJ, Butler K, Winocur J, Pritchard K, Brazel S, Gao J, Miller A: Multidisciplinary weight management in locoregional breast cancer: results of a phase II study. *Breast Cancer Res Treat* 48:53–64, 1998
- Apridonidze T, Essah PA, Iuorno MJ, Nestler JE: Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 90:1929–1935, 2005
- Glueck CJ, Papanna R, Wang P, Goldenberg N, Sieve-Smith L: Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. *Metabolism* 52:908–915, 2003
- Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH: Screening women with polycystic ovary syndrome for metabolic syndrome. *Obstet Gynecol* 106:131–137, 2005
- Korhonen S, Hippelainen M, Vanhala M, Heinonen S, Niskanen L: The androgenic sex hormone profile is an essential feature of metabolic syndrome in premenopausal women: a controlled community-based study. *Fertil Steril* 79:1327–1334, 2003
- Vural B, Caliskan E, Turkoz E, Kilic T, Demirci A: Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome. *Hum Reprod* 20:2409–2413, 2005
- Talbott EO, Zborowski JV, Rager JR, Boudreaux MY, Edmundowicz DA, Guzick DS: Evidence for an association between metabolic cardiovascular syndrome and coronary and aortic calcification among women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 89:5454–5461, 2004
- Solomon CG, Hu FB, Dunaif A, Rich-Edwards JE, Stampfer MJ, Willett WC, Speizer FE, Manson JE: Menstrual cycle irregularity and risk for future cardiovascular disease. *J Clin Endocrinol Metab* 87:2013–2017, 2002
- Dunaif A, Segal KR, Futterweit W, Dobrjansky A: Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 38:1165–1174, 1989
- Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Tapanainen JS: Insulin sensitivity, insulin secretion, and metabolic and hormonal parameters in healthy women and women with polycystic ovarian syndrome. *Hum Reprod* 15:1266–1274, 2000
- Vrbikova J, Cibula D, Dvorakova K, Stanicka S, Sindelka G, Hill M, Fanta M, Vondra K, Skrha J: Insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 89:2942–2945, 2004
- Ciampelli M, Fulghesu AM, Cucinelli F, Pavone V, Caruso A, Mancuso S, Lanzano A: Heterogeneity in beta cell activity, hepatic insulin clearance and peripheral insulin sensitivity in women with polycystic ovary syndrome. *Hum Reprod* 12:1897–1901, 1997
- Dunaif A, Green G, Futterweit W, Dobrjansky A: Suppression of hyperandrogenism does not improve peripheral or hepatic insulin resistance in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 70:699–704, 1990
- De Leo V, la Marca A, Petraglia F: Insulin-lowering agents in the management of polycystic ovary syndrome. *Endocr Rev* 24:633–667, 2003
- Ciaraldi TP, Morales AJ, Hickman MG, Odom-Ford R, Olefsky JM, Yen SS: Cellular insulin resistance in adipocytes from obese polycystic ovary syndrome subjects involves adenosine modulation of insulin sensitivity. *J Clin Endocrinol Metab* 82:1421–1425, 1997
- Dunaif A, Xia J, Book CB, Schenker E, Tang Z: Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle: a potential mechanism for insulin resistance in the polycystic ovary syndrome. *J Clin Invest* 96:801–810, 1995
- Li M, Youngren JF, Dunaif A, Goldfine ID, Maddux BA, Zhang BB, Evans JL: Decreased insulin receptor (IR) autophosphorylation in fibroblasts from patients with PCOS: effects of serine kinase inhibitors and IR activators. *J Clin Endocrinol Metab* 87:4088–4093, 2002
- Dunaif A, Wu X, Lee A, Diamanti-Kandarakis E: Defects in insulin receptor signaling in vivo in the polycystic ovary syndrome (PCOS). *Am J Physiol Endocrinol*

- mol Metab* 281:E392–E399, 2001
36. Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F: Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. *J Clin Endocrinol Metab* 83:2001–2005, 1998
  37. Zhang G, Garmey JC, Veldhuis JD: Interactive stimulation by luteinizing hormone and insulin of the steroidogenic acute regulatory (StAR) protein and 17 $\alpha$ -hydroxylase/17,20-lyase (CYP17) genes in porcine theca cells. *Endocrinology* 141:2735–2742, 2000
  38. Gilling-Smith C, Story H, Rogers V, Franks S: Evidence for a primary abnormality of thecal cell steroidogenesis in the polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 47:93–99, 1997
  39. Nestler JE, Jakubowicz DJ: Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *N Engl J Med* 335:617–623, 1996
  40. Ehrmann DA, Schneider DJ, Sobel BE, Cavaghan MK, Imperial J, Rosenfield RL, Polonsky KS: Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 82:2108–2116, 1997
  41. Baillargeon JP, Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Nestler JE: Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. *Fertil Steril* 82:893–902, 2004
  42. Mathiesen UL, Franzen LE, Fryden A, Foberg U, Bodemar G: The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. *Scand J Gastroenterol* 34:85–91, 1999
  43. Daniel S, Ben-Menachem T, Vasudevan G, Ma CK, Blumenkehl M: Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. *Am J Gastroenterol* 94:3010–3014, 1999
  44. Suzuki A, Angulo P, Lymp J, St Sauver J, Muto A, Okada T, Lindor K: Chronological development of elevated aminotransferases in a nonalcoholic population. *Hepatology* 41:64–71, 2005
  45. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J: NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 35:373–379, 2002
  46. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ: Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 37:1286–1292, 2003
  47. Adams LA, Lindor KD, Angulo P: The prevalence of autoantibodies and autoimmune hepatitis in patients with non-alcoholic fatty liver disease. *Am J Gastroenterol* 99:1316–1320, 2004
  48. Al-Osaimi AM, Berg CL, Caldwell SH: Intermittent disconjugate gaze: a novel finding in nonalcoholic steatohepatitis and cryptogenic cirrhosis (Letter). *Hepatology* 41:943, 2005
  49. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P: The natural history of non-alcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 129:113–121, 2005
  50. Caldwell SH, Hespdenheide EE: Subacute liver failure in obese women. *Am J Gastroenterol* 97:2058–2062, 2002
  51. Caldwell SH, Crespo DM: The spectrum expanded: cryptogenic cirrhosis and the natural history of non-alcoholic fatty liver disease. *J Hepatol* 40:578–584, 2004
  52. Hui JM, Kench JG, Chitturi S, Sud A, Farrell GC, Byth K, Hall P, Khan M, George J: Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology* 38:420–427, 2003
  53. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ: Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348:1625–1638, 2003
  54. El-Serag HB, Tran T, Everhart JE: Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 126:460–468, 2004
  55. Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE: Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 42:641–649, 2005
  56. Diraison F, Moulin P, Beylot M: Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab* 29:478–485, 2003
  57. Bray GA, Nielsen SJ, Popkin BM: Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr* 79:537–543, 2004
  58. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS: Apolipoprotein synthesis in nonalcoholic steatohepatitis. *Hepatology* 35:898–904, 2002
  59. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN: Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 120:1183–1192, 2001
  60. Perez-Carreras M, Del Hoyo P, Martin MA, Rubio JC, Martin A, Castellano G, Colina F, Arenas J, Solis-Herruzo JA: Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology* 38:999–1007, 2003
  61. Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C: Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 27:128–133, 1998
  62. Riley NE, Li J, Worrall S, Rothnagel JA, Swagell C, van Leeuwen FW, French SW: The Mallory body as an aggresome: in vitro studies. *Exp Mol Pathol* 72:17–23, 2002
  63. Feldstein AE, Canbay A, Angulo P, Tanai M, Burgart LJ, Lindor KD, Gores GJ: Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology* 125:437–443, 2003
  64. Schroder M, Kaufman RJ: ER stress and the unfolded protein response. *Mutat Res* 569:29–63, 2005
  65. Crespo J, Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, Fernandez-Escalante JC, Pons-Romero F: Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 34:1158–1163, 2001
  66. Joy D, Thava VR, Scott BB: Diagnosis of fatty liver disease: is biopsy necessary? *Eur J Gastroenterol Hepatol* 15:539–543, 2003
  67. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ, Nonalcoholic Steatohepatitis Clinical Research Network: Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 41:1313–1321, 2005
  68. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N: Metformin in non-alcoholic steatohepatitis. *Lancet* 358:893–894, 2001
  69. Uygun A, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A, Yesilova Z, Gulsen M, Dagalp K: Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 19:537–544, 2004
  70. Nair S, Diehl AM, Wiseman M, Farr GH Jr, Perrillo RP: Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. *Aliment Pharmacol Ther* 20:23–28, 2004



71. Schwimmer JB, Middleton MS, Deutsch R, Lavine JE: A phase 2 clinical trial of metformin as a treatment for non-diabetic paediatric non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 21: 871–879, 2005
72. Bugianesi E, Gentilecore E, Manini R, Natale S, Vanni E, Villanova N, David E, Rizzetto M, Marchesini G: A randomized controlled trial of metformin versus vitamin E or prescriptive diet in non-alcoholic fatty liver disease. *Am J Gastroenterol* 100:1082–1090, 2005
73. Caldwell SH, Hespeneheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL: A pilot study of a thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. *Am J Gastroenterol* 96:519–525, 2001
74. Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling RK, Stravitz RT, Shiffman ML, Clore J, Mills AS: A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2:1107–1115, 2004
75. Promrat K, Lutchman G, Uwaifo GI, Freedman RJ, Soza A, Heller T, Doo E, Ghany M, Premkumar A, Park Y, Liang TJ, Yanovski JA, Kleiner DE, Hoofnagle JH: A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 39:188–196, 2004
76. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang SY, Saiki RK, Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffery DA, Lee AT, Batliwalla F, Remmers E, Criswell LA, Seldin MF, Kastner DL, Amos CI, Sninsky JJ, Gregersen PK: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 75:330–337, 2004
77. Goh PY, Tan YJ, Lim SP, Tan YH, Lim SG, Fuller-Pace F, Hong W: Cellular RNA helicase p68 relocalization and interaction with the hepatitis C virus (HCV) NS5B protein and the potential role of p68 in HCV RNA replication. *J Virol* 78:5288–5298, 2004
78. Gobin S, Thuillier L, Jogl G, Faye A, Tong L, Chi M, Bonnefont JP, Girard J, Prip-Buus C: Functional and structural basis of carnitine palmitoyltransferase 1A deficiency. *J Biol Chem* 278:50428–50434, 2003
79. Wilcox I, McNamara SG, Collins FL, Grunstein RR, Sullivan CE: “Syndrome Z”: the interaction of sleep apnoea, vascular risk factors, and heart disease. *Thorax* 53 (Suppl. 3):S25–S28, 1998
80. Bresnitz EA, Goldberg R, Kosinski RM: Epidemiology of obstructive sleep apnea. *Epidemiol Rev* 16:210–227, 1994
81. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S: The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 328:1230–1235, 1993
82. Al-Delaimy WK, Manson JE, Willett WC, Stampfer MJ, Hu FB: Snoring as a risk factor for type II diabetes mellitus: a prospective study. *Am J Epidemiol* 155: 387–393, 2002
83. Leproult R, Copinschi G, Buxton O, Van Cauter E: Sleep loss results in an elevation of cortisol levels the next evening. *Sleep* 20:865–870, 1997
84. Spiegel K, Leproult R, Van Cauter E: Impact of sleep debt on metabolic and endocrine function. *Lancet* 354:1435–1439, 1999
85. Ip MS, Lam B, Ng MM, Lam WK, Tsang KW, Lam KS: Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med* 165:670–676, 2002
86. Punjabi NM, Shahar E, Redline S, Gottlieb DJ, Givelber R, Resnick HE; Sleep Heart Health Study Investigators. Sleep-disordered breathing, glucose intolerance, and insulin resistance: the Sleep Heart Health Study. *Am J Epidemiol* 160: 521–530, 2004
87. Vgontzas AN, Papanicolaou DA, Bixler EO, Hopper K, Lotsikas A, Lin HM, Kales A, Chrousos GP: Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab* 85:1151–1158, 2000
88. Coughlin SR, Mawdsley L, Mugarza JA, Calverley PM, Wilding JP: Obstructive sleep apnoea is independently associated with an increased prevalence of metabolic syndrome. *Eur Heart J* 25: 735–741, 2004
89. Harsch IA, Schahin SP, Radespiel-Troger M, Weintz O, Jahreiss H, Fuchs FS, Wiest GH, Hahn EG, Lohmann T, Konturek PC, Ficker JH: Continuous positive airway pressure treatment rapidly improves insulin sensitivity in patients with obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 169:156–162, 2004
90. Brooks B, Cistulli PA, Borkman M, Ross G, McGhee S, Grunstein RR, Sullivan CE, Yue DK: Obstructive sleep apnea in obese noninsulin-dependent diabetic patients: effect of continuous positive airway pressure treatment on insulin responsiveness. *J Clin Endocrinol Metab* 79:1681–1685, 1994
91. Babu AR, Herdegen J, Fogelfeld L, Shott S, Mazzone T: Type 2 diabetes, glycemic control, and continuous positive airway pressure in obstructive sleep apnea. *Arch Intern Med* 165:447–452, 2005
92. Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG: Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest* 103:1763–1768, 1993
93. Somers VK, Dyken ME, Clary MP, Abboud FM: Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest* 96:1897–1904, 1995
94. Bratel T, Wennlund A, Carlstrom K: Pituitary reactivity, androgens and catecholamines in obstructive sleep apnoea. Effects of continuous positive airway pressure treatment (CPAP). *Respir Med* 93:1–7, 1999
95. Bingham EM, Hopkins D, Smith D, Pernet A, Hallett W, Reed L, Marsden PK, Amiel SA: The role of insulin in human brain glucose metabolism: an 18fluorodeoxyglucose positron emission tomography study. *Diabetes* 51:3384–3390, 2002
96. Figlewicz DP, Szot P, Israel PA, Payne C, Dorsa DM: Insulin reduces norepinephrine transporter mRNA in vivo in rat locus coeruleus. *Brain Res* 602:161–164, 1993
97. Skeberdis VA, Lan J, Zheng X, Zukin RS, Bennett MV: Insulin promotes rapid delivery of N-methyl-D-aspartate receptors to the cell surface by exocytosis. *Proc Natl Acad Sci USA* 98:3561–3566, 2001
98. Banks WA, Jaspan JB, Kastin AJ: Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. *Peptides* 18:1257–1262, 1997
99. Marfaing P, Penicaud L, Broer Y, Mraovitch S, Calando Y, Picon L: Effects of hyperinsulinemia on local cerebral insulin binding and glucose utilization in normoglycemic awake rats. *Neurosci Lett* 115:279–285, 1990
100. Craft S, Asthana S, Cook DG, Baker LD, Cherrier M, Purganan K, Wait C, Petrova A, Latendresse S, Watson GS, Newcomer JW, Schellenberg GD, Krohn AJ: Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer’s disease: interactions with apolipoprotein E genotype. *Psychoneuroendocrinology* 28:809–822, 2003
101. Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, Kern W: Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* 29: 1326–1334, 2004
102. Schwartz MW, Figlewicz DF, Kahn SE, Baskin DG, Greenwood MR, Porte D Jr: Insulin binding to brain capillaries is reduced in genetically obese, hyperinsulinemic Zucker rats. *Peptides* 11:467–472, 1990
103. Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM: Diabetes mellitus and the risk of dementia: the Rotterdam Study. *Neurology* 53:1937–1942, 1999
104. Luchsinger JA, Mayeux R: Cardiovas-

- cular risk factors and Alzheimer's disease. *Curr Atheroscler Rep* 6:261–266, 2004
105. Watson GS, Craft S: The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs* 17:27–45, 2003
106. Gasparini L, Gouras GK, Wang R, Gross RS, Beal MF, Greengard P, Xu H: Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling. *J Neurosci* 21:2561–2570, 2001
107. Zhao L, Teter B, Morihara T, Lim GP, Ambegaokar SS, Ubeda OJ, Frautschy SA, Cole GM: Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: implications for Alzheimer's disease intervention. *J Neurosci* 24:11120–11126, 2004
108. Watson GS, Cholerton BA, Reger MA, Baker LD, Plymate SR, Asthana S, Fishel MA, Kulstad JJ, Green PS, Cook DG, Kahn SE, Keeling ML, Craft S: Preserved cognition in patients with early Alzheimer disease and amnesic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *Am J Geriatr Psychiatry* 13:950–958, 2005