

Insulin Resistance Concepts

ZACHARY T. BLOOMGARDEN, MD

Perspectives on the News commentaries are now part of a new, free monthly CME activity. The Mount Sinai School of Medicine, New York, New York, is designating this activity for 2.0 AMA PRA Category 1 credits. If you wish to participate, review this article and visit www.diabetes.procampus.net to complete a posttest and receive a certificate. The Mount Sinai School of Medicine is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

This is the first in a series of four articles on presentations given at the World Congress on the insulin resistance syndrome (IRS), reviewing concepts pertaining to insulin resistance.

Clinical aspects of insulin resistance

Yehuda Handelsman (Tarzana, CA) discussed the clinical implications of insulin resistance. He reminded listeners that Gerald Reaven introduced the concept of Syndrome X with his 1988 Banting Lecture, leading to increasing recognition of the importance of the IRS by the World Health Organization (WHO), the American College of Endocrinology, the International Diabetes Federation (IDF), and the American Heart Association. With new definitions, there have been new approaches to treatment, and areas of controversy as well, with the IDF and American Heart Association suggesting that the syndrome exists and is clinically important, while the American Diabetes Association and European Association for the Study of Diabetes have suggested this not to be the case.

Handelsman offered a synthesis of the apparently opposing positions. "The syndrome," he said, "is not a disease. It is

distinguished from type 2 diabetes and CVD [cardiovascular disease]. The concept [of an IRS] is designed to predict and prevent [the development of illness]." In this context, it may be particularly important to redefine the "metabolic syndrome" as the "insulin resistance syndrome," allowing one to group together the multitude of seemingly diverse conditions, affecting skin, the reproductive system, liver, cancer, the brain, breathing/sleeping disorders, coagulation disorders, hypertension, and atherosclerosis, with abnormality in one of these areas suggesting the need to look in others. Increased alanine transaminase (ALT) may, for example, predict the development of CVD. Handelsman pointed out that among individuals with breast and prostate cancer, the second leading cause of death, after the malignancies themselves, is CVD. Insulin resistance increases the likelihood of microalbuminuria in individuals with hypertension, further increasing CVD risk. Sleep apnea increases insulin resistance and continuous positive airway pressure treatment reduces it, further evidence of the bidirectional links between all these conditions. Insulin resistance is linked to CVD by dyslipidemia, with elevated triglyceride and small LDL particles and low HDL cholesterol, and by direct interactions between insulin resistance and atherosclerotic end points, with evidence that the insulin sensitizer pioglitazone may reduce CVD as suggested by the PROactive Study. The DREAM Study, among others, suggests improvement of liver function with rosiglitazone and shows marked reduction with this agent in the development of diabetes among individuals with impaired glucose tolerance (IGT). Handelsman concluded that we need to develop new clinical diagnostic

algorithms, being particularly careful to screen individuals with some manifestations of the IRS for the myriad of other associated conditions.

Gerald Reaven (Stanford, CA) offered a reappraisal of aspects of the relationship between insulin resistance and the insulin resistance syndrome. Early studies of insulin resistance were carried out by Himsforth in the 1930s (1), and Reaven's original studies characterizing insulin sensitivity with the steady-state plasma glucose methodology were carried out more than three decades ago (2). He described a study of 490 apparently healthy individuals with up to eightfold variability in insulin sensitivity, of which, he suggested, approximately half is likely genetic, and one quarter each related to the presence or absence of obesity and of regular physical activity. Insulin resistance should, he suggested, be distinguished from hyperinsulinemia, which causes many of the manifestations of the IRS in tissues that remain responsive to insulin. As an example, he pointed out that hypertension in insulin resistant individuals in part reflects the occurrence of this phenomenon in the kidney and in the sympathetic nervous system. Discussing three popular definitions of the metabolic syndrome, those of the WHO, Adult Treatment Panel III, and IDF, he pointed out that they all use criteria and cut points that are essentially arbitrary, including the WHO requirement of a glycemic marker, the Adult Treatment Panel III requirement for three of five criteria, and the IDF ethnic-specific waist circumference criteria.

Insulin acts on the liver to set the level of triglyceride production from free fatty acid (FFA). Among insulin-resistant individuals with hypertriglyceridemia, these lipid levels progressively increase during the day with accumulation of remnant lipoproteins, leading to the clustering of high insulin and high triglyceride, along with low HDL cholesterol, elevated blood pressure, and multiple additional abnormalities characterizing those at greatest risk of developing CVD. Reaven referred to excess adiposity as "the most confusing component" of the IRS, as obesity modulates insulin action. The degree of insulin resistance and BMI vary independently as well: for a given degree of insulin resistance, BMI predicts, while for a given BMI

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: 11 β HSD, 11 β OH steroid dehydrogenase; AgRP, Agouti-related peptide; AICAR, 5-aminimidazole-4-carboxamide-1- β -D-ribofuranoside; apo, apolipoprotein; ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CVD, cardiovascular disease; FFA, free fatty acid; IDF, International Diabetes Federation; IGT, impaired glucose tolerance; IL, interleukin; IRS, insulin resistance syndrome; NPY, neuropeptide Y; PGC, peroxisome proliferator-activated receptor- γ coactivator; PI3K, phosphatidylinositol 3-kinase; POMC, proopiomelanocortin; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X-receptor; WHO, World Health Organization.

DOI: 10.2337/dc07-zb05

© 2007 by the American Diabetes Association.

the degree of insulin resistance predicts CVD risk factors. Considering total versus abdominal adiposity, Reaven noted that BMI correlates with waist circumference and that neither is particularly more useful in predicting insulin sensitivity. He further put forward the concept that in many studies the correlations of visceral, subcutaneous, and total fat with clamp insulin sensitivity are similar, suggesting that the concept of visceral adiposity may be overstated and that the simple calculation of BMI is as likely to be helpful. Addressing the assessment of inflammation, he showed data suggesting that the measurement of the leukocyte count is as useful as that of C-reactive protein. Finally, he questioned whether there is a benefit to making the diagnosis of the metabolic syndrome, suggesting rather that one should simply treat all CVD risk factors.

Mechanisms of insulin resistance

Neil Ruderman (Boston, MA) reviewed the role of AMP-activated protein kinase (AMPK) in the development of insulin resistance and its complications. He defined the IRS as a disorder in which a group of genetic factors, inactivity, diet, and obesity lead to a set of metabolic dysregulatory conditions, causing conditions including diabetes, hypertension, malignancies, atherosclerosis, dyslipidemia, nonalcoholic steatohepatitis, and polycystic ovarian syndrome. AMPK was discovered ~20 years ago in a study of the control of acetyl-CoA carboxylase. AMPK is a stress kinase responding to depleted energy states, activated when the AMP-to-ATP ratios increases, with AMPK causing increased ATP generation and decreased ATP usage. Exercise also activates AMPK, helping to restore the energy utilized. AMPK has α , β , and γ subunits, with ATP binding to the γ domain in the resting state. When the AMP-to-ATP ratio increases, AMP displaces ATP, leading to a conformational change in AMPK. AMPK is activated by phosphorylation mediated by AMPK kinases, such as calmodulin-dependent protein kinase kinase and LKB1, with inactivation of the latter in Peutz-Jeghers syndrome promoting the development of a variety of tumors.

Malonyl-CoA serves two purposes in the cell, acting both as an intermediate in fatty acid synthesis and also as an allosteric inhibitor of carnitine palmitoyl-transferase 1 α (Cpt1 α), which controls entrance of long-chain fatty acid-CoA into the mitochondria, where it is oxidized. The alternative pathways lead to

fatty acid esterification to diacyl glycerol and triglyceride. AMPK inhibits the malonyl-CoA effect by activating malonyl-CoA decarboxylase and inhibiting acetyl-CoA carboxylase (ACC) and thus plays a major role in these aspects of energy metabolism. AMPK also acts on fatty acid-CoA metabolism to inhibit glycerol phosphate acyl transferase, inhibiting triglyceride and diacyl glycerol synthesis, and inhibits ceramide synthesis via serine palmitoyl transferase and, by less clear pathways, reduces oxidative stress and inhibits nuclear factor- κ B activation.

Decreased AMPK and/or increased malonyl-CoA are found in animal models of IRS and in humans. This is seen in the leptin receptor-deficient *fa/fa* rat, the Zucker diabetic fatty (ZDF) rat with both leptin receptor abnormality and β -cell dysfunction leading to diabetes, the fat-fed rat, the acute glucose-infused rat, the Dahl Salt-sensitive rat, and mice not expressing interleukin (IL)-6. Animals lacking AMPK are also predisposed to atherosclerosis in appropriate models, such as those not expressing the LDL receptor or apolipoprotein (apo)E. Furthermore, treatment of streptozotocin-induced diabetic rats with AMPK activators appears to improve insulin sensitivity.

A variety of approaches exist to AMPK activation, including caloric restriction, exercise, metformin, thiazolidinediones, adiponectin, the adenosine analog 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR), α -lipoic acid, estrogen, and polyphenols such as those found in red wine. All diminish insulin resistance, hyperglycemia, endothelial cell dysfunction and other manifestations of the IRS in animal models. There is also evidence that reduction in malonyl-CoA may increase insulin action. ACC-deficient mice have increased insulin sensitivity and decreased liver and muscle fat. ACC inhibition increases insulin sensitivity in fat-fed mice (3), with evidence of a reduction in hepatic steatosis with administration of ACC antisense RNA in this model (4), and in vitro ACC inhibition increases insulin action in muscle incubated in a hyperglycemic medium.

AMPK and AcCoA carboxylase, then, are potential therapeutic targets for improvement in insulin sensitivity. Insulin-stimulated glucose uptake is increased in AICAR-treated rats (5). With exercise, in part because of increase in circulating catecholamine and IL-6 levels, AMPK is activated in muscle, liver, and epididymal fat. In AMPK-deficient animals such as

the ZDF rat, ectopic lipid deposits in the islets, liver, and skeletal muscle, and diabetes develops, with prevention by caloric restriction or thiazolidinedione administration. AICAR administration and exercise prevent hyperglycemia as well, including AMPK activation associated with preservation of β -cell morphology and function, as well as decreasing liver, muscle, and β -cell lipid deposition (6,7). A number of cells have AMPK-malonyl-CoA fuel-sensing and signaling mechanisms, including muscle, liver, β -cells, adipocytes, and glucose-sensing cells in the brain, and there is emerging evidence that this mechanism is present in endothelial cells (8). In the absence of glucose, AMPK activation and fatty acid oxidation are increased at least threefold (9), while hyperglycemia causes insulin resistance, apoptosis, and mitochondrial dysfunction, with AMPK inhibiting these effects.

Jeremy Tomlinson (Birmingham, U.K.) discussed prereceptor cortisol metabolism as a therapeutic target. He noted that Cushing's disease has many features of the IRS but that, if anything, cortisol levels are slightly reduced in IRS. 11 β OH steroid dehydrogenase (11 β HSD)-type 2 inactivates cortisol to cortisone in the kidney, placenta, and colon, preventing activation of the mineralocorticoid receptor, while 11 β HSD-type 1 activates cortisone by catalyzing its conversion to cortisol, an energy-requiring process utilizing NADPH. 11 β HSD1 is expressed in adipose tissue, to twice as great an extent in omental as in subcutaneous fat. This can be demonstrated in cultured human preadipocytes, with induction of 11 β HSD1 by cortisol, leading to the IRS being referred to as "Cushing's Disease of the Omentum" (10). Cortisol reduces adipocyte proliferation but promotes differentiation. Mice overexpressing 11 β HSD1 in adipocytes have increased adiposity, predominantly in abdominal depots, and develop hypertension, obesity, and insulin resistance, while animals overexpressing 11 β HSD2 have the opposite effects with decreased total body fat. 11 β HSD1 is regulated, with decreased expression in rodents given a high fat diet, while lean individuals have greater production of cortisol from cortisone. In human obesity hepatic 11 β HSD1 is decreased, potentially causing chronic HPA axis overactivity. Downregulation of 11 β HSD1 is not seen in type 2 diabetes, potentially promoting hyperglycemia and worsening adiposity. 11 β HSD1 is expressed in liver, pancreas, fat, muscle, and the vasculature. In adipo-

cyte incubations, carbenoxalone, which inhibits both 11 β HSD1 and 11 β HSD2, can be used to demonstrate reduction in cortisol generation with consequent decrease in lipolysis. Compounds are being developed to specifically reduce 11 β HSD1 activity, including BVT2733, which increases insulin sensitivity in animal models, and Merck compound 544, which reduces fasting glucose, insulin, triglyceride and cholesterol, body weight, and, in mice not expressing apoE, atherosclerosis.

Mary Elizabeth Patti (Boston, MA) described the role of mitochondrial dysfunction in the IRS. Changes in gene and protein expression mediate insulin resistance and β -cell dysfunction causing type 2 diabetes. Human muscle samples show reduced insulin-stimulated signal transduction, with decreased insulin-stimulated glycogen and lipid storage in pre-diabetic individuals. Expression of genes involved in mitochondrial oxidative metabolism is reduced in individuals with diabetes and in nondiabetic insulin-resistant individuals with a family history of diabetes, the latter individuals also having reduced ATP synthesis. These observations have led to the hypothesis that the pathogenesis of diabetes involves decreased levels and/or activity of nuclear respiratory factors, transcription factors binding to DNA, which promote transcription of nuclear-encoded mitochondrial genes, with consequent reduction in fatty acid oxidation, causing lipid accumulation. An important nuclear regulatory factor is peroxisome proliferator-activated receptor (PPAR) γ coactivator (PGC)-1, which also coactivates PPAR α and hepatic nuclear factor-4, with levels both of PGC-1 α and - β reduced by half in diabetic individuals and insulin-resistant nondiabetic individuals, playing an important metabolic role in adipocytes and skeletal muscle. PGC-1 has limited effects on muscle glucose metabolism but has marked action on muscle lipid metabolism, enhancing ¹⁴C palmitate disposal and decreasing lipid synthesis. PGC-1 is involved in gene-environment interactions, with polymorphisms in PGC-1 influencing responses to a variety of lifestyle factors as well as to medications such as acarbose. Lack of physical activity is associated with decreased PGC-1 expression. Patti reviewed a study comparing low- with high-capacity running mice, the former having increased blood pressure, increased insulin levels, and decreased PGC-1 and PPAR γ , suggesting a link between aerobic fitness and mitochondrial

function. There is a strong relationship between BMI and PGC-1 expression, with both genetic (*ob/ob* mouse) and high-fat diet-induced obesity decreasing PGC-1 expression in animal models, and with 3-day high-fat feeding in humans also associated with reduced PGC-1 expression. Tissue culture models show reduced PGC-1 expression with palmitate but not with oleate incubation, suggesting that saturated fatty acids may decrease expression of PGC-1, potentially mediating their effect on tricarboxylic acid cycle genes, as animals overexpressing PGC-1 α or - β fail to show this palmitate effect. Palmitate decreases multiple genes in the oxidative phosphorylation cascade, consistent with its effect to reduce mitochondrial function. Rosiglitazone, metformin, and AICAR also appear to increase PGC-1 expression. Patti noted that there are other, PGC-1 independent, transcriptional regulators that play similar roles and asked whether PGC-1 deficiency causes insulin resistance or whether insulin resistance causes PGC-1 deficiency. Regardless, there is intriguing evidence that PGC-1 normally plays a role in the fasting state in ensuring fuel availability, decreasing with food ingestion. Chronic deficiency caused by chronic overnutrition and saturated fat associated, with roles of genetic factors or inactivity, may lead to a state of "metabolic inflexibility," linked to insulin resistance and ultimately to the development of diabetes. Patti observed that the paradoxical inhibition (rather than stimulation) of mitochondrial gene expression by insulin in states of insulin resistance cannot be explained by changes in PGC-1, suggesting that there are other mechanisms of metabolic dysregulation that must be present in the IRS.

Insulin resistance and the brain

Michael Schwartz (Seattle, WA) discussed neuronal mechanisms linking obesity and insulin resistance. The prevailing concept that obesity causes insulin resistance, which in the setting of β -cell defect causes diabetes, may be incomplete, as insulin resistance and obesity may have specific causal linkage with β -cell dysfunction. Energy homeostasis is the biological process whereby energy intake is matched to expenditure to promote stability of fuel storage as fat. The regulation of fat stores must involve the brain, controlling both energy intake and expenditure. Two major adiposity feedback signals are insulin and leptin, both circulating in proportion to body fat and both crossing the blood-

brain barrier into areas related to food intake and autonomic control, with blockage of neuronal signaling by either insulin or leptin, which leads to increased food intake with consequent weight gain. Most forms of obesity show normal to increased food intake with increased fat stores in the face of normal leptin levels, suggesting leptin resistance.

The arcuate nucleus of the hypothalamus contains neuropeptide Y (NPY) and proopiomelanocortin (POMC) neurons, the former also secreting Agouti-related peptide (AgRP), which increases food intake by blocking the neuronal melanocortin-4 receptor. Leptin and insulin both inhibit the orexigenic NPY/AgRP neurons and stimulate the anorexigenic POMC neurons, acting in a coordinate fashion to promote negative energy balance. During weight loss, leptin and insulin levels decrease, activating NPY/AgRP neurons and inhibiting the POMC neurons. Gut factors are also important, with ghrelin stimulating and protein YY inhibiting NPY neurons, as well as fatty acid and glucose having indirect effects on hypothalamic function.

The actions of leptin at the arcuate nucleus enhance the sensitivity of the hindbrain to input from satiety signals, such as gastric distension. Food reward, the pleasure of eating, can be triggered by olfactory, taste, and cognitive inputs. Leptin inhibits food reward, reducing motivation for food ingestion. Conversely, with food deprivation and reduction in leptin levels, there is increased food reward as well as decreased effect of satiety signals.

Insulin acts in the brain via the insulin receptor substrate phosphatidylinositol 3-kinase (PI3K) pathway, converting phosphatidylinositol diphosphate to the triphosphate, which activates protein kinase-B and other mediators, promoting glucose transport and glycogen and protein synthesis, and exerting mitogenic and antiapoptotic effects. Metabolic stresses and, in particular, food excess decrease PI3K signaling.

Expression of insulin receptor is decreased in islets in states of insulin resistance, leading in type 2 diabetes to a cascade of worsening insulin resistance as well as contributing to insulin secretory defects. Given this perspective, it is likely that insulin receptor signaling plays a role in the central regulation of food intake. Neuronal insulin receptor substrate-2 deletion is associated with obesity in mouse models. Schwartz described a study in which infusing a PI3K inhibitor into the

3rd ventricle decreased the ability of intracerebroventricular insulin to reduce food intake. He pointed out that insulin hyperpolarizes glucose-responsive arcuate nucleus neurons via a mechanism that requires PI3K. The leptin receptor, a cytokine receptor, potentiates activation of the insulin receptor pathway and PI3K. Impaired hypothalamic PI3K signaling occurs in rats with high-fat diet–induced obesity. Thus, the same process that impairs peripheral insulin action may underlie the weight gain of the IRS. Furthermore, there may be peripheral effects of central insulin resistance, as insulin, FFA, glucose, and amino acids infused into the arcuate nucleus increase hepatic insulin sensitivity and decrease hepatic glucose production. Similarly, leptin action in the hypothalamus may change insulin sensitivity, as the Koletsky fa^k/fa^k rat, with an abnormal leptin receptor, is insulin resistant, with adenoviral expression of the leptin receptor in the hypothalamus improving insulin sensitivity, which can be blocked with central PI3K inhibitor administration. Reduced action of insulin, of leptin, or of other humeral inputs in the brain may then lead to increased food ingestion, leading to a vicious cycle of worsening insulin sensitivity and appetite dysregulation.

David Busija (Winston-Salem, NC) discussed mechanisms of cerebral vascular dysfunction related to insulin resistance, which is associated with vascular dysfunction in general. Vascular responsiveness of the cerebral arteries is severely impaired in insulin resistance, with endothelial and vascular smooth muscle abnormalities, which are nitric oxide dependent, and involving potassium channel function. Superoxide anion is one of the factors causing oxidant stress in insulin resistance, with Busija showing evidence of improved vascular function with superoxide dismutase and with rosuvastatin. The vascular dysfunction of insulin resistance leads to increased cerebral infarction in stroke insulin receptor models, suggesting a mechanism of the benefit of interventions.

Suzanne Craft (Seattle, WA) discussed the relationship of insulin resistance to cognitive impairment and neurodegenerative disease, based on observations that insulin plays a role in normal brain function and that insulin resistance increases risk for cognitive impairment, Alzheimer's disease, and other neurodegenerative disease, with potential mechanisms including inflammation, in-

creased β -amyloid, and decreased cerebral glucose metabolism. Furthermore, treatment of insulin resistance may have effects on cognition in individuals with diabetes, in those with insulin resistance, and perhaps in all individuals with Alzheimer's disease.

There is close linkage between peripheral and central insulin levels. Insulin crosses the blood-brain barrier by saturable receptor–mediated transcytosis. With increased peripheral insulin, there is increased insulin binding to numerous brain regions, including the hippocampus and the cortex, playing roles in memory and cognition. Insulin increases glucose use in specific brain regions; increases the levels of neurotransmitters such as dopamine, acetylcholine, and norepinephrine; modulates firing of neuronal cells and membrane expression of N-methyl-D-aspartate receptors; protects against oxidative stress and mitochondrial dysfunction in neurons and glial cells; and enhances memory under certain experimental circumstances. Insulin is typically secreted and cleared quickly, Craft said, but she noted that “high chronic elevations are problematic,” associated with decreased brain insulin uptake and action and with inflammation and mitochondrial dysfunction. Although insulin may be anti-inflammatory at low levels, high chronic levels are associated with increased FFA, cytokines, and F2-isoprostane, reflecting central free radical–mediated oxidation of arachidonic acid.

In a study of 16 individuals (mean age 69 years) with saline versus hyperinsulinemic-euglycemic clamp, insulin increased spinal fluid IL-1 α , -1 β , and -6; tumor necrosis factor- α ; and F2-isoprostane levels. One of the key histological features of Alzheimer's disease is the deposition of β -amyloid, which has neurotoxic and acute memory-inhibiting effects in aging and in Alzheimer's disease. Hyperinsulinemia promotes the release of intracellular β -amyloid and inhibits its degradation by insulin degrading enzyme. In the study, increased β -amyloid was seen only in individuals aged ≥ 70 years and correlated with the change in CSF F2-isoprostane levels.

Insulin resistance and inflammation, then, may increase the risks of memory impairment and Alzheimer's disease. In part, this appears to be mediated by FFA elevations, as FFA correlates with β -amyloid levels and stimulates assembly of β -amyloid oligomers, as well as of tau fil-

aments, an additional pathological feature of Alzheimer's disease. Excess FFA and its inefficient oxidation appear to be primary causes of mitochondrial dysfunction, and there is evidence of oxidative stress and mitochondrial dysfunction in the brain in early stages of Alzheimer's disease. β -Amyloid impairs brain mitochondrial function, and brain mitochondrial from type 2 diabetes rat models show age-related vulnerability to β -amyloid toxicity.

There are therapeutic implications of these associations between insulin and clinical symptoms, β -amyloid, and inflammation. Craft reviewed a 4-month study of individuals age >55 years with IGT or mild type 2 diabetes treated with 30 mg pioglitazone daily versus 120 mg nateglinide three times daily versus placebo. Brain positron emission tomography scanning showed decreased metabolism in the left temporal, frontal, and parietal cortex, a pattern that has been observed in individuals subsequently developing Alzheimer's disease, in diabetes, and, to a lesser extent, with IGT. Similarly, the normal frontal cortex activation pattern with a memory task was particularly decreased with diabetes and also decreased with IGT. Pioglitazone improved memory, correlating with the improvement in glycemia; high responders had an $\sim 40\%$ improvement on memory tasks. In a study of some 500 nondiabetic individuals with mild to moderate Alzheimer's disease treated with placebo or rosiglitazone for 6 months, the Alzheimer's Disease Assessment Scale showed stability with active treatment among the insulin-resistant subset, compared with worsening in those receiving placebo (11). If confirmed, these findings may support treatment directed at improving insulin action in individuals at risk for Alzheimer's disease and memory impairment, as well as in those with established Alzheimer's disease.

Biological rhythms and insulin resistance

Peter Grant (Leeds, U.K.) opened a symposium on biological rhythms and their relationships to insulin sensitivity by stressing the myriad circannual and circadian rhythms regulating biological processes (12). Sandy Martin (Aurora, CO) discussed metabolic changes occurring in response to seasonal variation in hibernation as a model for insulin resistance in humans. Hibernation is a seasonal switch of phenotype. Hibernating squirrels have a rather sudden drop in body temperature from 35 to 5°C. In the summer, they are

homeothermic, with a rich supply of food, but in winter “instead of fighting the cold they escape the cold” and become heterothermic, with 12-day cycles of torpor with low body temperature, heart rate, respirations, and metabolism, interrupted by interbout arousal to briefly regain metabolic/biochemical hyperactivity. All hibernating species display this pattern of cycling. There is a transition period in the spring, with shortening of the bouts of torpor before the animals finally enter the nonhibernating state. Torpor is not the same as hypothermia: hibernating animals defend the new core temperature by increasing or decreasing the metabolic rate. There are hibernating species in all the mammalian lineages, monotremes (egg-laying mammals), marsupials, and eutherians (placental mammals). Primates (lemurs) also hibernate, as do bats, bears, and hedgehogs, suggesting that all mammals share the genes for hibernation.

Martin considered the relevance of hibernation in ground squirrels to human physiology and insulin resistance. Hibernators are a remarkable model of human obesity, fueling the torpor cycle with adipocyte hypertrophy, hyperphagia, hyperinsulinemia and reduced energy expenditure to nearly double body weight by storing vast depots of fat. There is marked insulin resistance during the period of fat storage, with Martin suggesting that the patterns of fat acquisition follow endogenous circannual rhythms entrained by ambient light. Interestingly, weight gain does not occur during the period of maximal food intake, suggesting variations in energy expenditure also mediate the changes in body weight.

The animals first change from summer to winter phenotype and only then can express torpor. Thus, animals must gain weight to express torpor; this is modulated by dietary fatty acid composition. Interestingly, in the winter phase, hibernating animals are resistant to a wide variety of ischemia reperfusion injuries, both in brain and in the gastrointestinal tract, both in the torpor and in the interbout arousal states, while during the weight-gain phase, when insulin resistance is greatest, the animals may be more prone to such ischemia-related injury. The torpor to arousal switch involves reversible metabolic depression different from the homeothermy to heterothermy switch. Although the mechanism of these changes are not currently known, there is speculation that heat shock proteins and vitamin D may play roles. Among many

areas of potential medical relevance is the use of such an approach in storage of organs for transplantation. Various animals make different uses of the biochemical changes associated with hibernation, with bats using torpor throughout the winter but also during the night in summer, with the hypometabolism perhaps related to their unusually long life spans. Some but not all mammals use brown adipose tissue for heat production via activation of uncoupling protein. The purpose of developing insulin resistance in hibernators has not been fully explained and may play a role in energy storage or in maintaining glucose stores for brain utilization, while other tissues become insulin resistant. The ability to turn on fat storage, leading to an obesity-like state, and then to reverse that, so that on similar diets there may be great differences in weight gain in different portions of the cycle, should also lead to insight into potential approaches to treatment of human disease.

Bart Staels (Lille, France) discussed central and peripheral clocks and the regulation of metabolism in humans. Circadian controls affect heart rate, blood pressure, the sleep-wake cycle, bile acid metabolism, cholesterol synthesis (greatest during the night, hence the recommendation to take short-acting statins in the evening), body temperature, gut activation, renal activity, and, of course, endocrine systems. The diurnal pattern of blood pressure, pulse, epinephrine, and norepinephrine is related to the increased risk of myocardial infarction and stroke in early morning (13). These circadian phenomena are regulated by factors including exercise, feeding behavior, and light, all acting to entrain central and peripheral biological “clocks” to the external environment. These systems suggest that there is an ability to develop metabolic flexibility, that circadian rhythmicity perturbations may cause metabolic perturbation, and that circadian dysregulation may be associated with metabolic disorders. Such conditions are clinically seen with shift working, travel-related change in cycle, and nighttime binge eating syndrome with excess calorie intake, as well as with tissue-specific circadian gene dysregulation. All are associated with the development of metabolic syndrome and obesity. Changes in sleep duration and quality are related to development of obesity, with particular association of chronic sleep loss with obesity, type 2 diabetes, and CVD (14). Sleep deficiency has a number of effects on metabolic and endo-

crine function, reducing glucose tolerance, and is associated with decreased thyroid-stimulating hormone, increased sympathetic nervous system activity, increased evening cortisol (15), increased ghrelin, decreased leptin, and increased BMI (16). IL-6 is increased after sleep restriction (17), suggesting a relationship to inflammation.

The negative and positive limbs of feedback loops of the circadian clock are interconnected, with the molecular clock “positive limb” genes *Bmal 1* and *Clock*, and “negative limb” *Per* and *Cry* genes expressed under the control of a number of central and systemic cues, as well as by the nuclear orphan receptor “regulator of virion” (Rev)-*erbα* (18,19) and by autonomous cellular rhythmicity. Isolated hepatocytes also display a circadian rhythm, suggesting that clock processes exist in the periphery, presumably with the central clock serving to synchronize peripheral clocks in various tissues. The transcription factors of *clock* also regulate metabolic transcription factors such as sterol regulatory element-binding protein-1 and PPAR, which exhibit circadian patterns. Mice not expressing *Clock* develop obesity and metabolic syndrome.

Rev-*erbα* is an atypical nuclear receptor, its ligand pocket occupied by amino acid side chains so that it may not have ligand regulation, appearing to function as a repressor of gene transcription. Its circadian variation is related to effects of the clock genes, and it exhibits negative autoregulation. Rev-*erbα* is controlled by PPAR α /retinoid X-receptor (RXR) complex, with which it competes for DNA binding. It appears to play a role in the circadian pacemaker, in lipid metabolism, in decreasing myocyte differentiation, and in adipocyte function, suggesting that it functions to integrate a number of metabolic pathways. In the liver, Rev-*erbα* regulates ApoC3 and LPL activity, with mice not expressing Rev-*erbα* having high triglyceride levels and decreased PPAR γ -stimulated adipocyte differentiation. Adipogenesis is controlled by a cascade centered on the effect of the PPAR γ /RXR complex central. Rosiglitazone induces Rev-*erbα* RNA in adipocytes, and Staels suggested that Rev-*erbα* acts in a negative feedback fashion to repress adipocyte gene transcription events. Rev-*erbα*-deficient mice have increased adiposity, although not developing obesity, with their adipocytes having decreased insulin-stimulated glucose uptake and with abnormal glucose toler-

ance. Rev-erb α also plays a role in the control of immunity, of thrombosis, and of inflammation, repressing in a circadian fashion the transcription of plasminogen activator inhibitor 1 (20). As well as PPAR/RXR, the liver X-receptor and farnesoid X receptor have roles in Rev-erb α expression, and there are species differences; thus, the rodent studies will need to be replicated in humans, but this appears to be of great importance as a controlling mechanism of a variety of metabolic pathways related to the IRS.

Eleanor Scott (Leeds, U.K.) presented a unifying hypothesis of the interrelationships between insulin resistance, type 2 diabetes, and CVD based on these considerations. Obesity is linked to insulin resistance and associated risk factors with subsequent development of diabetes and CVD. She recalled the common soil hypothesis of Michael Stern that CVD and diabetes represent similar processes stemming from insulin resistance (21). In 1962, Neel suggested the thrifty genotype hypothesis that survival during famine favored individuals with efficient fat storage, although in times of nutrient excess this leads to unfavorable consequence (22). States of famine are infrequent, however, with mortality related to diseases such as infection rather than malnutrition per se, so that it is not clear how such environmental stresses would perpetuate "thrifty genes." The role of the adipocyte should also be considered, as the fat-loaded adipocyte produces a wide variety of cytokines and related products, as well as reducing adiponectin production with fat excess; a coherent theory would explain the physiological benefit of these adipocyte responses to fat loading (23). Fat loading toward the end of summer prepares the hibernating organism for lack of nutrient availability during the fall and winter. Regulation of body weight involves the day/night light stimulus, which sends messages to the suprachiasmatic nucleus, synchronizing clock genes, influencing melatonin as well as other neural and hormonal signals, with effects on peripheral oscillators (also utilizing the clock gene). Clock synchronization keeps the body's physiological processes in time with itself and the environment, ensuring physiological function and behavior appropriate to the time of day, synchronized to the day/night cycle and relative lengths of day and night. If this is disrupted, mice gain weight, develop fatty liver, and have raised glucose, triglyceride, cholesterol, and leptin, fur-

ther suggesting the importance of this regulation in metabolism (24).

Pinelectomy or long-day light exposure causes the summer phenotype, which can be treated with melatonin replacement. In a study comparing a 16-h day with a 10-h day for 4-week periods, the long day is associated with increased melatonin and the short day with increased prolactin levels (25), with the responses attenuated by artificial light exposure (26). Shift work is now a way of life for >20% of the industrialized world; however, it is associated with obesity, increased triglycerides, low HDL and abdominal obesity (27), diabetes (28), and CVD (29,30). Postprandial metabolic responses in shift workers show disrupted circadian rhythmicity of the melatonin profile, with increased glucose, insulin, and triglyceride responses (31).

Scott pointed out that the amount of sleep has declined by 1.5 h over the past century, in association with increases in obesity, dyslipidemia, hypertension, and diabetes. In children, obesity is associated with decreased sleep (32). Sleep duration has been associated with the development of type 2 diabetes in numerous studies (33,34), and, experimentally, <1 week of sleep restriction is associated with decreased glucose tolerance and insulin receptor (35). In Scott's study of 537 individuals from 89 families, three common polymorphisms in the clock gene were associated with the metabolic syndrome. Thus, in the 21st century, with decreasing exposure to normal seasonal and daily cycles, excess light and food have promoted risk clustering causing abnormality of insulin sensitivity, with consequent CVD and diabetes development. Scott suggested that Neel's hypothesis is actually related to survival in winter and that loss of seasonal adipocyte triggers lead to adoption of the summer phenotype in preparation for a "winter that never comes." The absence of seasonal variation leads to chronic insulin resistance, diabetes, and CVD.

References

1. Himsworth H: Diabetes mellitus: a differentiation into insulin-sensitive and insulin-insensitive types. *Lancet* 1:127-130, 1936
2. Shen SW, Reaven GM, Farquhar JW: Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. *J Clin Invest* 49:2151-2160, 1970
3. Harwood HJ Jr, Petras SF, Shelly LD, Zac-

caro LM, Perry DA, Makowski MR, Hargrove DM, Martin KA, Tracey WR, Chapman JG, Magee WP, Dalvie DK, Soliman VF, Martin WH, Mularski CJ, Eisenbeis SA: Isozyme-nonselective N-substituted bipiperidylcarboxamide acetyl-CoA carboxylase inhibitors reduce tissue malonyl-CoA concentrations, inhibit fatty acid synthesis, and increase fatty acid oxidation in cultured cells and in experimental animals. *J Biol Chem* 278:37099-37111, 2003

4. Savage DB, Choi CS, Samuel VT, Liu ZX, Zhang D, Wang A, Zhang XM, Cline GW, Yu XX, Geisler JG, Bhanot S, Monia BP, Shulman GI: Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J Clin Invest* 116:817-824, 2006
5. Iglesias MA, Ye JM, Frangioudakis G, Saha AK, Tomas E, Ruderman NB, Cooney GJ, Kraegen EW: AICAR administration causes an apparent enhancement of muscle and liver insulin action in insulin-resistant high-fat-fed rats. *Diabetes* 51:2886-2894, 2002
6. Yu X, McCorkle S, Wang M, Lee Y, Li J, Saha AK, Unger RH, Ruderman NB: Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. *Diabetologia* 47:2012-2021, 2004
7. Pold R, Jensen LS, Jessen N, Buhl ES, Schmitz O, Flyvbjerg A, Fujii N, Good-year LJ, Gotfredsen CF, Brand CL, Lund S: Long-term AICAR administration and exercise prevents diabetes in ZDF rats. *Diabetes* 54:928-934, 2005
8. Dagher Z, Ruderman N, Tornheim K, Ido Y: The effect of AMP-activated protein kinase and its activator AICAR on the metabolism of human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 265:112-115, 1999
9. Dagher Z, Ruderman N, Tornheim K, Ido Y: Acute regulation of fatty acid oxidation and amp-activated protein kinase in human umbilical vein endothelial cells. *Circ Res* 88:1276-1282, 2001
10. Bujalska IJ, Kumar S, Stewart PM: Does central obesity reflect "Cushing's disease of the omentum?" *Lancet* 349:1210-1213, 1997
11. Risner ME, Saunders AM, Altman JF, Ormandy GC, Craft S, Foley IM, Zvartau-Hind ME, Hosford DA, Roses AD; Rosiglitazone in Alzheimer's Disease Study Group: Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J* 6:246-254, 2006
12. Grant PJ: Obesity, adipocytes and squirrels. *Diab Vasc Dis Res* 1:67, 2004
13. Giles T: Relevance of blood pressure variation in the circadian onset of cardiovascular events. *J Hypertens Suppl* 23:S35-

- S39, 2005
14. Taheri S, Lin L, Austin D, Young T, Mignot E: Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med* 1:e62, 2004
 15. Spiegel K, Leproult R, Van Cauter E: Impact of sleep debt on metabolic and endocrine function. *Lancet* 354:1435–1439, 1999
 16. Spiegel K, Tasali E, Penev P, Van Cauter E: Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Ann Intern Med* 141:846–850, 2004
 17. Vgontzas AN, Zoumakis E, Bixler EO, Lin HM, Follett H, Kales A, Chrousos GP: Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *J Clin Endocrinol Metab* 89: 2119–2126, 2004
 18. Alvarez JD, Sehgal A: REV-ving up the clock. *Dev Cell* 3:150–152, 2002
 19. Torra IP, Tsibulsky V, Delaunay F, Saladin R, Laudet V, Fruchart JC, Kosykh V, Staels B: Circadian and glucocorticoid regulation of Rev-erb α expression in liver. *Endocrinology* 141:3799–3806, 2000
 20. Wang J, Yin L, Lazar MA: The orphan nuclear receptor Rev-erb α regulates circadian expression of plasminogen activator inhibitor type 1. *J Biol Chem* 281: 33842–33848, 2006
 21. Stern MP: Diabetes and cardiovascular disease: the “common soil” hypothesis. *Diabetes* 44:369–374, 1995
 22. Neel JV: Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? *Am J Hum Genet* 14:353–362, 1962
 23. Scott EM, Grant PJ: Neel revisited: the adipocyte, seasonality and type 2 diabetes. *Diabetologia* 49:1462–1466, 2006
 24. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J: Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 308:1043–1045, 2005
 25. Wehr TA, Moul DE, Barbato G, Giesen HA, Seidel JA, Barker C, Bender C: Conservation of photoperiod-responsive mechanisms in humans. *Am J Physiol* 265:R846–R857, 1993
 26. Wehr TA, Giesen HA, Moul DE, Turner EH, Schwartz PJ: Suppression of men’s responses to seasonal changes in day length by modern artificial lighting. *Am J Physiol* 269:R173–R178, 1995
 27. Karlsson BH, Knutsson AK, Lindahl BO, Alfredsson LS: Metabolic disturbances in male workers with rotating three-shift work: results of the WOLF study. *Int Arch Occup Environ Health* 76:424–430, 2003
 28. Kroenke CH, Spiegelman D, Manson J, Schernhammer ES, Colditz GA, Kawachi: Work characteristics and incidence of type 2 diabetes in women. *Am J Epidemiol* 165:175–183, 2007
 29. Kawachi I, Colditz GA, Stampfer MJ, Willett WC, Manson JE, Speizer FE, Hennekens CH: Prospective study of shift work and risk of coronary heart disease in women. *Circulation* 92:3178–3182, 1995
 30. Knutsson A, Akerstedt T, Jonsson BG, Orth-Gomer K: Increased risk of ischaemic heart disease in shift workers. *Lancet* 2:89–92, 1986
 31. Lund J, Arendt J, Hampton SM, English J, Morgan LM: Postprandial hormone and metabolic responses amongst shift workers in Antarctica. *J Endocrinol* 171:557–564, 2001
 32. Chaput JP, Brunet M, Tremblay A: Relationship between short sleeping hours and childhood overweight/obesity: results from the ‘Quebec en Forme’ Project. *Int J Obes (Lond)* 30:1080–1085, 2006
 33. Yaggi HK, Araujo AB, McKinlay JB: Sleep duration as a risk factor for the development of type 2 diabetes. *Diabetes Care* 29: 657–661, 2006
 34. Kawakami N, Takatsuka N, Shimizu H: Sleep disturbance and onset of type 2 diabetes. *Diabetes Care* 27:282–283, 2004
 35. Spiegel K, Knutson K, Leproult R, Tasali E, Van Cauter E: Sleep loss: a novel risk factor for insulin resistance and type 2 diabetes. *J Appl Physiol* 99:2008–2019, 2005