Adiposity and Diabetes

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A t a symposium titled “Adipose Tissue as a Secretory Organ” at the American Diabetes Association (ADA), San Francisco, June 2002, Jeffrey Flier (Boston, MA) discussed the effects of visceral obesity. While reviewing the differing components of the metabolic syndrome, which to large extent are driven by insulin resistance, he asked, “Where does the insulin resistance come from?” The existence of a common underlying factor related to visceral obesity, which is more strongly associated with the syndrome than total obesity, might be important. A role for abnormal glucocorticoid metabolism in adipose tissue as the mechanism of the syndrome would explain many of its features. Glucocorticoids regulate adipose tissue function and distribution and in excess produce Cushing’s syndrome, which is characterized by visceral obesity, insulin resistance, and diabetes. The prevalent forms of obesity, however, are not associated with increased circulating glucocorticoid levels. Flier pointed out that the adipocyte is an active endocrine cell that produces hormones, in particular leptin, cytokines, complement factors, and important enzymes, such as angiotensinogen. Cortisol, produced in the adrenal gland, acts on enzymes, such as angiotensinogen. Cortisol, which is regulated by circulating glucocorticoids from activation by circulating glucocorticoids, while HSD-1 in liver, adipose tissue, and brain leads to increased glucocorticoid action. HSD-1 is expressed to a greater extent in visceral adipose tissue, which also express greater levels of cortisol receptors. HSD-1 in adipose tissue may in fact promote visceral obesity. The initial recognizers of this phenomenon characterized visceral obesity as “Cushing’s disease of the omentum” (1).

A transgenic model overexpressing rat HSD-1 in mouse visceral fat shows central obesity with particular increase in mesenteric fat, with cell size rather than cell number increasing. Circulating levels of corticosterone, the active glucocorticoid in rodents, are normal, but visceral fat corticosterone levels are increased. The mice have hyperphagia. Increased free fatty acid (FFA), triglyceride, and leptin levels are seen, with glucose intolerance, particularly when given high-fat diets, and extreme insulin resistance. telemetry blood pressure measurements show development of hypertension as well, particularly on high-salt and high-fat diets, with response to low-dose angiotensin receptor antagonist treatment. The increased blood pressure may be caused in part by the insulin-resistant state, but also of importance is the induction of adipose tissue angiotensinogen production, which is regulated by circulating glucocorticoids. Adipocyte angiotensinogen doubles, with increased circulating levels.

Aldosterone levels are increased, while renin appears to be decreased. Levels of adiponectin are decreased in these animals, while tumor necrosis factor (TNF)-α increases. Other models of increased angiotensinogen expression lead to hypertension, further increasing the plausibility of this model.

Flier concluded that there might be an “intracrine” rather than an endocrine cause of the insulin resistance syndrome, with increased HSD-1, thereby leading to local increases in cortisol producing adipogenesis and lipogenesis, induction of insulin resistance, and modification of a number of secreted adipocyte proteins contributing to the metabolic syndrome. A study on humans with fat biopsy suggests potential clinical relevance, with increased HSD-1 associated with obesity (2). More understanding of the regulation of HSD-1 is critical, and the development of specific HSD-1 inhibitors may lead to novel approaches to treatment. Interestingly, thiazolidinediones (TZDs) inhibit the activity of the enzyme. Flier described mice not expressing HSD-1 that are resistant to development of obesity and diabetes and also showed resistance to age-related cognitive decline, thus suggesting potential for clinical use. An interesting question asked by an audience member was whether individuals who have undergone adrenalectomy and are treated with noncortisol glucocorticoids not metabolized by HSD-1 might be less likely than those receiving cortisol to develop the metabolic syndrome.

In a study presented at the ADA meeting, Yu et al. [1700-P] found 7% lesser increases in food intake and body weight, a 28% lesser increase in epididymal fat pad weight, and 33 and 24% lower serum leptin and triglycerides levels, respectively, after injecting an antisense oligonucleotide to murine HSD-1 mRNA versus saline twice weekly in a model of high-fat diet–induced obesity, although glucose tolerance did not improve (abstract numbers refer to the abstracts of the 62nd ADA Scientific Sessions, Diabetes 51 [Suppl. 2], 2002). Alberts et al. [172-OR] reported studies with a selective inhibitor of HSD-1 in an animal model of type 2 diabetes, showing a decrease in blood glucose with a decrease in hepatic glucose
production, suggesting the potential of this as a therapeutic target. Ortsater et al. [1602-P] reported the presence of HSD-1 in β-cells, correlating with the degree of insulin secretion, suggesting additional role of the enzyme. Lindsay et al. [354-P] measured adipose HSD-1 in subcutaneous biopsies from 10 Pima and 10 Caucasian persons, showing correlation with BMI, percentage fat, and fasting insulin, suggesting a relationship with insulin sensitivity and obesity in humans.

Philipp Scherrer (Bronx, NY) discussed adiponectin, continuing the theme that adipocyes are more than just storage compartments for triglycerides. Too much fat causes insulin resistance. Indeed, the adipocyte expresses a number of factors, including leptin and resistin, as well as molecules involved in the innate immune response such as TNF-α and acute-phase reactants, the circulating levels of which are increased in subjects with diabetes. Too little fat, however, as seen in states of lipodystrophy or lipoatrophy, is associated with deficiency of insulin-sensitizing adipocyte mediators.

Adiponectin, an adipocyte-specific secretory protein, has come to increasing attention over the past 12–18 months. It is similar in structure to complement factor C1Q and to TNF-α. It binds calcium, leading to conformational change in the interaction of the molecule with its receptor. Rather than increasing in individuals with diabetes, there is an inverse correlation of adiponectin levels with adiposity and with insulin sensitivity. In a diabetic mouse model, TZD administration increases adiponectin levels, while metformin, though acting at the liver in a similar fashion, does not increase adiponectin. Recombinant adiponectin administered to mice decreases serum glucose levels, in normal and both type 1 and type 2 diabetes models, with dramatic sensitization of the hepatic response to insulin, and decreasing levels of hepatic markers of gluconeogenesis such as phosphoenolpyruvate carboxykinase and glucose 6 phosphatase. These effects can also be seen in vitro in hepatocyte cell culture systems, with little effect of adiponectin alone but a marked increase in response to insulin. Adenosine monophosphate (AMP) kinase and protein kinase B are activated by adiponectin, although the specific receptors have not yet been characterized.

Adiponectin is produced constitutively and then regulated by proteasomal degradation. It appears that large molecular weight forms of adiponectin are activated by cleavage of certain portions of the protein, suggesting further complexity to its regulation. Adiponectin is predominantly expressed in the abdominal perigonadal rather than subcutaneous fat deposits. In animal models of type 2 diabetes, these fat deposits, which are important sites of TZD action, are the first to downregulate adiponectin expression. Similarly, in humans, there is an inverse relationship between adiponectin and insulin sensitivity. With HIV lipodystrophy, there is redistribution of fat to the central abdominal region with dramatic decrease in adiponectin production. In the mouse, leptin levels peak around 2 A.M. and adiponectin levels peak ~12 h later, at the time of the nadir in insulin levels. Males have most adiponectin in a low molecular weight form, while in females half is in a higher molecular weight form. Male mice have higher adiponectin levels than females, and there is a peripubertal increase in levels. Levels decrease during pregnancy and remain low during lactation, suggesting roles of prolactin and of estrogen. In a transgenic mouse producing adiponectin at levels approximately three times those of controls, insulin sensitivity increases, with particular increase in hepatic response to insulin. These mice actually have increased body weight in association with increased fat mass, located in a dorsal fat pad composed primarily of white adipose tissue. In contrast, mice not expressing adiponectin show marked postprandial hypertriglyceridemia and insulin resistance.

Scherrer noted that resistin is another fat cell–specific candidate protein influencing insulin sensitivity. It is not a cytokine classically responsive to inflammatory stimuli. Three forms of resistent are seen in mice, with one of these not expressed in humans, again suggesting a complex regulatory system. A related protein, resistin-like molecule (relm) α, is expressed in periadipocyte stroma, with similar regulation, with a differently regulated colon-specific form, relm β.

Philippe Froguel (Lille, France) noted although we are uncertain as to the molecular determinants of the transition from normal to type 2 diabetes, adipocyte factors including TNF-α, plasminogen activator inhibitor (PAI)-1, resistin, and interleukin (IL)-6, which may mediate insulin resistance, and leptin and adiponectin, which are related in increasing insulin sensitivity, may play important roles. Polymorphisms in TNF-α may relate to variation in levels of insulin sensitivity in the population. Resistin and leptin as well as leptin receptor polymorphisms have not been shown to play important roles in genetic studies in humans. PAI-1, Froguel stated, may play a role in type 2 diabetes and in obesity. In a study in the French population, his group has screened for genetic polymorphisms associated with diabetes and obesity. A sequence in exon 2 of the PAI-1 gene is associated with dyslipidemia, perhaps independent of diabetes, suggesting an effect in modifying triglyceride levels.

Adiponectin protects against high dietary fat–induced diabetes and reverses the diabetes of lipatophropy and the insulin resistance of obesity in animal models, and adiponectin levels correlate with insulin sensitivy, being low in persons with diabetes and with obesity. Several genome scans have suggested linkage with diabetes, as well as with coronary disease. In his studies of French and Japanese populations, adiponectin levels increase with increasing age and are higher in early puberty with a decrease toward the end of puberty, potentially mediating the decrease in insulin sensitivity with progression of pubertal stage, and are perhaps caused by changes in sex hormones during puberty. Genotype analyses in a group of families with type 2 diabetes in France show a significant association of type 2 diabetes and of insulin resistance with the adiponectin promoter region. In obese subjects, adiponectin levels were decreased in individuals with the G/G genotype at position 276 of the adiponectin gene. A rare missense mutation in exon 3 is associated with decreased adiponectin levels and may be another related potential cause of diabetes. Studying a population with BMI >40 kg/m², Froguel found that those persons with the G/G genotype and low adiponectin have higher fasting insulin levels, further suggesting a role of this hormone in insulin resistance in humans. Polymorphisms of the adiponectin promoter may decrease its levels, or may specifically decrease levels of the active form of the hormone, leading to an increase in diabetes risk. Abnormalities in adiponectin may increase risk of athero-
sclerosis, with associations shown in his studies, particularly in obese subjects.

Adiponectin received a great deal of attention in studies presented at the ADA meeting. Ito et al. [1241-P] compared 125 subjects with magnetic resonance imaging measurement of subcutaneous fat, visceral fat, hepatic lipid content, and intramyocellular lipid content, showing negative correlation of serum adiponectin level with hepatic but not muscle lipid and positive correlation with insulin sensitivity, suggesting that adiponectin may affect insulin resistance in liver. Those individuals with the G/G genotype had 48% greater degree of insulin resistance.

The relationship of lifestyle change to adiponectin levels is not entirely certain. Takanami et al. [248-OR] followed 28 middle-aged persons participating in a 3-month exercise program, showing that their increase in adiponectin, averaging 11%, did not correlate with the changes in total and visceral fat, which decreased 13 and 12%, respectively. Ishii et al. [1006-P] compared 14 subjects with type 2 diabetes after 4–6 weeks of aerobic exercise with diet therapy versus 13 individuals on diet therapy alone. Insulin sensitivity improved in the former group, correlating with the change in adiponectin levels. Havel et al. [1867-P] placed 21 normal weight men and women on 800 and 600 kcal/day diets, respectively, for 7 days, showing decreases in serum insulin from 10 to 6 µU/ml in women and from 7 to 2 µU/ml in men. In addition, there were 6 and 5% decreases in body fat mass, but there was an 11% increase in adiponectin in women and a 20% decrease in men. These results suggest a modest effect of caloric restriction and gender differences in adiponectin regulation, with adiponectin apparently not playing a role in the increase in insulin sensitivity with weight loss in men.

Kriketos et al. [23-LB] showed adiponectin to be positively associated with insulin sensitivity; both were negatively associated with total and—to a greater extent—central adiposity in 39 healthy males and in 20 HIV-seropositive males, 10 of whom acquired lipodystrophy following protease inhibitor treatment and 10 of whom were without lipodystrophy and had not received protease inhibitors. Motoshima et al. [357-P] reported that insulin and rosiglitazone increased omental fat adiponectin secretion in vitro by 37 and 39%, respectively, with a 67% increase with combined administration, while neither agent affected subcutaneous fat adiponectin secretion. Yu et al. [367-P] studied nine diabetic and nine obese and eight lean nondiabetic subjects; the latter group had a higher baseline adiponectin level, and all groups showed doubling of plasma adiponectin following troglitazone 600 mg daily for 12 weeks. Adiponectin showed a positive correlation with glucose disposal and HDL cholesterol and a negative correlation with triglyceride and insulin levels. A potential mechanism of action of TZD may be by this potentiation of adiponectin secretion.

A number of studies presented at the ADA meeting assessed the physiologic and pathogenic roles of leptin. In a study of its mechanism of action, Minokoshi et al. [1375-P] showed peripheral as well as central mechanisms of leptin stimulating fatty acid oxidation in white adipose tissue through activation of AMP-activated protein kinase. Yang et al. [1702-P] demonstrated suppression of glucose-stimulated insulin in intravenous leptin in 3-month-old but not in 22-month-old rats, but showed that both groups had similar insulin suppression with intracerebroventricular leptin, suggesting a central mechanism of action, with the blood-brain barrier causing resistance to the leptin effect with aging. Muzumdar et al. [1601-P] showed intracerebroventricular leptin inhibited insulin secretion in a dose-dependent manner, and that a specific inhibitor of the melanocortin 3/4 receptors prevented the effect. Mizuno and Mobb’s [163-OR] studied leptin-deficient mice, which had decreased hypothalamic proopiomelanocortin (POMC) expression and a transgene expressing the POMC gene under control of the neuron-specific enolase promoter, thereby leading to a sixfold increase in hypothalamic POMC mRNA. Glucose tolerance normalized with improvement in obesity, hyperphagia, and hypothermia. When leptin-deficient mice (with decreased POMC expression) matched with the transgenic group for body weight or for food intake were studied, the improvement in glucose tolerance was not demonstrated, suggesting direct effects of POMC in improving glucose homeostasis.

Walker et al. [1786-P] assessed 137 and 63 newborns of women with and
without type 1 diabetes, respectively, showing higher cord insulin and leptin levels that were associated with birth weight only in the infants of mothers with diabetes, whose mean weight was 0.3 kg greater than the infants of non-diabetic mothers. Cord IGF-1 levels were associated with birth weight in both groups. The authors suggest that insulin and leptin may be additional mediators of growth in infants of mothers with type 1 diabetes. Soderberg et al. [119-OR] found that leptin was a risk marker for diabetes and impaired glucose tolerance in 9,693 men and women followed for 11 years after adjustment for age, ethnicity, and obesity, with greater association in men. The relationship between food intake and insulin therapy, a common concern for patients, was studied by Packianathan et al. [1687-P]. Twenty patients with type 2 diabetes and HbA₁c >8.5% starting insulin treatment increased weight by 5.2 kg, with increased hunger rating by the patients following a standard liquid meal and a 340-kcal/day increase in habitual food intake assessed by 4-day food diary. In a potential explanation of this phenomenon, Birkenland et al. [1674-P] found that, in 21 individuals with type 2 diabetes before initiation of insulin treatment, the increase in leptin during a hyperinsulinemic-euglycemic glucose clamp correlated with the weight gain after 3, 6, 9, and 12 months, with correlation increasing in strength over time.

The role of resistin appears less clear based on studies presented at the meeting. Yang et al. [366-P] showed no evidence of resistin gene expression in brain, heart, muscle, kidney, intestine, liver, and lung and what they termed “marginal” expression in fat, but they did find high expression in human monocytes and particularly high levels in human acute monocytic leukemia cells. They found that mouse resistin localized to chromosome 8, and the human resistin gene localized to chromosome 19p13.3, and the authors suggested that in humans “resistin may be more related to the function or differentiation of myeloid cells.” Smith et al. [1238-P] also reported that resistin expression was very low in adipose tissue and most abundant in bone marrow, and showed that macrophage resistin expression was decreased 80% by incubation with rosiglitazone, suggesting a role of PPAR-γ in the regulation of human resistin expression. In contrast, McTernan et al. [1229-P] found resistin production by both abdominal subcutaneous and omental adipose tissue, with secretion from both preadipocytes and adipocytes. Serum resistin levels were higher in persons with than in those without type 2 diabetes, and nondiabetic subjects showed correlation between adiposity and resistin levels. Degawa-Yamauchi et al. [1660-P] similarly reported serum resistin levels 2.6-fold higher in 44 obese than in 11 lean individuals, which correlated with the degree of insulin resistance. In further studies from McTernan et al. [356-P], insulin increased resistin protein secretion by abdominal adipocytes from nonobese, nondiabetic women, and coadministration of rosiglitazone decreased resistin production. Shojima et al. [1237-P], however, reported that pretreatment of mice with dexamethasone increased adipocyte resistin expression, as did incubation with high glucose concentrations, while insulin and troglitazone decreased resistin expression. Kawashima et al. [352-P] also showed that in vitro adipocyte resistin synthesis was decreased by incubation with insulin, an effect blocked by the protein synthesis inhibitor cycloheximide. The inhibition was not affected by administration of inhibitors of known intracellular pathways of insulin action, PD98059, an inhibitor of the extracellular signal-regulated kinase1/2 pathway, or SB203580, an inhibitor of p38 mitogen-activated protein (MAP) kinase, while LY294002, an inhibitor of phosphatidylinositol 3-kinase (PI3K), paradoxically potentiated the insulin effect on resistin. Addressing the mechanism of resistin action, both Moon et al. [1231-P] and Gravelle et al. [1256-P] showed evidence that resistin leads to a decrease in glucose transporter levels.

Perry Bickel (St Louis, MO) discussed syndecan ectodomains in the delivery of lipoprotein lipase (LPL) in adipose tissue and their relationship to the classic adipocyte function of energy storage and to control of systemic lipid metabolism. LPL is a glycoprotein enzyme that catalyzes the rate-limiting state in the hydrolysis of lipoprotein triglycerides and is required for clearance of chylomicrons and for conversion of VLDL to its remnants. LPL is secreted most abundantly by adipocytes and by muscle cells in adipocytes resulting in the liberation of FFA for ultimate conversion to triglyceride for storage and in muscle used for energy substrate. LPL is secreted as a dimer that binds and is stabilized by heparan sulfate, whose strong negative charges interact with basic residues in the dimer. Insulin enhances release of active LPL from adipocytes by a uncertain mechanism. High-carbohydrate meals, which increase insulin, increase LPL activity by a posttranslational mechanism. Similarly, in euglycemic clamp studies, insulin increases LPL.

Heparan sulfate proteoglycans (HSPs) are a major source of heparan sulfate in tissues, including perlecans, components of basement membranes, glypicans, cell surface proteins anchored to the outer membrane by a lipid anchor, and the transmembrane syndecan. The syndecan family of HSPs consists of four members, expressed in most adherent cells in a developmentally consistent manner. The linear polysaccharide side chains bind to a number of extracellular molecules, including extracellular matrix components such as fibronectin and soluble molecules such as LPL, participating in their uptake, degradation, and recycling, and acting as coreceptors for molecules such as the fibroblast growth factor. Pools of LPL on the adipocyte and on muscle cells are attached to heparan sulfate side chains of proteins such as the syndecans. These molecules can be shed from the adipocyte cell surface via proteolytic cleavage. LPL is synthesized by the adipocyte, but its site of action is the lumen of the capillary endothelium. LDL is inherently unstable in solution, and its ability to remain active in this process may depend on the activity of an extracellular chaperone.

Bickel reviewed a number of studies addressing the questions of which HSPs are shed from the cell surface and whether these serve as extracellular chaperones for LPL. White adipose tissue expresses syndecan 1 and 4. Shedding of the ectodomains of both of these syndecans requires insulin and can be blocked by the PI3K inhibitor wortmannin or by inhibitors of MAP kinase, which block postreceptor pathways of insulin action. Similar mechanisms are involved in secretion of other adipocyte proteins, such as TNF-α. A complex of the syndecans with LPL can be shown in adipocyte cell cultures. Syndecan 1 ectodomains stabilize LPL activity in a dose-dependent manner. The ability to secrete LPL is seen early in the differentiation of adipocytes and may involve a metalloproteinase, with proteolytic deliv-
ery appearing to be an important means of insulin-mediated regulation of LPL. Many questions about this process of LPL secretion are not yet answered. Fatty acids modulate expression of HSP in fibroblasts and other tissues, and it is not known whether this is relevant to the effect of insulin. LPL and heparan-containing proteoglycans have a role in apolipoprotein binding, and insulin may similarly affect this. Bickel noted that some of the actions of insulin he observed may be mediated via the IGF-1 receptor.

Inflammation and diabetes

Joshua Barzilay (Tucker, GA) reviewed the association of markers of inflammation with diabetes. Inflammation includes a large number of responses to noxious stimuli, such as fever, increased cortisol levels, leukocytosis, thrombocytosis, anemia, and muscle wasting. Inflammation is strongly related to insulin resistance, although the question of whether treatment directed at the inflammatory process could lead to benefits, such as decreasing the development of diabetes, has yet to be answered.

Acute-phase reactants, part of the innate immune system, either increase or decrease in levels and result in activation of monocytes as well as complement and other effector systems. The greatest increases are in C-reactive protein (CRP) and serum amyloid levels, with an increase also seen in fibrinogen and a decrease seen in serum albumin levels. These in turn respond to cytokines, particularly IL-6 and TNF-α, released by monocyte/macrophages. Studies on the association of inflammation and diabetes began to be performed as knowledge accumulated on the relationship between inflammatory markers and atherosclerosis and of the association between adipocytes and cytokine production.

Before the 1990s, haptoglobin and fibrinogen were studied because of the thought that diabetes was a hypercoagulable state. Subsequent studies showed that patients with diabetes had higher sialic acid levels than nondiabetic control subjects and also found a correlation of sialic acid with blood pressure levels (3) and with heart disease among persons with diabetes. Subjects with high triglyceride levels, obesity, cardiovascular disease (CVD), and hypertension—features of the insulin resistance syndrome—show modest increases in sialic acid and cortisol levels and a marked increase in CRP levels (4).

The Insulin Resistance Atherosclerosis Study (IRAS) studied 1,008 individuals, one-third of whom were with impaired glucose tolerance, to assess the relationship between inflammatory markers and insulin sensitivity. The study showed that CRP levels increased with increasing features of the insulin resistance syndrome and was particularly associated with insulin sensitivity, BMI, and systolic blood pressure (5). Family studies of subjects with type 2 diabetes showed that factor VII, fibrinogen, and von Willebrand factor levels were elevated in relatives who had higher insulin levels, lower HDL cholesterol levels, higher triglyceride levels, and higher PAI-1 levels. Barzilay pointed out that in this setting these should be considered inflammatory factors, rather than being related to coagulation pathways (6). Other studies have shown increased levels of TNF-α in insulin-resistant persons (7) and an association of hyperglycemic states with elevated ferritin levels (8).

A number of prospective studies have shown evidence of inflammation to be associated with development of type 2 diabetes. The Atherosclerosis Risk in Communities (ARIC) followed 12,330 individuals initially without diabetes for 7 years; of these subjects, 1,335 developed diabetes. Having a leukocyte count in the highest quartile was associated with almost a doubling of risk (9).

Studies in Pima Indians have also shown this association of diabetes with mild degrees of leukocytosis (10) as well as an association of diabetes with gamma globulin levels (11). The Women’s Health Study of 27,628 women followed for a mean of 4 years (188 developed diabetes) showed that those with CRP >75th percentile had a fourfold increase in adjusted risk of diabetes, that elevated IL-6 led to doubling of risk, and that those with elevated CRP and elevated IL-6 had a sixfold increase in risk of developing diabetes (12). In the Cardiovascular Health Study of 4,481 nondiabetic adults, 45 of whom developed diabetes during 3–4 years of follow-up, CRP in the highest quartile doubled the risk of diabetes (13). Increased risk of diabetes was noted in subjects with increased CRP in the West of Scotland Coronary Prevention Study (WOSCOPS) (14).

In a study reported at the ADA meeting, Mohanty et al. [441-P] treated 11 obese individuals with type 2 diabetes with rosiglitazone 4 mg daily, showing decreases in fasting glucose and insulin from 157 to 127 mg/dl and from 33 to 16 μU/ml, respectively, at 6 weeks. Nuclear factor (NF)κB binding activity in mononuclear cells decreased by two-thirds and monocyte reactive oxygen species generation decreased by one-third, suggesting both an anti-inflammatory and antioxidant effect. Diamant et al. [675-P] reported correlation of carotid stiffness with both CRP and IL-6 in 17 normotensive persons with type 2 diabetes and 17 control subjects, as well as correlation of carotid stiffness with visceral fat in the diabetic group. Avignon et al. [622-P] found elevation of fibrinogen, leukocytes, and CRP in 46 diabetic subjects having no cardiac symptoms, but with severe coronary stenosis on angiography, in comparison to 20 without and 19 with moderate coronary stenosis, which is suggestive of the utility of inflammatory markers in deciding which patients to screen for coronary disease.

Hepatic steatosis may be relevant to diabetes. Tiikkainen et al. [1425-P] studied 21 obese women with previous gestational diabetes; the subjects were grouped into those with high and low liver fat, and the two groups showed fasting insulin of 15 vs. 9 μU/l and triglycerides of 150 vs. 115 mg/dl, respectively. Liver fat, insulin, and triglyceride decreased in both groups with weight loss but remained significantly higher in the former group of women. Ito et al. [932-P] reported levels of serum alanine and aspartate aminotransferase above twice normal in 3.2 and 1.8% of individuals with diabetes, but in 1.4% of those without diabetes, in a group of 31,175 Japanese persons. McKolanis et al. [94-OR], using computed tomography, showed that 47 of 75 subjects with type 2 diabetes had fatty liver, which correlated with visceral adiposity to a somewhat greater extent than with total fat mass, as well as with insulin resistance, with FFAs, and to a lesser extent with triglyceride, fasting glucose, CRP, TNF-α, and IL-6 levels. Weight loss was associated with improvement in both liver steatosis and CRP.

Diabetic dyslipidemia and inflammation

Vivian Fonseca (New Orleans, LA) discussed evidence for the acute-phase pro-
tein CRP and homocysteine (Hcs) as CVD risk factors. Individuals with diabetes have increased mortality and have not experienced the decrease in CVD risk reported in the U.S. population without diabetes. Markers of inflammation may be relevant to this, with CRP showing additive risk with traditional factors, such as lipid levels, and having higher predictive value than fibrinogen, PAI-1 activity, IL-6, and ICAM-1. CRP is derived from the liver and stimulated by a number of adipocyte cytokines. CRP levels may increase 1,000-fold in response to stresses such as infections. Considerably lower levels, which require measurement with a high-sensitivity assay, predict future CVD events. Most healthy subjects have CRP levels <2 mg/dl; higher levels are associated with tripling of CVD risk, and CRP levels >3.9 mg/l are associated with the highest risk. CRP is of prognostic value in acute coronary syndrome, and among individuals with stable angina, events occur more frequently in those with higher CRP levels. CRP levels are also associated with increased risk of sudden death. High CRP is associated with increased risk among persons with normal cholesterol, and high CRP with increased cholesterol is associated with greatly increased risk. Those subjects whose HDL-to-total cholesterol ratio and CRP are both increased are at particularly high risk.

CRP is not associated with increased risk of malignancy, suggesting it to be an important and specific marker, rather than a nonspecific marker of any underlying disease. CRP also clusters with other risk factors, such as cigarette use; the greater the number of risk factors, the higher the CRP level. In the IRAS, the number of metabolic disorders was proportional to the CRP level.

“All of these people are obese,” Fonseca commented, so an important question is whether CRP is a marker of obesity. There is a close association between CRP and obesity, and a high–glycemic index diet is also associated with higher CRP levels. CRP, however, appears to predict diabetes risk, independent of degree of obesity, in the Cardiovascular Health Study, the IRAS, and the WOSCOPS. A variety of treatment approaches, including diet, vitamins, aspirin in high doses, and insulin, lower CRP. Metformin lowers CRP 10–15%, and TZDs do so by 30–40%. Statins reduce CRP levels, possibly underlyling their “pleotropic” effect. Individuals with LDL cholesterol below median and with CRP above median had considerable benefit from lovastatin in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/ TexCAPS), suggesting an important role of CRP measurement in determining whether to initiate statin treatment (15). Those with high cholesterol and CRP in this study did not show benefit from the statin, Fonseca remarked, so that the interpretation of the study is somewhat uncertain.

Turning to Hcs, Fonseca reviewed the metabolic sequence from methionine, present in the diet, via transmethylation to Hcs, a toxic substance which is remethylated to methionine in a vitamin B12– and folate-dependent reaction via 5,10-methylene tetrahydrofolate reductase, with genetic abnormalities in this enzyme associated with increased thrombosis and atherosclerosis and treatable by administration of folate. Classical homocystinuria, however, is caused by a genetic abnormality in the pyridoxine-dependent enzyme cystathionine β-synthase (CBS), which is operative in the postprandial state. Much of Hcs is protein bound, with a much smaller fraction—the reduced sulfhydryl form of Hcs—more strongly associated with CVD risk. Furthermore, postmethionine load Hcs is a much better marker of risk than is the fasting level. Hcs levels increase with nutritional deficiency of vitamin B12, folate, and pyridoxine and with hypothyroidism. Increased Hcs is seen in persons with renal insufficiency and is associated with albuminuria, even with normal glomerular filtration rate. Treatment with fibrates, particularly fenofibrate, also increases Hcs, and levels increase to a lesser extent with metformin, perhaps because of development of mild B12 deficiency.

Hcs is a CVD risk factor, particularly for subjects with established CVD, with a continuous relationship of levels to risk. In diabetes, postmethionine Hcs predicts increased CVD risk, and individuals with diabetes may be particularly at increased risk for a given increase in Hcs. In persons with the metabolic syndrome and glucose intolerance, Hcs levels are particularly high, and there are further increases with hypertension. In animal models of insulin resistance, CBS activity decreases and Hcs levels increase. High Hcs is associated with endothelial dysfunction, and folate treatment may improve this. In an important study, treatment with folic acid (1 mg), B12 (400 μg), and pyridoxine (10 mg) was shown to be of benefit following angioplasty (16), but there are as yet no large outcome studies. As Fonseca summarized, “The exact role of homocysteine is unclear.”

Mohamed Navab (Los Angeles, CA) discussed functional assays of HDL bioactivity. He reviewed the hypothesis that circulating LDL can penetrate the arterial wall, and that when the balance between pro-oxidant and anti-oxidant levels is disturbed, unsaturated fatty acids on LDL lipids can undergo oxidation. The modified LDL stimulates endothelial production of monocyte adhesion molecules, inducing monocyte chemotactic protein and factors allowing monocyte differentiation into macrophages, which convert mildly modified LDL into highly modified particles which can lead to macrophage lipid accumulation, thus producing foam cells. If this continues, the internal elastic lamina frays, and a subpopulation of smooth muscle cells migrate toward the subendothelial space, picking up lipid and becoming foam cells. Calcifying vascular cells appear, and the shoulder area of the plaque becomes prone to rupture, leading to thrombus formation and occlusion of the lumen. Thus, LDL seeded with oxidation products, either from reactions in the circulation or from subendothelial oxidized LDL returning to the circulation, initiates the atherosclerotic process.

HDL can prevent many of the steps leading to LDL modification. Trimmings of aorta from donor heart used for cardiac transplant have been used to construct arterial wall cell cultures and to assess the inflammatory effect of LDL and protective effect of HDL. Normal HDL or apolipoprotein (apo) A1 prevent the generation of mildly modified LDL in this system, while HDL from animals that have been maintained on atherogenic diets fail to prevent this process. Analysis of the Framingham data suggests that close to half of CVD events occur in subjects with HDL cholesterol considered normal. Using a cell-free assay for detection of dysfunctional HDL, sera from individuals with normal or high HDL and atherosclerosis do not show the normal protective effect of these particles. Discontinuation of statin treatment similarly appears to cause loss of the protective capacity of HDL. ApoA1 and ApoA1-mimetic peptides in animal models can be shown to...
Perspective on the News

decrease evidence of atherosclerosis and improve HDL function; oral administration of a modified peptide also appears to be effective. Their action seems to involve the binding of circulating oxidized lipids.

James Otvos (Raleigh, NC) discussed the roles of various lipoprotein subclasses in diabetes. He differentiated between lipid and lipoprotein subfractions, and pointed out that hypercholesterolemia alone may not explain the increased risk associated with diabetes and that delineation of abnormal lipoproteins may improve the understanding of this phenomenon. Elevated triglyceride, low HDL, and small LDL size (a measure of LDL quality) explain much of the risk not explained by the LDL cholesterol level alone. LDL cholesterol as measured commonly, either by the Friedewald equation or by direct assay, is actually not a very good estimate of LDL quantity, particularly in persons with the metabolic syndrome or with type 2 diabetes.

Lipoproteins have tremendous variability of core cholesterol content. For a given LDL size and LDL cholesterol level, individuals with a lower amount of cholesterol in each particle will have a greater number of particles. The Quebec cardiovascular study showed that apoB, a measure of LDL particle quantity, was of greater importance than was the measurement of LDL size per se, which can be considered a modulator of particle number rather than a direct risk mediator (17). Subjects with small LDL size may have 70% more LDL particles for a given LDL cholesterol level. Metabolically, when triglyceride levels are elevated, due to the presence of large VLDL particles, there is pairwise exchange of triglyceride and cholesterol ester via the action of cholesterol ester transfer protein (CETP). This causes the LDL particle to become relatively cholesterol deficient and triglyceride rich, with CETP also causing exchange between HDL and VLDL, which leads HDL particles also to become cholesterol deficient and triglyceride rich. Hepatic lipase then recognizes these triglyceride particles as their substrate and produces small dense LDL and HDL particles. These two steps drive the production of atherogenic LDL and HDL particles, and interindividual variation in CETP, hepatic lipase, or other processes may greatly modify the extent of the process. LDL particles with low cholesterol levels are found in ~30% of nondiabetic populations and considerably more frequently in individuals with diabetes. A relatively low LDL cholesterol does not, then, indicate that a person with diabetes has low LDL particle numbers. “It’s the number of particles at the artery wall that is driving the disease,” Otvos pointed out, “not the amount of cholesterol in the particles.”

Using a nuclear magnetic resonance spectroscopic process, it is possible to measure the number of particles of each subtype. This allows rapid measurement of the number of large, medium, and small VLDL, LDL, and HDL particles, from which one can calculate average particle size and total LDL particle concentration, a measure accurate in specimens having triglyceride levels <1,000 mg/dl. Analysis of frozen samples from a large number of previously conducted clinical trials may allow greater appreciation of the importance of these measures. In a study of a population of individuals with and without diabetes, triglyceride levels were the most important differentiating factors between insulin-sensitive and insulin-resistant subjects without diabetes, while LDL cholesterol was not elevated in any of the groups. Analysis of subclasses, however, showed that the increase in triglycerides was due to elevation in the large VLDL subclass, that small LDL particles were seen in insulin-resistant individuals and that large particles were seen in insulin-sensitive individuals, and that large HDL showed the greatest association with the degree of insulin sensitivity. Persons with low HDL and high triglyceride levels then have smaller LDL and hence greater LDL particle numbers. As an example, Otvos discussed a subject with type 2 diabetes whose LDL cholesterol was 112 mg/dl, around the 30th percentile of the population, while the subject’s LDL particle number was actually in the 95th percentile. Only by using such measurements, he suggested, can one properly assess lipid levels and initiate appropriate treatment. Individuals with LDL between 95 and 105 mg/dl may have a highly elevated LDL particle number, for which LDL-lowering treatment would be indicated.

Elizabeth Leary (Seattle, WA) discussed the role and measurement of remnant lipoproteins in CVD, pointing out that the controversy as to whether triglyceride is a risk factor may be due in part to the use of triglyceride levels as a surrogate measurement for a variety of triglyceride-rich lipoproteins. Lipoprotein remnants do not have a single unifying characteristic, but rather are derived from VLDL and from chylomicrons, and include intermediate-density lipoproteins (IDLs). In the catabolism of chylomicrons, which contain apoA1, A4, C, and B48, a large triglyceride core, and a small amount of cholesterol ester, apoA1, A4C, and triglyceride decrease and cholesterol ester levels increase. Catabolism of LDL, containing apoB100, leads to the addition of apoE, with loss of cholesterol and increase in levels of cholesterol ester, with subsequent hepatic uptake via the LDL receptor, which leads to the production of IDL and LDL. Remnant levels are elevated in persons with obesity, coronary artery disease, type 2 diabetes, insulin resistance, hypertension, dyslipidemia with and without increased triglyceride, renal insufficiency, and menopause. Because of their relatively rapid catabolism, the concentrations of remnant particles are fairly low. Measurement of LDL levels has been most frequently used, requiring ultracentrifugation. A new procedure is the measurement of remnant-like particle cholesterol (RLPC) based on apolipoprotein affinity measurement. Leary described a number of studies performed with this assay, showing elevated RLPC in patients with diabetes, with hypertension, with insulin resistance, and with atherosclerosis, and suggested that such measurements might be included in atherosclerotic risk assessment.

A number of studies at the meeting addressed aspects of lipid abnormality in subjects with diabetes. The MRC/BHF Heart Protection Study Collaborative Group [82-OR] presented an analysis of 5,963 individuals with diabetes whose physicians did not consider statin therapy indicated and who were treated with simvastatin 40 mg or placebo. There was a reduction in major vascular events of at least one-third in those treated with the statin, so that those persons who did not have CHD at entry avoided ~70 major vascular events per 1,000 treated for about 5 years. Yee et al. [982-P] identified 10,996 new users of oral hypoglycemic agents in Saskatchewan, of whom 1,221 eventually used insulin and 484 used a statin for at least 1 year. The mean duration of diabetes treatment before requiring insulin was 6.0 years for those treated with a statin versus 4.8 years for those not receiving such treatment, suggesting gly-
cemic benefit. Aguilar et al. [604-P] reported a survey of 2,209 subjects from 417 Mexican cities, of whom 195 had diabetes and 116 had impaired fasting glucose (IFG) (110–125 mg/dl). Of those with IFG, 70% had two or more CVD risk factors and 2.5% had a history of coronary disease, a frequency identical to that in the group with diabetes and exceeding that of 0.9% among the 1,898 control subjects. The NCEP-ATP III guidelines, however, recommended pharmacologic lipid treatment for 41% of those with diabetes but only for 16% of those with IFG, suggesting that more aggressive treatment recommendations might be appropriate.

McCormack et al. [79-OR] reported that, among men, NMR lipoprotein subclass profiles in the national DCCT/EDIC cohort showed that the TT genotype of the hepatic lipase gene is associated with higher HDL levels and more than a doubling of the large-to-small HDL ratio, suggesting this to be an important genetic determinant of CVD risk. Thomas et al. [334-OR] showed, in vitro, that human monocyte glycated protein–stimulated TNF-α release decreased 29% after incubation with pravastatin. Khan et al. [419-P] analyzed data from a number of double-blind studies of rosiglitazone, showing that 2.1% of 1,635 individuals treated with rosiglitazone in 328 control subjects receiving placebo plus a statin had an increase in serum alanine aminotransferase above 1.5 times normal, further supporting the lack of an effect of this glitazone on hepatic dysfunction. No report of frequency of creatinine phosphokinase elevation was included.

References
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