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Dyslipidemia, endothelial dysfunction, and glycosylation

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This is the 7th of 8 reports on the American Diabetes Association (ADA) Annual Meeting and Scientific Sessions held in San Diego, California, in June. It covers topics related to lipid-lowering treatment and to endothelial dysfunction and glycosylation in diabetes.

Lipids

Whether statins should be the primary agents used in the treatment of dyslipidemia in diabetes was debated at the Metropolitan Diabetes Society, New York, NY, on 14 September 1999. Hayes Dansky, New York, NY, argued for treatment with statins as the primary agents, emphasizing that approximately three-quarters of the incidents of mortality of diabetic patients are a result of cardiovascular disease (CVD) and that three-quarters of these incidents are a result of coronary heart disease (CHD). It is clear that lipid-lowering treatment is effective in patients with diabetes. Dansky pointed out that the Helsinki Heart Study (HHS) showed a 10.5 vs. 3.4% 5-year CHD incidence in diabetic patients treated with placebo vs. gemfibrozil, respectively; these prevalences exceed the 7.4 vs. 3.3% frequency found in individuals without diabetes (1). The benefit of lipid-lowering treatment was more dramatically shown in the Scandinavian Simvastatin Survival Study (4S), which consisted of patients with prior CVD and baseline LDL cholesterol levels averaging 180 mg/dl. After treatment with simvastatin, the prevalence of CVD events decreased by 55 vs. 32% in patients with vs. those without diabetes, respectively, and the prevalence of CHD events decreased by 43 vs. 29%, although patients with triglyceride levels >350 mg/dl or with poor diabetic control were excluded from the study (2). Similar results were reported for the larger group of patients in the 4S, in which diabetes was diagnosed on the basis of having fasting blood glucose levels >126 mg/dl or impaired fasting glucose based on levels >110 mg/dl (3). In the Cholesterol and Recurrent Events (CARE) Study (4) and the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Trial (5), baseline LDL cholesterol levels were ~130 mg/dl, and significant benefit of treatment remained in those with and without diabetes.

Donald Smith, New York, NY, suggested that the use of treatments other than statins may often be preferable. Smith stressed the importance of assessing triglyceride levels as the predominant indicator of lipid abnormality in diabetic patients (6) and suggested that even mild degrees of fasting hypertriglyceridemia are associated with marked increases in postprandial triglyceride levels (7). The presence of postprandial hypertriglyceridemia is also a characteristic of first-degree relatives of patients with diabetes (8), which, according to Smith, possibly explains why the development of atherosclerosis antedates the development of hyperglycemia in type 2 diabetic patients. The Quebec Heart Study showed convincingly that both high LDL cholesterol levels and hypertriglyceridemia are significant CVD risk factors (9). Furthermore, Stampfer et al. (10) reported that men in the Physicians’ Health Study showed an association between hypertriglyceridemia and risk of CVD. They also showed evidence suggesting that postprandial hypertriglyceridemia has direct effects on endothelial function (11). The association of small-dense LDL with myocardial infarction (MI) is lost when correcting for hypertriglyceridemia, which makes it difficult to determine which is the causative agent (12). Small-dense LDL may act as a risk factor by decreasing the particle size per unit of LDL cholesterol or by increasing LDL entry into the endothelium or the macrophage. Thus, Smith suggested that 3 lipid types commonly seen in diabetic patients may benefit from treatment with niacin or with fibric acid derivatives. These lipid types consist of 1) those in patients with mildly high triglyceride levels who have low HDL cholesterol levels and experience a mild increase in LDL cholesterol levels, 2) those in patients with markedly high triglyceride levels, and 3) those in patients who have more severe elevations in LDL cholesterol levels and develop hypertriglyceridemia while receiving statin treatment. Smith stated that the risk of combination fibrate-statin treatment has been overstated and that this approach can have significant benefits for the latter group of patients.

Dansky pointed out that the Heart and Estrogen/Progestin Replacement Study showed that estrogen levels in postmenopausal women with CVD indicated the need for proof of reasonable hypotheses, such as nonstatin therapy for diabetic dys-
administration of and triglyceride effects, which are seen with
Moreover, Smith noted that such high niacin
4.9 with an increase in the mean HDL cho-
218 mg/dl and a reduction in total choles-
drop in triglyceride levels from 397 to
increase from 141 to 164
had fasting glucose levels
5.54 (treated vs. untreated patients, respectively,
benefits of niacin and fibrates. In the
Smith responded by specifically addressing
alone may increase LDL cholesterol levels.
Dansky also mentioned that fibrates given
statins similar to those in subjects with
vention Study experienced benefits from
The statin trials," he stated,
with low HDL cholesterol levels in the Air
and diabetes were treated with niacin. Treatment
administration of fenofibrate. There
mean LDL particle size; all of these effects
cholesterol level; from 261 to 161 mg/dl, and increases
administration of gemfibrozil increases on
mean HDL cholesterol level from 397 to
35 mg/dl and a triglyc-
level from 261 to 161 mg/dl, and increases
mean LDL particle size; all of these effects
increase from 141 to 164
administration of ~2 g daily of immediate-
or intermediate-release niacin products.
Smith mentioned several fibrate studies.
Administration of gemfibrozil increases on
average the mean HDL cholesterol level from
50 mg/dl, decreases the triglyceride level from 397 to
218 mg/dl and a reduction in total choles-
tol-to-HDL cholesterol ratios from 8.6 to
4.9 with an increase in the mean HDL cho-
Moreover, Smith noted that such high niacin
doses may not be needed for maximal HDL
triglyceride effects, which are seen with
administration of ~2 g daily of immediate-
or intermediate-release niacin products.
Smith mentioned several fibrate studies.
Administration of gemfibrozil increases on
average the mean HDL cholesterol level from
45 to 50 mg/dl, decreases the triglyceride level from
261 to 161 mg/dl, and increases
the mean LDL particle size; all of these effects
contribute to a decrease in "atherosclerotic potential" (16). Similar benefit has been
reported with administration of fenofibrate.
In addition, fibrates appear to decrease lev-
els of small-dense LDL cholesterol (17).
Finally, the recently reported Veteran’s
Administration HDL Intervention Trial (VA-
the exciting findings of which will
later be discussed in detail) showed that
modest changes in HDL and triglyceride
levels—without changes in LDL cholesterol
levels—decreased CVD outcomes.

At a symposium on dyslipidemia in
diabetes at the 1999 ADA Annual Meeting,
Steven Hafler, San Antonio, TX, pointed
out that both diabetic men without history
of MI and nondiabetic men with history of
MI showed an annual CVD event rate of
3%, which is 6 times greater than the risk
for men with neither illness. Those men
with both diabetes and a history of MI have
a 6-7% annual risk of experiencing a CVD
event (18). This finding implies that the
goals for lipid treatment for patients with
diabetes should be at least as strict as those
for nondiabetic patients with CVD. Because
approximately half of patients with dia-
betes have LDL cholesterol levels >130
mg/dl (19), lipid treatment is an important
aspect of the management of diabetes.

In a work presented at the meeting,
Haffner et al. (abstract 300) compared 50
insulin-resistant individuals with 28 insulin-
sensitive individuals who developed dia-
betes during the 7-year follow-up of the
San Antonio Heart Study of 1,734 persons
(abstract numbers refer to the Abstracts of
the 59th Annual Meeting and Scientific
Sessions of the ADA, Diabetes 48 [Suppl. 1]:
A1–A550). Only the former group had
hypertriglyceridemia, low HDL cholesterol
levels, and hypertension, suggesting that
this subset of patients at risk of diabetes
also requires intensive lipid treatment.
Melissa Austin, Seattle, WA, discussed
triglycerides and small-dense LDLs. There
is epidemiological evidence suggesting that
hypertriglyceridemia increases CVD risk
independently of HDL cholesterol levels
and other risk factors (20). In a 20-year fol-
low-up of 101 families with familial hyper-
triglyceridemia in Seattle, WA, 435
first-degree relatives and 283 spouses were
identified, showing a 1.7-fold increase in
CVD risk. A decrease in LDL size is associ-
ated with hypertriglyceridemia and is
therefore another risk factor. These particles
may more readily permeate the arterial
wall, may be more susceptible to oxidation,
or may have altered LDL-receptor binding,
which leads to increased atherogenicity.
The adverse effect of small LDL size may be
particularly important among women.

Markku Laakso, Kuopio, Finland, dis-
cussed insulin resistance and atherosclero-
sis. Although the question of whether
endogenous hyperinsulinemia, insulin
resistance, or exogenous insulin treatment
are risk factors was raised by Robert Stout
in 1968 (21), Laakso pointed out that "we
do not have any final answer." Clamp stud-
ies show an association of CVD with higher
fasting insulin levels and lower insulin sen-
sitivity. In the Insulin Resistance Athero-
sclerosis Study of 1,397 patients whose
insulin sensitivity was determined,
Hispanic and non-Hispanic Caucasian sub-
jects showed an association between
insulin resistance and atherosclerosis, but
African-American subjects did not (22).
The insulin level is regulated by insulin
secretion, extraction, and the glucose level,
and it only predicts one-third to one-half of
the variance in insulin sensitivity. The Que-
bec Heart Study, the 22-year follow-up of the
Helsinki Policemen’s Study (23), and a
meta-analysis of 19 studies (24) showed that
the fasting insulin level is a significant
predictor of CVD. It may be inappropriate,
however, to ask whether insulin is an inde-
dependent risk factor, because it so strongly
clusters with metabolic abnormalities.
Laakso concluded by pointing out that the
Diabetes Mellitus–Insulin Glucose Infusion
in Acute Myocardial Infarction Study, the
University Group Diabetes Program, and the
U.K. Prospective Diabetes Study found
no adverse CVD effect of exogenous
insulin, which suggests that insulin resis-
tance, rather than insulin itself, is the risk
factor for CVD complications.

Sander Robins, Boston, MA, discussed
the VA-HIT, addressing the treatment of
low levels of HDL cholesterol and high lev-
els of triglycerides with gemfibrozil (25).
Analysis of the findings of the HHS sug-
gested that the lowering of triglyceride lev-
els was not independently associated with
benefit (26). The VA-HIT studied 2,531
men who had an average HDL cholesterol
level of 32 mg/dl, an average triglyceride
level of 161 mg/dl, and an average LDL cho-
lesterol level of 111 mg/dl; the mean age of
the study participants was 64 years, and the
mean BMI was 29 kg/m². Of the study sub-
cets, 25% had diabetes and 57% had
hypertension. Those subjects randomized
to gemfibrozil showed a 22% decrease in
prevalence of cardiac mortality and nonfatal
MI at a 5-year follow-up; these benefits
were similar to those of pravastatin treat-
mint in the CARE and LIPID studies.
Patients with and without diabetes showed
similar benefits; the degree of benefit was
greatest for those who achieved an HDL
cholesterol level >35 mg/dl and a triglyc-
eride level <160 mg/dl. The triglyceride
level was an independent risk factor for the
subgroup with the lowest HDL levels.
George Steiner, Toronto, ON, Canada, dis-
cussed the Diabetes Atherosclerosis Inter-
vention Study (DAIS) (abstract 5) and other
lipid studies in diabetes. An important
question concerns whether triglyceride-
lowering treatment is beneficial or acts
merely as a marker of the insulin-resistant
state. Analysis of the diabetic subgroups of
a number of studies showed benefits simi-
lar to those seen in nondiabetic patients. In
the 4S, there was a trend to greater benefit
with triglyceride levels >1.7 mmol/l (150 mg/dl) and HDL cholesterol levels <1.1 mmol/l (43 mg/dl). The Post-Coronary Artery Bypass Graft Trial showed the same benefit of intensive cholesterol-lowering in patients with and without diabetes (27), and the St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention Study showed a 21 vs. 9% frequency of MI or ischemic electrocardiogram changes in patients with diabetes who were treated with bezafibrate or placebo (28). The DAIS involved 418 patients who were treated with fenofibrate or placebo. Triglyceride levels decreased by 40 and 25% in the highest and middle tertiles, respectively (a 27% overall decrease with fenofibrate vs. a 2% increase with placebo), total cholesterol levels decreased by 17% in the highest tertile, and HDL cholesterol levels increased by 8 and 10% in the lowest and middle tertiles. Overall LDL cholesterol levels increased by 6 vs. 2%, overall HDL cholesterol levels increased by 7 vs. 1%, and overall levels of apolipoprotein(b) (apo[b]) increased by 11 vs. 1%, respectively in patients treated with fenofibrate vs. patients treated with placebo. Angiographic coronary artery diameter outcomes will be known in 2000. Additional studies involving the treatment with a variety of lipid-lowering agents of ~20,000 patients with diabetes are in progress.

In a study presented at the meeting, Hey-Hadavi et al. (abstract 351) reported that of 70 patients with type 2 diabetes who were referred to their center, 36 had LDL cholesterol levels >130 mg/dl, and only 22% of these patients were being treated. In addition, 24 patients had a history of coronary artery disease (CAD), and 83% of these patients had LDL cholesterol levels >100 mg/dl. After 6-12 months of aggressive treatment, it was possible to lower the mean LDL cholesterol level by >20 mg/dl. Therefore, Hey-Hadavi et al. concluded that more aggressive management of hyperlipidemia in type 2 diabetic patients is required for primary and secondary prevention of CAD. Nelson et al. (abstract 738) reported that among 724 patients undergoing coronary artery bypass grafting, 169 were known to have diabetes, and an additional 124 had previously undiagnosed diabetes; 81 and 74% of the 2 diabetic groups had dyslipidemia, for which more than half of the overall group had not received active treatment. Cook et al. (abstract 742) reported that 58% of 4,004 African-American vs. 54% of 327 Caucasian type 2 diabetic patients had LDL cholesterol levels >130 mg/dl, but that 24 vs. 44%, respectively, had HDL cholesterol levels <35 mg/dl, even though the African-American and the Caucasian groups had mean HbA1c values of 9.3 and 8.6%, respectively. The importance of reducing LDL cholesterol levels was stressed. Evans et al. (abstract 564) administered 100 mg ciprofibrate daily vs. placebo for 3 months to 20 patients with type 2 diabetes and showed that fasting and post–fatty meal triglyceride levels increased from 1.5 to 2.8 mmol/l with ciprofibrate vs. from 3.1 to 6.6 mmol/l with placebo. Administration of ciprofibrate also resulted in a lesser decrease in flow-mediated brachial artery dilation, which is a measure of endothelial function. Duncan et al. (abstract 751) described the relationship between lipid levels and the risk of developing diabetes, which had occurred in 1,288 of the 11,987 individuals in the Atherosclerosis Risk in Communities Study. The lowest vs. highest HDL cholesterol quartile had a significant 2.7-fold greater risk of diabetes after adjustment for age, sex, race, center, baseline fasting glucose level, physical activity, smoking habit, BMI, waist-to-hip ratio, and fasting insulin level. Lin et al. (abstract 1115) addressed treatment of dyslipidemia by reporting the results of administering niacin in an average dose of 2,580 mg a day to 28 diabetic patients. The agent proved to be effective for the treatment of diabetic dyslipidemia, as the mean triglyceride level decreased from 192 to 99 mg/dl, and the mean HDL cholesterol level increased from 41 to 57 mg/dl. The mean LDL cholesterol level showed little change, but there was an increase in LDL particle size and a decrease in the level of lipoprotein(a). The mean HbA1c value was stable. Lee et al. (abstract 1116) administered the nicotinic acid analog acipimox in doses of 250 mg 3 times a day for 3 months to 52 patients with type 2 diabetes. Compared with placebo, acipimox resulted in no change in HbA1c, fasting glucose levels, an ~75% increase in HDL cholesterol levels, a 46% decrease in triglyceride levels, and an 82% decrease in free fatty acid (FFA) levels. Montori et al. (abstract 3) reviewed 18 randomized controlled trials of dietary fish oil supplementation in doses of 1.7–10 mg for a mean of 12 weeks in 823 patients with type 2 diabetes. On average, the patients showed a 50 mg/dl decrease in triglyceride levels (those patients with higher baseline triglyceride levels experienced more pronounced declines) and an 8 mg/dl increase in LDL cholesterol levels. There were no significant changes in fasting glucose, HbA1c, or HDL cholesterol levels. Torres-Tamayo et al. (abstract 4) reported that, compared with those in nondiabetic control subjects, fasting and postmeal triglyceride levels were higher in diabetic women but not in diabetic men and that fasting HDL cholesterol levels were lower in both diabetic men and women. Moreover, there was no difference in retinyl palmitate clearance in either sex, which suggests that sex-specific effects of diabetes are related to defects in triglyceride lipolysis rather than to those in remnant clearance. Also, these findings may partly explain the greater impact of diabetes on CAD risk in women.

Carpenter et al. (abstract 1) compared 7 patients who had a successful kidney-pancreas transplant with systemic venous drainage to the ileal vein vs. 3 transplant patients who had portal venous drainage. All of the patients were treated with ~7.5 mg prednisone/day. Presumably, because of the loss of first-pass hepatic insulin clearance, patients with systemic drainage were hyperinsulinemic and insulin resistant. These patients also had a significantly higher VLDL triglyceride-to-apo(b) ratio, which suggests the presence of larger triglyceride-rich VLDLs. Lipid levels and VLDL-apo(b) production rates did not differ. Sibley et al. (abstract 2) studied 35 patients with type 1 diabetes who were followed-up after the Diabetes Control and Complications Trial (DCCT) and had measurements of intra-abdominal fat taken by use of a computed tomography (CT) scan and measurement of hepatic lipase activity and lipid levels. Abdominal fat was significantly correlated with triglyceride levels, apo(b) levels, LDL size, and hepatic lipase levels. There was a negative correlation between abdominal fat and HDL2 cholesterol levels, presumably because when the HDL2 cholesterol level is adjusted for the hepatic lipase level, it is no longer significantly related to intra-abdominal fat. These findings suggest that increased intra-abdominal fat is significantly related to hepatic lipase, which may be a mediator of dyslipidemia of central obesity in type 1 diabetes. Loeblein et al. (abstract 95) showed that subcutaneous adipose tissue is more sensitive than muscle to insulin-mediated inhibition of lipolysis. Taylor et al. (abstract 29) reported that expression of apolipoprotein CIII, a major constituent of chylomicrons and VLDLs and an important regulator of plasma triglycerides, is elevated...
in insulin-deficient animal models of type 1 diabetes and is decreased by insulin treatment. Hepatic nuclear factor (HNF)-3 is required for this insulin-mediated repression in hepatocytes. Stoffel et al. (abstract 297) demonstrated that diabetic and non-diabetic patients with mutations in HNF-4, which causes type 1 maturity-onset diabetes of the young, have decreased serum levels of apolipoprotein AlII.

Fatty Acids

David Kelley, Pittsburgh, PA, discussed the effects of fatty acids on skeletal muscle metabolism. He mentioned the long-standing evidence that fatty acids interfere with glucose metabolism over a period of several hours. In muscle, there is a marked decrease in glycogen formation with high FFA levels and a lesser decline in glucose oxidation. Muscle adiposity levels measured by CT scanning increase with obesity and insulin resistance, are somewhat greater in obese patients with diabetes, and show inverse proportionality to muscle glucose uptake. Kelley studied whether triglyceride accumulation in muscle was due to increased uptake or decreased oxidation by using regional indirect calorimetry and by measuring fractional FFA extraction; glucose uptake; and determination of muscle oxidative enzymes, fatty acid-binding proteins, and levels of uncoupling protein (UCP). In the fasting state, muscle uses fat. Insulin switches muscle to glucose oxidation. Patients with type 2 diabetes have decreased basal fat oxidation, which is, in part, the result of hyperglycemia-induced muscle glucose oxidation, an effect that is particularly pronounced with co-existing obesity. FFA levels are slightly increased in obesity, showing stable levels of uptake but decreased oxidation with decreased muscle carnitine palmitoyltransferase levels that together result in increased net storage. Muscle oxidative capacity increases with increasing insulin sensitivity and decreases with increasing muscle adiposity. Muscle malonyl CoA levels are associated with the degree of insulin resistance and may underlie the decrease in oxidative capacity. Kelley also found evidence of increased muscle membrane-bound fatty acid-binding proteins with obesity. Finally, levels of UCP2 increases in obesity, correlating with the increase in respiratory quotient (RQ) (a measure of glucose metabolism rather than fat metabolism). The higher basal RQ decreases the ability of muscle to increase this parameter in response to stimuli, leading to the concept of rigidity of tissue metabolism as a factor in both diabetes and obesity.

J. Dennis McGarry, Dallas, TX, discussed interrelationships between glucose and fatty acids. He recalled the observation of Malaisse et al. (29) that insulin secretion by β-cells from fed animals exceeds that of β-cells from fasted animals without relationship to islet insulin content. In addition, there is a much greater insulin response to glucose when fatty acid levels are increased, whereas niaclin, by lowering fatty acid levels, decreases insulin secretion, an effect that can be reversed by simultaneous administration of heparin and fat emulsions to raise FFA levels. He asked whether all fatty acids are of equal insulino-tropic potency. In a study of polyunsaturated fats (PUFAs) from soybean oil versus saturated fats (SFAs) from lard, the latter caused greater insulin secretion. In the perfused pancreas, longer and more saturated fatty acids are more potent insulin secretagogues, with octanoic, then linoleic, then oleic, then palmitic, and finally stearic acids having the greatest effect. Might “too much of a good thing not be good?” McGarry asked. Elevation of FFA levels for 6 h in a rat model reduced glucose-stimulated insulin release, whereas a 48-h elevation decreased basal insulin release. McGarry termed this effect “chronic lipotoxicity.” Thus, it appears that a fatty acid-derived moiety is needed for insulin release, and that FA in the fasted state “sees to its own clearance” with feeding by potentiating the response to glucose and a variety of other insulin secretagogues. The greater effect of SFA than PUFA may contribute to their effect in causing hyperinsulinemia and dyslipidemia, and chronic elevations may also cause β-cell dysfunction. When asked if glucotoxicity might have an additive effect with lipotoxicity, McGarry speculated about interactions between the hexosamine pathway and fatty acid metabolites.

Gary Lewis, Toronto, ON, Canada, discussed human studies of FFA regulation of pancreatic insulin secretion. He noted the dual effect of fatty acids in causing insulin resistance and affecting its secretion, and he cited epidemiological evidence that elevations in FFAs precede the development of glucose intolerance and type 2 diabetes. One needs to take into account the insulin resistance in assessing the insulin response to glucose with fatty acid administration. In a study of 16 lean men with and without a 48-h heparin plus fat infusion that doubled FFA levels, fasting glucose levels increased with evidence of insulin resistance to glucose disposal. Insulin levels increased, and insulin secretion rates were similar in the 2 groups; however, the group with the heparin plus fat infusion showed a decrease in insulin clearance. Acute elevations in FFA levels caused a 50% greater insulin secretion with increased insulin levels. Thus, one may conclude that the chronic increase in FFA levels “disables β-cell compensation.” Is diabetes associated with a greater susceptibility to inhibition of insulin secretion by FFAs? The answer here is less clear, as Lewis showed a decline in insulin secretion during heparin plus fat infusion in obese individuals but not in those with diabetes. Lewis explained this to be the result of the diabetic subjects already having maximal fatty acid–induced insulin resistance, whereas the obese subjects became more insulin resistant with increases in FFA levels. Peter Arner, Karolinska, Sweden, discussed depot-specific control of FFA turnover, noting that visceral adipose tissue (VAT) has a “direct connection to the liver from the portal vein.” In humans, the main hormones that control fatty acid metabolism are catecholamines and insulin. VAT shows an increased lipolytic response to catecholamines. There are 3 adipocyte catecholamine receptors: β1, which is antilipolytic and is increased in subcutaneous adipose tissue (SCAT); β2, which is decreased in SCAT and mediates lipolysis via hormone-sensitive lipase (HSL); and β3, which is increased in VAT. The antilipolytic effect of insulin is less potent in VAT than in SCAT. Insulin receptor affinity is greater in SCAT, with increased degrees of autophosphorylation of the insulin receptor, increased levels of insulin receptor substrate (IRS)-1, and increased phosphatidylinositol 3-kinase activity. IRS-2 and IRS-3 are unchanged. Thus, more fatty acids are mobilized from VAT than from SCAT, resulting in greater portal fatty acid delivery to the liver, decreased hepatic insulin degradation, and increased hepatic VLDL triglyceride production. An important question concerns the role of VAT in overall metabolic homeostasis. VAT shows increased turnover with net fat accumulation, presumably because of increased triglyceride synthetic capacity. In obese patients undergoing gastric-banding surgery, omental fatty acid turnover correlates with triglyceride levels. Men showed increased activities of VAT, increased levels of insulin, and greater catecholamine-induced
fatty acid release, but the metabolic activities of SCAT did not differ between sexes. Studies that examined nonobese patients with polycystic ovarian syndrome (PCOS) showed that the SCAT of these patients increases catecholamine-induced lipolysis and decreases β2 receptors and HSL activity; this pattern is similar to that seen in obesity. VAT from individuals with PCOS shows increased catecholamine-induced lipolysis with evidence of increased β3 and HSL activity. Thus, the insulin-resistant state is associated with greater catecholamine-induced lipid mobilization from VAT and is related to increased VAT mass, but it is also related to an increased catecholamine response, which may offer a target for future pharmacotherapy.

Mittelman et al. (abstract 218) showed greater suppression of hepatic glucose production with infusion of insulin into the omentum via the superior mesenteric artery than with direct hepatic infusion via the portal vein, suggesting that a major action of insulin in controlling hepatic glucose production is indirect via its effect on VAT fatty acid release. Balon et al. (abstract 411) administered lisofylline, which decreases circulating fatty acids and fatty acid oxidation products, to insulin-resistant diabetic rats. The results showed improved glucose tolerance with increased skeletal muscle insulin-stimulated glucose uptake and suggested that lisofylline may provide a therapeutic approach to improving insulin-mediated glucose disposal. Lamarche et al. (abstract 740) showed that among 114 men with CAD and without diabetes in the Quebec Cardiovascular Study, FFA levels were 11% higher than those in a control group.

Homocysteine

In a lecture on 16 December 1999 at the Mount Sinai Diabetes Conference, Killian Robinowitz, Cleveland, OH, discussed the relationship between homocysteine (Hcy) and CVD. Hcy exists as the free monomer, as a dimer, or as a mixed disulfide with albumin to comprise 70–80% of total plasma levels or as a mixed disulfide with cysteine to comprise ~20%. Hcy metabolized to methionine and both folate and vitamin B12 are cofactors for methylene tetrahydrofolate reductase (MTHFR), the enzyme that remethylates Hcy to methionine, whereas vitamin B6 is a cofactor for cystathionine-β-synthase, the enzyme that transulfurates Hcy to cystathionine. Serum folate shows a negative correlation with Hcy in all groups, and high levels of Hcy are associated with low levels of vitamins B6 and B12. Treatment with theophylline reduces B6 and increases Hcy levels; hypothyroidism is also associated with elevated levels. Normal total Hcy levels are 5–15 µmol/l, and levels >15 µmol/l are seen in 5% of normal populations, in 30% of patients with CVD, in 60% of those who underwent renal transplantation, and in 85% of patients with renal failure. Levels exceed 400 µmol/l in homocysteinuria, the inherited disorder of Hcy metabolism that occurs once in every 60,000–400,000 people and is associated with premature CVD. Hcy increases with renal insufficiency; conversely, the hyperfiltration seen in early diabetes is associated with low levels of Hcy (30). Hcy is a risk factor for CVD, regardless of cholesterol levels, smoking habit, and hypertension, and there is a dose-response effect between Hcy and risk. This effect may partly be due to low folate and vitamin B6 levels (31). Furthermore, in patients with CVD and in patients with diabetes, higher levels of Hcy are associated with worsening survival rates (32,33), with Hcy being a more potent risk factor in patients with than in those without diabetes. Potential CVD-causing mechanisms include direct endothelial cytotoxicity of Hcy, which decreases nitric oxide (NO)-dependent vasodilation and decreases tissue plasminogen activator binding to the endothelium. Folate treatment decreases Hcy by ~25% with 400 µg of folate appearing as an effective dose in normal populations (34). Sometimes higher doses (1–2 mg a day) may be needed to treat those patients who also have renal insufficiency. A number of ongoing trials will study the roles of folate and B6 treatment in CVD prevention.

At the ADA Annual Meeting, Hofmann et al. (abstract 132) reported that levels of receptors for advanced glycosylation end products (RAGEs) increased in the renal vasculature in streptozotocin (STZ)-induced diabetic mice that were fed a diet high in methionine and low in pyridoxine, vitamin B12, and folic acid; the diet increased Hcy levels 8-fold. Similarly, RAGE and RAGE-mRNA increased ~3- and 6-fold in cultured endothelial cell preparations exposed to increased L-Hcy, perhaps by mechanisms linked to increased oxidative stress. Hcy may have a causal relationship to both macrovascular and microvascular diseases. Vaccaro et al. (abstract 785) measured Hcy levels in 68 patients with type 1 diabetes without evidence of CVD and with normal serum cre-atinine levels, showing a 50–60% increase in patients with albuminuria and with retinopathy. Of patients with microalbuminuria and/or proliferative retinopathy, 55.5 and 16.6%, respectively, were homozgyous for the C677T mutation in the MTHFR gene, which causes moderate hyperhomocysteinemia. Murata et al. (abstract 573) reported on a MTHFR genotype associated with elevated Hcy in diabetic patients with serum folate levels <7.0 nmol/l. Those patients with this genotype had a lower ankle pressure index. Dicker-Brown et al. (abstract 580) reported that both hyperglycaemia and hyperinsulinemia increase activity of MTHFR and decrease activity of cystathionine-β-synthase; these effects potentially worsen macrovascular disease in type 2 diabetes. Meigs et al. (abstracts 715 and 716) showed that worsening glucose tolerance was not itself associated with higher Hcy levels in 2,220 individuals free of CVD in the Framingham Offspring Study, but that increased levels of Hcy were seen in individuals with the combination of hyperinsulinemia, obesity, and dyslipidemia and in those patients who suffered from hypertension. Their results suggested that insulin resistance plays a role in hyperhomocysteinemia. Kanda et al. (abstract 992) found that insulin resistance and impaired renal function were both associated with increased Hcy levels in patients with type 2 diabetes. Drzewoski et al. (abstract 1302) found mean Hcy levels in 28 poorly controlled vs. 18 well controlled patients with type 2 diabetes were 27 and 17 µmol/l, respectively. In 57 well controlled nonhypertensive patients with type 1 diabetes, Ghirlanda et al. (abstract 1621) found a correlation between Hcy levels and Hba1c, C-reactive protein, and FFA levels.

Endothelial Dysfunction

At a lecture at the Mount Sinai Diabetes Conference on 23 September 1999, Richard Cohen, Boston, MA, discussed vascular reactivity and NO. He reviewed the mechanism of action of NO, the effects of insulin on NO, its relationship with CVD, and the relationship of NO and of CVD to oxidative stress, which Cohen described as “a very poorly defined term which came out because of the effects of vitamins.” Blood vessels relax in response to acetylcholine (ACh) in a fashion dependent on endothelial cell integrity, requiring synthesis by these cells of NO from arginine via NO synthase, activated by increased intracellular...
calcium either because of shear stress or in a receptor-mediated fashion. Nitrate vasodilators act in a similar fashion, but do not require intact endothelial cells. NO activates formation of the second messenger cGMP from GTP.

Endothelial cell function is impaired with hypercholesterolemia, diabetes, hypertension, cigarette smoking, aging, estrogen deficiency, and elevations in homocysteine (Hcy). This vast array of associated conditions leads one to wonder whether endothelial cell dysfunction is a marker or a mediator. With endothelial cell dysfunction, however, in addition to impaired vasodilatation, one can demonstrate increased platelet aggregation, leucocyte adhesion, smooth muscle cell proliferation, and acceleration of the atherosclerotic process. Thus, for example, cholesterol-fed rabbits administered the NO synthase (NOS) inhibitor L-NAME showed an impairment of the responses to ACh and to neointimal proliferation. Cohen described potential NO deficiency-mediated mechanisms of the increase in atherosclerosis in animal models with diabetes, which included decreased production and increased degradation, as by superoxide (SO) anion, or resistance to NO action at cellular targets. In alloxaan-diabetic rabbits, the decrease in the vasodilatory response to ACh is reversed by administration of an NO donor. In vitro, diabetic rabbit aorta without endothelium shows decreased relaxation in response to NO, with a dose effect of increasing degrees of hyperglycemia. The administration of SO dismutase (SOD) or the prostaglandin synthesis inhibitor indomethacin to decrease SO anion levels potentiates this effect. NADPH oxidase, which is present in neutrophils and in diseased blood vessels, increases SO anion production. NADPH oxide is a complex that consists of 6 different subunits; the vascular form is constitutively active, whereas the neutrophil form is inactive under basal conditions. SO anion is produced in the adventitia and in the endothelium, but not in the media. A paracrine effect of angiotensin II (A2) is increased SO anion production. A2-induced hypertension is associated with medial hypertrophy and adventitial proliferation with increased nitrotyrosine staining, which likely contributes to intimal proliferation. Atherosclerotic vessels show increased inducible NOS levels in intimal lesions, in media, and in adventitia. Consequently, these sites become areas of production of NO and SO anion. Levels of SO due to SOD indeed decreases atherosclerosis.

On a molecular level, NO interacts with vascular K+ channels and thereby decreases levels of intracellular Ca2+, an effect that can be blocked by inhibitors of the sarcoplasmic reticulum ATPase. Similarly, the insulin action of inducing arterial relaxation by increasing ACh levels can be blocked by these inhibitors, suggesting NO to be a mediator. Direct nonendothelial arterial relaxation by NO is also potentiated by insulin, apparently by direct insulin effects on Ca2+ uptake by the sarcoplasmic reticulum. Hypercholesterolemia decreases this Ca2+ pump activity, perhaps because of an increased nitrotyrosine effect on this molecule. In a related study at the ADA Annual Meeting, Reusch et al. (abstract 126) showed that the Ca2+/cAMP response element-binding protein level of vascular smooth muscle is increased in the mature quiescent state and is decreased in animal models of insulin-deficient or insulin-resistant diabetes and during incubation in cell culture with high insulin or high glucose levels. Jack et al. (abstract 558) showed that the protein kinase C (PKC)-β inhibitor, LY333531, decreased half ACH-induced (endothelium-dependent) relaxation of isolated perfused mesenteric vascular beds of STZ-diabetic rats, suggesting that PKC-β is involved in diabetes-induced endothelial dysfunction, which could contribute to the etiology of diabetic microvascular complications.

In a study of patients with diabetes, Ballesteros et al. (abstract 587) showed an association between loss of flow-associated (endothelium-dependent) vasodilatation measured with high-resolution ultrasound and insulin resistance in first-degree relatives of subjects with type 2 diabetes. Although higher levels of FFAs during the glucose clamp were associated with disturbed NO-dependent vasodilatation, Ballesteros et al. concluded that high FFA levels were related to the degree of insulin resistance. Lim et al. (abstract 612) showed decreased levels of endothelium-dependent vasodilatation and increased levels of soluble intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs) in 14 and 45 type 2 diabetic patients with and without microalbuminuria in comparison with 21 age-matched control subjects. Kim et al. (abstract 1584) did not show elevated VCAM levels but reported that the adhesion molecule E-selectin, which is expressed on activated endothelium only, was increased in patients with type 2 diabetes. Yamakita et al. (abstract 1628) showed that exhaled NO, a marker of endothelial function originating from pulmonary vascular endothelium, was decreased in patients with type 2 diabetes at rest and after exercise. Yeo et al. (abstract 1634) reported a decrease in endothelium-dependent vasodilatation in postmenopausal women with type 2 diabetes, with partial correction by estrogen supplementation, and Steinberg et al. (abstract 562) showed reduced blood flow increments in response to intra-arterial methacholine, a marker of endothelial function, and decreased levels of endothelium-derived NO in premenopausal women with type 2 diabetes. Sakuma et al. (abstract 588) analyzed DNA fragmentation and flow cytometry, showing that 25 vs. 5 mmol/l of glucose suppressed apoptosis in vascular smooth muscle cells, which potentially increases abnormal cell proliferation.

Antioxidants appear to have a relationship with endothelial function in diabetes. Devaraj et al. (abstract 455) treated 69 patients with type 2 diabetes with α-tocopherol (1,200 U daily). Plasma levels of E-selectin, P-selectin, monocyte chemotactic protein-1, ICAM-1, and VCAM-1 decreased by 20–50%, and monocyte adhesion to endothelium and tumor necrosis factor-α release decreased by 55 and 17%, respectively. Diabetic patients with and without CVD and control subjects showed similar responses. Ferber et al. (abstract 570) treated 36 patients with α-tocopherol 800 U daily, which resulted in decreased LDL oxidation and increased platelet-leukocyte coaggregation with a 10% reduction in the prothrombin time International Normalized Ratio. These findings suggest activation of the extrinsic coagulation pathway. Hu et al. (abstract 1338) reported on a 14-year follow-up study of 5,103 U.S. female nurses with diabetes. Those patients who took vitamin E and C supplements experienced respective decreases of 55 and 47% in CVD risk. Choi et al. (abstract 1515) compared 46 type 2 diabetic patients treated with continuous subcutaneous insulin infusion randomized to α-tocopherol (200 U daily) with 52 patients who received placebo. The former group showed an increase in serum and erythrocyte lipid peroxide levels.

A number of studies addressed interrelationships between PKC and both micro- and macrovascular disease. King et
al. (abstract 81) studied the mechanism of the effect of hyperglycemia on retinal capillary endothelial cells and pericytes in increasing endothelin-1, a mediator of decreased blood flow in early diabetic retinopathy and neuropathy. Their results suggested that enhanced endothelin-1 expression induced by hyperglycemia in diabetes is a result of activation of multiple PKC isoforms. Aiello et al. (abstract 82) showed an ~80% improvement in circulation time and retinal blood flow in 29 patients with diabetes and no or minimal retinopathy with oral administration of LY333531. Williamson et al. (abstract 420), however, showed that LY333531 increased vascular albumin permeation in retina, sciatic nerve, kidney, brain, and heart in non-diabetic rats and in brain and heart in STZ-induced diabetic rats. Takahara et al. (abstract 125) showed that rat carotid artery adenoviral transfection of the PKC-β isoform after balloon injury enhanced vascular smooth muscle cell migration and growth, which caused accelerated intimal hyperplasia that improved after administration of LY333531. Yamauchi et al. (abstract 131) reported that LY333531 reduced PKC activation due to hyperglycemia but not that caused by H₃O₂, or by AGEs in cultured rat aortic smooth muscle cells, the latter effects appearing to be mediated by phospholipase Cγ.

Glycation and AGEs

In this year's Banting lecture at the ADA Annual Meeting, Anthony Cerami, Tarrytown, NY, discussed the relationship of glycosylation with the complications of diabetes. In the early 1970s, transfusion of labeled erythrocytes into mice with and without diabetes showed that HbA₁c increased over time in both groups, particularly in the group with diabetes. Subsequent studies showed this to be due to the reaction of glucose with the NH₂-terminal valine of hemoglobin (35). Cerami pointed out that HbA₁c actually corresponds to average glucose levels over a period of ~<30 days and not to the longer lifespan of the erythrocyte, because of the partial reversibility of hemoglobin glycosylation. Koenig et al. (36) showed that HbA₁c levels decrease with establishment of good glycemic control, "the test that made everyone honest." This led to the Diabetes Control and Complications Trial's demonstration that intensive treatment to significantly lower HbA₁c levels decreases microvascular complications. Another important use of measuring HbA₁c levels is in preventing congenital malformations among infants of women with diabetes, since HbA₁c levels during the first trimester are strongly associated with adverse outcomes. The teratogenicity of hyperglycemia is related to the nonenzymatic reaction of glucose with the amino group of nucleic acids and with that of proteins, which is most clearly demonstrated by studies transplanting embryos into normal or diabetic rats. Food chemists have observed the reactions of glucose with proteins over the past century, as yellow-brown pigments are known to develop during cooking, with storage, and even with freezing. These reactions can cause cross-linking of proteins. In the lens, this process of pigment accumulation leads to cataract formation. Similar AGEs are seen in collagen and in human coronary arteries, with an increase with age, with renal insufficiency, and with diabetes. Some AGEs function as protein cross-links. Other glucose-derived molecules can also react with proteins. Injection of AGE peptides can lead to a pathology similar to that seen in diabetes. Interestingly, and once again in relation to the subjects food chemistry origins, consumption of foods that have formed brown pigmentation is associated with rapid uptake of AGE peptides. This may contribute to the adverse effects of high dietary protein intake in patients with renal failure. The reactive products formed during the preparation of tobacco may be another source of exogenous glycated toxins. To further substantiate the AGE hypothesis, chemicals that inhibit formation of AGEs and those that break cross-links have been studied. Aminoguanidine prevents AGE formation and cross-link formation and decreases complications in experimental models, including those associated with injecting AGE peptides, as described previously. A new compound, ALT-711, which regenerates during the course of its action, can break cross-links and improves vascular distensibility and left ventricular chamber and aortic stiffness.

In a lecture on 16 December 1999 at the Mount Sinai Diabetes Conference, Richard Bucala, Manhasset, NY, pointed out that the AGEs comprise a large number of different molecules, not resembling glucose. AGEs contain multiple double bonds causing fluorescence which were used in early assays. The lens, for example, is composed of specialized proteins synthesized at birth, with subsequent modifications being visible as the color changes from clear to brown. AGEs are toxic to the lens and thereby cause cross-linking and eventually cataract. Incubation of glucose with alumin can cause similar color and cross-linking changes. Other biological properties of AGEs include recognition by and chemotaxis of macrophages, induction of endothelial leakage and damage, growth-factor release, and quenching of NO, which leads to vasoconstriction. The half-life of hemoglobin AGEs, a circulating marker of advanced glycation, is 45 days, which is longer than that of HbA₁c (35 days) (37). Aminoguanidine prevents hemoglobin-AGE formation while not affecting HbA₁c levels. AGEs are present in the acellular or fibrous area of the atherosclerotic plaque and may contribute to the accelerated vascular disease of diabetes. The AGEs are eliminated principally by renal excretion; therefore, renal insufficiency is an important determinant of AGE levels. In particular, the combination of diabetes and renal insufficiency increases AGE levels, which, perhaps, explains the increased clinical risk of vascular disease. AGEs modify the primary amino groups of LDL phospholipids and apo(b), with both diabetes and renal insufficiency increasing LDL-apo(b) and LDL- lipid AGE formation.

Bucala hypothesized that AGEs enhance lipid oxidation by initiating the dehydration of unsaturated lipids; specific oxidation products, such as 4-hydroxy-2-nonenal, are present with high glucose incubations. AGE-LDL is not recognized by the LDL receptor, and its levels can be decreased by coincubation of LDL with aminoguanidine and glucose. Aminoguanidine administration to diabetic patients decreases VLDL levels by 26%. The "French paradox" of moderately consuming alcohol to protect against cardiovascular disease may be explained in this setting by the ability of the alcohol metabolite acetaldehyde to stabilize the unreactive cyclic form of the Amadori product. In vitro, acetaldehyde inhibits AGE formation. In vivo, ethanol administration decreases hemoglobin-AGE formation, without affecting HbA₁c, while increasing levels of the acetaldehyde-modified Amadori product of hemoglobin, a potential marker of chronic alcohol use. This may explain the benefit of alcohol in decreasing atherosclerosis in patients with diabetes, as recently confirmed (38). Al-Abed et al. (abstract 543) showed that the alcohol metabolite acetaldehyde reacts with protein-bound Amadori products to pro-
duce a chemically stabilized complex that can neither rearrange further nor progress to AGE formation. Diabetic rats that were fed ethanol showed a 52% decrease in hemoglobin-AGE levels without change in HbA₁c levels.

Kowluru et al. (abstract 79) showed that aminoguanidine administration to diabetic and to galactose-fed rats results in improvement in retinal PKC, NO, and oxidative stress, without change in blood glucose or galactose levels, which suggests the work of multiple mechanisms for the agent to improve renal microvascular disease in diabetes. Using a Chinese hamster ovary model, Sano et al. (abstract 133) showed that galactin-3, a β-galactoside-binding lectin, may act as a receptor for AGES in addition to other AGE-receptors, such as RAGE and macrophage scavenger receptor (class A, types 1 and 2). Because galactin-3 is localized in human atherosclerotic lesions and is upregulated in foam cells particularly, it may mediate the effects of AGE in atherosclerosis. Lopes-Virella et al. (abstract 134) reported that AGE-modified LDL, but not minimally oxidized LDL and native LDL, stimulated the surface expression of the monococyte-attracting factors ICAM-1, VCAM-1, and E-selectin in a concentration-dependent manner. Pyrroline-dithio-carbamate, a specific inhibitor of nuclear factor-κB (NF-κB), is involved in the regulation of the expression of adhesion molecules and was shown to block AGE-LDL-stimulated expression of the factors by 67, 31, and 22%, respectively. Rosen et al. (abstract 136) showed that AGE albumin, but not high glucose, stimulated expression of VCAM-1 in human umbilical vein endothelial cell cultures, although both activated the transcription factor NF-κB. NF-κB and RAGE antisense oligonucleotides inhibited VCAM-1 expression. Oturai et al. (abstract 151) studied the effects of an inhibitor of the AGE formation 2,3 diaminophenazine (NNC39-0028) and of the AGE cross-link breaker N-phenoxythiazolium bromide (PTB) on collagen solubility, albuminuria, and vascular dysfunction in diabetic rats. Diabetes was associated with 30 vs. 58% collagen solubility, respectively, in diabetic and control rats, and treatment with NNC39-0028 and PTB prevented this decrease by 48 and 36%. No significant effect in both rat models on albuminuria or vascular dysfunction was observed with long-term treatment of either agent. Brownlee et al. (abstract 312) studied thenoyltrifluoroacetone, an inhibitor of the mitochondrial FADH₂-dependent electron transport complex II. Sorbitol accumulation was reduced by 67%, and PKC and intracellular AGE formation were completely inhibited, suggesting that hyperglycemia-induced increased production of reactive oxygen species is the proximal initiating mechanism of diabetic complications. Nagai et al. (abstract 135) used an immunoassay to show that N'-[carboxymethyl]lysine (CML), a methylglyoxal-derived AGE structure, is present in AGE bovine albumin formed by incubation with glucose, suggesting that methylglyoxal plays a role as an intermediate for AGE formation. CML protein adducts are present in many tissues with aging and are further increased with diabetes. Shibayama et al. (abstract 535) showed the existence of CML autoantibodies in rats and patients with diabetes. A plasma fraction that bound to AGE-bovine serum albumin (BSA) but not to unmodified BSA showed a positive reaction to CML-BSA and to human lens proteins, which are known to undergo CML modification in vivo. Patients with renal failure had higher autoantibody activity than normal subjects or diabetic patients without renal failure. Koschinsky et al. (abstract 1649) showed that soluble AGE peptides from instant coffee and a cola soda stimulated human umbilical venous endothelial cell VCAM expression to the same degree as purified serum-AGES from fasting diabetic patients with nephropathy. Their findings suggest that these substances can induce endothelial dysfunction. Noting that AGES formed in cooked foods are absorbed, He et al. (abstracts 623 and 624) showed that an AGES-restricted diet decreased the incidence of diabetes from >80 to <40% in NOD mice, despite histologic evidence of insulin. In addition, NOD mice with diabetes that were fed a low AGE diet showed prevention of the glomerular hypertrophy, the mesangial expansion, and the arteriolar thickening that are seen in animals fed a regular diet. Moreover, at 6 and 14 months, urine albumin-to-creatinine ratios were 40 and 25% lower, respectively. Turk et al. (abstract 586) showed a correlation between mean HbA₁c levels and aortal collagen-linked AGES in diabetic rats without treatment or with treatment with insulin or phlorizin. Dihydroxyaceton phosphate and glyceraldehyde-3-phosphate are metabolized to the AGE precursor methylglyoxal, with levels increased in diabetes. Beisswenger et al. (abstract 611) showed marked variation among 20 patients with type 2 diabetes and normal renal function in levels of methylglyoxal and its precursors, a potential mechanism of differences in risk of complications. Kern and Kowluru (abstract 663) showed that aminoguanidine inhibited the development of acellular capillaries and pericyte ghosts in retinas of diabetic but not galactose-fed rats, although both groups showed decreased oxidative stress. In addition, aminoguanidine did not lower levels of hemoglobin AGES, which suggests that the mechanism of its action may not simply be from its inhibition of the formation of AGES. Kakamura et al. (abstract 1659) showed that an inhibitor of advanced glycation, OPB-9195, decreased CML accumulation in glomerular lesions in a rat model of nephropathy and decreased levels of urinary albumin excretion, further suggesting a potential approach to treatment.

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