Summary and Highlights of XIV International Diabetes Federation Satellite Symposium on Macrovascular Complications of Diabetes

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At the end of the symposium "Macrovascular Complications of Diabetes" organized by the American Diabetes Association Council on Complications as a satellite to the 14th International Diabetes Federation meeting, a few comments seem appropriate. The success of the symposium can be attributed both to the organizing committee, cochaired by Drs. Maria F. Lopes-Virella and John W. Baynes, and to the many speakers who delivered exciting, informative, and thoughtful presentations. Below, we provide a brief overview of the new and exciting information presented at the meeting. We try to identify underlying common themes and promising areas of future investigation that may lead to prevention or reversal of diabetic complications. Considerable progress has been made in recent years in understanding the pathogenesis of atherosclerosis in general, and this information is now being applied to understanding atherosclerosis in diabetes.

The Complications Council sponsored a program with a similar theme 3 yr ago. One major conclusion reached was that information on the unique mechanisms of atherogenesis in the diabetic person was lacking. Progress, specifically applicable to the diabetic patient, has now been made, but much, however, remains to be learned. A better understanding of atherosclerosis in people with diabetes should provide considerable insight into atherogenesis in general.

The recent symposium was remarkably broad, integrating epidemiologic information with basic pathological mechanisms involving the major elements of atherosclerosis. These elements comprised circulating soluble and formed elements, the arterial wall, and regulatory hormones.

The symposium began with a comprehensive overview of diabetic atherosclerosis by Dr. Edwin Bierman. He noted first that coronary artery disease is two to four times as prevalent in males and females with diabetes as in those without.

The epidemiology of atherosclerotic cardiovascular disease in the diabetic person was addressed in greater detail by Drs. R. John Jarrett and Barbara V. Howard. They made five points. First, atherosclerosis is accelerated in both IDDM and NIDDM. Second, not only coronary but also femoral and carotid disease are accelerated. Third, traditional risk factors alone cannot explain the increased incidence of coronary risk in people with diabetes. Fourth, Dr. Howard reviewed existing epidemiological studies in Native Americans to demonstrate the value of comparative studies of different racial and ethnic groups. The forthcoming Strong Heart Study described by Dr. Howard should provide additional insight into the role of heredity, lipoproteins, obesity, and hyperglycemia in diabetic atherogenesis. Fifth, Dr. Jarrett emphasized that proteinuria is the strongest recognized predictor of coronary disease and of overall mortality in the diabetic person. He also pointed out that proteinuria is associated with many traditional risk factors including hypertension, hypercholesterolemia, hypertriglyceridemia, low levels of HDL, and hyperfibrinogenemia.

Drs. Jarrett and Bierman both directed our attention to the importance of fat distribution and noted that abdom-

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HDL, high-density lipoprotein; LDL, low-density lipoprotein; NIDDM, non-insulin-dependent diabetes; TG, triglyceride; IDDM, insulin-dependent diabetes; apoE, apolipoprotein E; CE, cholesteryl ester; FC, free cholesterol; HDL, high-density lipoprotein; AGE, advanced glycosylation end product; LP-IC, lipoprotein-immune complex; MM-LDL, minimally modified low-density lipoprotein; RBC, red blood cell; MDA-LDL, malonedylaldehyde-LDL; F_c, crystallizable fragment of immunoglobulin; LP, lipoprotein; MCP-1, monocyte chemotactic protein–1; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor-α; CML, carboxymethyllysine; NC-1, carboxy terminal end of type IV collagen; PDGF, platelet-derived growth factor.

inal adipocity, even more than total adipocity, is associated with adverse atherogenic metabolic attributes including insulin resistance, hyperinsulinemia, hypertension, hypertriglyceridemia, and low levels of HDL-C. The unique effects of diabetes, hyperinsulinemia and insulin resistance, and hyperglycemia on hepatic triglyceride metabolism, regulation of lipoprotein production and degradation, and vascular wall remodeling are still poorly understood and are areas for productive future investigation.

Dr. Bierman's introductory overview also provided a framework for later speakers by reviewing current concepts of atherogenesis. He noted the role of oxidized LDL in foam-cell formation, one of the earliest events in atherogenesis. He described other lipoproteins that promote foam-cell formation in vitro and are potentially atherogenic in the diabetic person. He emphasized that hypertriglyceridemia is the most consistent and marked lipoprotein abnormality in people with diabetes. He described findings from his group and others characterizing the abnormalities of triglyceride metabolism in people with diabetes. They include both fasting and postprandial triglyceride enrichment of lipoproteins. Although hypertriglyceridemia has not emerged as an independent risk factor in the nondiabetic person, it is a potent independent risk factor in the diabetic person. Whether hypertriglyceridemia plays a causative role, as suggested by cellular studies, or is simply a marker for other causative factors can only be decided by randomized trials that seek to prevent coronary events by decreasing triglycerides.

The triglyceride enrichment of lipoprotein that occurs with diabetes was repeatedly emphasized by Drs. Marja-Riita Taskinen, Peter Alaupovic, Alan Chait, and Fredrick L. Dunn. Another element of lipoprotein metabolism that received considerable attention during the meeting was the enhanced postprandial lipemia that occurs in people with diabetes and other hypertriglyceridemic conditions. Drs. Bierman, Chait, and Angelike Georgopoulos all suggested that postprandial triglyceride-rich lipoproteins in the diabetic person are more atherogenic than postprandial lipoproteins from nondiabetic people. Both groups noted that postprandial lipoproteins from patients with NIDDM produce greater cholesterol ester accumulation when incubated with macrophages than do postprandial lipoproteins from nondiabetic people. Clearly, descriptive investigation of diabetic atherogenesis is reaching maturity, and investigation of diabetic atherogenesis has moved beyond descriptive science to hypothesis testing. Interactions among observations from diverse disciplines have facilitated development of testable hypotheses, with many examples evident throughout the meeting.

Presentations by Drs. Taskinen and Alaupovic further detailed the qualitative abnormalities that occur in the lipoproteins of patients with diabetes. Dr. Taskinen emphasized four factors that influence the TG abnormalities of people with IDDM: glycemic control, route of insulin administration, presence of nephropathy, and apoE genotype. She did note, however, that in the Finnish population, apoE isoforms are distributed similarly in diabetic and nondiabetic people. Dr. Taskinen also noted several compositional changes in diabetic lipoproteins in people with IDDM: a decreased CE-TG ratio in LDL, an increased FC-lecithin ratio in HDL and relative decreases in lysolecithin, lecithin, and spingomyelin in HDL. In addition to the four factors that influence TG levels in people with IDDM, Dr. Taskinen added obesity and insulin resistance as important factors that raise TG levels in people with NIDDM.

Dr. Alaupovic presented exciting new observations about the distribution and association of apoproteins in the diabetic person. The concept of "lipoprotein families" developed by Dr. Alaupovic is now well established and very important in our understanding of lipoprotein metabolism and the role of lipoproteins in atherogenesis. The occurrence of increased amounts of apoE across the entire lipoprotein density spectrum noted by both Dr. Bierman and Dr. Alaupovic could significantly affect intravascular lipoprotein metabolism and arterial wall lipid accumulation. It deserves further investigation in light of current models of atherosclerosis. Dr. Alaupovic also emphasized that the C-III ratio (the ratio of apoC-III in the heparin-manganese supernatant [HDL] to apoC-III in the heparin-manganese, precipitate [non-HDL-LPs]) reflected triglyceride clearance and the inverse relationship between TG and HDL-C. He noted that in people with NIDDM, apoB-containing lipoproteins are enriched in TG, free cholesterol, apoC-III, and apoE, whereas HDL particles are enriched in TG but depleted of apoE and apoC-III.

Dr. Jean-Charles Fruchart took up the role of lowered HDL in diabetic atherogenesis. He first reviewed the general concept of reverse cholesterol transport, noting both supporting observations and remaining uncertainties. He described the distribution of apoA-I–only lipoproteins across the HDL density range in people with diabetes and reviewed his recent finding that apoA-I–only HDL promotes cell cholesterol loss. These observations should lead to important hypothesis testing in the diabetic person.

The occurrence of modified lipoproteins in patients with diabetes and the role of modified lipoproteins in atherogenesis also received deserved attention at the meeting. Dr. Chait provided an overview of lipoprotein modification in people with diabetes. He listed five possible intravascular modifications including: oxidation, glycation, abnormal remnants enriched in apoE, small, dense LDL, and lipoprotein-immune complexes. He also noted five extravascular modifications including oxidation, glycation, formation of AGEs, lipase-mediated core lipid modifications, and aggregation. He provided evidence for the increased presence of these modified lipoproteins in people with diabetes and their potential contribution to accelerated atherosclerosis. Significantly, he considered the role of natural antioxidants in preventing atherosclerosis in the diabetic person.

Dr. Guy M. Chisolm also expanded on the role of oxidized LDL in atherogenesis, citing the cytotoxicity of oxidized LDL and noting that cytotoxicity is cell cycle–dependent, inhibited by HDL, caused by sterol oxidation products, and involves free radical–mediated pro-

cesses. He reported, as have Drs. Daniel Steinberg (University of California-San Diego), Joseph L. Witztum, and colleagues, that inhibition of the lipoxygenases but not of the cycloxygenase pathway prevents oxidative modification of LDL.

Dr. Timothy J. Lyons reviewed his findings on the structure of glycoxylated lipoproteins, those that have been both glycosylated and oxidized. This combination of modifications may produce particularly atherogenic lipoproteins. Moreover, glycosylation may increase the susceptibility of lipoproteins to oxidation.

Drs. Lopes-Virella, Witztum, and Christoph Gisinger together provided detailed insight into the formation and potential atherogenic attributes of lipoprotein-immune complexes in people with diabetes. Two common themes emerged. First, antibodies to oxidized LDL and to glycated LDL occur in people with diabetes. Dr. Witztum reported that levels of anti–MDA-LDL (a form of oxidized LDL) have predictive value for carotid lesions detected ultrasonographically. He provided further data indicating the relevance of LP-IC to atherogenesis in vivo, noting, among other observations, that immunoglobulins have been extracted from aortic lesions of Watanabe heritable hyperlipidemic rabbits. LP-IC may accelerate atherosclerosis in people with diabetics. Dr. Lopes-Virella provided evidence that lipid-immune complexes are taken up by macrophage F_c receptors, leading to enhanced cell cholesterol accumulation. She further provided a detailed discussion of the consequences of LDL-IC to macrophage, endothelial, and smooth muscle cell metabolism and function. Drs. Gisinger and Lopes-Virella both reviewed and compared the actions of soluble and RBC-bound LP-immune complexes, noting that RBC binding inhibits LP-IC degradation and promotes the adverse effects of these complexes.

Several investigators emphasized the importance of lipoprotein modifications in altering lipoprotein interaction with other elements in atherogenesis, particularly macrophages, endothelial and smooth muscle cells, and fibrous elements on the arterial wall. Dr. Judith Berliner reviewed her findings of the effects of minimally modified LDL on monocyte-endothelial interaction in people with diabetics. Her previous studies showed that MM-LDL enhances monocyte binding to the extracellular matrix laid down by endothelial cells, whereas polymorphonuclear leukocyte binding is unaffected. The enhanced binding is due to monocyte elaboration of specific adhesion molecules and chemotractants (for example, MCP-1). She also noted that MM-LDL enhanced the production of cytokines including GM-CSF. Thus, MM-LDL can accelerate atherogenesis in the diabetic person by increasing arterial wall macrophage content and the release of cytokines that may further enhance elaboration of extracellular matrix. These and other observations noted below raise the possibility that natural or pharmacological antioxidants could retard accelerated development of atherosclerosis in the diabetic person. Interestingly, Dr. Berliner noted that MM-VLDL– and MM-TG–rich VLDL had adverse effects like those of MM-LDL.

Dr. Fredric B. Kraemer further elaborated on the potential for lipoprotein-macrophage interactions in atherogenesis. He noted that macrophages possess multiple distinct cell-membrane receptors including receptors for LDL, scavenger receptors, recognition sites for oxidized LDL, FC receptors, and specific receptors for glycated proteins. Binding to any of these receptors can lead to release of cytokines, growth factors, proteases, prostaglandins, and oxygen-linked free radicals. Each of these receptor interactions could contribute to development of atherosclerotic lesions. In addition, he noted that macrophages secrete proteins important to lipoprotein metabolism: lipoprotein lipase and apolipoprotein E.

The roles of platelets and the coagulation abnormalities associated with diabetes were considered by Drs. Peter D. Winocour and Hau C. Kwaan. Dr. Winocour noted that platelets from diabetic people, compared with those from nondiabetic control subjects, show increased aggregation in response to ADP, collagen, and thrombin; increased granule release; and increased thromboxane production. He further considered the roles of platelet membrane glycosylation and of changes in membrane fluidity in producing these abnormalities.

Dr. Kwaan presented a particularly valuable discussion of the many abnormalities in soluble coagulation factors and function that occur in people with diabetes. He pointed out particularly the nexal elevation of plasma fibrinogen, which is linked to platelet aggregation, blood coagulation, blood flow, and atherogenesis.

The phenomena of protein glycation and formation of advanced glycosylation end products and the physiological consequences of these phenomena on the arterial wall were addressed by several speakers. Drs. Vincent M. Monnier and Aristidis S. Charonis noted the effects of glycation on collagen and laminin structure. Dr. Monnier discussed pentosidine, a fluorescent AGE, and reported that diabetic people have increased amounts of pentosidine compared with age-matched nondiabetic people. Furthermore, diabetic patients with renal failure have more pentosidine than those without.

Dr. Baynes expanded on the formation of AGEs, noting that oxidation plays a role in the fixation of AGEs (glycoxylation), particularly in formation of both carboxymethyllysine and pentosidine. Like pentosidine, carboxymethyllysine is more abundant in patients with diabetes than in those without. In short-term studies (17 wk), Drs. Lyons and Baynes reported that improved glycemic control did not decrease CML content of skin collagen in 14 patients with IDDM, lending support to the concept that oxidation serves as a fixative for glycosylation. Dr. Charonis specifically addressed glycosylation of laminin and type II collagen, reporting that glycosylation decreased laminin cross-links and the lateral association of collagen in vitro. Furthermore, glycosylation of NC-1, a domain of type IV collagen, blocks its ability to inhibit aggregates.

Dr. Helen Vlassara elaborated on the role of AGE in activating macrophages. She provided a model for AGEs in normal tissue remodeling, emphasizing the role of the macrophage. Occupancy of unique macrophage AGE receptors stimulates production of various cytokines including IL-1β and TNF-α. These in turn, or perhaps concurrently, result in release of proteolytic enzymes and

growth factors. She noted that AGE induces TNF release, and, conversely, treating macrophages with TNF increases AGE binding. Thus, the accelerated atherosclerosis in people with diabetes may reflect a disturbed regulation of normal tissue remodeling by increased amounts of AGEs. AGEs when present in normal amounts may serve to regulate normal tissue remodeling but, when overly abundant (as in diabetic people), may result in accelerated atherogenesis. Glycosylation and later formation of AGEs occurs not only in the soluble circulating elements (for example, lipoprotein) but also in circulating formed elements (for example, macrophages and platelets) and in the arterial wall, producing both structural and functional changes.

Dr. Michael Brownlee reviewed and updated his exciting approaches to pharmacological prevention of AGE formation. The use of aminoguanidine appears extremely encouraging in animal models and has entered early human testing. He also described the difficulties in this transition.

Two major processes contributing to atherogenesis in the diabetic person were emphasized during the symposium: the role of AGEs in tissue remodeling and the role of macrophages, particularly as influenced by modified lipoproteins and AGEs in arterial wall biology. A consequence of these processes is enhanced vascular permeability, which probably contributes not only to diabetic microvascular complications but to macrovascular complications as well. The idea that common mechanisms cause both micro- and macrovascular complications of diabetes is appealing. Techniques exist for measuring modified lipoproteins in the circulation and in the arterial wall. Demonstration of altered lipoproteins and in vivo inhibition studies could provide evidence that the in vitro observations are relevant in vivo.

The functional consequences of lipoprotein modification received considerable attention. The role of minimally modified LDL in the production of vasoactive molecules represents a superb example of multidisciplinary interaction leading to progress in understanding atherogenesis in the diabetic person. The role of immune complexes likewise raises many new questions. The relation of modified lipoproteins and LP-IC to the regulatory molecules that may be altered in atherogenesis may be of particular importance. Both circulating hormones and local autocrine and paracrine hormones within the arterial wall were noted as promising areas for future investigation. Traditional hormones are no longer restricted to endocrine function but must be viewed in the context of their autocrine and paracrine activities as well. This development presents an additional challenge because it is extremely difficult, as pointed out by several presenters, to quantitate the levels of these regulators at the site of their function as well as to be able to intervene therapeutically at a sufficiently local level.

Dr. Elaine Raines focused on the role of platelet-derived growth factor in atherogenesis. She noted several actions by which PDGF may play a role in atherogenesis including directing cell migation, promoting matrix deposition, and promoting cell lipid accumulation and vasoconstriction. She noted, based on the rat carotid artery balloon injury model, that these activities may be more important than the mitogenic effects of PDGF on arterial smooth muscle cells.

Dr. Kenneth R. Feingold elaborated on the potential role of cytokines, particularly IL-1, IL-6, and TNF, in atherogenesis of diabetes. He noted that AGEs stimulate macrophage production of TNF and IL-6, which have been reported to be increased in the circulation of diabetic people. He then elaborated on the effects of TNF on lipid metabolism in subjects with diabetes, noting that TNF increases TG levels when administered to normal or streptozocin diabetic rats. He presented a thoughtful, thorough discussion of the mechanism by which TNF and IL-1 increase TG levels. This important area should prove fruitful in future investigation.

The concluding session of the symposium was devoted to the effects of treatment on the development of macrovascular disease. Dr. Dunn summarized the consequences of improved glycemic control on lipoprotein abnormalities. Whereas Dr. Abhimanyu Garg specifically addressed lipid-lowering therapy in the diabetic patient, both Dr. Dunn and Dr. Taskinen noted differences in the effects of i.p. versus peripherally administered insulin on lipid levels in people with IDDM. Dr. Dunn also reviewed data from Dr. Bagdade's lab dealing with HDL (Dr. Bagdade was unable to attend).

The symposium brought together a great deal of fundamental information and indicated that progress is being made in understanding atherogenesis in the diabetic person. Dr. Bierman concluded his introduction by citing Dr. Elliott Joslin, who said, "I believe the chief cause of premature development of arteriosclerosis in diabetes, save for advancing age, is an excess of fat, excess of fat in the body, and an excess of fat in the blood. With an excess of fat diabetes begins, and from an excess of fat diabetics die, formerly of coma, recently of arteriosclerosis." The proceedings of this symposium will provide the reader with confirmation of Dr. Joslin's wisdom as well as with new insights into the mechanisms of atherogenesis in the diabetic person.