Glycation, Oxidation, and Lipoxidation in the Development of Diabetic Complications

A. Laurence Kennedy and Timothy J. Lyons

THE RISK OF MORTALITY from ischemic heart disease is about two to four times higher in people with non–insulin-dependent diabetes mellitus (NIDDM) than in the general population, so ischemic heart disease accounts for approximately 40% of deaths in NIDDM.[1] There are similar increases in the risk of stroke and lower-limb arterial disease in NIDDM,[2,3] and it is reasonable to suggest that some common factor(s) may be at play in increasing macrovascular disease at each of these sites. Hyperglycemia is the defining abnormality in diabetes mellitus, and it is natural to speculate that increased levels of glucose are in some way responsible for the excess morbidity and mortality that diabetic patients suffer. Glycation, the nonenzymatic adduction of glucose to proteins, represents one possible mechanism by which excessive levels of glucose in plasma, in interstitial fluid, and within cells could lead to pathophysiological change.[4] However, all diabetologists know patients who, despite prolonged periods of poor glycemic control, have escaped the worst of vascular complications, and others who quickly develop complications even when glycemic control is good. Therefore, one must invoke additional modifying factors if the "glycation hypothesis" is to be credible. One such additional modifying factor is 'oxidative stress', defined by Baynes[5] as "the steady state level of reactive oxygen or oxygen radicals in a biologic system." Oxidation reactions are closely associated with glycation, and together they cause permanent and irreversible modification of proteins ("glycoxidation"). Oxidation reactions may also damage both glucose and lipids directly, generating reactive carbonyl-containing compounds (a carbonyl group consists of a carbon and an oxygen atom linked by a double bond). These reactive fragments can then cause irreversible modification of proteins. The term glycoxidation is used to cover this type of protein modification if the reactive fragments are derived from carbohydrate, but if they are derived from lipids, the term "lipoxidation" is used.

Since modification of proteins by all of these processes involves reactions with carbonyl-containing compounds, a "carbonyl stress hypothesis" for the pathogenesis of diabetic complications has been proposed.[6,7] This states that the extent of damage to macromolecules is a function of cumulative exposure to reactive carbonyl compounds and determines the risk for development of diabetic complications. This hypothesis represents a modification of the glycation hypothesis, taking into account the modulating effects of oxidative stress, including oxidation of lipids. In this brief review, we will outline the basic processes involved in glycation and the closely linked processes of free radical oxidation, glycoxidation, and lipoxidation, with particular reference to the development of macrovascular disease.

From the Diabetes Care Center, Division of Endocrinology, University of North Carolina, Chapel Hill, NC; and Division of Endocrinology, Diabetes and Medical Genetics, Medical University of South Carolina, Charleston, SC.

Address reprint requests to Timothy J. Lyons, MD, FRCP, Division of Endocrinology, Diabetes and Medical Genetics, Medical University of South Carolina, 171 Ashley Ave, Charleston, SC 29425.

0026-0495/97/4601-1004$03.00/0

GLYCATION, GLYCOXIDATION, LIPOXIDATION, AND CARBONYL STRESS

Figure 1 illustrates the reactions involved in early and advanced glycation. Covalent bonds formed between aldehyde groups of glucose molecules and reactive-amino groups of proteins, usually located on lysine side-chains and *N*-terminal amino acid residues, constitute the early part of the process. The aldimine-linked Schiff base is unstable and its formation is readily reversible, whereas the fructoselysine (FL) compound yielded by an Amadori rearrangement of the Schiff base is relatively stable. Even so, FL can partake in further reactions yielding advanced glycation end products (AGEs). Stable end products that have been unequivocally identified include N^{ϵ}-carboxymethyllysine[8] ([CML] formed in several ways described later) and a fluorescent cross-link, pentosidine.[9,10] Some others have been identified in model systems and by immunologic techniques in vivo, eg, pyrraline[11] and "crosslines."[12-14] Pyrraline has recently been identified in skin collagen in vivo by chromatographic methods[15]: it is a glucose-lysine adduct that may form cross-links by interaction with other amino acids.[16]

AGE formation from FL involves either oxidative modification of FL or nonoxidative dissociation of FL to form new reactive intermediates that again modify proteins. For this reason, in long-lived proteins such as insoluble collagen or lens crystallins, FL does not accumulate over time beyond a few weeks, and there is a good correlation between FL levels in such tissues and glycated hemoglobin.[17] FL levels in collagen respond over several weeks of improved glycemic control (AGE levels do not),[18] and it is logical to expect that the effects of increased levels of FL, as opposed to AGEs, are most likely to predominate in short-lived circulating proteins in which there is insufficient time for high levels of AGEs to accumulate.

As mentioned, AGEs may be formed from FL not only by direct oxidative damage to FL, but also nonoxidatively by interactions between proteins and dissociation products of FL.[19,20] Instead of regenerating glucose, FL dissociation yields other more reactive carbonyl-containing intermediaries such as 3-deoxyglucosone,[19] a potent modifier of lysine residues. An additional pathway for AGE formation is through direct oxidative damage (autoxidation) to glucose and other sugars, bypassing FL altogether.[21,22] Reactive fragments of the sugar, such as glyoxal, are formed, which then bind to proteins. As already detailed, the term glycoxidation is again used to describe this reaction sequence, even though in this case oxidation precedes "glycation." Thus, CML may be formed either from oxidative

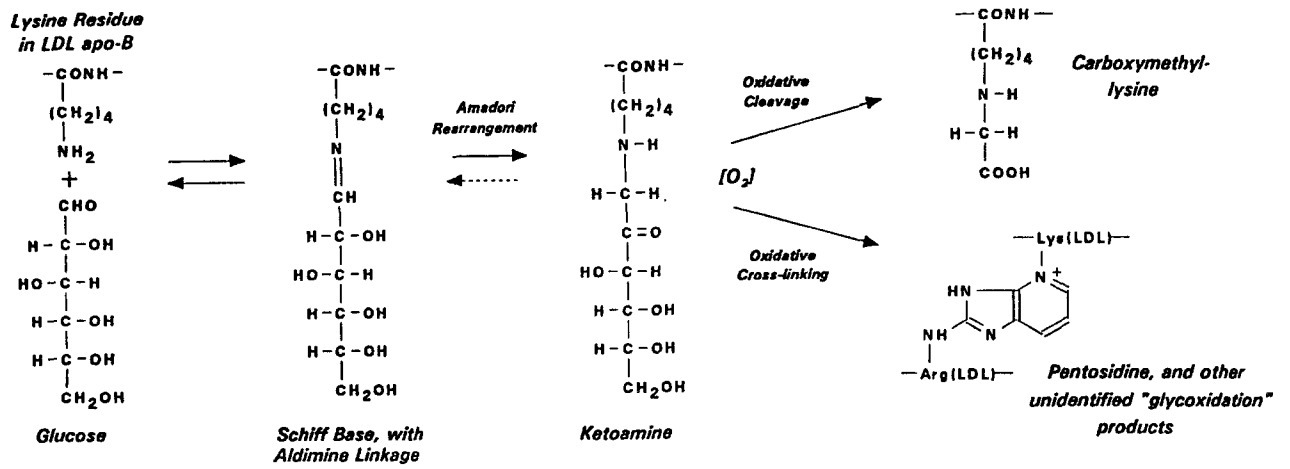

Fig 1. Early and advanced stages of glycation involving a lysine residue in LDL. The initial reaction results in formation of a Schiff base. An Amadori rearrangement leads to formation of the first stable product, FL, a compound containing a ketoamine link. Subsequently, oxidative reactions result in formation of the AGEs CML and pentosidine.

decomposition of FL or from reactions of glyoxal, the main dicarbonyl-containing autoxidation product of glucose, with protein.[22]

Yet another source of reactive carbonyl-containing species is (per)oxidation of lipoproteins. Compounds generated in this way include glyoxal,[23] malondialdehyde, and 4-hydroxynonenal,[24] and lipoxidation adducts formed by these compounds on lysine residues in low-density lipoprotein (LDL) have recently been demonstrated.[24] Since glyoxal can be derived from oxidation of either glucose or lipids, CML may be either a glycoxidation or lipoxidation product.[23]

To encompass all of these related processes, the term "carbonyl stress" has been introduced.[6,7] This describes potentially pathogenic reactions of reactive carbonyl-containing species with macromolecules. The reactive carbonyl groups may be (1) located on glucose itself, (2) generated oxidatively by free radical damage to carbohydrates or lipids, or (3) generated nonoxidatively during FL dissociation. Carbonyl stress may affect both short- and long-lived proteins as reviewed herein. Many issues remain to be clarified, including (1) the identity and relative importance of the final reaction products, (2) the relative contributions of the various glycoxidation and lipoxidation pathways to the formation of these products, (3) the question of whether oxidative stress is increased in diabetes per se (glycation stress clearly is) or only as a function of vascular disease, and (4) the role of the various products in the pathogenesis of diabetic complications.

LIPOPROTEINS

In NIDDM, plasma lipoprotein levels may be normal, although modest elevations of LDL cholesterol and triglycerides accompanied by reduced high-density lipoprotein (HDL) cholesterol are not uncommon.[25] Qualitative changes in lipoproteins, including modification by glycation and oxidation, may be as important as quantitative abnormalities in determining atherogenicity.

LDL

Studies of glycoxidation and lipoxidation of LDL are facilitated by the fact that each particle has only one apoprotein, apoB100. LDL isolated from diabetic patients exhibits a twofold increase in apoB glycation compared with levels in nondiabetic subjects,[26-28] and LDL glycation correlates with other indices of glycemic control.[28] In vitro glycation of LDL impairs its binding and degradation by human fibroblasts in proportion to the extent of glycation, and severe modification (> 6% to 8% lysine residues) may completely abolish recognition by the classic LDL receptor.[29-31] In vivo[32] and in vitro[33] glycation of LDL in insulin-dependent diabetes mellitus (IDDM) patients correlated with cholesteryl ester accumulation in human monocyte-derived macrophages. Using boronate affinity chromatography, LDL from both diabetic and nondiabetic subjects can be separated into two fractions, one "bound" and highly glycated, the other "unbound" and much less glycated.[34] Degradation of the bound LDL fraction via the classic LDL receptor pathway is, as one might predict, greatly impaired in fibroblasts in comparison to the unbound fraction.[35] However, with human monocyte-macrophages (the main precursors of the foam cells characteristic of atherosclerotic plaques) marked increases in the accumulation and degradation of highly glycated LDL occur, along with increased accumulation of cholesteryl ester.[35] When LDL is glycated in vitro under antioxidant conditions, facilitating even higher levels of glycation (but not lipoxidation), proportionately greater effects on macrophage cholesteryl ester synthesis, accumulation, and degradation are observed together with decreased recognition by the classic LDL receptor.[33] Competition studies with acetylated LDL have ruled out the scavenger pathway receptor as the conduit for increased uptake of glycated LDL. These observations suggest the existence of a separate low-affinity, high-capacity receptor on human monocyte-macrophages that is specific for glycated LDL and accelerates foam cell formation and atherosclerosis.

It has been shown recently by Bucala et al[36,37] that modifica-

tion of LDL by exposure to AGE-containing peptides reduces its clearance from plasma, and the site of modification of apoB that determines impaired recognition by the classic LDL receptor has been identified.[36,37]

Lipoproteins contain varying amounts of unsaturated fatty acids at their core that are vulnerable to oxidative damage. Oxidized LDL is a potent stimulator of foam cell formation by macrophages,[38-40] and in the vascular wall it is chemotactic to monocytes.[41,42] Oxidized LDL also inhibits endothelial cell migration into areas of endothelial injury[43] and is cytotoxic to nearby cells.[44,45] As stated earlier, the processes of glycation and oxidation are closely linked. Autoxidation of both FL and simple monosaccharides under physiological conditions in the presence of trace amounts of metal ions generates superoxide radicals, in turn promoting oxidative damage to lipoprotein core lipids.[46] However, there is at present no definite evidence that uncomplicated diabetes per se increases plasma lipoprotein oxidation. The situation may be different once vascular damage has become established, with extravasated lipoproteins sequestered in vessel walls or outside capillaries. Then, concomitant glycoxidation of connective tissue proteins may encourage further glucose-mediated binding of lipoproteins, permitting greater generation of free radicals and the establishment of vicious cycles of vascular injury.[47,48]

Immunologic consequences of LDL modification. Enhanced modification of LDL in diabetes may provoke an immune response.[49-55] The existence of antibodies against glycated LDL, even at low levels, may be important because LDL immune complexes (LDL-ICs) can stimulate foam cell formation and are potently atherogenic, as previously reviewed.[51,53,54] This atherogenicity of LDL-ICs is mediated by altered behavior of vascular cells, including altered cytokine release, coagulant activity, vascular permeability, and expression of vascular growth factors.

In diabetes, the formation of LDL-ICs in situ in vessel walls is probably enhanced by the presence of sequestered glycoxidized lipoproteins.[56] However, it is unclear whether significant formation of LDL-ICs occurs before or only after vascular disease has developed. Higher titers of antibodies against oxidized LDL have been observed in patients with NIDDM,[51] but in this particular study, it is unclear whether the nondiabetic control group had vascular disease. Recent study from Baynes' group suggests that CML may be the dominant epitope for antibody formation against glycoxidation products,[57] and it is therefore conceivable that CML may also be an important epitope on modified LDL.

Very–low-density lipoprotein, HDL, and Lipoprotein(a)

Increased glycation of very–low-density lipoprotein (VLDL) and HDL apoproteins has been demonstrated in diabetes,[58] and may affect the cellular interactions, function, and metabolism of the particles so as to promote atherogenesis. VLDL from normolipemic NIDDM and IDDM patients stimulates increased cholesteryl ester synthesis and accumulation in cultured human monocyte-macrophages.[59,60] However, subtle alterations in VLDL composition occur in diabetes,[61] so these effects cannot be confidently attributed to increased glycation. In vivo, the various VLDL apoproteins undergo differing degrees of glycation because of differing plasma residence times. This issue complicates assessment of the effects of glycation, and unfortunately, the differential glycation cannot be readily reproduced in vitro. Increased glycation of VLDL may therefore have important atherogenic consequences, but this has not yet been convincingly proven.

Similar problems afflict studies of glycated HDL. Mild glycation of HDL in vitro accelerates its clearance from plasma in guinea pigs,[62] an effect possibly contributing to low plasma levels of HDL in diabetic patients. Glycation of HDL may also compromise its reverse cholesterol transport function: impaired high-affinity binding to fibroblasts[63] and ability to remove cholesterol from peripheral cells has been demonstrated.[64]

The unusual apoprotein of lipoprotein(a) [Lp(a)], apo(a), has structural homology with plasminogen. Lp(a) may contribute to macrovascular disease both by an antifibrinolytic effect (competitive inhibition of plasminogen activation) and because it is a cholesterol-rich particle.[65] However, apo(a) has relatively few lysine residues, and a recent study investigating the effects of in vitro glycation showed no evidence of increased atherogenicity, at least in terms of interactions with macrophages.[66]

GLYCATION OF OTHER PLASMA CONSTITUENTS

Glycated Albumin

Glycation of albumin has been implicated in the pathogenesis of vascular disease, especially glomerular damage, in diabetes. In vitro-glycated albumin inhibited proliferation of aortic endothelial cells in culture, and the effect could be blocked by antibodies reacting specifically with Amadori products in albumin.[67] Similar effects were observed in renal mesangial cells,[68] and these cells also exhibited abnormal collagen gene expression. Again, these effects could be inhibited by the specific antibody against glycated albumin.[69] Indeed, administration of this antibody to diabetic mice was found to prevent the development of nephropathy[70] and to inhibit retinal capillary basement membrane thickening.[71]

Glycation of Antithrombin III

Glycation of antithrombin III may impair its thrombin-inhibiting activity,[72] thereby enhancing thrombosis. Suggesting that this effect may be relevant in vivo, an inverse correlation of antithrombin III activity with both hemoglobin A_{1c} and plasma glucose independent of plasma concentrations of antithrombin III has been described.[73] In contrast, glycation of fibrinogen has no effect on its function, and is therefore unlikely to promote thrombosis.[74]

COLLAGEN AND VASCULAR TISSUE

Increased early glycation (FL content) of collagen in many tissues, including the aorta, has been observed in diabetes.[17,75,76] As discussed, this is reversible (at least in skin) by a relatively short period of improved glycemic control, and is less likely to have pathological effects than the more advanced modifications involving glycoxidation and lipoxidation.[18,76]

With advancing age and the progressive formation of stable cross-links,[77] collagen becomes more insoluble, thermally stable, and resistant to enzymatic digestion. This biochemical "aging" of collagen is greatly increased in people with diabetes,[78] and is accompanied by an accelerated accumulation of AGEs includ-

ing CML and pentosidine.[79] Once formed, these products are essentially permanent, and unlike FL, the levels do not decrease in response to improved glycemic control. Note that in vitro in the presence of glucose, antioxidant conditions inhibit the formation of CML, pentosidine, and collagen fluorescence, but not FL.[80]

There is now much evidence demonstrating associations between collagen AGE formation and the presence and severity of diabetic microvascular complications.[81-83] For macrovascular disease, there are several possible mechanisms by which glycoxidation and lipoxidation of vascular wall structural proteins may be implicated. Glycoxidation products in collagen have been shown to damp the activity of nitric oxide (endothelium-derived relaxing factor), which could in turn cause abnormal vascular tone and perfusion and conceivably contribute to hypertension.[84] Both in vivo[81] and autopsy[85] studies have shown increased aortic stiffness in diabetic subjects that may be associated with increased cross-linking of connective tissue proteins. Brownlee et al[47] found that glycated collagen binds more LDL than control collagen, and in diabetic animals, cross-linking of LDL to aortic collagen is increased 2.5-fold. AGEs in vessel walls have been localized immunologically to intracellular sites in macrophages, smooth muscle cells, and foam cells,[86] and there are specific receptors for AGEs on monocyte/macrophages.[87] Macrophages expressing such receptors can phagocytose protein molecules and even entire cells with AGEs on their surface.[88] Receptors for AGEs have also been identified and characterized on endothelial cells.[89] Reaction of AGE products with these various receptors can (1) induce prothrombotic tissue factor[90] and (2) stimulate release of cytokines that may accelerate the atherosclerotic process[91] and promote collagen production mediated by platelet-derived growth factor and transforming growth factor beta.[92] In animals, infusion of AGEs caused increased expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 in endothelial cells, associated with accelerated atherogenesis.[93]

CONCLUSIONS, AND MEANS TO REDUCE CARBONYL STRESS

In conclusion, there is evidence that enhanced modification of proteins by carbonyl-containing species, in the course of glycation, glycoxidation, and lipoxidation reactions, is implicated in diabetic vascular disease. Atherogenesis may be accelerated by damage both to short-lived plasma constituents such as LDL and to long-lived structural proteins in the arterial wall. Reduction of carbonyl stress could slow disease progression by mitigating damage to successive generations of short-lived species and cumulative damage to long-lived species. Measures to inhibit the glucose-mediated, lipid-mediated, and "oxidative" components of carbonyl stress may be considered separately.

Glucose-Mediated Stress

This may be minimized by optimizing glycemic control, thus minimizing FL formation and the quantity of glucose vulnerable to autoxidation. Existing FL levels in structural proteins may be reduced.[18] Optimal glycemic control is already an established goal in the management of diabetic patients.

Lipid-Mediated Stress

This may be mitigated by treatment of hyperlipidemia, if present (using dietary and/or pharmacologic measures), and by improving the "quality" of lipoproteins, ie, making them more resistant to oxidation. The latter goal may be addressed by dietary measures to minimize the oxidizability of fatty acid constituents of lipoproteins and cell membranes. Substitution of monounsaturated for polyunsaturated or saturated fats results in LDL that is less susceptible to oxidative damage,[94] and consumption of fruits and vegetables containing natural antioxidants may be beneficial.

Oxidative Stress

There is little direct evidence concerning the efficacy of antioxidant supplementation in diabetes. Vitamins C and E are the most important aqueous and fat-soluble antioxidants, respectively, and plasma levels of both tend to be abnormally low in diabetic patients.[95-97] Supplements of these vitamins therefore appear to provide a cheap, low-risk intervention. However, under some circumstances, vitamin C may act as a pro-oxidant,[98] and there are insufficient grounds to recommend its routine use. For vitamin E, there is significant circumstantial but no direct evidence that it may be beneficial. Like vitamin C, vitamin E may have pro-oxidant effects under some circumstances.[99] Probucol may also reduce lipid peroxidation[100] and have vasoprotective effects,[101-103] but it seems to have few advantages over vitamin E. Similar considerations apply to butylated hydroxytoluene.[104,105] Coenzyme Q (ubiquinone) detoxifies the oxidation product of vitamin E (tocopheroxyl radical), but any beneficial effect as a dietary supplement has yet to be clearly defined.[106] New agents to inhibit lipid peroxidation or its consequences are likely to be developed as the understanding of the chemistry involved improves.[107]

Scavenging Reactive Carbonyls

Aminoguanidine scavenges reactive carbonyl groups, and especially dicarbonyl compounds (eg, glyoxal formed by oxidative decomposition of FL, Schiff base, or fatty acids, or 3-deoxyglucosone formed by decomposition of FL). It can therefore block the formation of glycoxidation and lipoxidation products and interrupt vicious cycles of vascular damage. In vitro, it inhibits both collagen cross-linking[108] and lipid peroxidation,[109,110] and in cell culture, we have shown that at concentrations as low as 1 μmol/L it can inhibit cytotoxicity development in LDL exposed to glycoxidative stress.[111] Similar concentrations inhibit the toxicity of simulated hyperglycemia (25 mmol/L glucose) for retinal vascular cells (T.J. Lyons, unpublished observations, March 1997). Thus, the observed toxicities of both modified LDL and glucose may be mediated by their oxidation products at very low concentrations. In vivo, aminoguanidine inhibits the development[112,113] and progression[114] of diabetic retinopathy in streptozotocin-diabetic rats and the development of diabetic nephropathy[115,116] and neuropathy.[117,118] A multicenter phase III study is in process assessing its efficacy in diabetic nephropathy in humans, and other similar agents with greater potency are under development.[119]

Possibilities for Direct Removal of AGEs From Serum or Dialysis Fluid

AGEs are not effectively removed during hemodialysis, but studies investigating the AGE receptor have demonstrated that they bind to lysozyme.[120] Matrix-bound lysozyme is being assessed as a means of clearing AGEs from serum and dialysis fluid, and the initial results are encouraging.[121] Finally, compounds that may cleave existing AGE-mediated cross-links are under investigation.[122]

Some or all of these measures may, in the future, contribute to preventive strategies to abolish the increased risk for macrovascular disease in NIDDM.

REFERENCES

1. Geiss LS, Herman WH, Smith PJ: Mortality in non–insulin-dependent diabetes, in Diabetes in America (ed 2). Bethesda, MD, National Diabetes Data Group, NIH Publication No. 95-1468, 1995, pp 233-257
2. Stamler J, Vaccaro O, Neaton JD, et al: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 16:434-444, 1993
3. Melton LJ III, Macken KM, Palumbo PJ, et al: Incidence and prevalence of clinical peripheral vascular disease in a population-based cohort of diabetic patients. Diabetes Care 3:650-654, 1980
4. Kennedy L, Baynes JW: Non-enzymatic glycosylation and the chronic complications of diabetes: An overview. Diabetologia 26:93-98, 1984
5. Baynes JW: Role of oxidative stress in development of complications in diabetes. Diabetes 40:405-412, 1991
6. Lyons TJ, Jenkins AJ: Glycation, oxidation and lipoxidation in the development of the complications of diabetes mellitus: A "carbonyl stress" hypothesis. Diabetes Rev (in press)
7. Lyons TJ, Jenkins AJ: Lipoprotein glycation and its metabolic consequences. Curr Opin Lipidol (in press)
8. Ahmed MU, Thorpe SR, Baynes JW: Identification of carboxymethyllysine as a degradation product of fructose-lysine in glycosylated protein. J Biol Chem 261:4889-4994, 1986
9. Sell DR, Monnier VM: Structure elucidation of a senescence cross-link from human extracellular matrix. Implication of pentoses in the aging process. J Biol Chem 264:21597-21602, 1989
10. Grandhee SK, Monnier VM: Mechanism of formation of the Maillard protein cross-link pentosidine. Glucose, fructose and ascorbate as pentosidine precursors. J Biol Chem 266:11649-11653, 1991
11. Hayase F, Nagaraj R, Miyata S, et al: Aging of proteins: Immunological detection of a glucose-derived pyrrole formed during Maillard reaction in vivo. J Biol Chem 263:3758-3764, 1989
12. Nakamura K, Hasegawa T, Fukunaga Y, et al: Crosslines A and B as candidates for the fluorophores in age- and diabetes-related cross-linked proteins, and their diacetates produced by Maillard reaction of α-*N*-acetyl-L-lysine with D-glucose. J Chem Soc Chem Commun 992-994, 1992
13. Ienaga K, Nakamura K, Hochi T, et al: Crosslines, fluorophores in the AGE-related crosslinked proteins. Contrib Nephrol 112:42-51, 1995
14. Yoshimori K, Kakita H, Nakano K, et al: Presence of crosslines, a novel advanced glycosylation end products (AGEs) in diabetics. Diabetes 45:260A, 1996 (suppl, 2, abstr)
15. Portero-Otin M, Nagaraj RH, Monnier VM: Chromatographic evidence for pyrraline formation during protein glycation in vitro and in vivo. Biochim Biophys Acta 1247:74-80, 1995
16. Nagaraj RH, Portero-Otin M, Monnier VM: Pyrraline ether crosslinks as a basis for protein crosslinking by the advanced Maillard reaction in aging and diabetes. Arch Biochem Biophys 325:152-158, 1996
17. Lyons TJ, Kennedy L: Non-enzymatic glycosylation of skin collagen in patients with limited joint mobility. Diabetologia 28:2-5, 1985
18. Lyons TJ, Bailie K, Dunn JA, et al: Decrease in skin collagen glycosylation with improved glycemic control in patients with insulin-dependent diabetes mellitus. J Clin Invest 87:1910-1915, 1991
19. Wells-Knecht KJ, Lyons TJ, McCance DR, et al: 3-Deoxyfructose concentrations are increased in human plasma and urine in diabetes. Diabetes 43:1152-1156, 1994
20. Zyzak DV, Richardson JM, Thorpe SR, et al: Formation of reactive intermediates from Amadori compounds under physiological conditions. Arch Biochem Biophys 316:547-554, 1995
21. Wolff SP, Dean RT: Glucose autoxidation and protein modification: The potential role of "autoxidative glycosylation" in diabetes mellitus. Biochem J 245:243-250, 1987
22. Wells-Knecht MC, Thorpe SR, Baynes JW: Pathways of formation of glycoxidation products during glycation of collagen. Biochemistry 34:15134-15141, 1995
23. Requena JR, Fu M-X, Ahmed MU, et al: Lipoxidation products as biomarkers of oxidative damage to proteins during lipid peroxidation reactions. Nephrol Dial Transplant 11:48-53, 1996 (suppl 5)
24. Fu M-X, Requena JR, Jenkins AJ, et al: The advanced glycation end-product, N^{ε}-(carboxymethyl)lysine (CML), is a product of both lipid peroxidation and glycoxidation reactions. J Biol Chem 271:9982-9986, 1996
25. Kennedy L, Lappin TRJ, Lavery TD, et al: Relation of high density lipoprotein cholesterol concentration to type of diabetes and its control. Br Med J 2:1191-1194, 1978
26. Scheicher E, Deufel T, Wieland OH: Non-enzymatic glycosylation of human serum lipoproteins. FEBS Lett 129:1-4, 1981
27. Gonen B, Baenziger J, Schonfeld G, et al: Non-enzymatic glycosylation of low-density lipoproteins in vitro. Diabetes 30:875-878, 1981
28. Lyons TJ, Patrick JS, Baynes JW, et al: Glycosylation of low density lipoprotein in patients with type 1 diabetes: Correlations with other parameters of glycaemic control. Diabetologia 29:685-689, 1986
29. Witztum JL, Mahoney EM, Branks MJ, et al: Nonenzymatic glucosylation of low-density lipoprotein alters its biologic activity. Diabetes 31:283-291, 1982
30. Sasaki J, Cottam GL: Glycosylation of LDL decreases its ability to interact with high-affinity receptors of human fibroblasts in vitro and decreases its clearance from rabbit plasma in vivo. Biochim Biophys Acta 713:199-207, 1982
31. Steinbrecher UP, Witztum JL: Glucosylation of low density lipoproteins to an extent comparable to that seen in diabetes slows their catabolism. Diabetes 33:130-134, 1984
32. Lyons TJ, Klein RL, Baynes JW, et al: Stimulation of cholesteryl ester synthesis in human monocyte-derived macrophages by low-density lipoproteins from type 1 (insulin-dependent) diabetic patients: The influence of non-enzymatic glycosylation of low-density lipoprotein. Diabetologia 30:916-923, 1987
33. Lopes-Virella MF, Klein RL, Lyons TJ, et al: Glycosylation of low-density lipoprotein enhances cholesteryl ester synthesis in human monocye-derived macrophages. Diabetes 37:550-557, 1988
34. Jack CM, Sheridan B, Kennedy L, et al: Non-enzymatic glycosylation of low-density lipoprotein. Results of an affinity chromatography method. Diabetologia 31:126-128, 1988
35. Klein RL, Laimans M, Lope-Virella MF: Isolation, characterization and metabolism of the glycated and non-glycated sub-fractions of

low density lipoproteins isolated from type 1 diabetic patients and non-diabetic subjects. Diabetes 44:1093-1098, 1995

36. Bucala R, Makita Z, Vega G, et al: Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. Proc Natl Acad Sci USA 91:9441-9445, 1994

37. Bucala R, Mitchell R, Arnold K, et al: Identification of the major site of apolipoprotein B modification by advanced glycosylation end products blocking uptake by the low density lipoprotein receptor. J Biol Chem 270:10828-10832, 1995

38. Henriksen T, Mahoney EM, Steinberg D: Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: Recognition by receptors for acetylated low density lipoproteins. Proc Natl Acad Sci USA 78:6499-6503, 1981

39. Steinbrecher UP, Parthasarathy S, Leake DS, et al: Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density phospholipids. Proc Natl Acad Sci USA 81:3883-3887, 1984

40. Morel DW, DiCorleto PE, Chisolm GM: Endothelial and smooth muscle cells alter low density lipoprotein in vitro by free radical oxidation. Arteriosclerosis 4:357-364, 1984

41. Quinn MT, Parthasarathy S, Fong LG, et al: Oxidatively modified low density lipoproteins: A potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci USA 84:2995-2998, 1987

42. Cushing SD, Berliner JA, Valente AJ, et al: Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. Proc Natl Acad Sci USA 87:5134-5138, 1990

43. Murugesan G, Chisolm GM, Fox PL: Oxidized low density lipoprotein inhibits the migration of aortic endothelial cells in vitro. J Biol Chem 120:1011-1019, 1993

44. Hessler JR, Morel DW, Lewis J, et al: Lipoprotein oxidation and lipoprotein-induced cytotoxicity. Arteriosclerosis 3:215-222, 1983

45. Cathcart MK, Morel DW, Chisolm GM III: Monocytes and neutrophils oxidize low density lipoprotein making it cytotoxic. J Leukoc Biol 38:341-350, 1985

46. Mullarkey CJ, Edelstein D, Brownlee M: Free radical generation by early glycation products: A mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 173:932-939, 1990

47. Brownlee M, Vlassara H, Cerami A: Nonenzymatic glycosylation products on collagen covalently trap low-density lipoprotein. Diabetes 34:938-941, 1985

48. Lyons TJ: Oxidised low density lipoproteins—A role in the pathogenesis of atherosclerosis in diabetes? Diabet Med 8:411-419, 1991

49. Witztum JL, Steinbrecher UP, Kesaniemi YA, et al: Autoantibodies to glucosylated proteins in the plasma of patients with diabetes mellitus. Proc Natl Acad Sci USA 81:3204-3208, 1984

50. Curtiss LK, Witztum JL: A novel method of generating region-specific monoclonal antibodies to modified proteins. Application to the identification of human glucosylated low density lipoproteins. J Clin Invest 72:1427-1438, 1983

51. Lopes-Virella MF, Virella G: Immune mechanisms of atherosclerosis in diabetes mellitus. Diabetes 41:86-91, 1992 (suppl 2)

52. Bellomo G, Maggi E, Poli M, et al: Autoantibodies against oxidatively modified low-density lipoproteins in NIDDM. Diabetes 44:60-66, 1995

53. Lyons TJ, Lopes-Virella MF: Glycosylation-related mechanisms, in Draznin B, Eckel RH (eds): Diabetes and Atherosclerosis—Molecular Basis and Clinical Aspects. New York, NY, Elsevier, 1993, pp 169-189

54. Lopes-Virella MF, Klein RL, Virella G: Modification of lipoproteins in diabetes. Diabetes Metab Rev 12:69-90, 1996

55. Witztum JL, Steinbrecher UP, Fisher M, et al: Nonenzymatic glucosylation of homologous low density lipoprotein and albumin renders them immunogenic in the guinea pig. Proc Natl Acad Sci USA 80:2757-2761, 1983

56. Brownlee M, Pongor S, Cerami A: Covalent attachment of soluble proteins by non-enzymatically glycosylated collagen: Role in the in situ formation of immune complexes. J Exp Med 158:1739-1744, 1983

57. Reddy S, Bichler J, Wells-Knecht KJ, et al: *N* epsilon-carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. Biochemistry 34:10872-10878, 1995

58. Curtiss LK, Witztum JL: Plasma apo-lipoproteins A-I, A-II, B, C-I and E are glucosylated in hyperglycemic diabetic subjects. Diabetes 34:452-461, 1985

59. Klein RL, Lyons TJ, Lopes-Virella MF: Metabolism of very low- and low-density lipoproteins isolated from normolipidaemic type 2 (non–insulin-dependent) diabetic patients by human monocyte-derived macrophages. Diabetologia 33:299-305, 1990

60. Klein RI, Lyons TJ, Lopes-Virella MF: Interaction of very–low-density lipoprotein isolated from type I (insulin-dependent) diabetic subjects with human monocyte-derived macrophages. Metabolism 38:1108-1114, 1989

61. Klein RL, Lopes-Virella MF: Metabolism by human endothelial cells of very low density lipoprotein subfractions isolated from type 1 (insulin-dependent) diabetic patients. Diabetologia 36:258-264, 1993

62. Witztum JL, Fisher M, Pietro T, et al: Nonenzymatic glucosylation of high-density lipoprotein accelerates its catabolism in guinea pigs. Diabetes 31:1029-1032, 1982

63. Duell PB, Oram JF, Bierman EL: Nonenzymatic glycosylation of HDL resulting in inhibition of high-affinity binding to cultured human fibroblasts. Diabetes 39:1257-1263, 1990

64. Duell PB, Oram JF, Bierman EL: Nonenzymatic glycosylation of HDL and impaired HDL-receptor–mediated cholesterol efflux. Diabetes 40:377-384, 1991

65. Jenkins AJ, Best JD: The role of lipoprotein(a) in the vascular complications of diabetes mellitus. J Intern Med 237:359-365, 1995

66. Makino K, Furbee JW, Scanu AM, et al: Effect of glycation on the properties of lipoprotein(a). Arterioscler Thromb Vasc Biol 15:385-391, 1995

67. Cohen MP, Hud E, Wu V-Y, et al: Glycated albumin modified by Amadori adducts modulates aortic endothelial cell biology. Mol Cell Biochem 143:73-79, 1995

68. Ziyadeh FN, Cohen MP: Effects of glycated albumin on mesangial cells: Evidence for a role in diabetic nephropathy. Mol Cell Biochem 125:19-25, 1993

69. Cohen MP, Ziyadeh FN: Amadori glucose adducts modulate mesangial cell growth and collagen gene expression. Kidney Int 45:475-484, 1994

70. Cohen MP, Sharma K, Jin Y, et al: Prevention of diabetic nephropathy in db/db mice with glycated albumin antagonists. J Clin Invest 95:2338-2345, 1995

71. Clements R, Robison WG, Cohen MP: Amelioration of retinal capillary basement membrane material accumulation in the db/db mouse with anti-glycated albumin treatment. Diabetes 45:16A, 1996 (suppl 2, abstr)

72. Brownlee M, Vlassara H, Cerami A: Inhibition of heparin-catalyzed antithrombin III activity by non-enzymatic glycosylation: Possible role in fibrin deposition in diabetes. Diabetes 33:532-535, 1984

73. Ceriello A, Guigliano D, Quatraro A, et al: Daily rapid blood glucose variations may condition antithrombin biological activity but not its plasma concentration in insulin dependent diabetes: A possible role for labile non-enzymatic glycation. Diabete Metab 13:16-19, 1987

74. McVerry VA, Thorpe S, Gaffney JP, et al: Non-enzymatic glycosylation of fibrinogen. Haemostasis 10:261-270, 1981

75. Rosenberg H, Modrak JB, Hassing JM, et al: Glycosylated collagen. Biochem Biophys Res Commun 91:498-501, 1979

76. Cohen MP, Urdanivia E, Surma M, et al: Increased glycosylation of glomerular basement membrane collagen in diabetes. Biochem Biophys Res Commun 95:765-769, 1980

77. Hamlin CR, Kohn RR: Evidence for progressive, age-related structural changes in post-mature human collagen. Biochim Biophys Acta 236:458-467, 1971

78. Hamlin CR, Kohn RR, Luschin JH: Apparent accelerated aging of human collagen in diabetes mellitus. Diabetes 24:902-904, 1975

79. Sell DR, Lapolla A, Odetti P, et al: Pentosidine formation in skin correlates with the severity of complications in individuals with long-standing IDDM. Diabetes 41:1286-1292, 1992

80. Fu M-X, Knecht KJ, Lyons TJ, et al: Role of oxygen in the cross-linking and chemical modification of collagen by glucose. Diabetes 43:676-683, 1994

81. Monnier VM, Vishwanath V, Frank KE, et al: Relations between complications to type 1 diabetes mellitus and collagen-linked fluorescence. N Engl J Med 314:403-408, 1986

82. Makita Z, Radoff S, Rayfield EJ, et al: Advanced glycosylation end-products in patients with diabetic nephropathy. N Engl J Med 325:836-842, 1991

83. McCance DR, Dyer DG, Dunn JA, et al: Maillard reaction products and their relation to complications in insulin dependent diabetes mellitus. J Clin Invest 91:2470-2478, 1993

84. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 87:432-428, 1991

85. Oxlund H, Rasmussen LM, Andreassen TT, et al: Increased aortic stiffness in patients with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 32:748-752, 1989

86. Kume S, Takeya M, Mori T, et al: Immunochemical and ultrastructural detection of advanced glycation end products in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. Am J Pathol 147:654-667, 1995

87. Vlassara H, Brownlee M, Cerami A: Novel macrophage receptor for glucose-modified proteins is distinct from previously described scavenger receptors. J Exp Med 164:1301-1309, 1986

88. Vlassara H, Valinsky J, Brownlee M, et al: Advanced glycosylation end products on erythrocyte cell surface induce receptor-mediated phagocytosis by macrophages. J Exp Med 166:539-549, 1987

89. Schmidt AM, Vianna M, Gerlach M, et al: Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. J Biol Chem 267:14987-14997, 1992

90. Ichikawa K, Yoshinari M, Wakisaka M, et al: Advanced glycosylation end products induced tissue factor expression in human monocytes. Diabetes 45:128A, 1996 (suppl 2, abstr)

91. Vlassara H, Brownlee M, Manogue KR, et al: Cachectin/TNF and IL-1 induced by glucose-modified proteins: Role in normal tissue remodeling. Science 240:1546-1548, 1988

92. Throckmorton DC, Brogden AP, Min B, et al: PDGF and TGF-beta mediate collagen production by mesangial cells exposed to advanced glycosylation end products. Kidney Int 48:111-117, 1995

93. Vlassara H, Fuh H, Donnelly T, et al: Advanced glycation endproducts promote adhesion molecule (VCAM-1, ICAM-1) expression and atheroma formation in normal rabbits. Mol Med 1:447-456, 1995

94. Reaven P, Parthasarathy S, Grasse BJ, et al: Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects. J Clin Invest 91:668-676, 1993

95. Frei B, England L, Ames BN: Ascorbate is an outstanding antioxidant in human plasma. Proc Natl Acad Sci USA 86:6377-6381, 1989

96. Jennings PE, Chirico S, Jones AF, et al: Vitamin C metabolites and microangiopathy in diabetes mellitus. Diabetes Res 6:151-154, 1987

97. Karpen CW, Cataland S, O'Dorisio TM, et al: Production of 12 HETE and vitamin E status in platelets from type 1 human diabetic subjects. Diabetes 34:526-531, 1985

98. Young IS, Torney JJ, Trimble ER: The effect of ascorbate supplementation on oxidative stress in the streptozotocin diabetic rat. Free Radic Biol Med 13:41-46, 1992

99. Bowry VW, Stocker R: Vitamin E in human low-density lipoprotein: When and how this antioxidant becomes a pro-oxidant. Biochem J 288:341-344, 1992

100. Parthasarathy S, Young SG, Witztum JL, et al: Probucol inhibits oxidative modification of low density lipoprotein. J Clin Invest 77:641-644, 1986

101. Carew TE, Schwenke DC, Steinberg D: Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci USA 84:7725-7729, 1987

102. Kita T, Nagano Y, Yokode M, et al: Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc Natl Acad Sci USA 84:5928-5931, 1987

103. Kuzuya M, Naito M, Funaki C, et al: Probucol prevents oxidative injury to endothelial cells. J Lipid Res 32:197-204, 1991

104. Björkhem I, Henriksson-Freyschuss A, Breuer O, et al: The antioxidant butylated hydroxytoluene protects against atherosclerosis. Arterioscler Thromb 11:15-22, 1991

105. Freyschuss A, Stiko-Rahm A, Swedenborg J, et al: Antioxidant treatment inhibits the development of intimal thickening after balloon injury of the aorta in hypercholesterolemic rabbits. J Clin Invest 91:1282-1288, 1993

106. Thomas SR, Neuzil J, Stocker R: Cosupplementation with coenzyme Q prevents the prooxidant effect of α-tocopherol and increases the resistance of LDL to transition metal–dependent oxidation initiation. Arterioscler Thromb Vasc Biol 16:687-696, 1996

107. Coffey MD, Cole RA, Colles SM, et al: In vitro cell injury by oxidized low density lipoprotein involves lipid hydroperoxide-induced formation of alkoxyl, lipid, and peroxyl radicals. J Clin Invest 96:1866-1873, 1995

108. Brownlee M, Vlassara H, Kooney A, et al: Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science 232:1629-1632, 1986

109. Bucala R, Cerami A: Phospholipids react with glucose to initiate advanced glycosylation and fatty acid oxidation: Inhibition of lipid advanced glycosylation and oxidation by aminoguanidine. Diabetes 41:23A, 1992 (suppl, abstr)

110. Bucala R, Cerami A, Makita Z, et al: Inhibition of advanced glycosylation by aminoguanidine reduces plasma LDL levels in diabetes. Clin Res 41:183A, 1993 (abstr)

111. Lyons TJ, Li W: Aminoguanidine and the cytotoxicity of modified LDL to retinal capillary endothelial cells. Diabetes 43:112A, 1994 (suppl 1, abstr)

112. Hammes HP, Martin S, Federlin K, et al: Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. Proc Natl Acad Sci USA 88:11555-11558, 1991

113. Hammes HP, Brownlee M, Edelstein D, et al: Aminoguanidine inhibits the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat. Diabetologia 37:32-35, 1994

114. Hammes H-P, Strodter D, Weiss A, et al: Secondary intervention with aminoguanidine retards the progression of diabetic retinopathy in the rat model. Diabetologia 38:656-660, 1995

115. Soulis-Liparota T, Cooper M, Papazoglou D, et al: Retardation by aminoguanidine of development of albuminuria, mesangial expan-

sion, and tissue fluorescence in streptozotocin-induced diabetic rat. Diabetes 40:1328-1334, 1991

116. Edelstein D, Brownlee M: Aminoguanidine ameliorates albuminuria in diabetic hypertensive rats. Diabetologia 35:96-97, 1992

117. Cameron NE, Cotter MA, Dines K, et al: Effects of aminoguanidine on peripheral nerve function and polyol pathway metabolites in streptozotocin diabetic rats. Diabetologia 35:946-950, 1992

118. Kihara M, Schmelzer JD, Poduslo JF, et al: Aminoguanidine effects on nerve blood flow, vascular permeability, electrophysiology, and oxygen free radicals. Proc Natl Acad Sci USA 88:6107-6111, 1991

119. Bhatt I, Terleckyj I, Manjula B: Demonstration of in vivo efficacy of aminoguanidine and novel AGE-formation inhibitors: Effects on tissue AGE content and microalbuminuria. Diabetes 45:130A, 1996 (suppl 2, abstr)

120. Li YM, Mitsuhashi T, Baviello G, et al: Opsonization and removal of serum AGEs by coupling to lysozyme. Diabetes 45:239A, 1996 (suppl 2, abstr)

121. Mitsuhashi T, Li YM, Fishbane S, et al: Depletion of reactive advanced glycation endproducts (AGEs) from diabetic uremic sera by a lysozyme-linked matrix. Diabetes 45:47A, 1996 (suppl 2, abstr)

122. Vasan S, Zhang X, Zhang X, et al: Design, synthesis, and pharmacological activity of a novel class of thiazolium-based compounds that cleave established AGE-derived protein crosslinks. Diabetes 45:28A, 1996 (suppl 2, abstr)