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Diabetic Atherosclerosis and Glycation of LDL(Low Density Lipoprotein)

-Review-

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Abstract

Diabetes carries an increased risk of atherosclerotic disease that is not fully explained by known cardiovascular risk factors. There is accumulating evidence that advanced glycation of structural proteins, and oxidation and glycation of circulating lipoproteins, are implicated in the pathogenesis of diabetic atherosclerosis. Reactions involving glycation and oxidation of proteins and lipids are believed to contribute to atherogenesis. Glycation, the nonenzymatic binding of glucose to protein molecules, can increase the atherogenic potential of certain plasma constituents, including low density lipoprotein(LDL). Glycation of LDL is significant increased in diabetic patients compared with normal subjects, even in the presence of good glycemic control. Metabolic abnormalities associated with glycation of LDL include diminished recognition of LDL by the classic LDL receptor ; increased covalent binding of LDL in vessel walls ; enhanced uptake of LDL by macrophages, thus stimulating foam cell formation ; increased platelet aggregation ; formation of LDL-immune complexes ; and generation of oxygen free radicals, resulting in oxidative damage to both the lipid and protein components of LDL and to any nearby macromolecules. Oxidized lipoproteins are characterized by cytotoxicity, potent stimulation of foam cell formation by macrophages, and procoagulant effects. Combined glycation and oxidation, "glycoxidation" occurs when oxidative reactions affect the initial products of glycation, and results in irreversible structural alterations of proteins. Glycoxidation is of greatest significance in long lived proteins such as collagen. In these proteins, glycoxidation products, believed to be atherogenic, accumulate with advancing age : in diabetes, their rate of accumulate is accelerated. Inhibition of glycation, oxidation and glycoxidation may form the basis of future antiatherogenic strategies in both diabetic and nondiabetic individuals.

Key words: diabetic atherosclerosis, glycated-LDL, lipoprotein, glucose, glycation

INTRODUCTION

Diabetes is associated with accelerated atherosclerosis, with the incidence of cardiovascular disease in diabetic patients three to four times that of nondiabetic individuals(1). The increase in cardiovascular risk is partly explained by the high prevalence of lipid abnormalities in diabetes, but even when all known cardiovascular risk factors are accounted for, diabetes remains as an independent risk factor(2). Attention has therefore focused on potential cardiovascular risk factors that are specific to the diabetic metabolic milieu, and considerable evidence now exists to suggest that the processes of oxidation and glycation are of major importance. Other reviewers have examined in detail the biochemical processes involved in advanced glycation (3), oxidation(4), and lipoprotein glycation(5).

The importance of atherosclerosis in diabetes is clear. Since the beginning of the insulin era, the proportion of total deaths from coronary heart disease(CHD) in diabetes has progressively increased to the point where now almost three fourths of deaths among diabetics are directly attributable to CHD. Recent data from the Joslin Clinic indicate that by age 50, fully one third of male and female individuals with insulin-dependent diabetes mellitus(IDDM) have already died from CHD, a proportion far exceeding that observed in an age-matched nondiabetic cohort(5). Although CHD mortality cannot be directly equated with atherogenesis, such data are compelling nevertheless.

Of the risk factors for atherosclerosis in the general population, most of the potentially reversible ones are more prevalent among the diabetic population. However, an epidemiological analysis suggests that the contribution of all of the commonly measured risk factors together can account for no more than about 25% of

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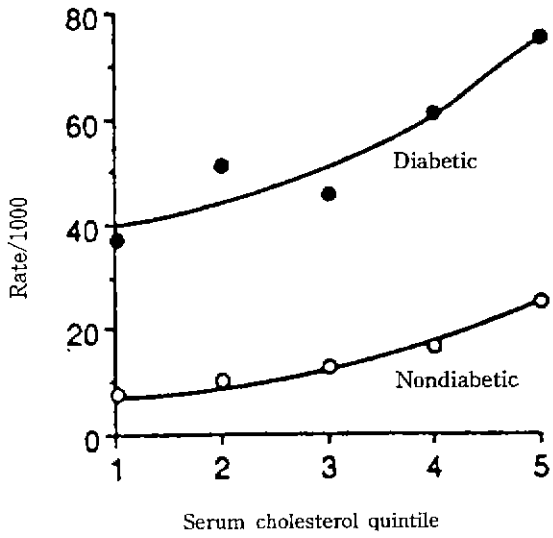

Fig. 1. Plot of coronary heart disease mortality rate in nondiabetic and diabetic men.

the excess CHD in diabetics(6). Recent data from the Multiple Risk Factor Intervention Trial, which included more than 5,000 middle-aged diabetic men among the more than 350,000 participants, indicate that while the mortality rate for CHD increases exponentially as a function of serum cholesterol levels in diabetics just as it does in nondiabetics, for every cholesterol level diabetics have threefold to fivefold higher CHD mortality rates(7)(Fig. 1).

The individual with diabetes often develops complications of the disease which are a major threat to both the quality and length of life. These complications are a wide group of clinical disorders which affect the vascular system, the kidney, the retina, the peripheral nerves, the lens, and the skin. The individual with diabetes has a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene, and a 2- to 6-fold increased risk of coronary heart disease and ischemic brain damage(8).

This review discusses recent work that implicates these processes in the pathogenesis of diabetic atherosclerosis, and examines the role of potential atherogenic agent of glycated LDL currently under investigation.

DYSLIPIDEMIA IN DIABETES

With regard to plasma lipid and lipoprotein levels, it is clear that high plasma triglyceride levels, usually increased in persons treated for diabetes(both IDDM and non-insulin-dependent diabetes mellitus[NIDDM]), have been consistently shown to be a risk factor for CHD among diabetic individuals in cross-sectional studies(9). This is in contrast to the controversy over the role of hypertriglyceridemia as a CHD risk factor populations. There is now an 11-year prospective study from Paris indicating that higher triglyceride levels among diabetics increase their risk of developing CHD (10). Hypertriglyceridemia in diabetes can be associated with a variety of changes in circulating lipoproteins (Table 1). Chylomicronemia may be seen in poorly controlled diabetics, but from the work of Nordestegaard et al.(11), it appears that chylomicrons are too large to enter the arterial wall and hence need not be considered atherogenic per se. Very low density lipoprotein (VLDL) and remnants of VLDL and chylomicron catabolism have been associated with deposition of cholesterol ester in arterial wall cells and hence can be considered potentially atherogenic. When equal particle numbers of chylomicron remnants and low density lipoprotein(LDL) are incubated with human arterial smooth muscle cells, remnants are at least as LDL in increasing cholesterol esterification and producting cholesterol ester accumulation. In other studies, VLDL obtained from diabetic donors, whether hypertriglyceridemic of normotriglyceridemi, appears to be more avidly bound and degraded by mouse peritoneal macrophages(12) and results in more cholesterol ester accumulation in human monocyte-derived macrophages (13) than VLDL obtained from normal donors. These *in vitro* findings indicate that the increased VLDL and remnant concentrations seen in diabetics are potentially capable of increasing cholesterol ester deposition in arterial wall cells.

Table 1. Hypertriglyceridemia in diabetes

Chylomicronemia
Increased VLDL
Increased remnants(VLDL and chylomicrons)
TG-rich low density lipoprotein
TG-rich high density lipoprotein

VLDL: Very low density lipoprotein, TG: Triglycerides

GLYCATION OF LIPOPROTEINS

There are a number of lipoprotein modifications in diabetes that affect cell interaction(Table 2). For example, glycosylation of lipoproteins has been shown to

occur in diabetes, and glycated LDL may be functionally abnormal(14,15). Being immunogenic, glycated LDL accumulates in plasma and may enhance cholesterol ester accumulation in macrophages(16). Glycation of HDL impairs its functional ability to bind to the HDL receptor binding site on cells and to promote intracellular cholesterol efflux(17)(Fig. 2). Thus, glycated HDL may by another factor potentially contributing to arterial cell cholesterol ester accumulation. However, the concentration of circulating glycated lipoproteins is relatively small, and their role in the arterial wall *in vivo* needs to be elucidated. Glycation of lipoproteins and other proteins involves nonenzymatic formation of Amadori products. As a result of the browning(Maillard) reaction, these products can be further processed with the formation of cross-links to advanced glycosylated end products(AGEs)(18). A particular cross-link involving pentosidine has been described by Sell and Monnier(19), cross-linking arginine to lysine on proteins yielding fluorescent products. Other AGEs such as carboxymethyllysine can by formed by oxidation, and because Amadori adducts are a ready source of superoxide, it appears that glycation of protein enhances its potential for oxidative damage(20).

Collagen subjected to advanced glycosylation will avidly bind LDL as a function of the degree of glycosylation(21). Vlassara and colleagues(22) have described in a series of studies the multiplicity of ways in which AGEs could by involved in atherogenesis(Table 3). One of the more exciting possibilities is that aminoguanidine, which blocks formation of cross-links in AGEs proteins(23), can markedly inhibit the development of experimental atheroma in rabbits. Therefore, glycation and advanced glycation of protein in plasma and the arterial wall deserves to take its place among the factors that are potentially involved in atherogenesis in diabetes(Table 4). Although the concentration of AGE proteins normally increases with age in skin collagen, their formation is markedly accelerated in diabetes. A recent report documents an increase in the concentration of AGE proteins in the arterial wall of diabetics compared with matched nondiabetics(24).

Table 2. Lipoprotein modifications in diabetes affecting cell interactions

Glycosylation
Oxidation
Chemical modification
Alterations in lipid composition
Core
Increased triglyceride
Decreased cholesterol ester
Surface
Increased free cholesterol
Alterations in apolipoprotein composition

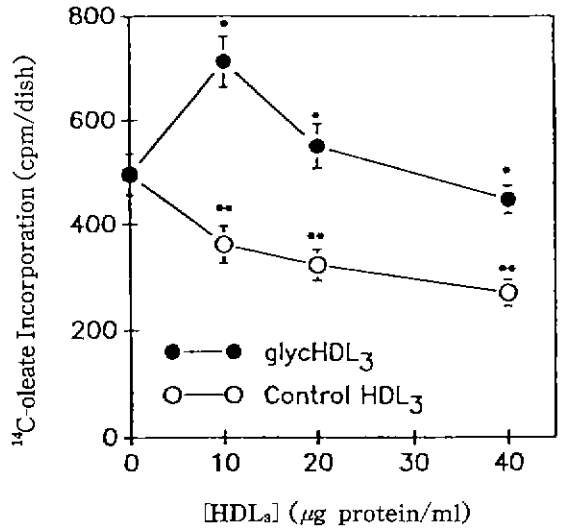

Fig. 2. Plot of effect of control and glycated high density lipoprotein subfraction 3(HDL$_3$) on cholesterol esterification.

Table 3. Potential role of AGE in atherogenesis

AGE accumulation in artery wall : Low density lipoprotein trapping
Endothelial cell changes
Permeability
Cell adhesion
Procoagulant state
Monocytes/macrophages
Chemotaxis and activation
Cholesterol ester accumulation
Cytokine/growth factor secretion
Smooth muscle cell proliferation
Prevention of experimental atheroma by aminoguanidine
Hyperinsulinemia : Decreased AGE receptor sites

AGE : advanced glycosylated end products

Table 4. Atherogenesis in diabetes

- Abnormalities of apoprotein and lipoprotein particle distribution("diabetic dyslipidemia")
- Procoagulant state
- Insulin resistance and hyperinsulinemia
- Glycation and advanced glycation of proteins in plasma and arterial wall
- "Glycoxidation" and oxidation
- Hormone, growth factor, and cytokine enhanced smooth muscle cell proliferation and foam cell formation

GLYCATION AND GLYCOXIDATION

Glucose reacts nonenzymatically with amino groups of amino acids of nucleic acids in a process known as nonenzymatic glycation(3)(Fig. 3). The initial products of the reaction are Schiff bases, which rearrange to form more stable Amidori products. These early glycation products are in reversible equilibrium with their precursors, and the levels will tend to rise and fall depending on the ambient glucose concentration. Amidori products are gradually degraded into reactive carbonyl compounds such as 3-deoxyglucosone, which can then further react with free amino groups to form advanced glycation endproducts(25). Advanced glycation endproducts(AGE) gradually accumulate on long lived proteins during normal aging and this process is significantly accelerated in diabetes, even by modest elevations in blood glucose(25). The process of advanced glycation end lead to marked changes in the structure and function of proteins(26) and the tissue concentration of AGE has been shown to correlate with the severity of diabetic microvascular complications. The formation of the presence of oxygen does not proceed under anaerobic conditions(27). The process is accelerated by the presence of transition metals and phosphate, and is inhibited by reducing compounds such as ascorbate(4,28). Thus some authors prefer the term "glycoxidation products" to describe the compounds that result from advanced glycation and oxidation.

GLYCATION AND OXIDATION OF LDL

LDL and other lipoprotein particles are designed to transport insoluble lipids through the aqueous plasma. The particles have protein on their surface that determine their interactions with cell-membrane receptors. The core of the particles contains esterified cholesterol, triglyceride, and other lipids. The surface protein can undergo glycative, oxidative, and glycoxidative damage. Additionally, the unsaturated fatty acids in the particle core can experience oxidative damage.

LDL GLYCATION

LDL glycation was first described by Schleicher et al.(29) more than 10 years ago. *In vitro* experiments conducted by these investigators showed that the extent of LDL glycation varied as a function both of the duration of LDL incubation and the concentration of glucose in the incubation mixture. These researchers were also the first to demonstrate that people with diabetes undergo increased *in vivo* glycation of apolipoprotein B(Apo B, the surface protein of LDL); this gave rise to the hypothesis that lipoprotein glycation contributes to the accelerated atherosclerosis of diabetes.

Studies have examined LDL glycation in nondiabetic subjects and in patients with well-controlled type I diabetes(30). This work demonstrated that since glucose is tightly controlled in nondiabetic subjects, so is glycation. In contrast, even diabetic patients with relatively good glycemic control exhibit a significant increase in LDL glycation *in vivo*. Metabolic abnormalities associated with glycated LDL may be relevant to accelerated atherosclerosis in diabetic patients, as well as in individuals with undiagnosed impaired glucose tolerance. A number of mechanisms may be involved. First, recognition of glycated LDL by the classic LDL receptor is impaired(31), and this may contribute to hyperlipidemia. Secondly, glycation results in increased sequestration and glucose-mediated covalent

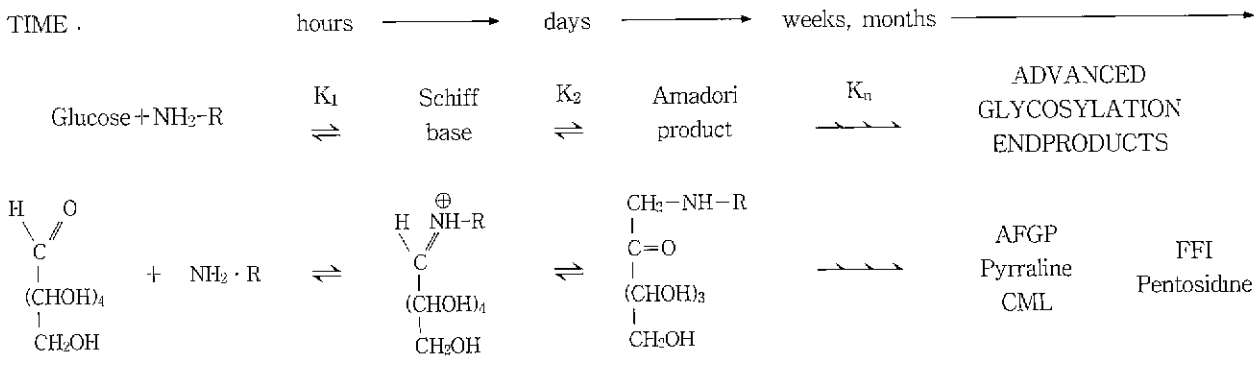

Fig. 3. Pathway of advanced glycation.

binding of LDL within vessel walls. Such binding will be further enhanced if the vessel wall structural proteins have themselves been modified by glycation and/or oxidation. A third alteration is enhanced uptake of LDL by monocyte-derived macrophages(32) stimulating the formation of foam cells, an early feature of atherosclerosis. Notably, however, glycated LDL does not enter the macrophage via the usual pathway for heavily modified particles(i.e. the scavenger receptor) ; our group has proposed the existence of a separate, low-affinity, high-capacity receptor pathway by which glycated LDL gains entry into the macrophage(32). Fourth, platelet aggregation is increased in the presence of glycated LDL(33). Fifth, there are theoretical reasons to believe that glycation of proteins generates free radicals(34), so glycated LDL particles may have increased susceptibility to subsequent oxidative damage. Finally, LDL modification can be extensive enough to provoke an antibody response, and the formation of potentially atherogenic LDL-immune complexes(35).

There is considerable evidence to support the notion that diabetes is a state of increased oxidatively reviewed by Baynes(4). Glucose can catalyze lipid peroxidation *in vitro*, and AGE may generate free radicals capable of oxidizing lipid in the arterial wall(36).

LDL GLYCOXIDATION

LDL and other circulating proteins persist in plasma for a relatively short time, so they are less likely than more long-lived, structural proteins(e.g., collagen) to experience combined glycation and oxidation. Nonetheless, LDL can undergo glycoxidation, and there are reasons to suspect that this occurs at an increased rate in people with diabetes. First, the increased glycative stress of diabetes increases the amount of glycated LDL available for potential oxidation. Second, impaired uptake of glycated LDL by the classic LDL receptor may increase its plasma half-life and, therefore, exposure to oxygen free radicals. Third, sequestration of lipoproteins, e.g., in vessel walls, prolongs their existence, allowing more time for glycoxidative damage.

Fig. 4 summarizes the effects of glycation and glycoxidation on LDL(5). Endothelial cell injury is caused by oxidized lipids in LDL, platelet aggregation, and cytotoxicity resulting from oxidation of lipid occurring within the vessel wall. In fact, most lipid oxidation probably occurs within the vessel wall, because, in plasma, oxidation is buffered by efficient antioxidant system, even in diabetic patients. As shown in Fig. 4, modified lipoproteins are covalently bound to vascular matrix proteins. Again, if the matrix proteins are themselves glycoxidized, adherence of the abnormal LDL will be even greater. Lipid peroxidation products were elevated in the diabetic patients, and subanalysis suggested that this elevation was predominantly in patients with microvasicular or macrovascular disease. It appears, therefore, that there is an association between lipid oxidation and complications of diabetes, but it is unclear whether or not oxidation plays a causative role. For example, recent work by Bucala et al.(37) suggests that the process of advanced glycation, which has been implicated in the pathogenesis of diabetic complications, may itself initiate lipid peroxidation.

It is now known that LDL exists in distinct subclasses that differ in size, density, and chemical composition. The smaller, denser LDL subfraction appears to be associated with increased risk of coronary artery disease(38). A preponderance of small dense LDL(termed "pattern B") is found in individuals with elevated plasma triglycerides and low levels of HDL cholesterol, a lipoprotein pattern observed commonly in patients with NIDDM(39).

Qualitative changes in LDL, consistent with a predominance of small dense particles, have been described in diabetic patients. Recent evidence suggests that small dense LDL is particularly susceptible to oxidative modification(40). Lipid peroxide levels in diabetic patients have been found to correlate with plasma triglyceride levels, and it is, therefore, reasonable to speculate that the presence of small dense LDL may be a contributing factor to increased lipid oxidation in diabetes.

GLYCATION OF CIRCULATING LIPOPROTEINS IN DIABETES

Glycation of human apolipoproteins was first described by Schleicher et al.(29), who were able to demonstrate *in vitro* glycation of several apoli poproteins, and identified glycated apolipoprotein B(apo-B) in the plasma of human diabetic subjects. *In vivo* glycation of apoproteins AI, AII, C and E in diabetes has also been studied, and LDL glycation in diabetic subjects has degree of glycemic control(30). Glycation of apo-B occurs on lysine groups that shield the LDL receptor-binding domain, thereby impairing its metabolism through the

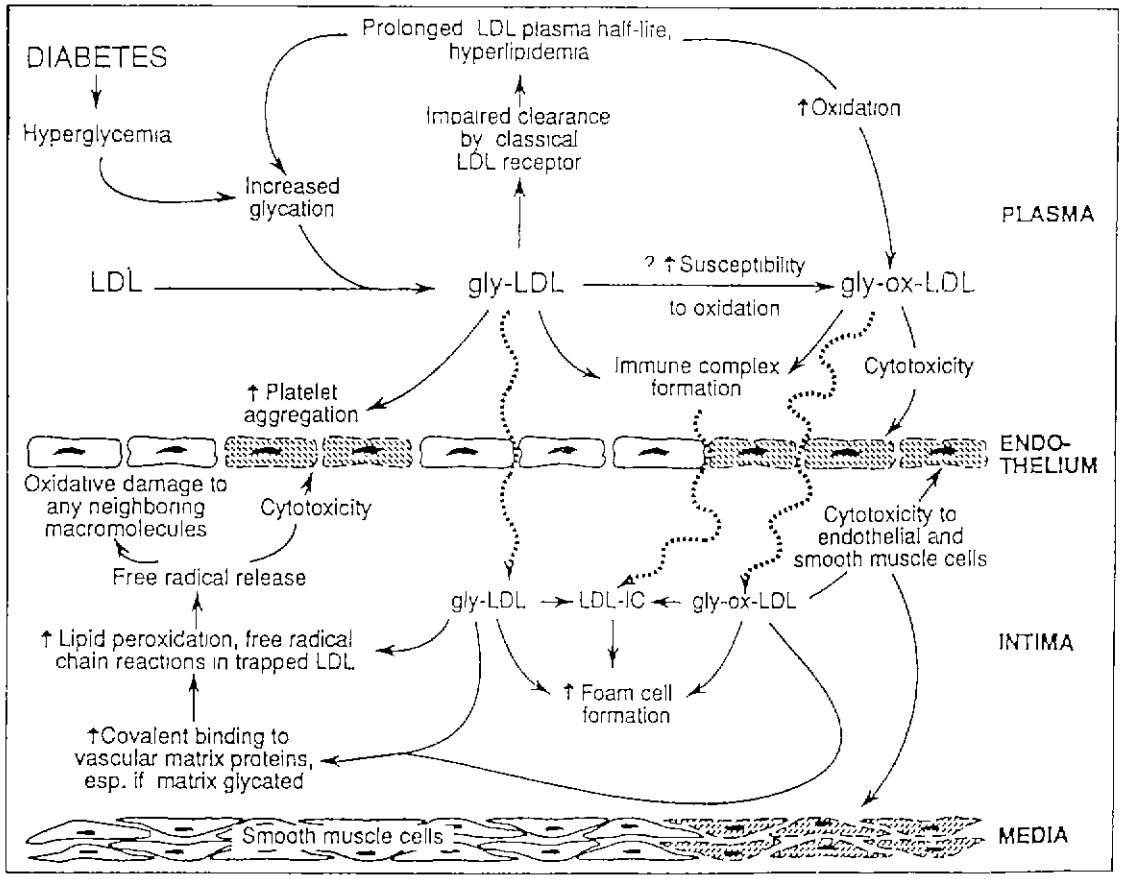

Fig. 4. Mechanism by which increased glycation and glycoxidation of low density lipoprotein(LDL).
(Ref : Lyons 1993)

classical LDL receptor pathway. This may lead to increased plasma levels, especially during time of poor glycemic control. Glycated LDL appears to enter human macrophages by a specific low affinity, high-capacity receptor pathway, which is separate from the scavenger receptor pathway and which may contribute to foam cell formation(41).

Glycation of LDL may render it immunogenic, to circulating antibodies and to glycated LDL, as well as immune complexes containing glycated LDL, have been found in diabetic patients(42). These immune complexes appear to be taken up avidly by macrophages and can induce foam cell formation. Glycated LDL isolated from diabetic subjects has also been found to stimulate the release of thromboxane B2 and aggregation of platelets(43), providing yet another mechanism by which it may stimulate atherosclerosis. There have been few studies that have examined in detail the biologic effects of glycation of the other lipoproteins; of particular interest is some recent evidence that glycation of HDL may impair its ability to participate in reverse cholesterol transport(44).

There is now evidence to suggest that advanced glycation of lipoproteins can occur *in vitro* and *in vivo*. When LDL is incubated with glucose, AGE are detectable within 3 days, linked to both the apo-B and the phospholipid components of the lipoprotein(37). The phospholipid AGE formed at a higher rate than the apo-B-linked AGE, and the formation of these compounds was associated with progressive oxidation of the lipid component of LDL. In the same study, AGE modification of LDL isolated from human diabetic subjects was identified, suggesting that this phenomenon occurs *in vivo*.

ADVANCED GLYCATION AND ATHEROSCLEROSIS

There are several mechanisms by which advanced protein glycation may be implicated in the pathogen-

esis of diabetic atherosclerosis. First. AGE are reactive compounds that can form into molecular cross-links with collagen and other structural proteins(3). Cross-linking increases the rigidity of collagen, and, therefore, may contribute to the decrease in arterial compliance and high incidence of hypertension reported in diabetic subjects. Futhermore, the products of lipid peroxidation, which are themselves increased in diabetes, can accelerate the cross-linking process. Second, advanced glycated collagen is capable of covalently trapping LDL (25), and the amount of trapping increases linearly with the degree of glycation. Once trapped in the arterial wall, LDL is susceptible to attack by free radicals(which can be generated by glycated proteins) and subsequent oxidative modification(36). Thirdly, human monocytes have AGE-specific receptors on their surface, migrate selectively in response to AGE, and respond by releasing cytokines(45). In tissue culture, selective migration of monocytes across endothelial cell monolayers is enhanced when the cells are grown on a matrix containing AGE (46). These *in vitro* studies suggest that AGE in the arterial wall may induce an inflammatory response, and they are supported by the finding that injection of AGE into rats induces a marked mononuclear infiltration of arterial walls and perivascular areas.

CONCLUSION

Atherosclerosis in diabetes is clearly multifactroial, but several potential mechanisms stand out and are in need of further focus. Foremost would be the unique effects of hyperglycemia mediated through the mechanisms of protein glycation and glycoxidation. There has not been an adequate clinical trial of glucose lowering and atherosclerosis outcomes.

Glycation glycoxidation are closely interrelated processes, each of which may contribute to atherogensis. Glycation alone may increase the atherogenicity of certain plasma constituents, notably lipoproteins. In long-lived proteins, glycative damage is fixed by oxidation reactions, and glycoxidation products accumulate. These products may contribute to the development of atherosclerosis by a variety of mechanisms, some of which have been briefly described. Glycative stress, leading to the first stage of a complex sequence of reactions, is constant in normal(non diabetic) individuals because circulating glucose levels are tightly controlled throughout their lives. In diabetic patients, however, glycative stress is increased. On the other hand, oxidative stress varies within both the normal and diabetic populations. Our data has not revealed any difference in the degree of variation in normal and diabetic individuals, suggesting that the diabetic state is not associated with any inherent increase in oxidative stress. Glycoxidative stress(the combined effect of glycation and oxidation) is increased in diabetics because of the increase in glycative stress. Therefore, in diabetics, the strength of oxidative defenses may be particularly important in modulating the consequences of hyperglycemia.

These observations raise questions concerning the possibility of interventions to slow or inhibit glycation and glycoxidation of lipoproteins and vascular wall proteins. Obviously, glycative stress can be modified by reducing glycemia, maintaining good diabetic control, and identifying patients in whom diabetes or impaired glucose tolerance has gone undiagnosed. Strategies for reducing oxidative stress may include use of antioxidant micronutrients(vitamins E and C, β-carotene) or medications such as probucol. Aminoguanidine, or a derivative, may have a future role in the inhibition of both glycation and oxidation. Future studies should shed more light on the antiatherogenic potential of such strategies.

Obviously much work needs to be done to validate these concepts. However, it is likely that both short- and long-term glycation of LDL, and possibly other lipoproteins, contributes to the increased incidence and severity of atherosclerosis that characterizes the hyperglyemic, diabetic patient.

REFERENCES

1. Kannel, W. B. and McGee, D. L : Diabetes and cardiovascular disease : Framingham study. *JAMA,* **241**, 0235 (1979)
2. Singer, D. E., Nathan, D M., Anderson, K M., Wilson, P. W. F. and Evans, J. C. : Association of HbAlc with prevalent cardiovascular disease in the original cohort of the Framingham Study. *Diabetes,* **41**, 202(1992)
3. Brownlee, M., Cerami, A. and Vlassara, H. : Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N. Engl. J. Med.,* **318**, 1315(1988)
4. Baynes, J. W. : Role of oxidated stress in development of complications in diabetes. *Diabetes.* **40**, 405(1991)
5. Lyons, T J. : Lipoprotein glycation and its metabolic

consequences. *Diabetes.* **41**, 67(1992)
6. Lyons, T. J., Thorpe, S. T. and Baynes, J. W. : Glycation and autoxidation of proteins in aging and diabetes. In "*Glucose metabolism, diabetes and the vascular wall*" Rudeman, N.(ed.), New York Oxford University Press(1992)
7. Stamlar, J. · Epidemiology, established major risk factor, and the primary prevention of coronary heart disease. In "*Cardiology*" Parmley, W. W. and Chatterjee, K.(eds.), Philadephia, Pa, JB Lippincott, p.1(1987)
8. Pyorala, K., Laakso, M. and Uusitupa, M. : Diabetes and atherosclerosis : An elpidemiologic view. *Diabetes Metab. Rev.,* **3**, 463(1987)
9. Laako, M., Pyorala, K., Sarlund, H. and Voutilainen, E. · Lipid and lipoprotein abnormalities associated with coronary heart disease in patients with insulin-dependent diabetes mellitus. *Atherosclerosis*, **6**, 679(1986)
10. Fontbonne, A., Eschwege, E., Cambien, F., Richard, J. L., Ducimetiere, P., Thibult, N., Warnet, J. M., Claude, J. R. and Rosselin, G. E. : Hypertriglyceridemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance or diabetes : Results from the 11-year follow-up of the Paris Prospective Study. *Diabetologia.,* **32**, 300(1989)
11. Nordestegaard, B. G., Stender, S. and Kjeldsen, K. : Reduced atherogenesis in cholesterol-fedn diabetic rabbits. *Arteriosclerosis,* **8**, 1(1988)
12. Kraemer, F. B., Chen, Y. D. I., Lopez, R. D. and Reaven, G. M. : Effects of noninsulin-dependent diabetes mellitus on the uptake of very low density lipoproteins by thioglycolate-elicited mouse peritoneal macrophages. *J Clin. Endocrinol. Metab.,* **61**, 335(1985)
13. Klein, R. L., Lyons, T. J. and Lopes-Virella, M. F. : Interaction of very-low-density lipoprotein isolated from Type I(insulin-dependent) diabetic subjects with human monocyte-derived macrophages. *Metabolism,* **38**, 1108 (1989)
14. Witztum, J. L., Mahoney, E. M., Branks, M. J., Fisher, M., Elam, R. and Steinberg, D. : Nonenzymatic glycosylation of low-density lipoprotein alters its biologic activity. *Diabetes,* **31**, 283(1982)
15. Susaki, J. and Cottam. G. L. : 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(1982)
16. Lopes-Virella, M. F., Klein, R. L., Lyons, T. J., Stevenson, H. C. and Witztum, J. L. : Glycosylation of LDL enhances CE synthesis in human monocyte-derived macrophages. *Diabetes,* **37**, 550(1988)
17. Duell, P. B., Oram, J. F. and Bierman, E. L. : Nonenzymatic glycosylation of HDL and impaired HDL-receptor-mediated cholesterol efflux. *Diabetes,* **40**, 377(1991)
18. Cerami, A., Vlassara, H. and Brownlee, M. : Role of advanced glycosylation products in complications of diabetes. *Diabetes Care,* **11**, 73(1988)
19. Sell, D. R. and Monnier, V. M. : End-stage renal disease and diabetes catalyze the formation of a pentose-derived cross link from aging human collagen. *J. Biol. Chem,* **85**, 380(1990)
20. Baynes. J. W. : Role of oxidative stress in development of complications in diabetes. *Diabetes,* **40**, 405(1985)
21. Brownlee, M., Vlassara, H. and Cerami, A. : Nonenzymatic glycosylation products on collagen covalently trap low-density lipoprotein. *Diabetes,* **34**, 938 (1985)
22. Kirstein, M., Brett, J., Radoff, S., Ogawa, S., Stern, D. and Vlassara, H. : Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor : Role in vascular disease of diabetes and aging. *Proc. Natl. Acad. Sci. U.S.A.,* **87**, 9010(1990)
23. Brownlee, M., Vlassara, H., Kooney, A., Ulrich, P. and Cerami, A. : Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science,* **232**, 1629(1986)
24. Makita, Z., Radoff, S., Rayfield, E. J., Yang, Z., Skolnik, E., Delaney, V., Friedman, E. A., Cerami, A. and Vlassara, H. : Advanced glycosylation end products in patients with diabetic nephropathy. *N. Engl. J. Med.,* **325**, 836(1991)
25. Brownlee. M. : Nonenzymatic glycosylation of macromolecules : prospects of pharmacologic modulation. *Diabetes,* **41**, 57(1992)
26. Charonis, A. S. and Tsilibary, F. E. C. : Structural and functional changes of laminin and type 4 collagen after nonenzymatic glycosylation. *Diabetes,* **41**, 49(1992)
27. Fu, M. X., Knecht, K. J, Thorpe, S. R. and Baynes, J. W. : Role of oxygen in cross linking and chemical modification of collagen by glucose. *Diabetes,* **41**, 42(1992)
28. Hunt, J. V., Bottoms, M. A. and Mitchinson, M. J. : Oxidative alterations in the experimental glycosylation model of diabetes mellitus are due to protein-glucose adduct oxidation. *Biochem J.,* **291**, 529(1993)
29. Schleicher, E., Deufel, T. and Wieland, O. H. : Nonenzymatic glycosylation of human serum lipoproteins. *FEBS Lett.,* **129**, 1(1981)
30. Lyons, T. J., Patrick, J. S., Baynes, J. W., Colwell, J. A. and Lopez-Virella, M. F. : Glycosylation of low density lipoprotein in patients with type I diabetes : correlations with other parameters of glycaemic control. *Diabetologia.,* **29**, 685(1986)
31 Steinbrecher, U. P. and Witztum, J. L. : Glycosylation of low density lipoproteins to an extent comparable to that seen in diabetes slows their catabolism. *Diabetes,* **33**, 130(1984)
32. Lopes-Virella, M. F , Klein, R. L., Lyons, T. J., Stevenson, H. C. and Witztum, J. L. : Glycosylation of low density lipoprotein enhances cholesteryl ester synthesis in human monocyte-derived macrophages. *Diabetes,* **37**, 1652(1988)
33. Klein, R. L., Lopes-Virella, M. F. and Colwell, J. A. : Enhancement of platelet aggregation by the glycosylated subfraction of low density lipoprotein(LDL) isolated from patients with insulin-dependent diabetes mellitus(IDDM). *Diabetes,* **39**, 173A(1990)
34. Wolff, S. P. and Dean, R. T · Glucose autoxidation and protein modification : the potential role of "autoxidative glycosylation" in diabetes mellitus. *Biochem. J.,* **245**, 243 (1987)
35. Zawadzki, Z., Milne, R. W. and Marcel, Y. L. : An immunochemical marker of low density lipoprotein oxidation. *J. Lipid Res.,* **30**, 885(1989)
36. Mullarkey, C. J., Edelstein, D and Brownlee, M. : Free radical generation by early glycation products : a mech-

anism for accelerated atherogenesis in diabetes. *Biochem. Biophys. Res. Commun.*, **173**, 932(1990)
37. Bucala, R., Makita, Z., Koschinsky, T., Cerami, A. and Vlassara, H. : Lipid advanced glycation ' pathway for lipid oxidation *in-vivo*. *Proc. Natl. Acad. Sci. USA*, **90**, 6434(1993)
38. Austin, M. A., Breslow, J. L., Hennekens, C. H., Buring, J. E., Willett, W C. and Krauss, R. M. : Low density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA*, **260**, 1917(1988)
39. Stern, M. P. and Haffner, S M. : Dyslipidaemia in type II diabetes · implications for therapeutic intervention. *Diabetes Care*, **14**, 1144(1991)
40. Chait, A., Brazg, R L., Tribble, D. L. and Krauss, R. M : Susceptibility of small, dense, low density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am. J. Med.*, **94**, 350(1993)
41. Lopez-Virella, M. F., Klein, R. L., Lyons, T. J., Stevenson, H. C. and Witztum, J. L. · Glycosylation of low density lipoprotein enhances cholesteryl ester synthesis in human monocyte-derived macrophages. *Diabetes*, **37**, 550(1988)
42. Lopez-Virella, M. F. and Virella, G. : Immune mechanisms for atherosclerosis in diabetes mellitus. *Diabetes*, **41**, 86(1992)
43. Watanabe, J., Wohltmann, H. J., Klein, R. L., Colwell, J. A. and Lopez-Virella, M. F. : Enhancement of platelet aggregation by low density lipoproteins from IDDM patients. *Diabetes*, **37**, 1652(1988)
44. Duell, P. B., Oram, J. F. and Bierman, E. L. : Non- enzymatic glycosylation of HDL and impaired HDL- receptor-mediated cholesterol efflux. *Diabetes*, **40**, 377(1991)
45. Vlassara, H., Brownlee, M., Manogue, K. and Pasagian, A : Cachectin/TNF and IL-1 induced by glucose-modified proteins : role in normal tissue remodeling. *Science*, **240**, 1546(1988)
46. Brett, J., Ogawa, S., Kirstein, M., Radoff, S., Vlassara, H. and Stern, D. : Advanced glycosylation end products selectively attract monocytes to migrate across endothelial cell monolayers, and induce activation and growth factor elaboration *Circulation*, **82**, 92(1990)

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