

Glycation as the glucose link to diabetic complications

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Hyperglycemia is considered a key causal factor in the development of diabetic vascular complications and can mediate its adverse effects through multiple pathways. This was confirmed for microangiopathy in the Diabetes Control and Complications Trial study for type 1 diabetes and corroborated for type 2 diabetes by the United Kingdom Prospective Diabetes Study published in 1998. Prevention of diabetic complications requires at least control of glycemia. This article briefly summarizes the evidence that strongly supports the role of hyperglycemia in vascular complications. After outlining the role of the polyol pathway, protein kinase C, and oxidative stress, the author focuses on one of the key biochemical mechanisms for this pathologic process: the direct deleterious action of glucose and other sugars on proteins, known as glycation or nonenzymatic glycosylation. Results of animal studies and phase III clinical trials reveal that the inhibition of this process attenuates the development of a range of these complications.

(Key words: diabetes, glycation, glomerulopathy, retinopathy, atherosclerosis, neuropathy, aminoguanidine)

Diabetes mellitus, a condition characterized mainly by a quantitative deficiency in insulin secretion or a resistance to insulin action, is estimated to afflict 5% to 7% of the population. This creates a huge economic burden related for the most part to the management of its complications, which are micro- and macroangiopathic.¹⁻⁵ Microangiopathy, the microvessel disease in diabetes, includes retinopathy, nephropathy, and neuropathy, and in patients with type 1 diabetes the first signs of these complications may develop in adolescence, particularly if insulin treatment has been inadequate. Similar complications occur later in life in patients with type 2 diabetes

and are frequently present at the time of diagnosis.

The precise mechanisms by which diabetic microangiopathy develops are not fully understood, but a consensus is emerging that points to a terrain of genetic influences onto which metabolic and hemodynamic derangements are superimposed.^{6,7} The anatomic hallmark of diabetic microangiopathy is the thickening of capillary basement membranes, which subsequently induces occlusive angiopathy, tissue hypoxia, and damage.^{8,9} The evolution of the numerous long-term complications of diabetes mellitus correlates well, in most cases, with the severity and duration of hyperglycemia. It is known that postprandial glucose levels greater than 200 mg/dL (11 mmol) are more frequently associated with renal, retinal, and neurologic complications that can commence 5 to 10 years after the onset of the disease.^{2,4,5} At the time of initial diagnosis of type 2 diabetes, many patients have postprandial glucose levels greater than 200 mg/dL and already have some diabetic compli-

cations. This evidence suggests that current diabetes care should be directed at earlier diagnosis of this condition and more effective control of the postprandial glucose excursions that may influence the development of long-term complications. To meet the first goal, in 1998, the American Diabetes Association lowered the cutoff point for the diagnosis of diabetes from 140 mg/dL to 126 mg/dL.¹⁰ These efforts are likely to delay the appearance and early progression of diabetic retinopathy, nephropathy, and neuropathy. However, findings of recent epidemiologic studies revealed that diabetic patients with poorly controlled glucose levels have a higher risk of cardiovascular disease (CVD) than those patients with well-controlled glucose levels.¹²

Some findings suggest that glycemia appears to be a continuous risk factor for CVD and that this association is not restricted to the diabetic range.¹³⁻¹⁵ Those authors believe glycemia represents a continuous risk factor for CVD comparable to that of dyslipidemia, smoking, or blood pressure. For instance, subjects with impaired glucose tolerance had a relative risk (RR) of death 30% higher than those with normal glucose; subjects with undiagnosed diabetes had an RR 80% higher and those with diagnosed diabetes had an RR 280% higher than those with normal glucose. A similar gradient in risks was found for CVD-specific mortality.

Evidence for a link between hyperglycemia and vascular complications in diabetes

A working knowledge of the intrinsic biochemical mechanisms, subjacent to diabetic long-term complications, should facilitate understanding of the basis for the current treatment guidelines, as well as the therapeutic agents under scrutiny that may become available soon. The rapid pace of research in the field makes any review outdated before publishing, but an attempt is made here to briefly summarize the evidence that strongly supports the role of hyperglycemia in vascular complications, the main focus being on one of the key biochemical mechanisms underlying this pathologic process: the direct effect of glucose and

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other sugars on proteins (known as glycation or nonenzymatic glycosylation) and its deleterious effect on the organism.

Apart from the key hemodynamic changes intervening in many tissue targets of diabetic complications, sound clinical and epidemiologic evidence exists that links hyperglycemia to vascular complications. Two controlled clinical trials stand out: (1) Diabetes Control and Complications Trial (DCCT), and (2) United Kingdom Prospective Diabetes Study (UKPDS).

Diabetes Control and Complications Trial

The DCCT was designed to answer the question of the association between hyperglycemia and vascular complications in a cohort large enough to permit incontestable conclusions.² The DCCT evaluated intensive insulin replacement and self-monitoring of blood glucose and used glycated hemoglobin assays to measure glycemic control over long periods.¹¹ It was conducted in subjects who had type 1 diabetes for a known duration and used well-established end-point criteria to address the glycemic hypothesis (retinopathy, nephropathy, and neuropathy).

Two groups of patients were followed-up for an average of 7 years—one treated conventionally with the goal of clinical well-being (standard treatment group) and another treated intensively to normalize blood glucose (intensive treatment group). The methods used to accomplish tight control in type 1 diabetes included three or more daily injections of insulin (66%) or use of programmable insulin-infusion pumps (34%). Data published in 1993 indicated that there was a 60% reduction in risk between the intensive treatment group and the standard treatment group in diabetic nephropathy, retinopathy, and neuropathy. The outcome showed that reduction of glycosylated hemoglobin (Hb A_{1c}) from levels of approximately 9% to approximately 7% reduced the progression and/or development of all microvascular complications. This change was due for the most part to the effect of therapy on glycemic control and, to some

extent, to the methods employed to achieve that control. All categories of patients benefited from intensive therapy, irrespective of age, sex, or duration of diabetes. The DCCT confirmed and expanded results from the analogously designed but smaller Stockholm Diabetes Intervention Study. These studies showed unequivocally that in type 1 diabetes, lowering the blood glucose delayed the onset and slowed the progression of microvascular complications. Secondary analyses in these studies showed strong relationships between the risks of developing these complications and glycemic exposure. Moreover, there was no clear-cut glucose threshold, but a continuous reduction in complications as glycemic levels approached the reference range.

The obvious major problems encountered in intensive treatment are the risks of hypoglycemia and weight gain, which must be taken into consideration; however, the benefits largely outweigh the risks. Patients should aim for the level of glucose control that can be achieved without undue risk for hypoglycemia. Any improved blood glucose control has been to slow the development and progression of microvascular complications. It can be argued that this constitutes an expensive treatment, but the cost-benefit ratio for intensive therapy is in a range comparable to other customarily accepted treatments in the United States.

United Kingdom Prospective Diabetes Study

The United Kingdom Prospective Diabetes Study (UKPDS) is a randomized trial of intensive treatment of patients with type 2 diabetes who were followed-up for an average of 10 years. The study recruited over 5000 patients with newly diagnosed type 2 diabetes in 23 centers in the United Kingdom between 1977 and 1991. The UKPDS started by analyzing the value of various strategies (diet and several orally administered hypoglycemic agents) to achieve tight blood glucose control compared with looser control.

Patients were followed-up to determine whether intensive use of pharmacologic therapy to lower blood glucose

levels result in reduced macro- and microvascular complications and whether use of different sulfonylurea drugs, metformin, or insulin have distinct advantages or disadvantages. In the subgroup of overweight subjects, metformin as monotherapy was compared with the control group and to the other three pharmacologic agents. The researchers soon became aware that high blood pressure may be an even stronger risk factor, and blood pressure treatment was accordingly included in the study.¹³⁻¹⁵

Even though it began as a randomized clinical trial, the UKPDS involved a considerable crossover among the subjects along the study period. The original design assigned patients to intensive therapy using one of four approaches—insulin, chlorpropamide, glyburide, or diet. Metformin was later added and compared with other modes of therapy. Nevertheless, monotherapy alone failed to achieve the glycemic goal. Initially, the diet group was intended to be the control for the intervention groups, but 80% of the diet group had to be moved to combination modes of therapy to prevent high blood glucose levels. These modes involved combining insulin or metformin with sulfonylurea drugs, which makes it more difficult to analyze the effect of each of the original interventions. These problems should not discredit the meaningful conclusions regarding the effect of tight control of complications.

The UKPDS provided answers to questions that plagued diabetes researchers and physicians for decades. Tightly controlling blood glucose concentration reduced the risk of complications in type 2 diabetes, and the overall microvascular complications rate was decreased by 25% in patients receiving intensive therapy versus conventional therapy.¹³⁻¹⁵ Confirming the DCCT data, the UKPDS showed a continuous relationship between the risk of microvascular complications and glycemia. For every percentage point decrease in Hb A_{1c}, there was a 35% reduction in the risk of microvascular complications. Sulphonylurea drugs and insulin produced equally good results in terms of

reducing the risk of microvascular complications.

The UKPDS showed that tight blood pressure control reduced the risk of diabetic complications. This study also showed a 16% reduction (not statistically significant) in the risk of myocardial infarction and sudden death in the intensively treated group. In the main trial, there were no significant differences with regard to diabetic complications or adverse cardiovascular events between therapy with insulin and with sulfonylurea drugs. Insulin should not be seen as the cause of atherosclerotic episodes, and sulfonylurea drugs should not be blamed for cardiovascular toxicity as previously was the case. Patients initially assigned to intensive therapy with metformin had decreased risks of combined diabetes-related end points, diabetes-related deaths, all-cause deaths, and myocardial infarction compared with the conventionally treated patients. In obese patients, no significant decrease in microvascular complications was observed with intensive metformin therapy or with combined insulin/sulfonylurea intensive therapy. The UKPDS results confirm and extend previous evidence supporting the hypothesis that hyperglycemia and its sequelae are a major cause of the microvascular complications of diabetes.

Implications in clinical practice

In its position statement, the American Diabetes Association expresses agreement with the design, protocols, and randomization of patients in this study. Clinical and laboratory tests were performed by recognized methodologies, and all end points were adequately documented. Moreover, the committee is confident that the results should apply to the US population of men and women with type 2 diabetes.

Thus, the hypothesis that it is glucose itself that is toxic in type 2 diabetes is confirmed, in line with the findings of the DCCT for type 1 diabetes: the mechanisms of this effect must be determined to better approach it therapeutically. The achievement of tight blood glucose control in type 2 diabetes is fea-

sible and should become the standard of care. The combination of pharmacologic agents used should be based on the individual evaluation of each patient. Notwithstanding the failure of diet therapy alone, diet remains an adjunct to pharmacologic therapy. The ability to prevent or at least slow down these complications may be made easier by the recently approved hypoglycemic agents that were not available to the UKPDS.

Hyperglycemia's role in diabetic complications (Figure 1)

No consensual framework has been found which encompasses all that is known about the link between hyperglycemia and complications. There are several equally defensible hypotheses on the roots of complications, including, but not limited to, the aldose reductase hypothesis,¹⁷ oxidative stress,^{18,19} the Maillard, or advanced glycation end product (AGE) hypothesis,²⁰⁻²³ modified protein kinase C activity,²⁴ pseudo-hypoxia, carbonyl stress,²⁵ altered lipoprotein metabolism,²⁶ and altered cytokine activities.²⁷

Of the aforementioned hypotheses, three of the favored pathways that are being investigated and that potentially explain the mechanisms by which high glucose levels can result in vascular damage require further attention: (1) the sorbitol theory, (2) modification of protein kinase C activity, and (3) the glycation hypothesis. It cannot be overemphasized that oxidative stress is generated in all these three pathways as well as in several others.

Sorbitol theory—The sorbitol hypothesis was proposed almost 3 decades ago.¹⁷ As shown in *Figure 2*, high glucose concentrations in non-insulin-dependent tissues may follow the pathway of aldose reductase. As this is a low-affinity enzyme, its activity is low when glucose concentrations are normal. Sorbitol is the product of this reaction, and nicotinamide adenine dinucleotide phosphate (NADPH) is used as a cofactor. In experimentally induced hyperglycemia in animals, increases in sorbitol formed through this reaction lead to altered cellular-energy metabolism, cell-membrane

integrity, and other functions. This is one possible biochemical mechanism by which hyperglycemia could impair the function and structure of the cells affected by diabetic complications.¹⁷ It has been proposed that increased sorbitol concentrations in certain tissues could result in osmotic changes promoting cell dysfunction. This mechanism seems to participate in cataract formation and nerve conduction impairment. Furthermore, the aldose reductase reaction uses NADPH, possibly shifting this coenzyme from other pathways (*Figure 2*). The recycling of the powerful antioxidant glutathione depends on NADPH supplies. In this way, an increase in the flow of metabolites through the sorbitol pathway may tilt the delicate balance of oxidants/antioxidants to the oxidative side.

Another reaction that uses NADPH is the synthesis of nitric oxide from arginine. Nitric oxide is the key vasodilator in the microcirculation; therefore, a shift in coenzyme availability might decrease nitric oxide synthesis and promote vasoconstriction and poor blood supply.^{17,28} Sorbitol can be further metabolized by sorbitol dehydrogenase, in which case the final products are fructose and the reduced form of nicotinamide adenine dinucleotide (NADH). Accumulation of NADH can be sensed as hypoxia by many cell pathways; the term *pseudo-hypoxia* has been coined for this situation. Further, high levels of NADH can keep pyruvate out of the mitochondrion by transforming it to lactate, and this is also a sign of pseudo-hypoxia.^{24,29} Based on these putative mechanisms, aldose reductase inhibitors (ARIs) such as tolrestat and others³⁰ have been used experimentally and in clinical trials for nearly 2 decades to treat hyperglycemia-related complications such as neuropathy. It is believed that ARIs most likely have a beneficial effect in the management of diabetic distal symmetrical polyneuropathy and autonomic neuropathy, but the clinical role of ARIs is to slow the progression of diabetic neuropathy rather than to reverse it. However, definitive evidence that ARIs prevent the development or progression of such complications is lacking, and none of the drugs is

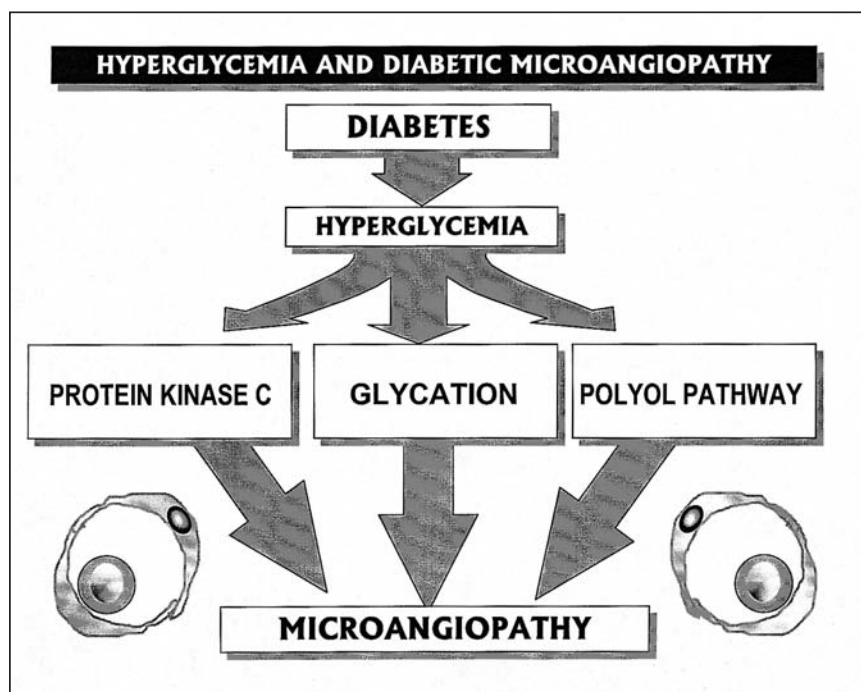


Figure 1. Author's illustration of three main pathways implicated in hyperglycemia-induced diabetic microvascular disease.

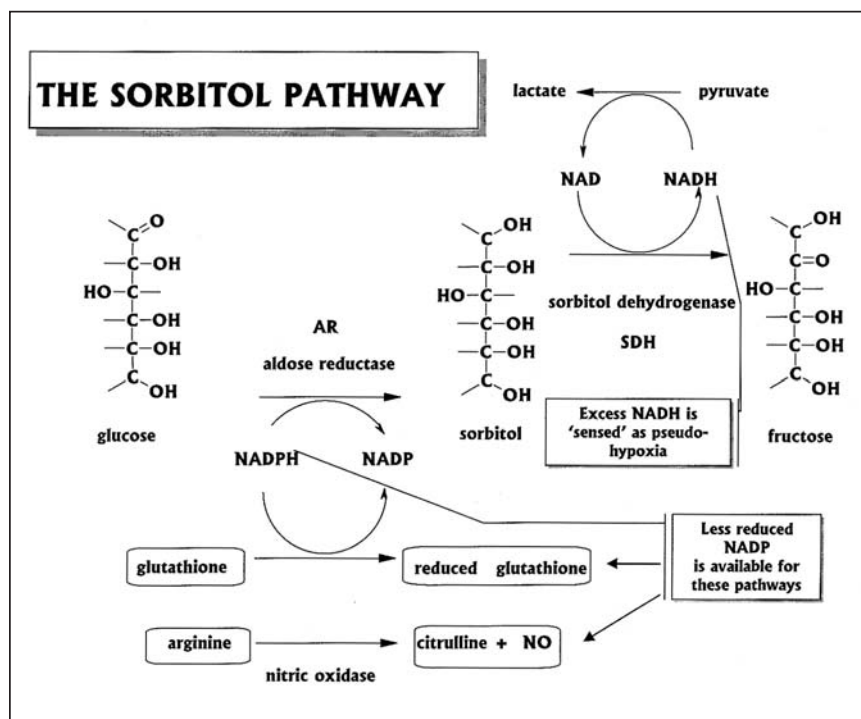


Figure 2. Author's illustration of the sorbitol (or polyol) pathway. In tissues where glucose uptake is nondependent on insulin, activation of this pathway may produce osmotic and metabolic changes implicated in the pathogenesis of cataracts and neuropathy.

available in the United States. While it is clear that the activity of this pathway is increased in humans with diabetes, evidence to support a clinical role for these effects is less definitive.^{18,19,30-32} It may be that ARIs need to be used for longer periods in better-defined populations before their efficacy is proven.

Protein kinase C activity—Another role of hyperglycemia appears to be the modification of protein kinase C (PKC) activity by hyperglycemia-induced increases in diacylglycerol, partly due to de novo synthesis. This chain of events should increase PKC activity.^{8,33,34} However, in tissues where aldose reductase levels are high, the opposite seems to be true (*Figure 3*). Decreased levels of myoinositol, probably shifted outside the cell when sorbitol levels increase, result in modification of phospho-inositol and diacylglycerol metabolism, which in turn affect PKC function.²⁴ Protein kinase C regulates various vascular functions by modulating enzymatic activities, such as cytosolic phospholipase A₂ and Na⁺/K⁺-ATPase, or gene expressions of extracellular matrix components and contractile proteins. When PKC activity is poorly regulated, some of the resulting vascular abnormalities include changes in retinal and renal blood flow, contractility, permeability, and cell proliferation.³⁵ Protein kinase C hyperactivity sensitizes vascular smooth muscle cells to vasoconstrictors and growth factors and thus promotes hypertension and atherogenesis. The first selective inhibitor of PKC has been produced and was used in animals in 1999.³⁶

Glycation hypothesis—The chemistry of early and advanced glycation end products (AGEs) is shown in *Figure 4*. In the glycation reaction, first discovered by Maillard in 1912 while studying foods, sugars react nonenzymatically with a wide range of proteins to form early glycation (Amadori or fructosamine) products.^{20,22} In humans, this process was first demonstrated for hemoglobin, but almost any protein can be affected.³⁷ Clinically, the measurement of the glycated form of hemoglobin, Hb A_{1c}, has revolutionized the monitoring and the study of patients with diabetes. Fruc-

tosamine (to be chemically correct, fructosamino-protein adduct) is the name given to any glycated plasma protein in this first stage. Measurement of glycated plasma proteins (usually called the “fructosamine assay”) is used as a tool for monitoring glycemic control (Figure 4) over a 3-week period.^{38,39}

The aforementioned reactions are considered “early glycation,” and they are by no means the end of the reaction cascade. In a second phase of the glycation pathway, a complex series of rearrangements and oxidative reactions leads to the formation of multiple, reactive species, collectively named AGEs,²¹ some of which are shown in Figure 4. Incidentally, a similar reaction, though more complete and produced by harsher conditions, occurs between sugars and proteins in foods—the final result being what we see in bread or piecrusts, for instance. The Maillard reaction also plays a part in the generation of brownish pigments in beer and cola drinks.

As mentioned, the reactive dicarbonyl intermediates, formed from Amadori products or from sugars, react with protein amino groups to form a variety of AGEs. Advanced glycation end products accumulate in vivo on vascular wall collagen and basement membranes as a function of age and levels of glycemia, and they are capable of producing cross-linking of proteins and have been shown to display diverse biological activities.⁴⁰⁻⁴⁴ Inherited differences in the ability to detoxify AGE intermediates might be one of the genetic factors responsible for the clinically observed large variability that the impact of a given level of glycemia has on diabetic complications.⁴⁵⁻⁴⁶

Advanced glycation end product molecules are found in plasma, cells, and tissues and accumulate in the arterial wall, the kidney mesangium, and glomerular and other basement membranes. Accumulation of AGEs in long-lived proteins contributes to the age-related increase in brown color, fluorescence, poor solubility of lens crystallins, and to the gradual cross-linking and decrease in elasticity of connective tissue collagens with age. These processes

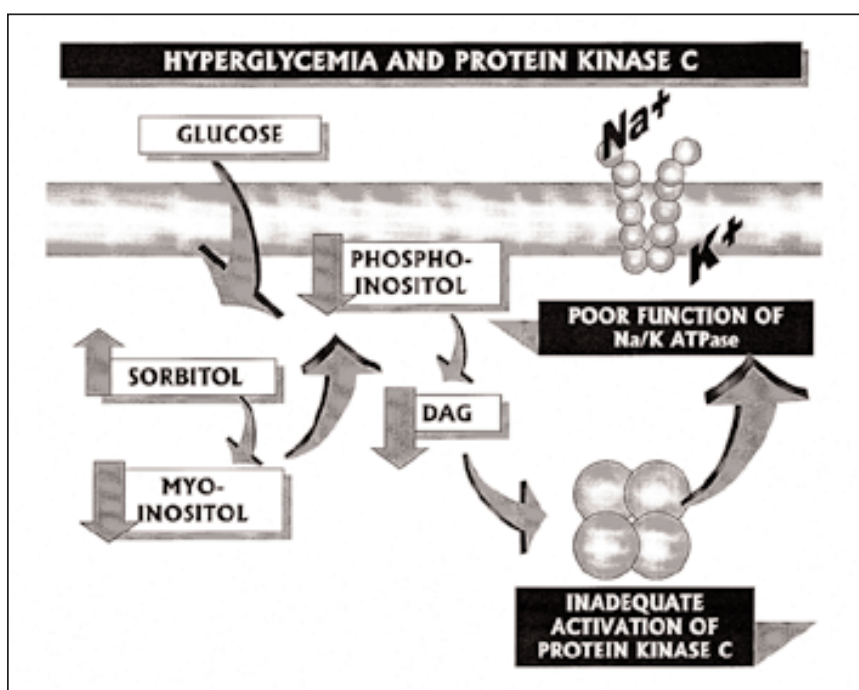


Figure 3. Author's illustration of protein kinase C in diabetes. Protein kinase C is a key component of an important cell-signaling pathway in tissues. High glucose may produce inadequate function of this enzyme that is involved in the pathogenesis of diabetic long-term complications.

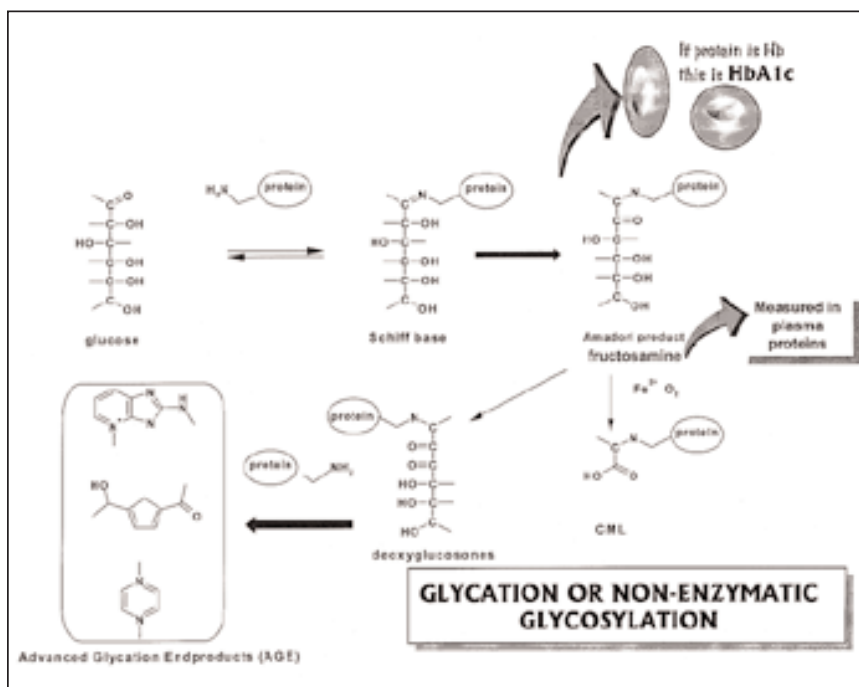


Figure 4. Author's illustration of glycation or nonenzymatic glycosylation. Glucose attachment to proteins occurs without intervention of enzymes; it depends on glucose concentrations. Used as an index of glycemic control in the form of Hb A_{1c} (glycated hemoglobin), this reaction is at the center of current theories proposed to explain diabetic complications.

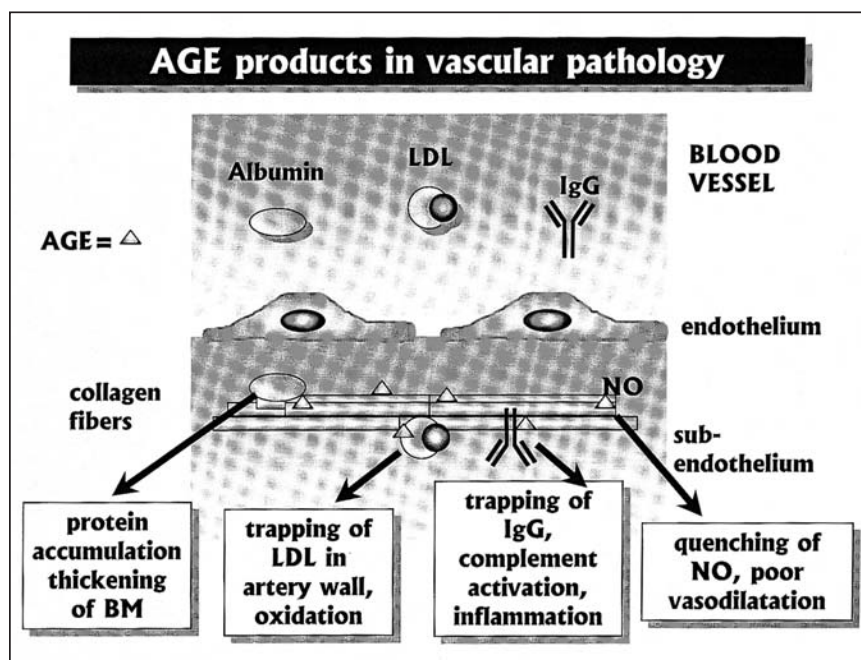


Figure 5. Author's illustration of advanced glycation products in vascular pathology. This diagram depicts some of the key points discussed in the text on the role of AGE products in microangiopathy as well as in macroangiopathy.

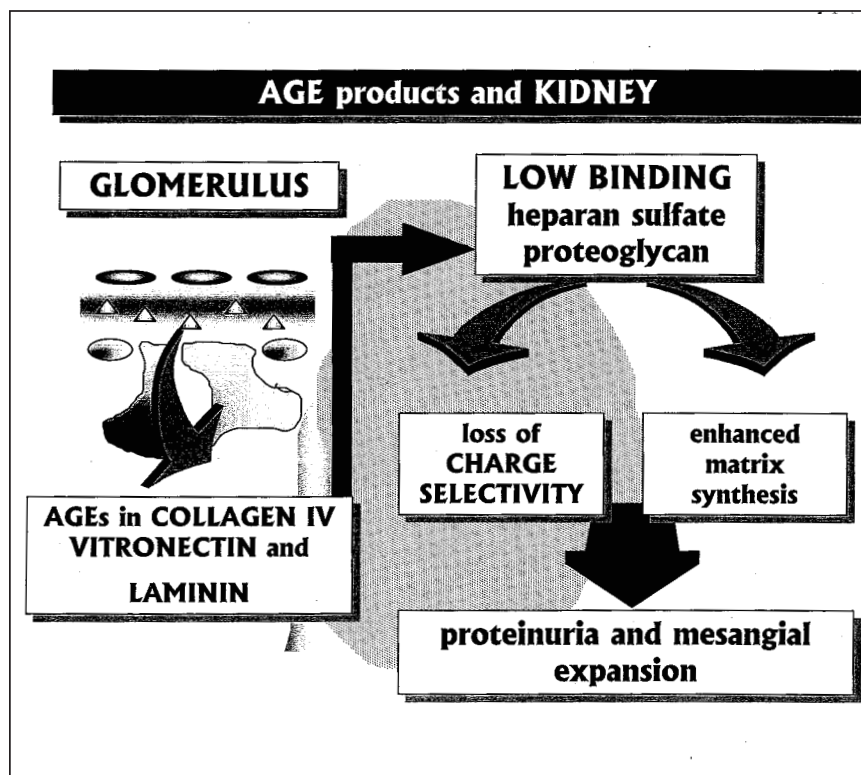


Figure 6. Author's illustration of advanced glycation products in nephropathy. This diagram outlines some of the main issues with regard to the role of AGE products in nephropathy.

are enhanced in patients with diabetes. Formation of AGEs increases at a greater rate than the increase in blood glucose; this suggests that even moderate elevations in diabetic blood glucose levels result in substantial increases in AGE accumulation.

As described, AGEs can be produced not only through direct action of sugars on proteins, but also via distinct oxidative reactions. Some authors coined a more general comprehensive term for these reactions: *carbonyl stress*.²⁵ The increase in glycoxidation and lipoxidation of tissue proteins in diabetes may accordingly be perceived as the consequence of enhanced carbonyl stress.⁴⁷

Direct effects of AGEs on proteins

Receptor-mediated effects

Direct effects of AGEs on proteins—Formation of AGEs modifies the functional properties of different key extracellular matrix molecules. In collagen (the most abundant protein in the body), AGEs form covalent, intermolecular bonds.^{48,49} As shown in Figure 5, luminal narrowing—a major feature in diabetic vessels—may arise in part from accumulation in the subendothelium of plasma proteins such as albumin, low-density lipoprotein (LDL), and immunoglobulin G (IgG). They may get trapped in basement membranes by covalently cross-linking to AGEs on collagen.^{50,51} It is known that the main features in diabetic glomerulopathy are proteinuria, mesangial expansion, and focal sclerosis.

Formation of AGEs on laminin (a key structural protein of the extracellular matrix) causes reduction in polymer self-assembly and decreased binding of the other major components of the molecular scaffolding of the basement membrane, namely type IV collagen and heparan sulfate proteoglycan.^{54,55} Heparan sulfate proteoglycan, which provides the negative charge of glomerular basement membrane, is the key factor impairing the leakage of plasma proteins and the resultant proteinuria.⁵⁶ As shown in Figure 6, diabetes-induced loss of matrix-bound heparan sulfate proteoglycan, secondary to AGE modification of glomerular base-

ment membrane proteins,⁵⁴ could prompt protein leaking and stimulate a compensatory overproduction of other matrix components in the vessel wall. This provides a strong molecular support to diabetic Kimmelstiel-Wilson nephropathy.⁵⁶ These AGE-induced abnormalities alter the structure and function of microvessels other than the renal microcirculation.⁵⁷ Finally, AGEs produce a dose-dependent quenching of nitric oxide (the major vasodilator), and in animals with diabetes, defects in the vasodilatory response to nitric oxide (see Figure 5) correlate well with the level of accumulated AGEs.⁵⁷⁻⁵⁹

Receptor-mediated effects⁶²⁻⁶⁸ (Figure 7)

Mononuclear cells—Monocytes and macrophages were first shown to bear specific receptors for AGEs. As shown on the right side of Figure 7, AGE proteins binding to these receptors⁶⁹ stimulate macrophage production of interleukin-1, insulin-like growth factor I, tumor necrosis factor α , and granulocyte/macrophage colony-stimulating factor at levels that have been shown to increase glomerular synthesis of type IV collagen and to stimulate proliferation of both arterial smooth muscle cells and macrophages.^{60,61}

Endothelium—As shown on the left side in Figure 7, reactive oxygen species are generated after AGE binding to endothelial cells, where they activate the free radical-sensitive transcription factor NF κ B, a multifaceted coordinator of numerous “response-to-injury” genes.⁶⁶⁻⁶⁷ These AGE-induced changes are involved in the modification of thrombomodulin and tissue factor production, and these alterations prompt two cumulative procoagulant changes in the endothelial membrane.⁷⁰ Concurrently, these AGE-induced alterations in endothelial cell function favor thrombus formation at sites of extracellular AGE accumulation.⁷⁰ The colocalization of receptors and AGEs at the microvascular sites of injury suggests that their interaction may play a significant role in the pathogenesis of diabetic vascular lesions.⁷¹⁻⁷³

Mesangial cells in kidney glomeruli—AGE receptors have also been described

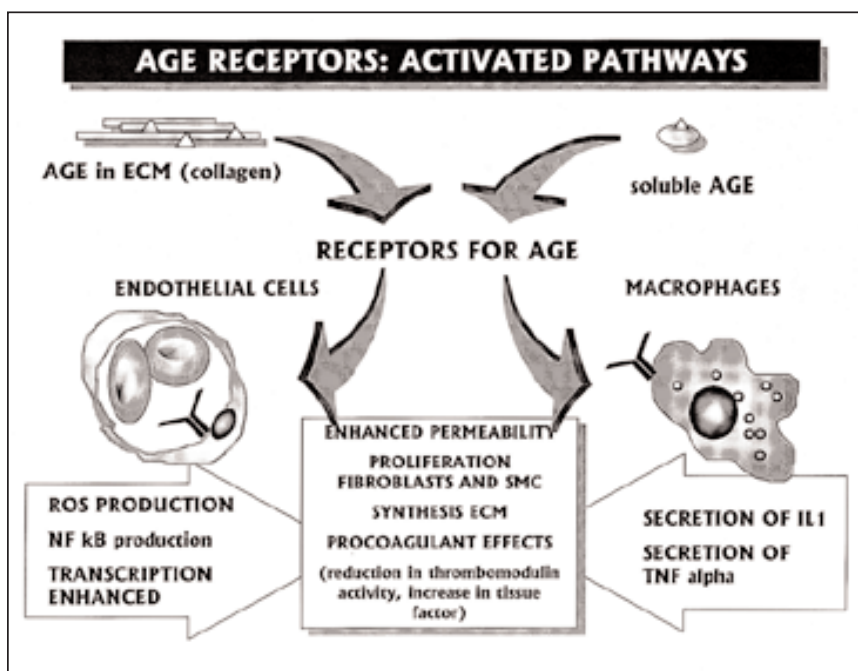


Figure 7. Author's illustration of the roles of receptors for advanced glycation products. Many cells bear receptors that recognize AGE. This diagram delineates some of the effects of this interaction with regard to microangiopathy.

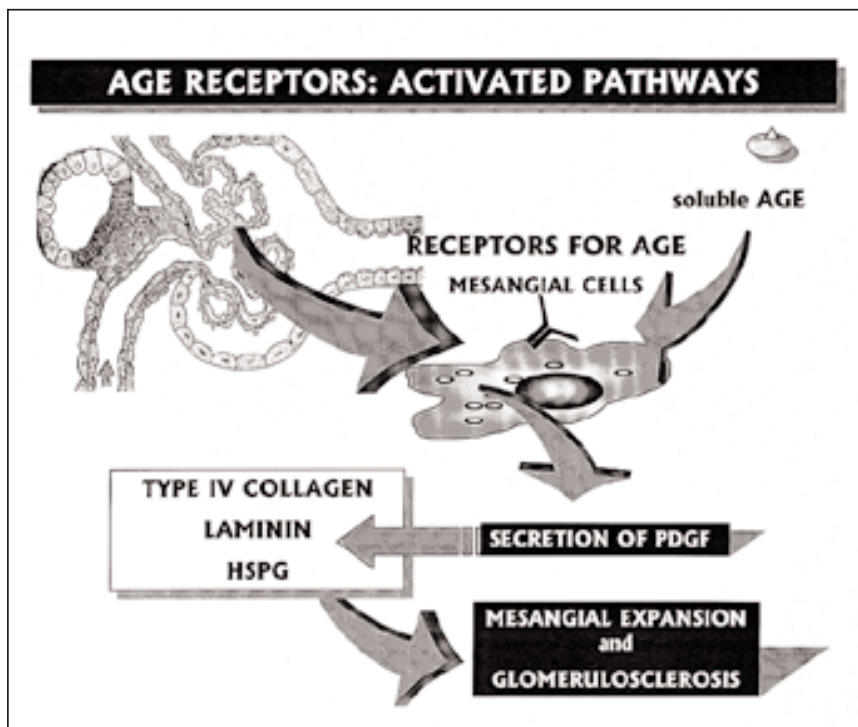
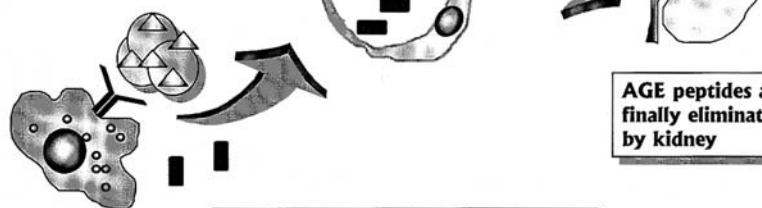


Figure 8. Author's illustration of receptors for advanced glycation products in kidney. Mesangial cells carry receptors that recognize AGE. This diagram depicts current knowledge on the effects of this interaction with regard to nephropathy.

AGE metabolism: AGE peptides as second generation glycating agents

Uptake of AGE proteins by macrophages

Partial degradation



Very reactive AGE peptides are liberated and circulate in plasma

They are **SECOND GENERATION** glycating agents, much more active than glucose

AGE peptides are finally eliminated by kidney

Figure 9. Author's illustration of advanced glycation products metabolism to small peptides. AGE peptides circulate and modify plasma and other proteins. This diagram summarizes in a schematic fashion current hypotheses on their metabolism.

RENAL HANDLING OF AGE-PEPTIDES

GLOMERULUS

AGE peptides in bloodstream

lumen
endothelium
GBM
podocyte

PROXIMAL TUBULE

AGE-peptides are filtered and then most of them are reabsorbed and catabolized by the proximal tubules

Figure 10. Author's illustration of advanced glycation peptides metabolism by the kidney. AGE peptides circulate and are filtered in the glomeruli.

on glomerular mesangial cells (Figure 8). AGE protein binding to their receptors on mesangial cells stimulates platelet-derived growth factor secretion, which in turn mediates mesangial expansion.^{54,74,75} In vivo, chronic administration of AGEs to otherwise healthy, euglycemic rats led to focal glomerulosclerosis, mesangial expansion, and proteinuria—the hallmarks of diabetic microangiopathy.⁷⁶

It has been demonstrated that AGEs form on cell proteins in vivo^{42,43,77} and on DNA in vitro.⁷⁸⁻⁸⁰ If AGEs also form on DNA in vivo, deleterious effects on gene expression may occur, and intracellular AGE formation on cell proteins may thus affect DNA function. The extremely rapid rate of AGE formation on liver histones points in this direction. The author has shown that histones from the livers of rats after 1 month of hyperglycemia showed AGE levels three times greater than those of their age-matched controls. Accumulation of AGEs on histones increased with the duration of the disease.⁸¹ This suggests a possible role for intracellular glycation in the increased teratogenesis associated with diabetes mellitus.⁸¹ Recently, increasing evidence suggests that glycation and oxidative stress may be linked to the sorbitol pathway, contributing to the development of diabetic complications. It must be remembered that fructose produced by the sorbitol pathway is a better glycation agent than glucose.⁸²⁻⁸⁵

"Second generation" glycating agents (Figure 9)

Glycation by glucose is slow when compared with many other monosaccharides. The emergence of glucose as the main circulating monosaccharide has indeed been proposed as an evolutionary advantage of higher forms of life, that is, we have the least toxic sugar in our circulation.⁸⁶ In the past few years, it has been shown that there are other reactive molecules in our bloodstream. Low-molecular-weight peptides that contain AGE circulate at increased levels in plasma from diabetic and kidney failure patients.^{87,88} These catabolic fractions of AGE-modified proteins bear dicarbonyl Maillard reaction intermediates, which

are a more aggressive menace to plasma and tissue proteins than the role formerly attributed to glucose.⁸⁹ Therefore, plasma proteins can become glycated by glucose itself or by the more potent “second generation” agents. For instance, AGE-peptides modified IgG in a rat with diabetes.⁹⁰ This change in IgG could lead to functional impairment of antibody molecules, and be linked to the well-known increase in susceptibility to infection seen in diabetic rats and humans. Further studies are needed to ascertain the correctness of this hypothesis.

It is believed that circulating AGE peptides are probably the result of incomplete catabolism of AGE proteins by macrophages and other cells that are on their way to be excreted by the kidneys (Figure 9). The author has shown that AGE peptides are filtered by the glomerulus and catabolized in part by the endolysosomal system of the proximal convoluted tubule, as shown in Figure 10. Reabsorption could represent an AGE-receptor-mediated mechanism triggering several cell responses, including cytokine secretion and oxidation reactions.⁸¹ It may be hypothesized that in diabetes, an increase in these processes could participate in the interstitial fibrosis reaction accompanying the characteristic glomerulosclerosis of end-stage renal disease.⁹²⁻⁹⁶ The increased tubular load of AGE peptides due to diabetes may overwhelm the whole process and lead to tubular disorders.⁹¹ AGE peptides increase in diabetes (excess of production) and in kidney failure (decreased excretion).

Finally, AGE peptides also bind covalently to phospholipids⁹⁷⁻⁹⁹ and react with membrane phospholipids if present in high local concentrations (such as shown in lysosomes) and if sufficient time is allowed. An accumulation of these adducts in tubular lysosomes might prove to be one further aggression to membranes and yet another process contributing to overall toxicity.

In addition to glucose-derived AGEs, the endogenously produced degradation products, AGE peptides, can amplify tissue damage and thus act as distinct toxins. The effects may particularly accel-

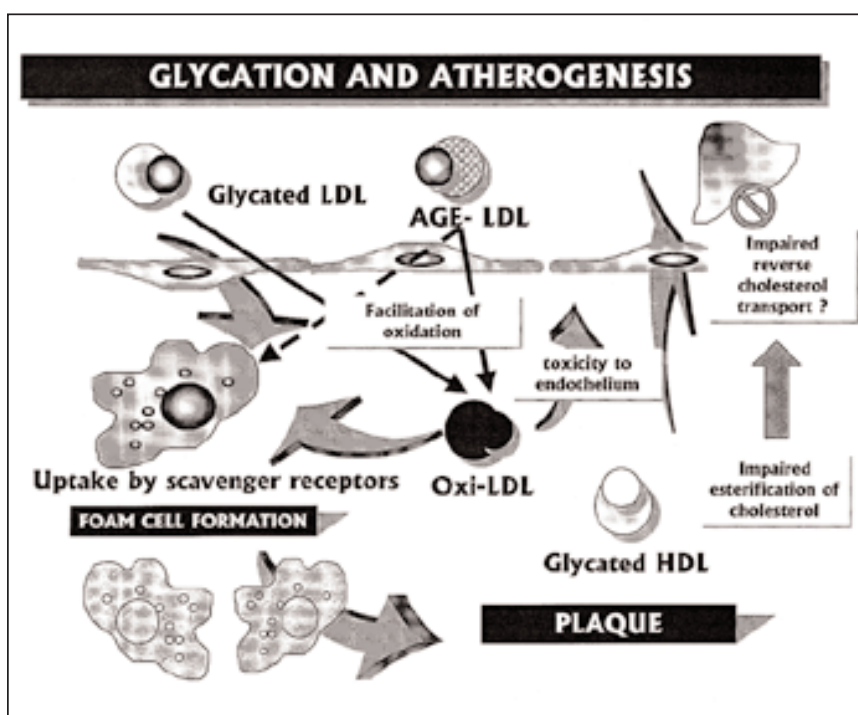


Figure 11. Author's illustration of glycation and macroangiopathy. Schematic view of the main pathways through which glycation of lipoproteins may enhance atherosclerosis.

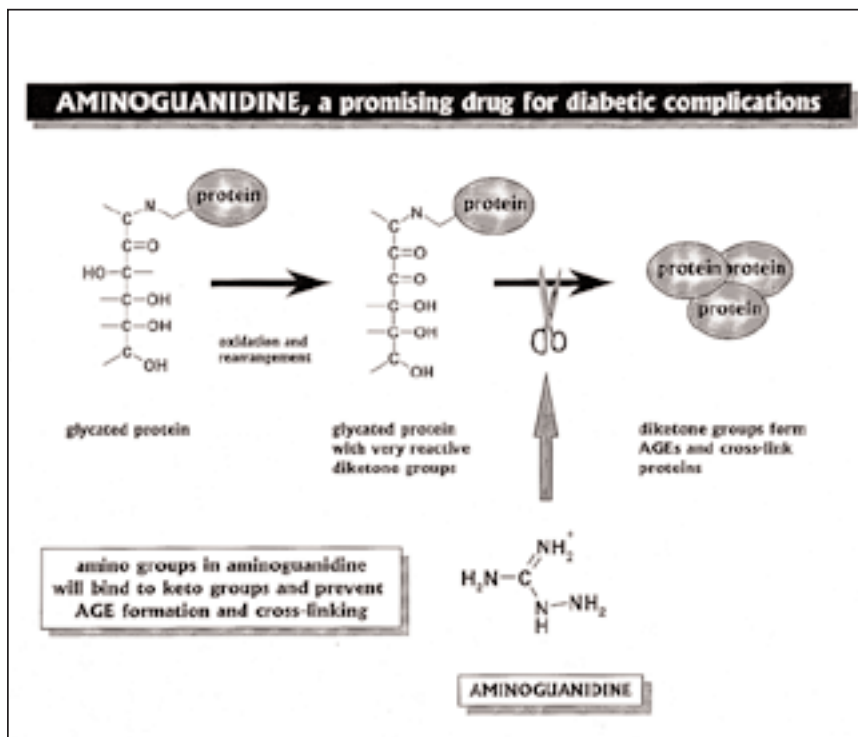


Figure 12. Author's illustration of how advanced glycation can be inhibited by aminoguanidine. This diagram illustrates the action of aminoguanidine as an effective agent preventing cross-linking of proteins by AGE.

erate the deleterious effect of glucose in certain individuals who are genetically susceptible to diabetic complications.

Macrovascular complications and hyperglycemia

Numerous questions remain unanswered with regard to the role of hyperglycemia in macrovascular complications seen in patients with types 1 and 2 diabetes and how treatment of hyperglycemia may affect these complications. With the ability to measure Hb A_{1c} levels, the DCCT found a 41% reduction in the risk for macrovascular events, which was not statistically significant because of the low frequency of these events in that population.^{11,12} Nevertheless, these data suggest a possible role for hyperglycemia in accelerating the atherosclerotic process in patients with type 1 diabetes. Epidemiologic analyses of UKPDS data have shown strong associations between blood glucose control and the risk of cardiovascular disease and all-cause mortality. There was a 16% reduction (not statistically significant) in the risk of myocardial infarction and sudden death in the intensively treated group. Nonetheless, these studies did not prove that high blood glucose causes these complications or that intensive treatment to lower glucose would reduce the risk.¹³⁻¹⁵ In the UKPDS, metformin decreased the risks of diabetes-related deaths and myocardial infarction when compared with other conventional treatments.

Proposed mechanisms linking hyperglycemia and atherosclerosis

Many of the pathways shown in *Figure 5* also apply to macroangiopathy. Arterial wall collagen bearing AGEs can trap LDL and IgG particles, which can accumulate in the intima. In this way, LDL particles would be prone to local oxidation and uptake by monocyte-macrophages. At the same time, endothelial cell activation may mediate the deposition of atheroma, since oxidized low-density lipoprotein causes endothelial cell activation.¹⁰⁰⁻¹⁰³ Moreover, activation of monocyte receptors by AGEs on vascular wall proteins (such as colla-

gen and elastin) would trigger the aforementioned sequence of cytokine-mediated inflammatory reactions.

Vascular diabetic complications may be due in part to chronic endothelial cell activation.^{102,104} The picture is incomplete as yet, for some mechanisms of endothelial cell activation have been observed only in vitro or in animals. Extensive literature points to a role for the glycation of lipoproteins in atherogenesis (*Figure 11*).¹⁰⁵⁻¹⁰⁷ Early glycation of apo B, apo A, and apo E has been described,¹⁰⁸ and abnormal metabolism of glycated forms of LDL and high-density lipoprotein (HDL) have been reported.^{105,107} Enhanced glycation may have direct effects and may also amplify the effects of oxidative stress on lipoproteins.^{63,97,109-111} Glycation has been shown not only to increase the susceptibility of LDL to oxidation, but also to enhance the propensity of vessel wall structural proteins to bind plasma proteins, including LDL, and thus to contribute to a more marked oxidative modification of LDL. Glycated and oxidized lipoproteins induce cholesteryl ester accumulation in human macrophages and may promote platelet and endothelial cell dysfunction.^{112,113}

With regard to HDLs, in vitro activation of lecithin-cholesterol acyltransferase by glycated apolipoprotein A-I (apo A-I is the major apoprotein in HDL) was lower than the activation by native apolipoprotein A-I.¹¹⁴ These data were confirmed by others in patients with diabetes.^{115,116} Because lecithin-cholesterol acyltransferase affords a driving force in reverse cholesterol transport, this abnormal activation may be associated with a reduction in reverse cholesterol transport and accelerated atherosclerosis in diabetic patients.

Even if it is too early to conclude that reduction of hyperglycemia has as great an impact on lowering macrovascular-disease risk as it has on microvascular-disease risk, these studies afford further stimulus to explore this issue. The Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction study showed that insulin-glucose infusion followed by intensive subcutaneous insulin

in diabetic patients with acute myocardial infarction improved long-term survival with an absolute reduction in mortality of 11%.¹¹⁷ More so, mortality in diabetic patients with acute myocardial infarction is predicted by age, previous heart failure, and severity of the glycometabolic state (Hb A_{1c}) at admission but not by conventional risk factors or gender.

The UKPDS showed that the impact of intensive pharmacotherapy in reducing cardiovascular complications remains unclear. Other factors that may predispose patients to cardiovascular complications, such as dyslipidemia, homocysteinemia, or hypertension, should also be contemplated in future studies. Aggressive treatment of blood pressure produces tangible benefits irrespective of the type of antihypertensive therapy.

Pharmacologic action against AGE?

Therapeutic agents that inhibit AGE formation have made it possible to investigate the role of AGEs in the development of diabetic complications in animals.¹¹⁸ The main AGE inhibitor is aminoguanidine, which has been studied in considerable detail. Aminoguanidine reacts mainly with dicarbonyl intermediates such as 3-deoxyglucosone, rather than with fructosamine products, on proteins (*Figure 12*).¹¹⁹ In addition to inhibiting AGE formation, aminoguanidine inhibits the inducible form of nitric oxide synthase in vitro. In vivo, however, concentrations ten times greater than those used to inhibit AGEs are needed to change nitric oxide concentrations significantly.¹¹⁹

The effects of aminoguanidine on the pathologic process of diabetes have been investigated in animals. The prevention of AGE formation by aminoguanidine treatment delays the evolution of the microvascular lesions in diabetic animals either in the retina or the glomeruli.^{120,121} Primary and secondary prevention with aminoguanidine has been successfully employed to ameliorate diabetic retinopathy in rats.^{122,123} In some studies, aminoguanidine reduced endothelial proliferation and completely arrested pericyte

dropout, but it did not completely attenuate progression of vascular occlusion.¹²⁴

When renal failure was produced in rats with streptozocin-induced diabetes by surgical reduction of renal mass and aminoguanidine was administered, the treated rats had significantly better survival rates than those of untreated, uremic rats with diabetes.^{125,126} The extended survival rate in the rats with uremia and diabetic nephropathy suggests that aminoguanidine may prove beneficial in humans with diabetes.

Other researchers investigated the effect of aminoguanidine on slowing of motor nerve conduction velocity of the sciatic nerve in rats with streptozocin-induced diabetes. Motor nerve conduction velocity was inversely correlated with AGE levels, and aminoguanidine improved nerve conduction probably through decreasing the AGE level in the peripheral tissues.¹²² Aminoguanidine may have therapeutic potential in controlling diabetic peripheral neuropathy.

As for whether AGE inhibitors also prevent diabetic complications in humans, clinical trials of inhibitors of this cross-linking have been inconclusive. The same caveats regarding interpretation of the results of trials of aldose reductase inhibitors thus apply to trials of inhibitors of AGE cross-linking. That is, longer trials in better-defined populations are needed before the effectiveness of these inhibitors can be proven. Some problems of toxicity have been encountered in a phase III clinical trial with aminoguanidine, so this drug should be considered a prototype for many new molecules that are being synthesized and tried in vitro at present.

Comments

Many factors are implicated in diabetic microangiopathy. However, the DCCT clearly showed in 1993 that strict glycemic control can delay the onset of complications or slow their evolution. This was corroborated for type 2 diabetes by the UKPDS published in 1998. Prevention of diabetic complications requires at least control of glycemia.

Hyperglycemia is regarded as a key (not the only) causal factor in the devel-

opment of diabetic vascular complications and can mediate its adverse effects through multiple pathways. A large body of evidence converges to point to glycation as one key molecular basis of diabetic complications due to hyperglycemia. Evidence from animal studies shows that the inhibitor of this process, aminoguanidine, attenuates the development of a range of diabetic vascular complications. When safe anti-glycation drugs become available, the root of the problem can be attacked instead of treating the end-stage pathologic process.

The evidence outlined in this article increasingly supports the contention that tight control of glucose results in fewer immediate and long-term complications, not only in type 1 but in type 2 diabetes. The first recommended glycemic goal for most patients with diabetes is to keep Hb A_{1c} to less than 2% above the upper limit of normal. Evidently, some patients cannot achieve this tight control. Furthermore, the intensity of therapy needs to be individualized and tailored to each patient. In this regard, the absolute benefits are substantial enough to warrant the intensive treatment necessary to achieve them.

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