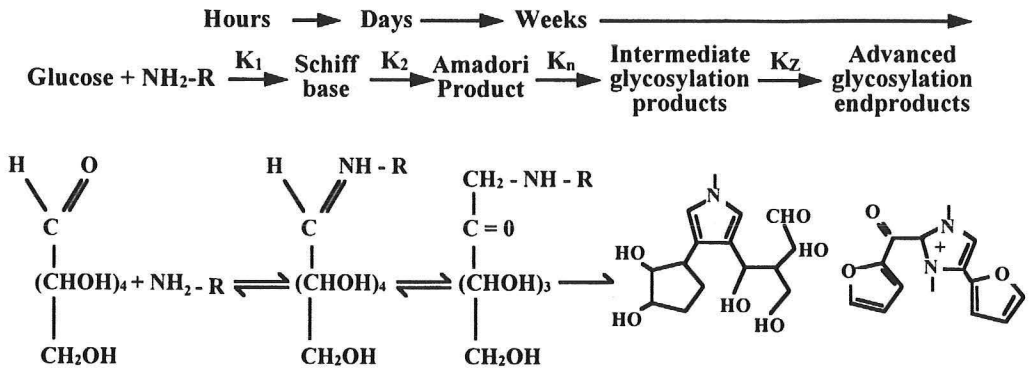
ROLE OF ADVANCED GLYCATION

END PRODUCTS (AGEs) IN DIABETIC

CARDIOVASCULAR AND RENAL DISEASE



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2) Regulation of Renal Phosphate Transport

3) Role of Cholesterol and Glycosphingolipids in Regulation of Renal Function

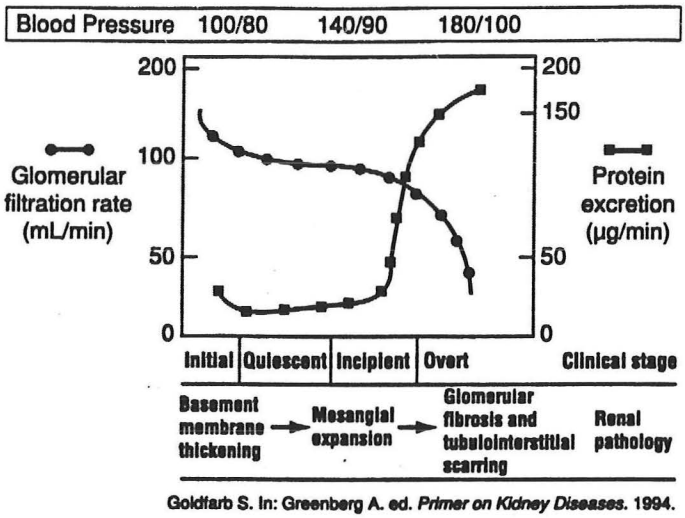
4) Early Detection of Peripheral Vascular Disease

INTRODUCTION

I) INCIDENCE OF DIABETIC CARDIOVASCULAR AND RENAL DISEASE

Diabetic nephropathy (defined as proteinuria, hypertension, and a decrease in glomerular filtration rate) develops in about 35% of patients with insulin-dependent diabetes mellitus (Type I, IDDM). The natural history of the renal disease is well established in IDDM and renal failure develops after 20 to 25 years after onset of the disease.

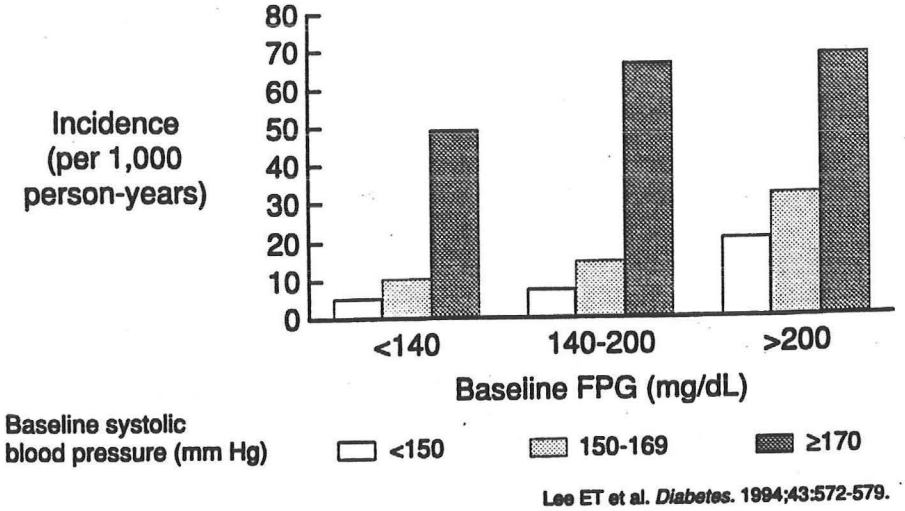
Progression of Untreated Diabetic Nephropathy



Goldfarb S. In: Greenberg A. ed. *Primer on Kidney Diseases.* 1994.

Non-insulin dependent diabetes mellitus (Type II, NIDDM) is also a major cause of renal disease; in fact, in the USA NIDDM accounts for more than 90% of subjects with chronic renal disease secondary to diabetes. Until recently although the course and determinants of renal failure in NIDDM were not clearly defined it was apparent that the time from apparent diagnosis to end stage renal disease (ESRD) was shorter than that for IDDM for two major reasons: **1)** The diagnosis of diabetes may not be made until 5 to 7 years after the true onset of disease; and **2)** Preexisting hypertension may accelerate the slope of progression to ESRD.

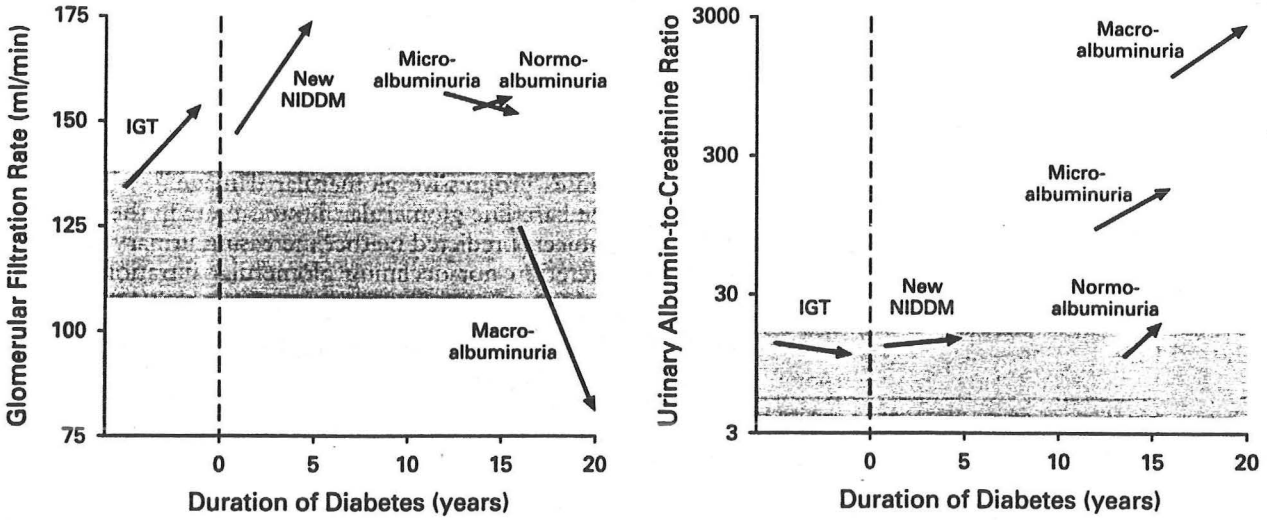
Incidence of Renal Failure 20 Years After Diagnosis of Type II Diabetes in Oklahoma Indians



Lee ET et al. *Diabetes*. 1994;43:572-579.

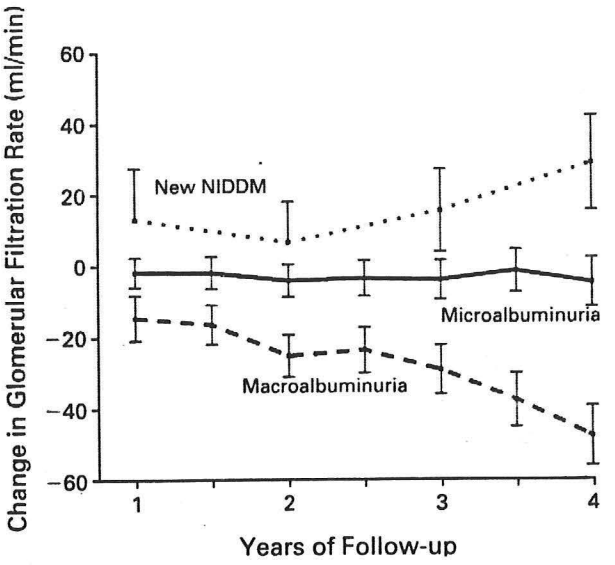
A recent study in Pima Indians with NIDDM has determined that the natural history of the renal disease in NIDDM is quite similar to that in IDDM. The study showed that the glomerular filtration rate is elevated at the onset of NIDDM and remains so while normal albumin excretion or microalbuminmia persists. However, it declines progressively after the development of macroalbuminuria (Nelson et al 1996).

RENAL DISEASE IN PIMA INDIANS WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS



Changes in the Mean Glomerular Filtration Rate and Median Urinary Albumin-to-Creatinine Ratio from Base Line to the End of Follow-up in Subjects with Impaired Glucose Tolerance (IGT), Newly Diagnosed Non-Insulin-Dependent Diabetes Mellitus (New NIDDM), NIDDM and Normal Urinary Albumin Excretion (Normoalbuminuria), NIDDM and Microalbuminuria, and NIDDM and Macroalbuminuria.

Each arrow connects the value at the base-line examination and the value at the end of follow-up. The dashed line indicates the time of diagnosis, and the shaded area the 25th through 75th percentiles of values in subjects with normal glucose tolerance. Albumin was measured in milligrams per liter and creatinine in grams per liter.

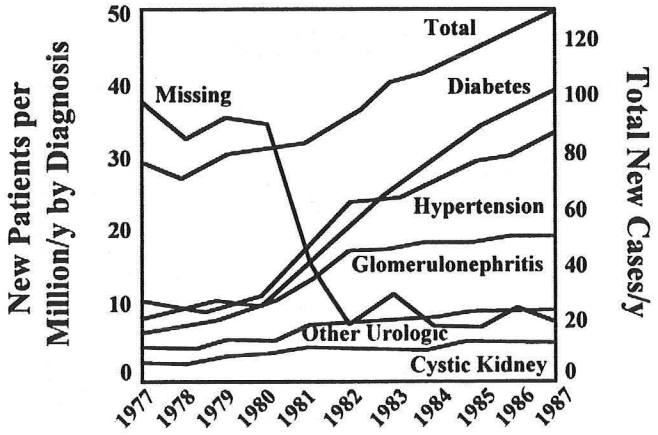


Mean (±SE) Change in the Glomerular Filtration Rate from Base Line during Four Years of Follow-up in Subjects with Newly Diagnosed NIDDM, NIDDM and Microalbuminuria, and NIDDM and Macroalbuminuria.

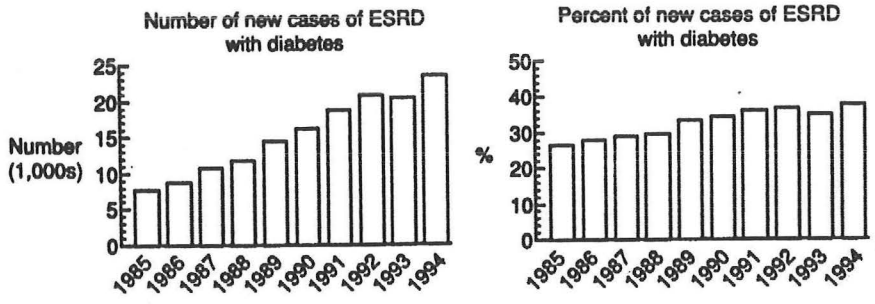
Lower error bars have been omitted where necessary to avoid overlap.

Diabetes mellitus (both IDDM and NIDDM) has become the leading and fastest growing cause of ESRD in the United States, requiring dialysis or transplantation for the maintenance of life.

Adjusted End-Stage Renal Disease Incidence Per Million by Primary Diagnosis, Adjusted for Age, Race, and Sex



Diabetes and End-Stage Renal Disease in the US*



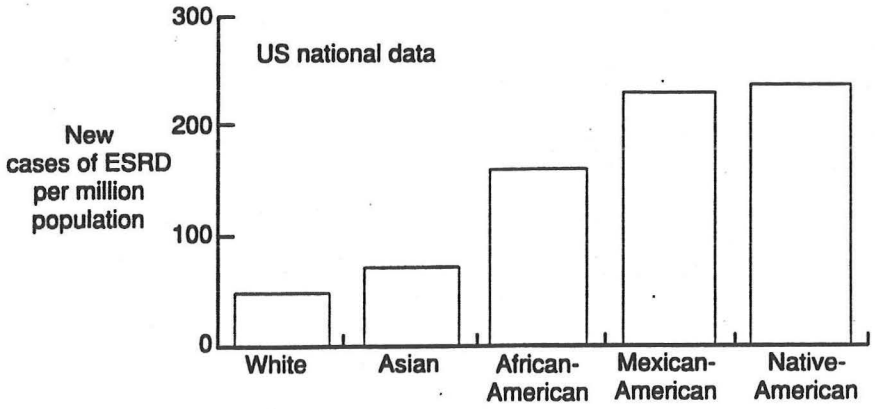
*Smaller studies indicate majority of increase is from Type II.

USRDS. *1994 Annual Data Report.* Renal Beneficiary and Utilization System, May 1996; Update 1989-94.

The data in Figure 6 reflect cases reported to the Health Care Finance Administration (HCFA) in the United States between 1985 and 1995. The data are for all types of diabetes. However, data from smaller studies indicated that type II diabetes (NIDDM) accounts for a majority of the disease-related increase in new cases of ESRD. The data in the left panel shows that the number of new cases of ESRD in diabetic patients tripled in one decade, reaching more than 23,000 per year in 1994. The data in the right panel shows that the percent of all ESRD cases that were accounted for by patients with diabetes rose from approximately 26% in 1985 to approximately 38% in 1994.

The United States Renal Data System (USRDS) indicates that ESRD is also more common in ethnic groups with high prevalence rates of NIDDM.

End-Stage Renal Disease and Diabetes: Five Ethnic Groups

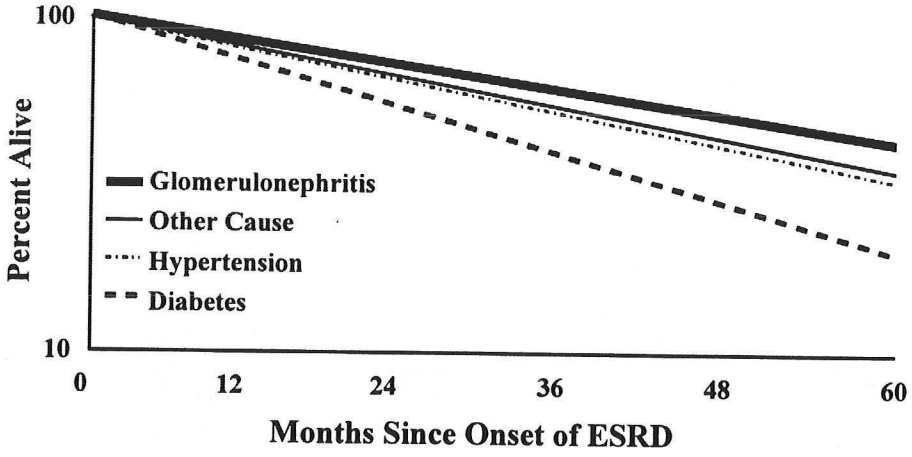


USRDS 1994.

The data in Figure 7 shows the number of new cases of ESRD in the population that were attributed to diabetes in five ethnic groups. Numbers are highest in-groups with the highest diabetes prevalence rates; however, a relative increase in diabetes-related ESRD in Mexican-Americans and African-Americans (3-5-fold the rate of whites) is greater than the relative increase in diabetes prevalence in these two groups (1.5-2-fold the prevalence in whites). This fact suggests that, in addition to higher prevalence rates of diabetes, other factors (for example, hypertension) contribute to the excess ESRD in these ethnic groups.

The importance of diabetes-induced renal disease is further amplified by the fact that USRDS data indicates that the survival of diabetic patients treated with any form of dialysis is greatly reduced compared with that of non-diabetic patients. Two-year survival rates are 51.4% for non-diabetics, and it is further reduced to 43.9% for diabetics.

Dialysis Patient Survival Estimates by Patient Age



The major cause of death in-patients with ESRD is cardiovascular disease, and the increased rate of mortality in diabetic patients may well be due to the fact that the relative risk of cardiovascular disease is markedly increased in diabetic compared with nondiabetic subjects.

Relative Risk of CVD in Diabetic vs Nondiabetic Persons: Framingham Heart Study

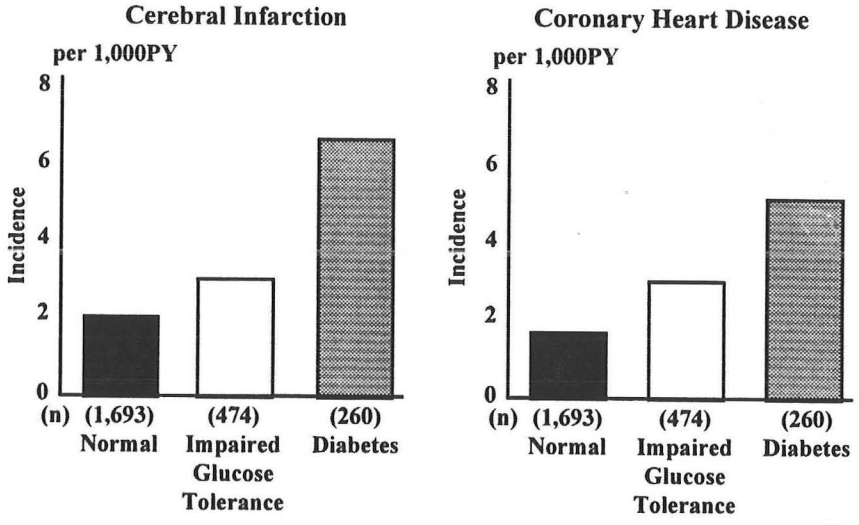
	Age-adjusted risk ratio	
Manifestation of CVD	Men	Women
Any CVD event	2.31*	2.47*
Stroke	1.51	1.82
Intermittent claudication	5.27*	2.60†
Cardiac failure	2.55†	4.92*
Coronary heart disease	1.73†	2.50‡
myocardial infarction	2.16‡	4.37*
angina pectoris	1.23	1.59
sudden death	2.51§	—
coronary mortality	2.38†	3.60†

*$P<0.001$, †$P<0.05$, ‡$P<0.01$, §$P<0.1$. Kannel WB et al. *Am Heart J.* 1990;120:672-674.

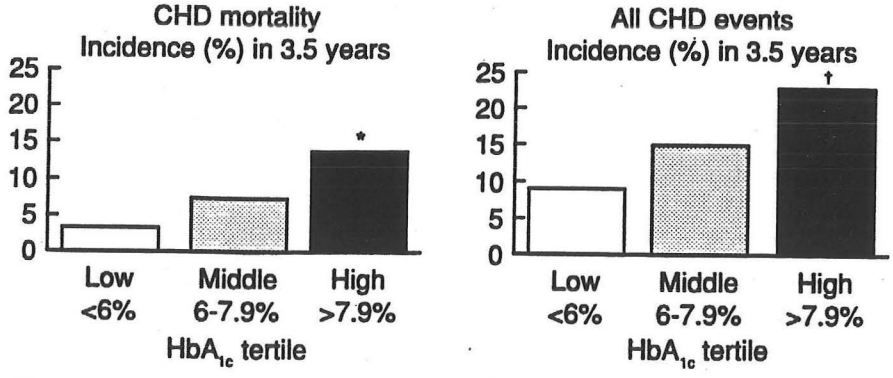
The Nature of Vascular Disease in Diabetes

- Microvascular disease (proven relationship to duration and severity of hyperglycemia)
  - nephropathy
  - retinopathy
  - neuropathy (related to altered metabolism in nerve)
  - peripheral microvascular disease and its contributions to limb loss
- Macrovascular disease (multifactorial determinants)
  - coronary artery disease
  - cerebrovascular disease
  - peripheral vascular disease

Age and Sex-Adjusted Incidence of Cerebral Infarction and Coronary Heart Disease per 1,000 Person-Years by Glucose Tolerance During the 5-Year Follow-up



HbA_{1c} Predicts Coronary Heart Disease in Type II Diabetes



*P<0.01 vs lowest tertile
†P<0.05 vs lowest tertile

Kuusisto J et al. *Diabetes.* 1994;43:960-967.

II) PATHOGENESIS OF DIABETIC CARDIOVASCULAR AND RENAL DISEASE

Extensive work in recent years, including in animal models of type I and type II diabetes mellitus, have shown that the pathogenesis of diabetic renal disease is multifactorial, as depicted in the following table.

MEDIATORS OF DIABETIC RENAL DISEASE

- **Genetic/Familial Predisposition**

- **Altered Intrarenal Hemodynamics**

- **Humoral Imbalance**
  Metabolic consequences of insulin deficiency
  Activation of Intrarenal cytokines or growth factors
    - Angiotensin II
    - Thromboxane
    - Nitric oxide
    - Insulin-like growth factor 1
    - Platelet-derived growth factor
    - Transforming growth factor-β

- **Activation of Pathways for Glucose Metabolism**
    - Aldose reductase-dependent polyol pathway (Increased sorbitol)
    - Pentose phosphate shunt (increased UDP glucose)
    - *De novo* synthesis of diacylglycerol and stimulation of Protein kinase C
    - Disordered cellular myo-inositol metabolism
    - Altered cellular redox state (Increased $NADPH/NADP^+$, $NADH/NAD^+$)
    - Altered glycosphingolipid metabolism
    - Renal tubular hypermetabolism and oxidant injury

- **Nonenzymatic Glycation of Circulating or Matrix Proteins**
    - Amadori-modified glucose adducts
    - Advanced glycosylation end-products (AGE)

In past Medicine Grand Rounds other members of the Nephrology Division and I have discussed the role of Lipids, Angiotensin II, and Growth Factors in the pathogenesis of diabetic renal disease. In this grand rounds I will discuss the role of Nonenzymatic Glycation of Circulating or Matrix Proteins, including Amadori-modified glucose adducts and advanced glycosylation end products (AGEs), in the pathogenesis of diabetic nephropathy and cardiovascular disease, as well as the role of AGEs in cardiovascular disease in diabetic and non-diabetic subjects with end stage renal disease ESRD. First though, I would like to include three cases, which will help illustrate the discussion, which will follow.

II) CASE PRESENTATIONS

Patient 1. M.R. is a 68-year-old Hispanic-American who has had Type II diabetes for 10 years. She has always been about 15%-20% above desirable body weight and has been unable to lose the extra pounds. She has been fairly well controlled on a sulfonylurea at gradually increasing doses. Hypertension was diagnosed about 3 years ago, and she was started on a generic thiazide (25 mg hydrochlorothiazide) that was covered by her health plan. Her control has deteriorated, and she is becoming symptomatic, with blurred vision, polyuria, nocturia, and fatigue despite weight loss of 5 lbs. over the last 6 weeks.

Physical Examination

Height: **5'3"**
Weight: **130 lbs.**
BP: **126/82 mm Hg**

On funduscopic examination, she has nonproliferative retinopathy. Cardiovascular examination reveals decreased pedal pulses and a right femoral bruit. On neurologic examination, she has absent ankle reflexes and decreased vibration sense. Touch sensation is normal.

Laboratory Tests

Urinalysis:	Normal
Microalbuminuria:	120 mg Protein/g Cr (normal < 30 mg/g)
Random plasma glucose	320 mg/dL
HbA_{1c}	10.8% (previously 7.8%)
Total cholesterol	240 mg/dL
LDL-C	160 mg/dL
HDL-C	30 mg/dL
Triglycerides	290 mg/dL

Patient 2. A 35-year old woman with a history of type-I diabetes mellitus since age 8 and diabetic nephropathy was evaluated for participation in a study of the relationship between levels of advanced glycation and renal-vascular complications of diabetes. Five years ago, uncontrolled diabetes (hemoglobin A_{1c} 11%) had been accompanied by mild hypertension, background retinopathy, proteinuria (1.5 g/24 hr), creatinine clearance 90 ml/min1.73 m^2, and an elevated serum cholesterol.

At the time of current evaluation, the patient had a blood pressure of 160/114 mm Hg and a regular heart rate of 72 beats/min. The rest of the physical examination was normal, as was her electrocardiogram. Laboratory evaluation revealed normal electrolytes with borderline hyperkalemia (5.1 mEq/liter); serum creatinine, 2.0 mg/dl; creatinine clearance, 70 ml/min; urinary albumin excretion (UAE) rate, 24 µg/min (normal < 15 µg/min or 22 mg/24 hr). Hemoglobin A_{1c} was 12.5%; serum LDL cholesterol, 208 mg/dl; HDL cholesterol, 49 mg/dl.

Laboratory Tests

- SK = 5.1 mEq/liter
- Scr = 2.0 mg/dl
- CrCl = 70 ml/min
- Urinary Albumin = 24µg/min (< 15 µg/min)

As part of the study, the level of hemoglobin-AGE was determined and was 9.7 U/mg hemoglobin (normal range: 3-5 U/mg). The patient agreed to participate in a 28-day double-blind placebo-controlled trial of an advanced glycation end product (AGE) inhibitor, aminoguanidine. After 28 days of treatment with aminoguanidine (400 mg orally twice daily), during which no adverse effects were noted, Hb A_{1c} was still 12%. Hb-AGE, however, had fallen to 5 U/mg and serum LDL cholesterol to 168 mg/dl (HDL cholesterol 52 mg/dl). (From Vlassara, Kidney International Nephrology Forum, 1996).

Laboratory Tests

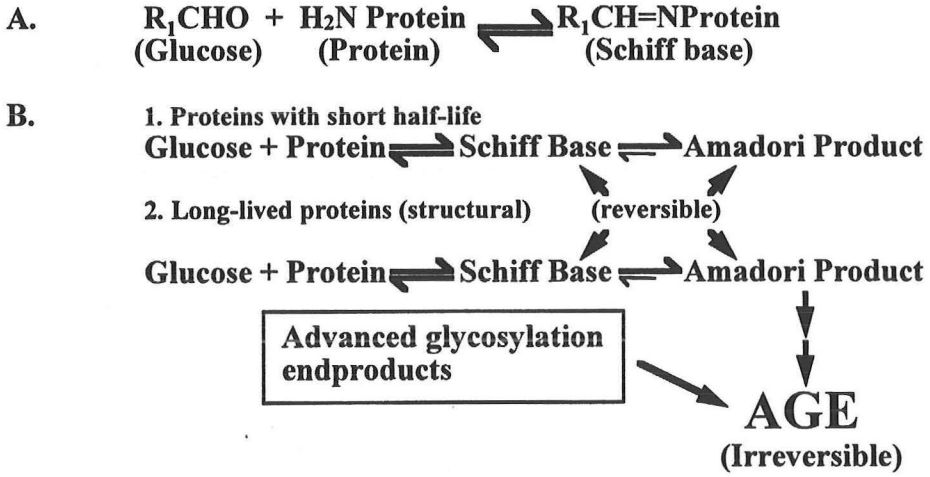
	Baseline	After Aminoguanidine
HbA_{1c} =	12.5%	12.0%
Hb-AGE =	9.7 U/mg	5.0 U/mg
LDL =	208 mg/dl	168 mg/dl
HDL =	48 mg/dl	52 mg/dl

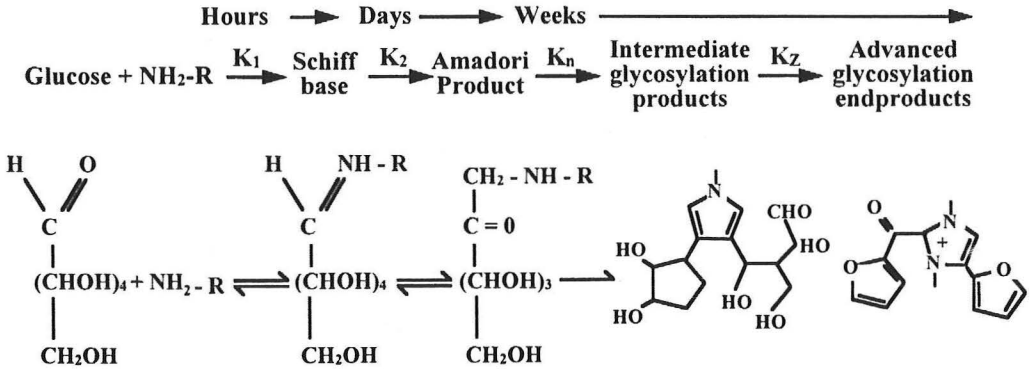
Patient 3. A 52-year-old non-diabetic woman with a history of chronic renal failure secondary to chronic pyelonephritis and hypertension had been treated with hemodialysis for one year. She was admitted to a study of the long-term effects of intensive hemodialysis on circulating levels of AGE-modified proteins and lipoproteins, known to be elevated in non-diabetic patients with end-stage renal disease (ESRD). At that time, the patient had a blood pressure of 165/100 mm Hg; pulse, 68 and regular; serum creatinine, 3.2 mg/dl; Hb A1, 4.1%; Hb-AGE, 5.5 U/mg; and serum AGE.88 U/ml (normal range 3-20 U/ml). Her serum LDL apolipoprotein B was elevated at 96 mg/dl, as was AGE-ApoB at 288 U/mg ApoB. Following a two-month period of therapy on a high-flux hemodialyzer (AN69), her Hb A1 and Hb-AGE were still within normal limits (3.6% and 4.0 U/mg, respectively). Total serum AGE was 60 U/ml and AGE-ApoB 210 U/mg ApoB. Interesting, serum LDL ApoB had fallen to within the normal range (50 mg/dl). (From Vlassara, Kidney International Nephrology Forum, 1996.)

III) ADVANCED GLYCOSYLATION END-PROUDUCTS

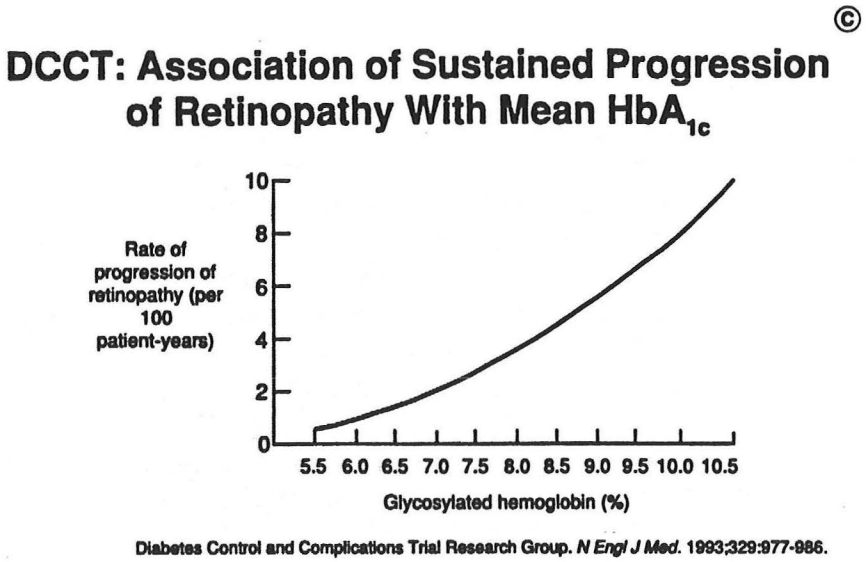
Much of the basis of advanced glycosylation chemistry has originated from the studies of the Maillard reaction (Maillard 1912). Even though the Maillard reactions have been of considerable interest to the food chemists since the turn of the century (also known as the Browning Reaction), an appreciation of Maillard-type reactions in living systems has occurred only over the last 15 years, and led directly from the realization that the products of advanced glycosylation form from the early products of nonenzymatic glycosylation. These early products are the Schiff bases and Amadori adducts that result from the covalent addition of reducing sugars to protein and phospholipid amino groups.

Outline of Nonenzymatic Glycation Reactions and How They lead to Formation of AGEs





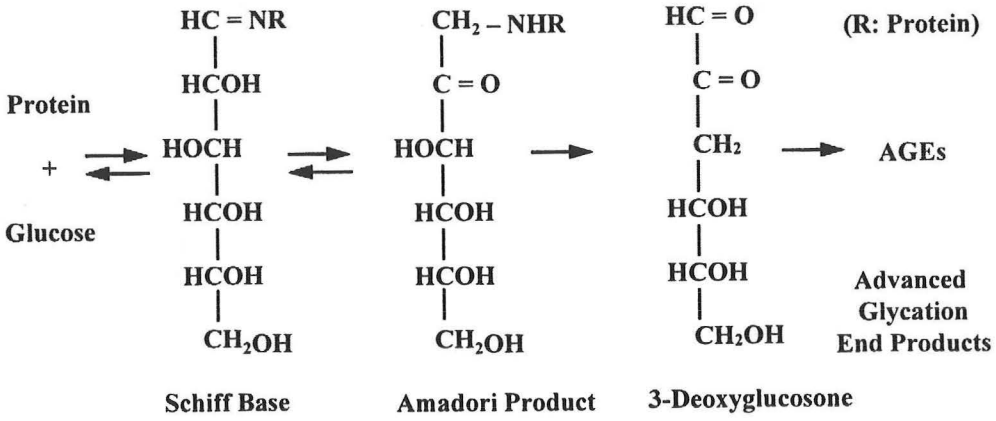
The hemoglobin Amadori product, HbA_{1c}, was the first protein identified to be modified by Amadori products *in vivo*, and its persistent elevation in diabetics has been utilized as an effective means to accurately monitor long-term glucose control (Bunn et al 1976; Bunn et al 1978; Higgins and Bunn 1981; Koenig et al 1982; Cohen 1986). Increasing HbA_{1c} values, indicating poor long-term glucose control have been associated with increased incidence of diabetic complications, including nephropathy, retinopathy, and neuropathy.



Overtime the Amadori product can undergo further rearrangement reactions. This leads to late products, AGEs, that have the capacity to covalently crosslink the proximate amino groups of proteins (Brownlee et al 1986, Brownlee et all 1988, Vlassara et al 1988, Kirstein et al 1990, Makita et al 1991, Monnier 1990, Monnier et al 1992, and Vlassara 1994) AGEs have been recently described also to form on aminophospholipids as well as on DNA bases (Bucala et al 1993, Papoulis et al 1995).

Although the precise identity of the major, reactive AGEs that form *in vivo* remains unsettled, increasing evidence has implicated α-diketone structures such as 1- and 3-deoxyglucosones and protein-bound dideoxyosones in the covalent crosslinks produced by the advanced glycosylation reaction (Bucala and Cerami 1992, Chen and Cerami 1993, Wells-Knecht et al 1994, Vasan et al, 1996).

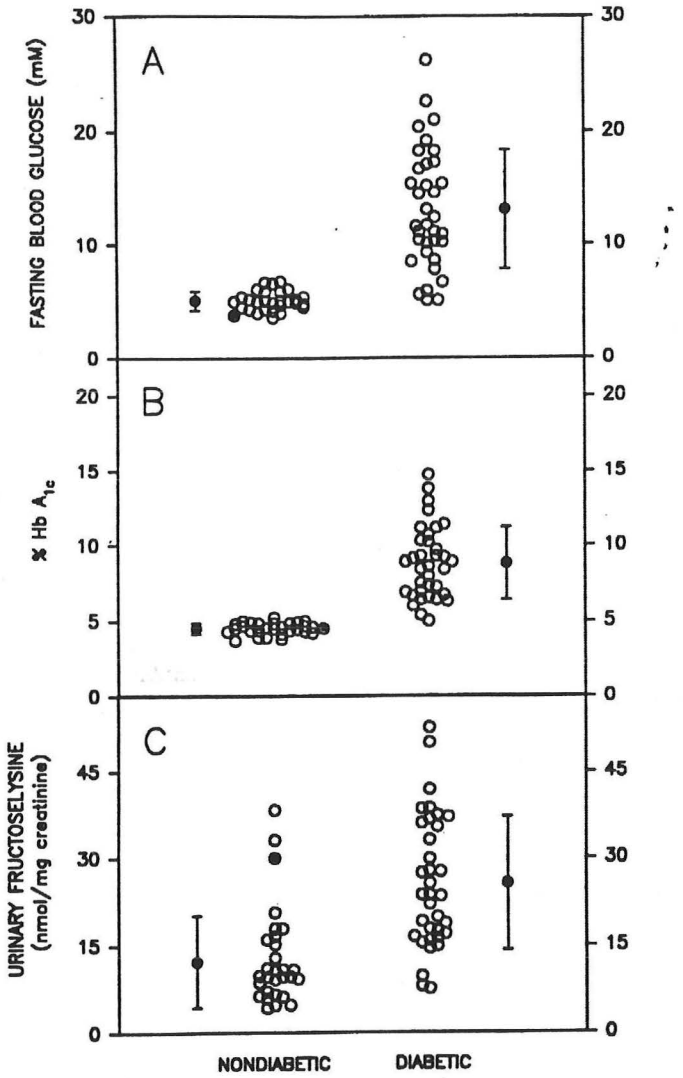
General Scheme of Glycation Reaction



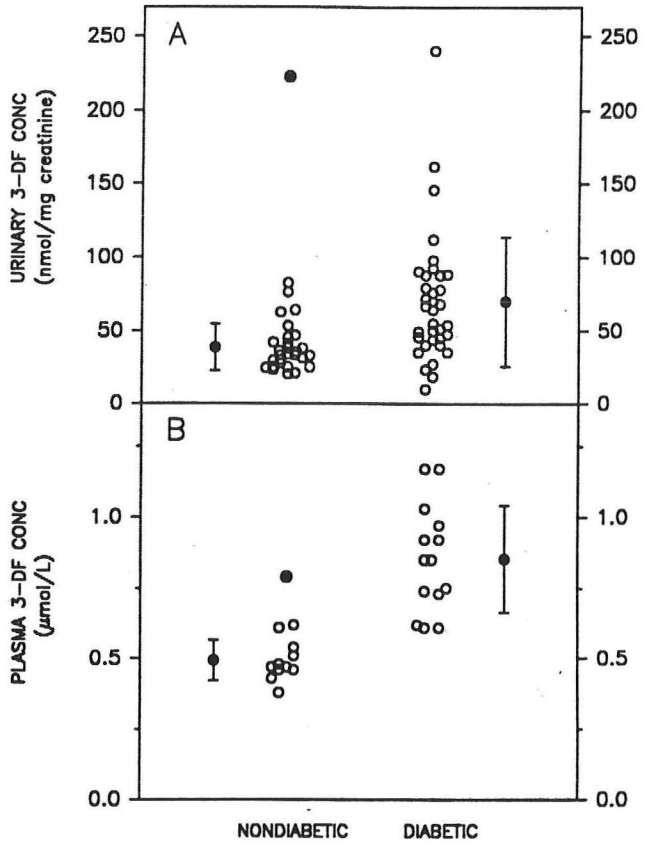
In vivo, the Amadori adduct appears to be the more significant precursor of AGE (Wells-Knecht et al, 1995), whereas *in vitro* it appears that about 50% of the AGE carboxymethyllysine originates from Amadori product oxidation, and 50% originates from other pathways, including from metal-catalyzed auto-oxidation of sugar, with glyoxal and arabinose as intermediates (Glomb and Monnier 1995). Interestingly, glucose has the slowest rate of glycosylation product formation of any naturally occurring sugar. The rate of AGE formation by such intracellular sugars as fructose, glucose-6-phosphate, and glyceraldehyde-3-phosphate is considerably faster than the rat for glucose (Monnier 1989). For this reason, the rate of intracellular AGE formation is muchmore rapid than the rate of AGE formation in the extracellular compartment.

3-deoxyglucosone (3-DG) may be formed *in vivo* from fructose, fructose 3-phosphate, or Amadori adducts to protein, such as N-fructolysine (FL), all of which are known to be elevated in body fluids or tissues in diabetes. Modification of proteins by 3-DG formed *in vivo* is thought to be limited by enzymatic reduction of 3-DG to less reactive species, such as 3-deoxyfructose (3-DF). In a recent study Wells-Knecht and coworkers measured 3-DF, as a metabolic fingerprint of 3-DG in plasma and urine from a group of diabetic patients and control subjects. (Wells-Knecht et al 1994). Plasma and urinary 3-DF concentrations were significantly increased in the diabetic compared with the control population (0.835 ± 0.189 vs. 0.494 ± 0.072 μM, $P < 0.001$, and 69.9 ± 44.2 vs. 38.7 ± 16.1 nmol/mg creatinine, $P < 0.001$, respectively). Plasma and urinary 3-DF concentrations correlated strongly with one another, with HbA_{1c} ($P < 0.005$ in call cases), and with urinary FL ($P < 0.02$ and $P = 0.005$, respectively). The overall increase in 3-DF

concentrations in plasma and urine in diabetes and their correlation with other indexes of glycemic control suggest that increased amounts of 3-DG are formed in the body during hyperglycemia in diabetes and then metabolized to 3-DF. These observations are consistent with a role for increased formation of the dicarbonyl sugar 3-DG in the accelerated browning of tissue proteins in diabetes.



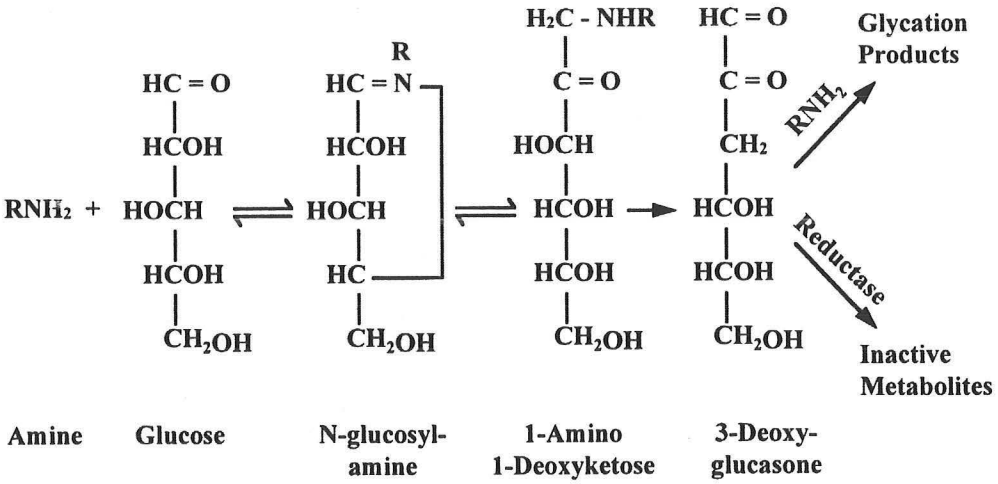
Fasting blood glucose, HbA_{1c}, and urinary FL concentrations in diabetic and nondiabetic subjects. Error bars and ● represent the mean ± 1 SD. An ICA-positive individual is classified on all graphs as nondiabetic, but with a unique symbol (⊙), and is not included in the statistical analyses. Results for diabetic patients (n = 35) compared with nondiabetic subjects (n = 29) were *A*: fasting blood glucose: 13.1 ± 5.3 vs. 5.1 ± 0.8 mM, $P < 0.001$; *B*: HbA_{1c}: 8.8 ± 2.4 vs. 4.5 ± 0.4%, $P < 0.001$; and *C*: urinary FL: 25.7 ± 11.4 vs. 12.2 ± 8.0 nmol/mg creatinine, $P < 0.001$.



Urinary and plasma 3-DF concentrations in diabetic and nondiabetic subjects. *A*: urinary 3-DF was elevated in diabetic patients (n = 35) compared with nondiabetic subjects (n = 29) (69.9 ± 44.2 vs. 38.7 ± 16.1 nmol/mg creatinine, $P < 0.001$). *B*: plasma levels of 3-DF were also elevated in diabetic subjects (n = 14) compared with nondiabetic subjects (n = 12) (0.853 ± 0.189 vs. 0.494 ± 0.072 μM, $P <$ 0.001).

As indicated above, increased levels of both 3-deoxyglucasone and methylglyoxal have been reported in diabetes (Thornally and Atkins 1989, Wells-Knecht et al 1994, Yamada et al 1994). Recently a 2-oxoaldehyde reductase has been isolated and cloned that reduces 3-deoxyglucasone to 3-deoxy fructose. This enzyme appears to be identical to aldehyde reductase (Takahashi et al 1993). Another enzyme, glyoxylose I, specifically converts methylglyoxal to D-lactate via the intermediate S-D-lactoylglulathione (Thornalley 1990).

Formation of AGEs from Glucose



The activity of these enzymes could be important determinants of the amount of AGEs that form at any given level of blood glucose in both diabetic and nondiabetic patients. It needs to be determined whether inherited differences in the ability to enzymatically detoxify AGE intermediates such as 3-deoxyglucosone may be an important genetic factor responsible for determining the impact of a given level of glycemia on diabetic complications.

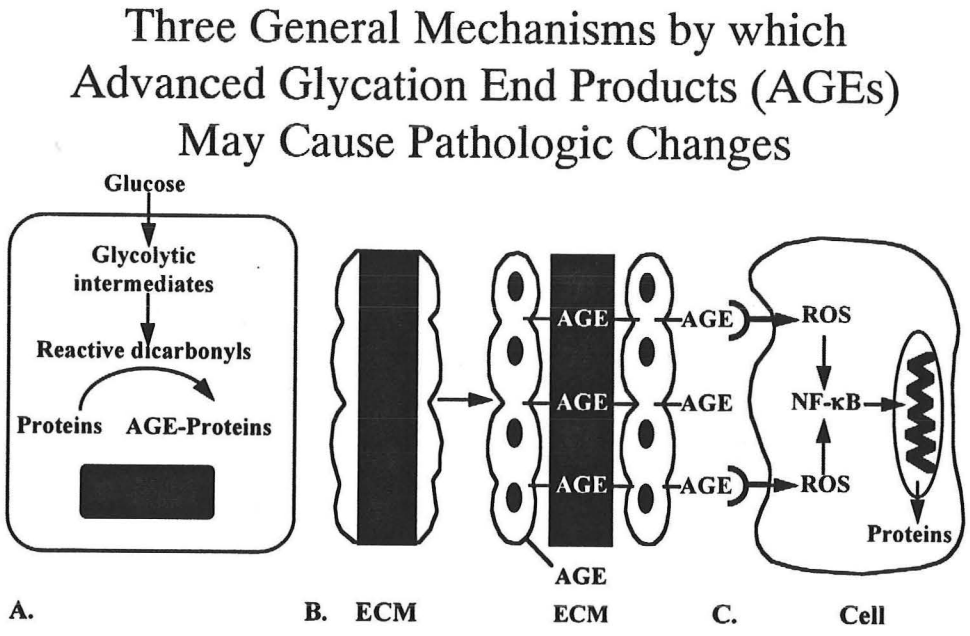
IV) MECHANISMS BY WHICH AGE FORMATION MAY CAUSE PATHOLOGIC CHANGES

There are at lease 3 general mechanisms by which AGE formation may cause pathologic changes (Brownlee 1996).

a) First, rapid intracellular AGE formation by glucose, fructose, and more highly reactive metabolic pathway-derived intermediates can directly alter protein function in target tissues.

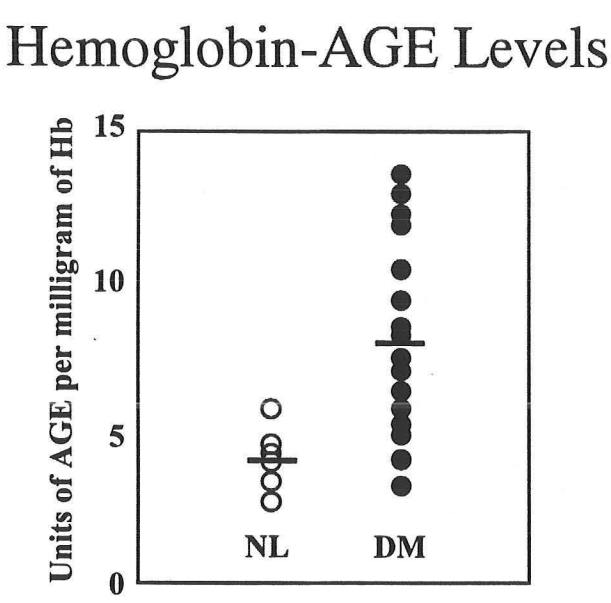
b) Second, extracellular AGEs alter matrix-matrix and matrix-cell interactions.

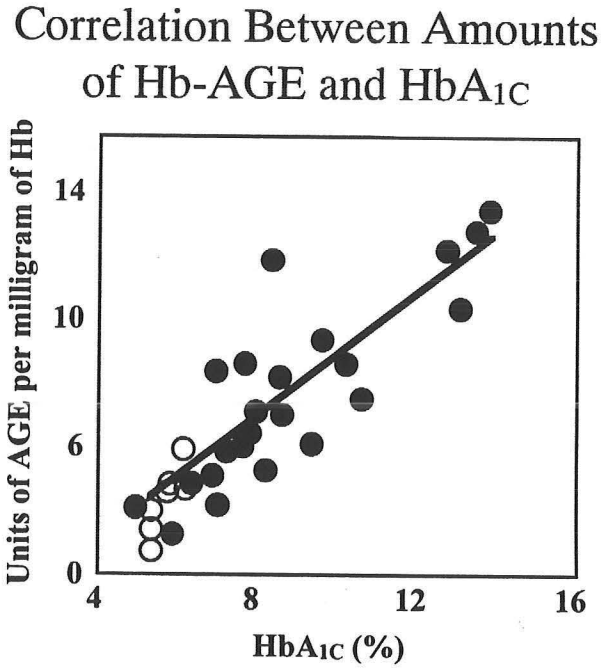
c) Third, AGEs alter the level of gene expression for a variety of molecules involved in the genesis of vascular pathology.



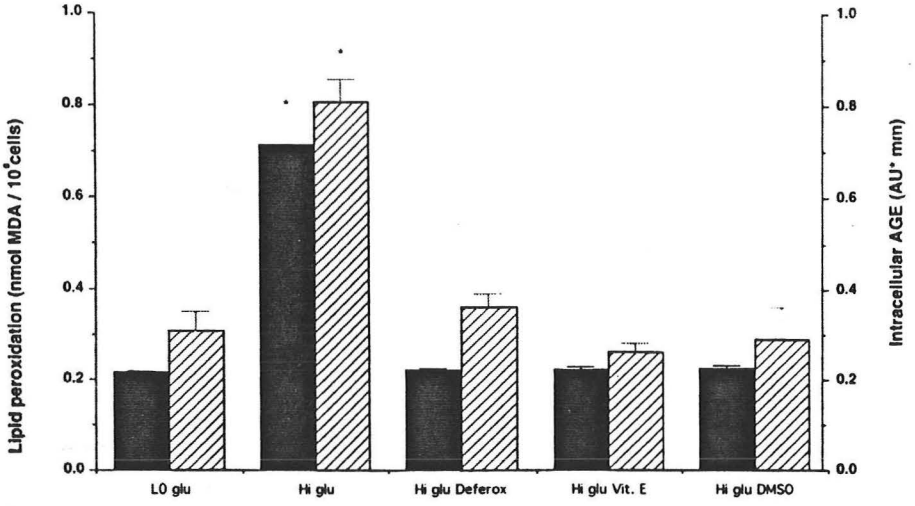
A) DIRECT ALTERATION IN PROTEIN FUNCTION BY INTRACELLULAR AGEs

The development of highly sensitive AGE-specific monoclonal and polyclonal antibodies and their use in immunohistochemistry and enzyme-linked immunosorbent assays have been highly instrumental in demonstrating that AGEs do form on proteins *in vivo*. In erythrocytes, AGE hemoglobin accounts for 0.42% of circulating hemoglobin in normal subjects and 0.75% in diabetics (Makita et al 1992).

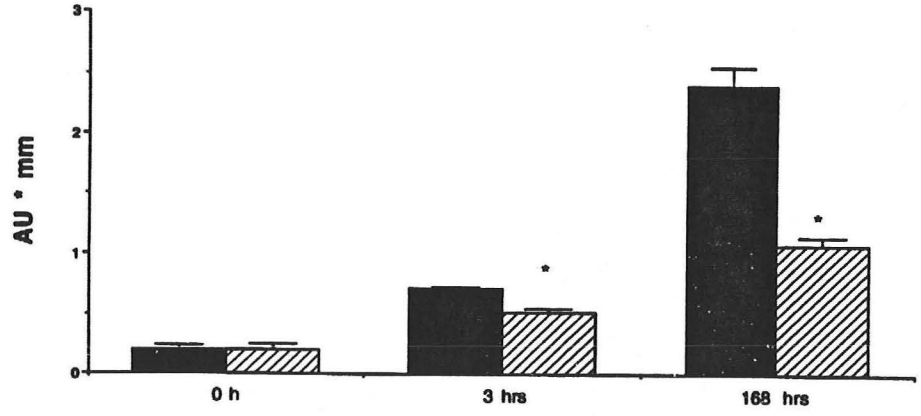




In endothelial cells, increase in AGE formation is even more pronounced. AGE content markedly increases in endothelial cells cultured in high glucose containing media. This extremely rapid rate of AGE formation most likely reflects hyperglycemia-induced increases in intracellular sugars, which are much more reactive than glucose, such as fructose, glucose-6-phosphate, and glyceraldehyde-3-phosphate. Interestingly, both antioxidants and/or the peroxidation-suppressing protoncogene bcl-2 profoundly inhibit hyperglycemia-induced intracellular AGE formation, demonstration that a reactive oxygen species-dependent process plays a central role in the generation of intracellular AGEs (Giardino et al 1996).



Effect of antioxidants on BAE intracellular ROS and AGE formation. BAE cells were incubated for 168 h in media containing either 5 mM glucose, 30 mM glucose or 30 mM glucose plus either 100 μM deferoxamine, 5 μg/ml α-tocopherol, or 100 mM DMSO. ROS levels (*solid bars*) were determined by FACS using the fluorescent probe DCF, and intracellular AGEs (*hatched bars*) were determined by scanning densitometry of immunoblots. The results expressed are mean±SE of three experiments. $*P < 0.001$, 30 vs. 5 mM glucose.



Effect of bcl-2 expression on intracellular AGE formation induced by 30 mM glucose. Gm7373 endothelial cells stably transfected with either Neo (*solid bars*) or Bcl-2 (*hatched bars*) were incubated for the indicated times in media containing 30 mM glucose. Intracellular AGE levels were determined by scanning densitometry of immunoblots. The results expressed are mean±SE of three experiments. $*P <$ 0.001, Neo vs. Bcl-2.

Intracellular AGE formation also effects DNA function. AGEs form on prokaryotic DNA *in vitro* and cause mutations and DNA transposition in bacteria and mammalian cells (Bucala et al 1984, Bucala et al 1985, Lee and Cerami 1987, and Bucala et al 1993).

B) INTERFERENCE WITH NORMAL MATRIX-MATRIX AND MATRIX-CELL INTERACTIONS

AGE formation alters the functional properties of several important matrix molecules including type I collagen, type IV collagen, and laminin. The functional and structural consequences of these alterations include **a)** increase in the permeability of the glomerular basement membrane, and **b)** the luminal narrowing of the blood vessels.

Formation of AGEs on extracellular matrix also interferes with matrix-cell interactions. AGE modification of type IV collagen's cell-binding domain decreases endothelial cell adhesion, and AGE modification of retinal basement membrane causes increased proliferation of retinal endothelial cells, the same pathology encountered in diabetic patients (Haitoglu et al 1992, Federoff et al 1993, Kalfa et al 1995, Brownlee 1996).

Effects of AGEs on matrix function

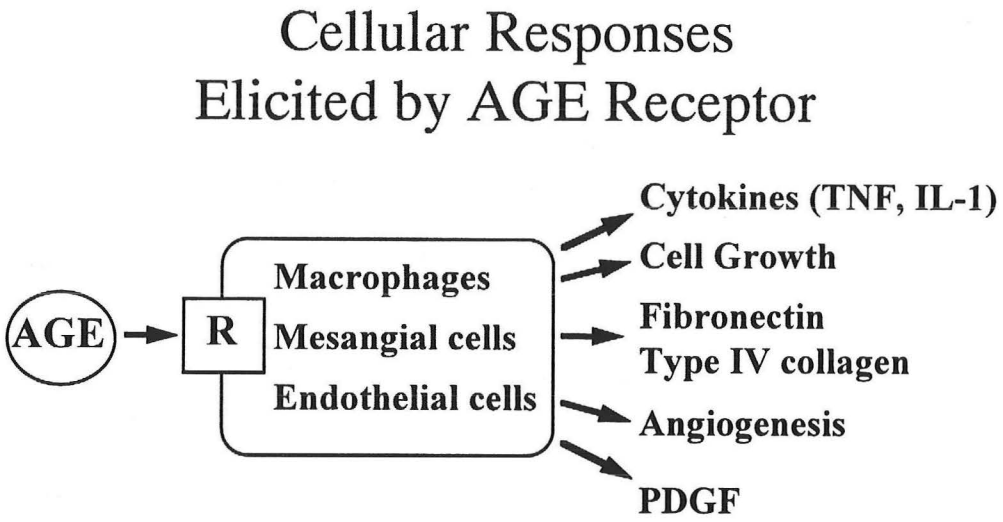
Collagen
Type IV ultrastructural assembly (↓)
Type I intermolecular spacing (↑)
Type I immobilization of soluble proteins (↑)
Type IV endothelial cell adhesion (↓)

Vitronectin
Binding of heparin (↓)
Binding of type IV collagen (↓)

Laminin
Polymerization/self-assembly (↓)
Binding of type IV collagen (↓)
Binding of heparin sulfate (↓)
Stimulation of neurite outgrowth (↓)

Matrix
Quenching of nitric oxide (↑)
Arterial wall elasticity (↓)
Arterial wall fluid filtration (↑)

C) MEDIATION OF PATHOLOGIC CHANGES IN GENE EXPRESSION BY ADVANCED GLYCATION END PRODUCT RECEPTORS



Specific receptors for AGEs were first identified on monocytes and macrophages. Two AGE binding proteins (60-kD and 90-kD) isolated from rat liver are present on monocytes and macrophages. AGE protein binding to this receptor stimulates 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 (Vlassara et al 1985; Yang et al 1991, Vlassara et al 1988, Kirstein et al 1992, and Yui et al 1994).

AGE RECEPTOR EXPRESSION AND FUNCTION

Cell type

Monocyte/macrophage

Species/AGE receptor components

Mouse, rat, human/p60/OST, p90/80K-H
galectin-3, RAGE

Function

- AGE-ligand binding, endocytosis, degradation
- Cytokine production (TNFα, IL-lα)
- Growth factor induction (PDGF, IGF-I)
- Chemotaxis
- Upregulation by TNFα
- Downregulation by insulin

Vascular endothelial cells also express AGE-specific receptors. A 35-KD and a 46-KD AGE binding protein have been isolated from endothelial cells (Schmidtetal 1992, Neeper et al 1992, and Schmidt et al 1996). In endothelial cells, AGE binding to its receptor induces changes in gene expression that include alterations in thrombomodulin, tissue factor, and vascular cell adhesion molecule 1 (VCAM-1). These changes induce procoagulatory changes in the endothelial surface and increase the adhesion of inflammatory cells to the vessel wall (Exposito et al 1992, Vlassara et al 1995, Schmidt et al 1995, and Nautler et al 1996).

AGE RECEPTOR EXPRESSION AND FUNCTION

Cell type

Endothelial cell

Species/AGE receptor components

Rat, human, bovine/p60/OST
P90/87K-H, galectin-3, RAGE

Function

- Ligand binding, transcytosis, degradation
- ↑Permeability
- ↑Tissue factor, ↓ thrombomodulin
- ↑VCAM-1, ↑ICAM-1

AGE receptors have also been identified on glomerular mesangial cells. AGE protein binding to its receptor on mesangial cells stimulates platelet-derived growth factor secretion, which in turn mediates production of type IV collagen, laminin, and heparin sulfate proteoglycan (Skolnik et al 1991 and Doi et al 1992). Long-term administration of AGEs to normal rats causes focal glomerulosclerosis, mesangial cell expansion, and alberminuria (Vlassara et al 1994). In addition AGEs cause an increase in glomerular type $\alpha 1$ (IV) collagen, laminin B1, and transforming growth factor β_1 mRNA levels (Yang et al 1994).

AGE RECEPTOR EXPRESSION AND FUNCTION

Cell type

Mesangial cells

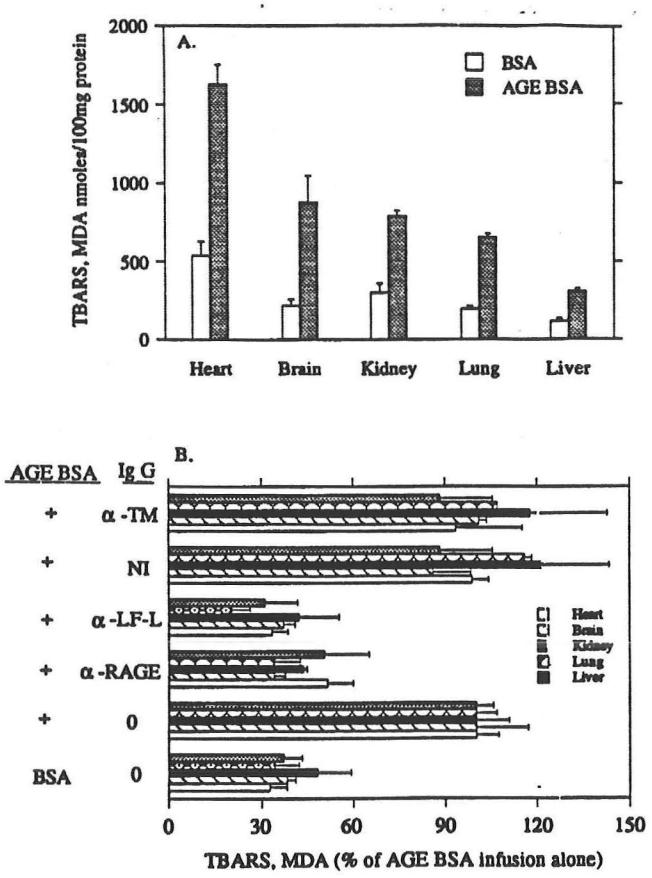
Species/AGE receptor components

Mouse, rat, human/p60/OST, p90/87K-H
Galectin-3, RAGE

Function

- Binding, endocytosis, degradation
- ↑Fibronectin, ↑collagen IV, ↑laminin
- Growth factor induction (PDGF, TGFβ1)

The AGE receptor appears to mediate signal transduction through the generation of oxygen free radicals. In cell culture reactive oxygen specific (ROS) are generated by AGE binding to endothelial cells (Giardino et al 1996). *In vivo* administration of AGE-modified BSA causes reactive oxygen species generation in all main target organs (Yan et al 1994).



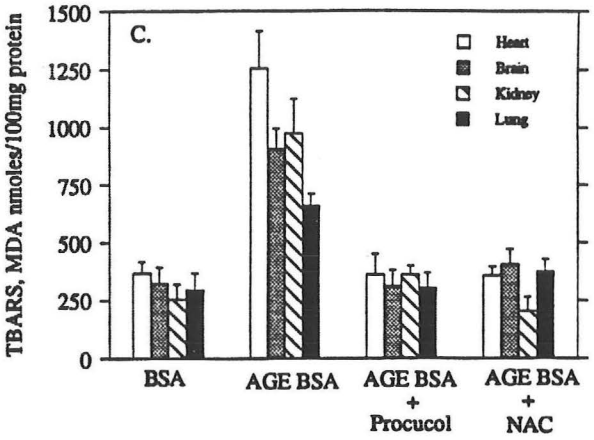
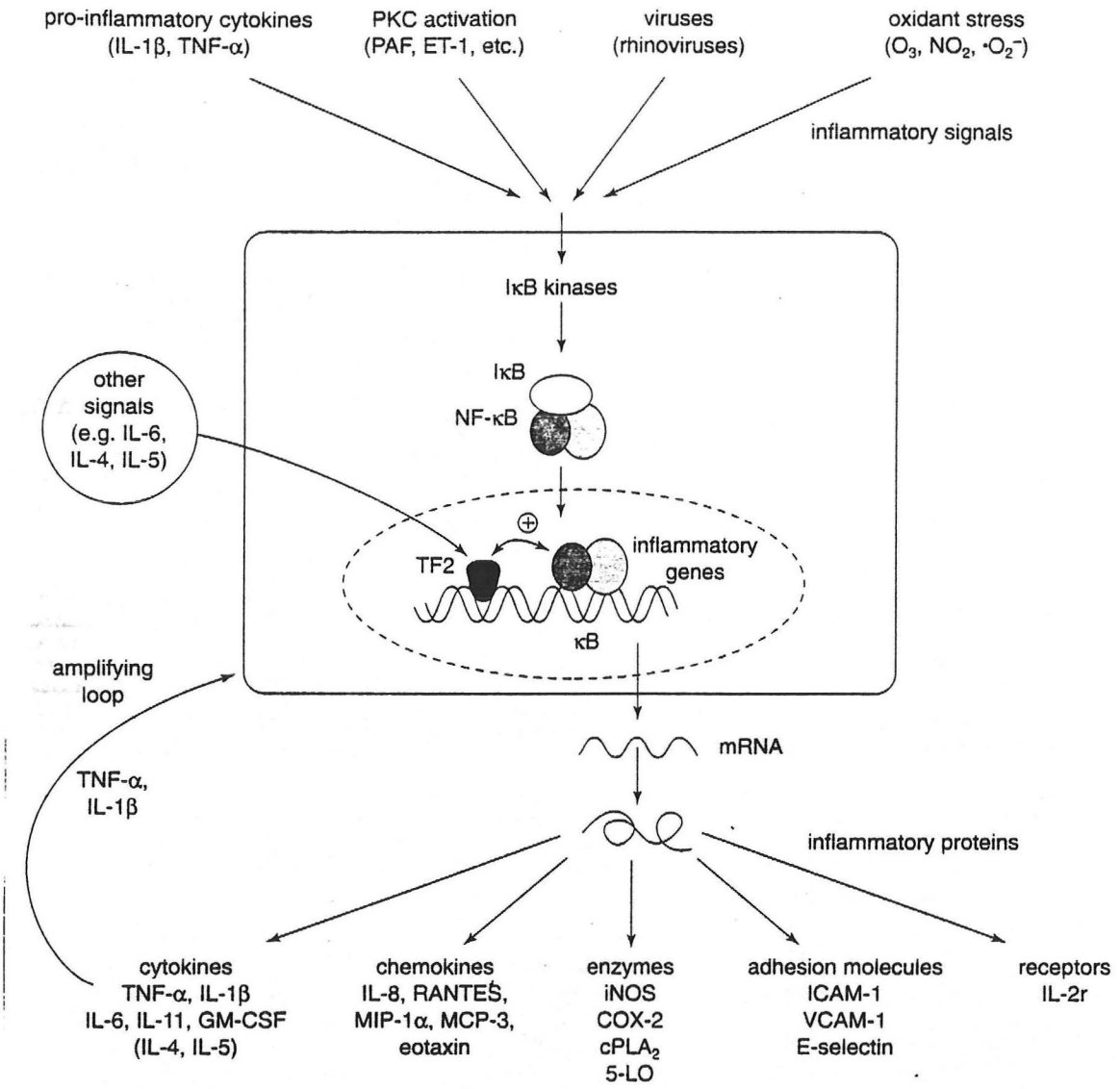


FIG. 7. **Generation of TBARS in mice infused with AGE albumin.** *A*, mice were infused with either AGE albumin (*AGE BSA*; 100 μg/animal; intravenous) or native albumin (*BSA*; 100 μg/animal; intravenous). Organs were harvested 60 min later for assessment of the formation of TBARS. *B*, mice were infused with AGE albumin as above, except, as indicated, animals were pretreated with anti-RAGE IgG (*α-RAGE*; 40 μg/animal; intravenous), anti-LF-L IgG (*α-LF-L*; 40 μg/animal; intravenous), anti-thrombomodulin IgG (*α-TM*; 40 μg/animal; intravenous), or nonimmune IgG (*NI*; 40 μg/animal; intravenous). The indicated organs were harvested 60 min later for determination of TBARS. *C*, mice were infused with AGE albumin (*AGE BSA*) as above, except, as indicated, animals were pretreated with probucol (50 μM; intravenous) or *N*-acetylcysteine (30 mM; intravenous). In each case, the mean ± S.E. of triplicate determinations is shown. Samples from animals infused with native albumin are indicated as BSA in *panels A–C*.

These reactive oxygen species (ROS) activate the free radical-sensitive transcription factor NF-κB, a pleiotropic regulation of many "response-to-injury" genes. The signal transduction cascade can be blocked by **a)** antioxidants, **b)** antibodies to the AGE receptor components, and **c)** antibodies to AGEs (Yan et al 1994, Schmidt et al 1994).



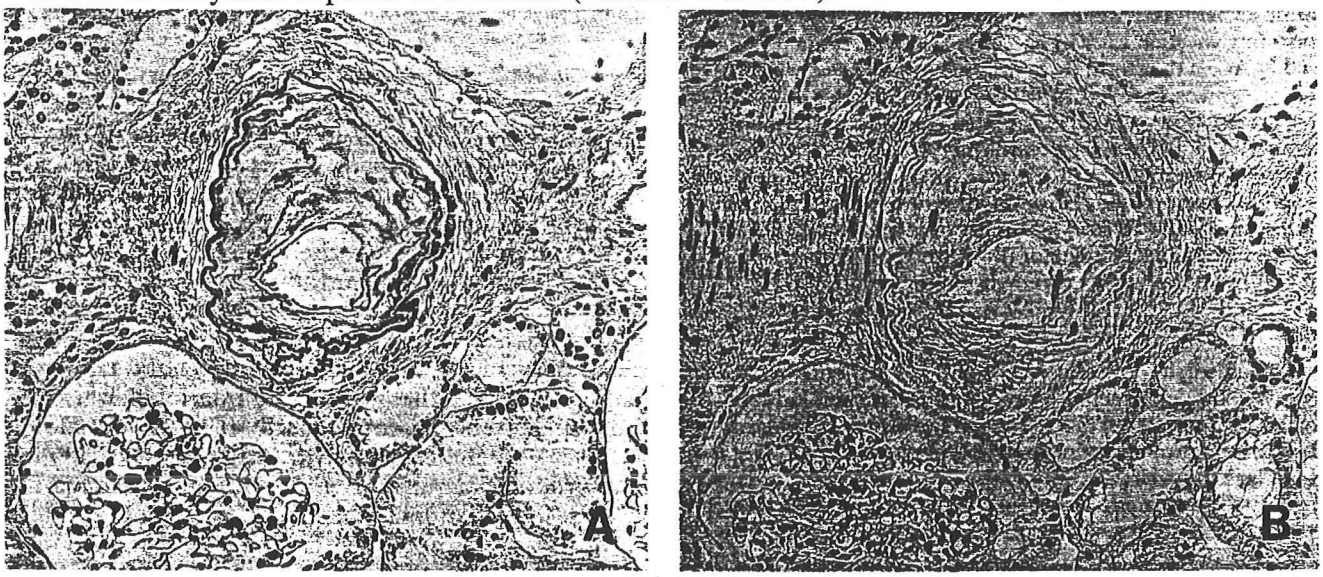
NF-κB may be activated in asthma by a variety of inflammatory signals, resulting in the coordinated expression of multiple inflammatory genes, including cytokines, chemokines, enzymes and adhesion molecules. Additional transcription factors (TF2) interact with NF-κB to amplify the expression of particular genes. The cytokines interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) both activate and are regulated by NF-κB and may there act as an amplifying feedforward loop. COX-2, cyclooxygenase-2; ET-1, $cPLA_2$, cytosolic phospholipase A_2; endothelin-1; GM–CSF, granulocyte–macrophage colony stimulating factor; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible nitric oxide synthase; 5-LO, 5-lipoxygenase; MCP-3, monocyte chemotactic protein-3; MIP-1α, macrophage inflammatory protein-1; PAF, platelet activating factor; RANTES, regulated on activation normal T-cell expressed and secreted; VCAM-1, vascular cell adhesion molecule-1.

IV. ROLE OF AGEs IN THE PATHOGENESIS OF RENAL DISEASE

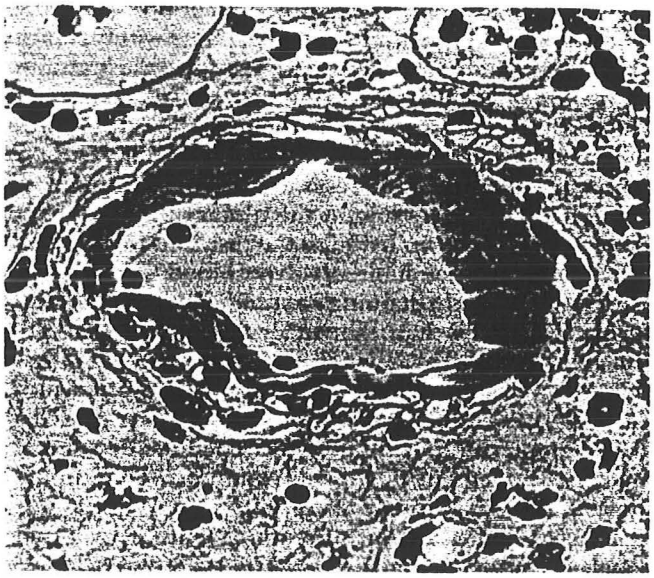
Three lines of evidence suggest an important role for AGEs in mediating diabetic renal disease:

A) Immunohistochemical Detection of Advanced Glycosylation End Products Within the Vascular Lesions and Glomeruli in Diabetic Nephropathy:

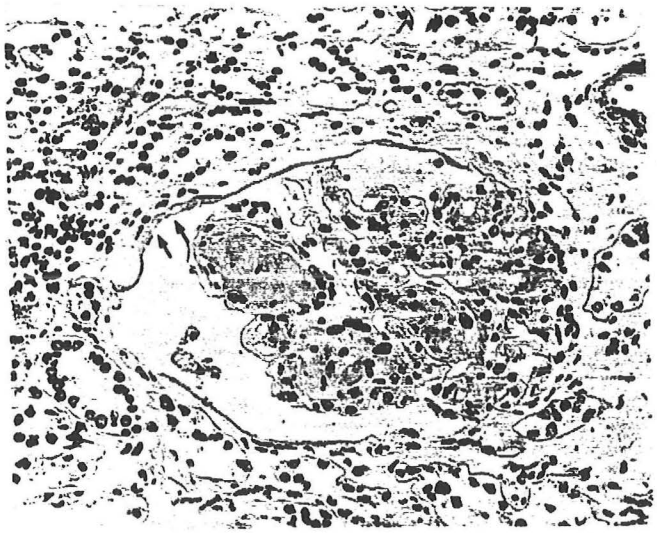
Immunohistochemical staining using anti-AGE antibody has showed a high level of AGE accumulation in diabetic vascular intima, particularly along the inner elastic layer of arteries. Positive staining has also been observed within nodular and severe diffuse lesions of glomeruli as well as in hyaline deposits of arterioles (Nishino et al 1995).



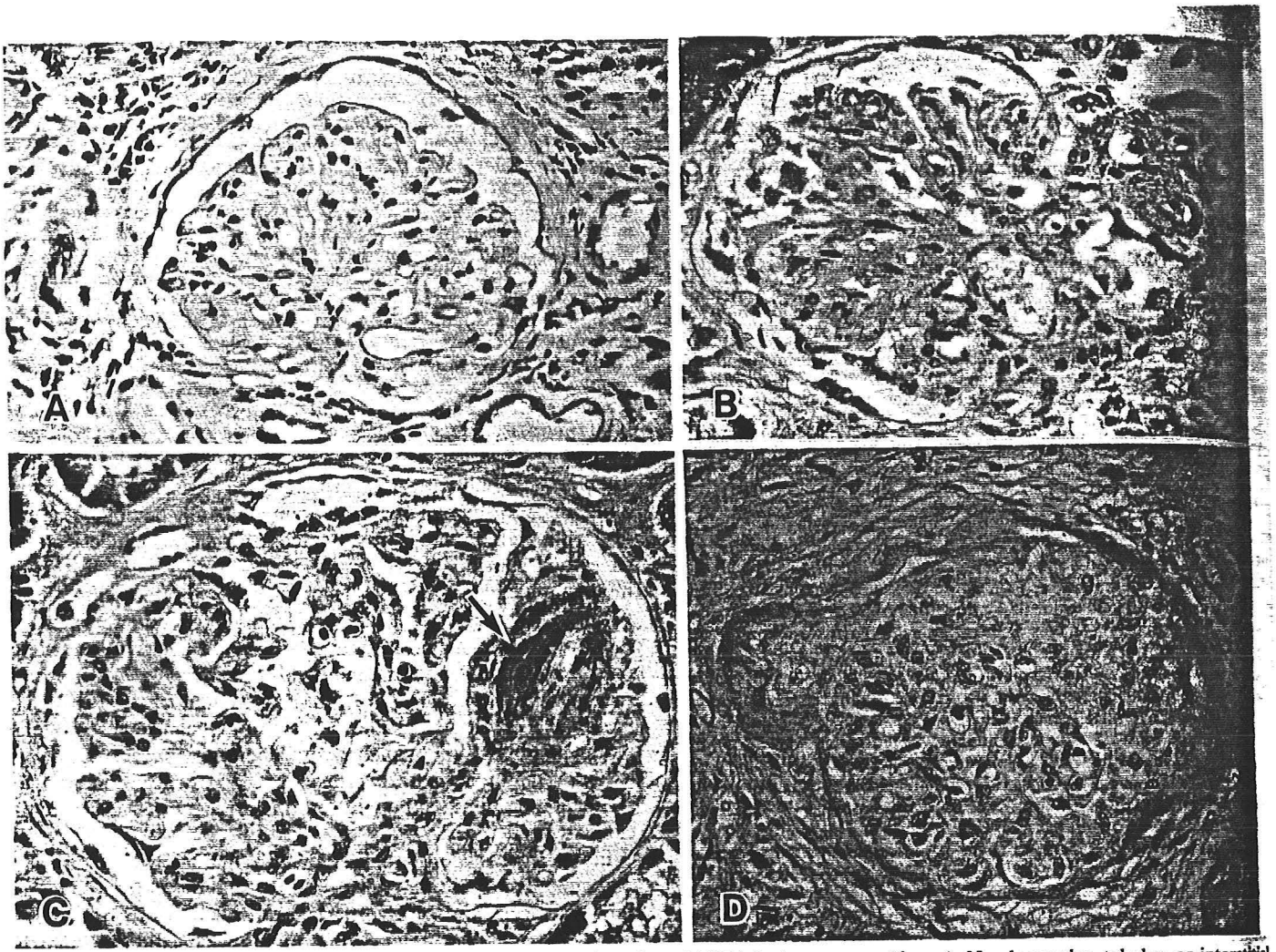
(A) Renal biopsy specimen obtained from a 69-year-old diabetic patient. Laminated staining of AGEs can be seen in the intima, particularly along the internal elastic lamina of the arcuate artery. Vascular endothelial cells and smooth muscle cells show almost no immunoreactivity (Indirect immunostaining; original magnification ×130.) (B) No immunoreactivity is observed in a consecutive section by using control serum (normal rabbit serum). (Indirect immunostaining; original magnification ×130.)



Renal biopsy specimen obtained from a 60-year-old diabetic patient. Hyaline deposits within an arteriole show homogenous intense anti-AGE staining. (Indirect immunostaining; original magnification ×340.)



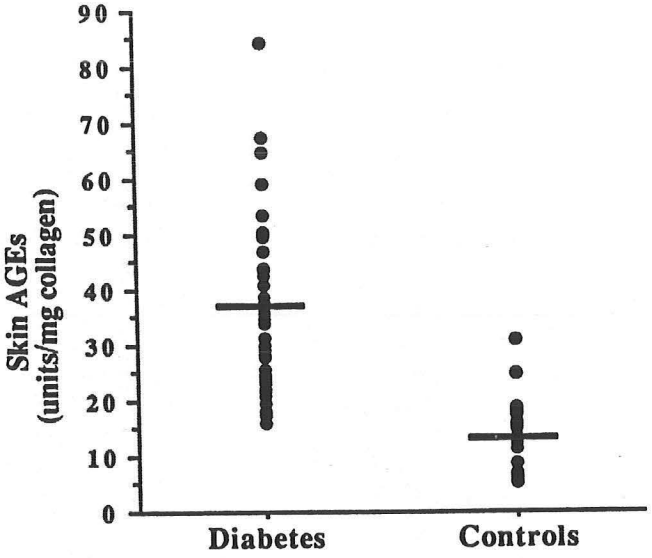
Renal biopsy specimen obtained from a 44-year-old diabetic patient. Weak and homogeneous staining of AGEs is seen in the nodular lesions and in enlarged mesangial areas. Arrows indicate positive staining of the capsular drop of Bowman's capsule. The thickened basement membrane of atrophic tubules also is stained weakly with anti-AGE antiserum. (Indirect immunostaining; original magnification ×130.)



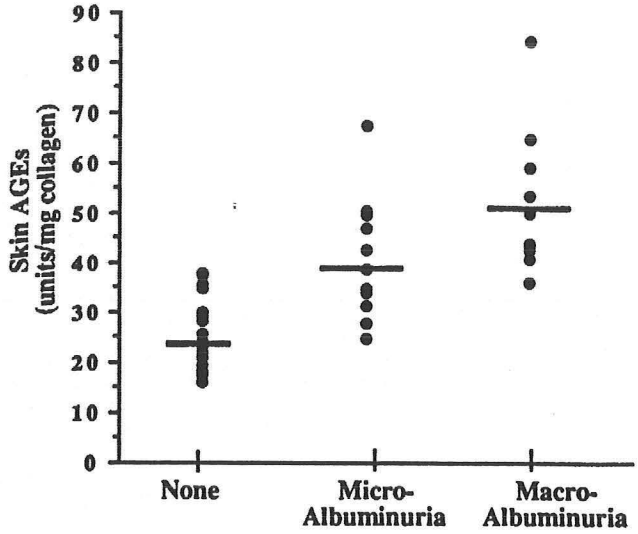
Immunohistochemical localization of advanced glycation end products (AGEs) by immunoperoxidase. **A.** No glomerular, tubular, or interstitial staining for AGEs in a specimen from a 62-year-old patients with membranous nephropathy without diabetes mellitus. **B.** AGEs immunoreactivity can be seen in the mesangial area of a kidney specimen from a 62-year-old diabetic patient. **C.** Intense AGEs immunoreactivity in a nodular lesion (arrow). AGEs immunoreactivity also can be observed in the tubulus and interstitium in a kidney specimen from a 85-year-old diabetic patient. **D.** AGEs immunoreactivity in the fibrous crescent of the Bowman's capsule of a sclerosed glomerulus in a kidney specimen from a 85-year-old diabetic patient.

Similarly, in streptozotocin-induced diabetes mellitus in the rat AGE accumulation has been detected in expanded mesangial area and glomerular basement membrane in the kidneys of diabetic rats (Shikata et al 1995).

A recent study used an AGE-specific enzyme-linked immunosorbent assay (ELISA) to measure skin AGEs to determine whether elevated levels can be detected before the onset of overt renal disease. Subjects with type I diabetes were graded for the degree of nephropathy: **a)** normal, **b)** microalbuminuria, **and c)** macroalbuminuria. Immunoreactive AGEs increased as subjects with no proteinuria advanced to have microalbuminuria and macroalbuminuria. This study suggests that levels of collagen-linked AGEs reveal a correlation with preclinical stages of diabetic nephropathy and may prove useful as early markers of microangiopathy in type I diabetes. This study also found that compared to subjects with good glycemic control (HbA1c < 8.5%), skin AGEs were progressively increased in subjects with fair (> 8.5%, ≤ 10.0%) and poor (> 10.0%) glycemic control (Beisswenger et al 1995).



AGE immunoreactivity (AGE-ELISA) in skin collagen for subjects with and without type I diabetes. Bar indicates mean value (P = 0.0001).



Relationship between skin collagen AGE immunoreactivity and severity of renal disease. Bar indicates mean value (P = 0.0001 by ANOVA across categories).

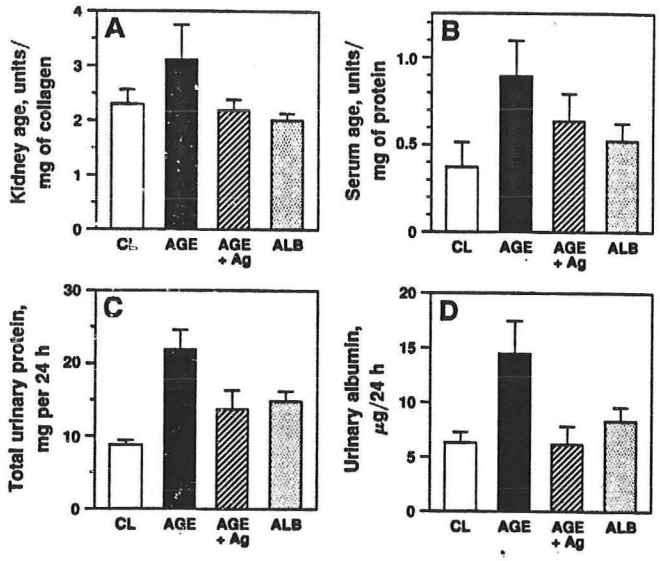
Relationship between glycemic control and AGEs

Glycemic control (mean HbA_{1c})	Levels of AGEs by ELISA (skin U/mg collagen)		
	3 years	1 year	Current
Good (≤ 8.5%)	26.0 ± 2.7	27.2 ± 2.5	30.6 ± 2.8
Fair (> 8.5, ≤ 10.0%)	34.3 ± 2.2	34.1 ± 2.4	33.5 ± 3.3
Poor (>10.0%)	49.0 ± 6.5	50.5 ± 5.3	44.2 ± 5.5
P value	0.0006	0.0001	0.055

Data are means ± SE.

B) Advanced Glycation end products induce Glomerular Sclerosis and Albuminuria in normal rats:

Administration of AGE-modified rat albumin to normal rats, sufficient to elevate circulating AGE levels to the range of diabetic serum, results in **a)** more than 50% increase in glomerular volume compared to controls, **b)** significant periodic acid/Schiff reagent-positive deposits, **c)** basement membrane widening, **d)** mesangial extracellular matrix increase, and **e)** significant glomerulosclerosis when compared to untreated or albumin-treated controls. These damages are also associated with significant proteinuria and albuminuria. Importantly, cotreatment with a pharmacological AGE inhibitor, aminoguanidine, markedly limits the AGE-induced structural and functional defects (Vlassara et al 1994).



Rat kidney tissue (*A*) and serum (*B*) AGE levels and total urinary protein (*C*) and albumin excretion (*D*) after treatment with AGE-modified RSA (AGE), unmodified RSA (ALB), or AGE-RSA/ aminoguanidine (AGE/Ag) for 5 months. CL, control. (*A*) Kidney AGE levels (mean ± SEM, $n = 6–12$ per group). Significant *P* values: AGE vs. CL, <0.05; AGE vs. ALB, <0.025; AGE vs. AGE/Ag, <0.025. (*B*) Serum AGE levels (mean ± SEM). *P* values: AGE vs. CL, <0.05; AGE vs. ALB, <0.025. Total urinary protein (*C*) and albumin (*D*) concentrations are expressed as the mean ± SEM. (*C*) *P* values: AGE vs. AGE/Ag, <0.025; AGE vs. ALB, <0.01; AGE vs. CL, <0.001; AGE/Ag vs. CL, <0.05. (*D*) *P* values: AGE vs. AGE/Ag, <0.005; AGE vs. ALB, <0.01; AGE vs. CL, <0.001.

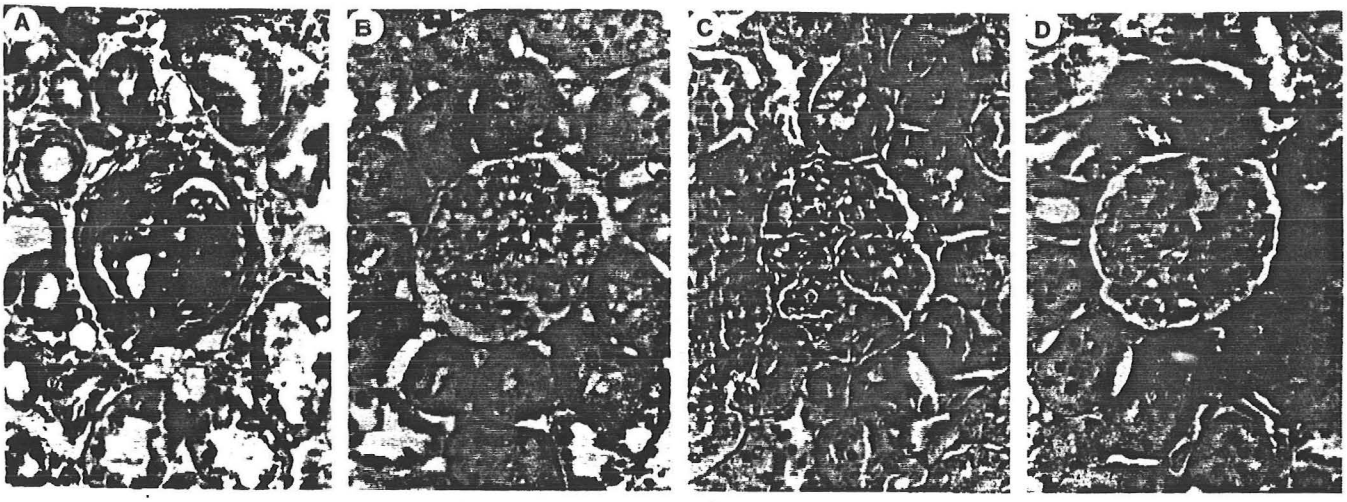
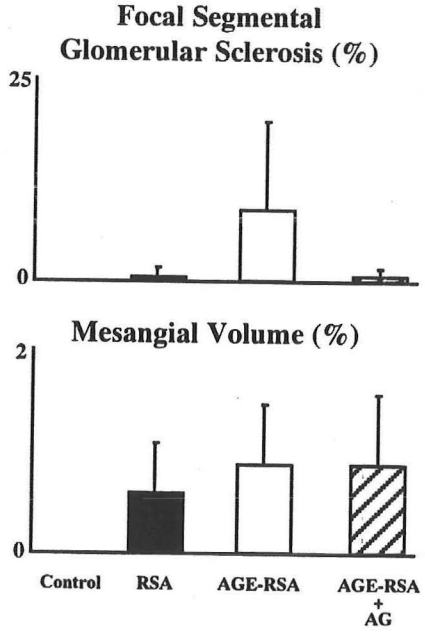
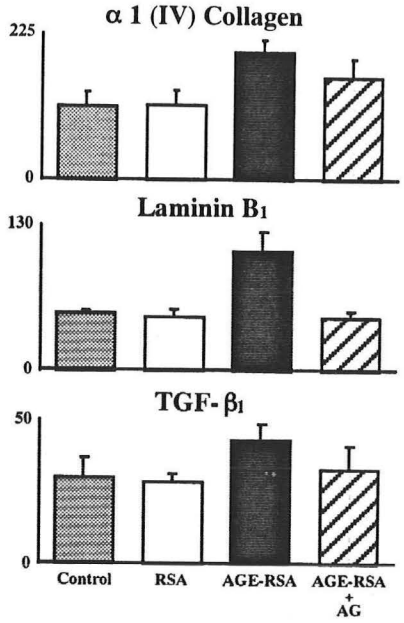


FIG. 2. Light microscopy of rat glomeruli from AGE-RSA-treated (*A*), untreated control (*B*), unmodified-RSA-treated control (*C*), and AGE-RSA/aminoguanidine-treated (*D*) rats. (PAS stain; ×75.)



Further studies attempted to determine how AGEs induced glomerulosclerosis and if the molecular events are similar to those found in diabetic kidneys. Administration of AGE-modified mouse serum albumin to normal mice resulted in development of glomerular hypertrophy and upregulation of glomerular $\alpha 1$(IV) collagen, laminin B1, and TGF-β_1 mRNA, similar to what has been observed in experimental models of diabetes. Cotreatment with aminoguanidine attenuated the effects of AGE-albumin on glomerular hypertrophy and collagen, laminin, and TGF-β_1 expression (Yang et al 1994).

Glomerular Gene Induction by AGEs



B) AMINOGUANIDINE AN INHIBITOR OF AGE FORMATION, PREVENTS OR AMELIORATES NEPHROPATHY IN EXPERIMENTAL DIABETES:

Pharmacological agents which inhibit AGE formation have made it possible to investigate the role of AGEs in the pathogenesis of diabetic complications in animal models. In addition, there are currently studies ongoing in type I and type II diabetic patients to determine the effect of inhibition of AGE formation on diabetic nephropathy and retinopathy.

Structure of Aminoguanidine, an Inhibitor of AGE Formation

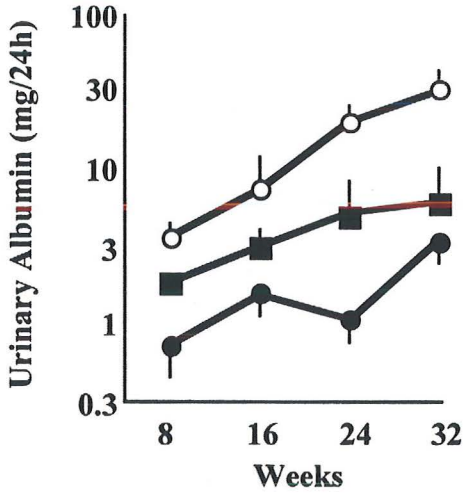
Aminoguanidine

$$H_2N - NH - C(=NH^+_2) - NH_2$$

The hydrazine compound aminoguanidine was the first AGE inhibitor to be identified (Brownlee et al 1986). Aminoguanidine reacts mainly with non-protein bound dicarbonyl intermediates such us 3-deoxyglucasone to form 3-amino-5- and 3-amino-6-substituted triazines, and with methylglyoxal to form 3-amino-5-methyl-1, 2,4, - and 3-amino-6-methyl-1, 2,4-triazines (Hirsch et al 1992, Lo et al 1994.

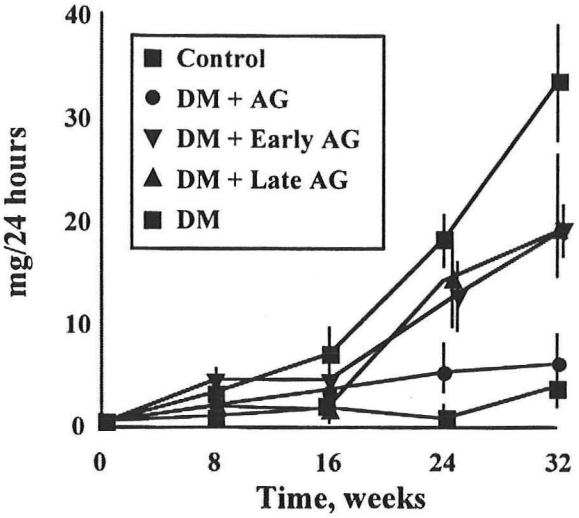
Administration of aminoguanidine to the streptozotocin-induced diabetes in the rat has been shown by several studies to decrease the AGE accumulation, the albuminuria, and the mesangial expansion (Soulis-Liparota et al 1991, Edelstein and Brownlee 1992, Soulis-Liparota et al 1005, Soulis et al 1996).

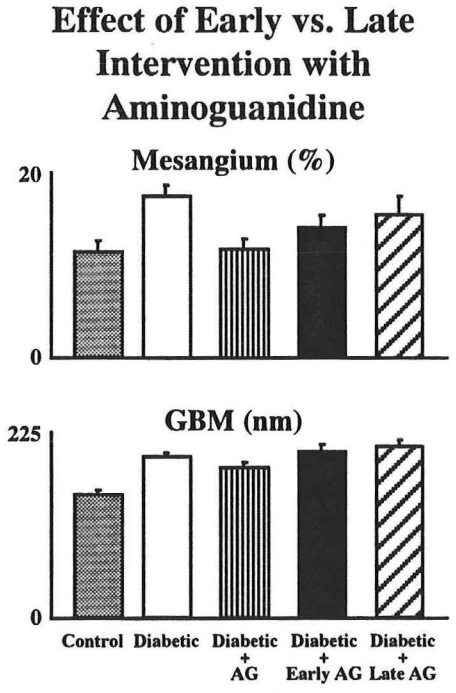
Serial Data for Albuminuria (y-axis, logarithmic Scale) at 8-week Intervals Over 32 Weeks Control (●), Diabetic (○) Diabetic + Aminoguanidine (■)



The beneficial effects of aminoguanidine on albuminuria and mesangial volume are seen **even** when aminoguanidine treatment is initiated after the establishment of diabetic nephropathy (Soulis et al 1996). This study provides promise for ongoing trials studying the efficacy of aminoguanidine in slowing progression of established diabetic nephropathy.

The Effect of Early vs Late Aminoguanidine Treatment on Proteinuria





While some studies have shown that aminoguanidine also prevents glomerular basement membrane thickening (Ellis and Good 1991), others have not been able to reproduce this finding (Soulis et al 1996, Oturai et al 1996).

In addition to inhibiting AGE formation, aminoguanidine has been shown to inhibit the inducible form of nitric oxide sythase (Corbett et al 1992). This effect of aminoguanidine could also account for its beneficial effects in diabetic nephropathy. The early phase of diabetic renal disease is characterized by glomerular hyperfiltration, a process which eventually has delitirious effects in the kidney. Studies streptozocin-induced diabetes in the rat revealed increased levels of NO_2-/NO_3- in diabetic rats and increased excretion of these amions in the urine. Furthermore, NO was shown to mediate the early hyperfiltration (Bank and Aynedjian 1993). Interestingly, infusion of glycated serum proteins (Amadori protein adducts) to normal rats produces hyperfiltration typical of early diabetes (Sabbatini et al 1992).

In endothelial cells glycated albumin enhances nitric oxide and TNF-α synthase activity and gene expression (Amore et al 1997).

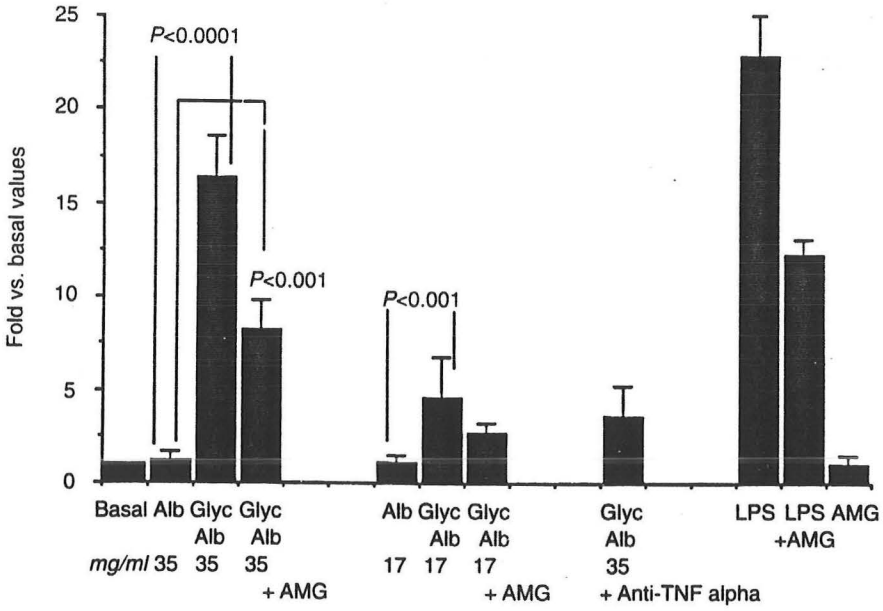


Fig. 1. *NOS activity of murine endothelial cells after incubation with Glycated albumin and normal albumin.* Results are expressed as the fold of basal values (mean ± SD of 5 experiments). *P* values refer to the ANOVA test. Abbreviations are: Basal, EC cultured in unsupplemented medium; Alb, native human serum albumin; Glyc Alb, glycated human serum albumin; AMG, aminoguanidine 0.05 M; LPS, lipopolysaccharide 10 μg/ml; L-NAME, N ω nitro-L-arginine methyl ester 0.01 M; anti-TNF-α, antibodies to tumor necrosis factor alpha 10 pg/ml.

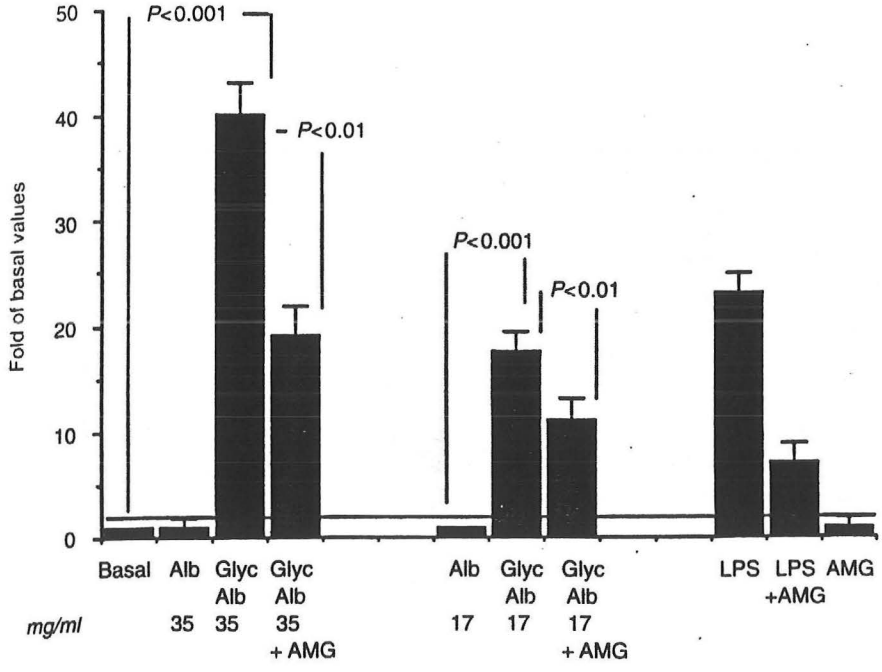
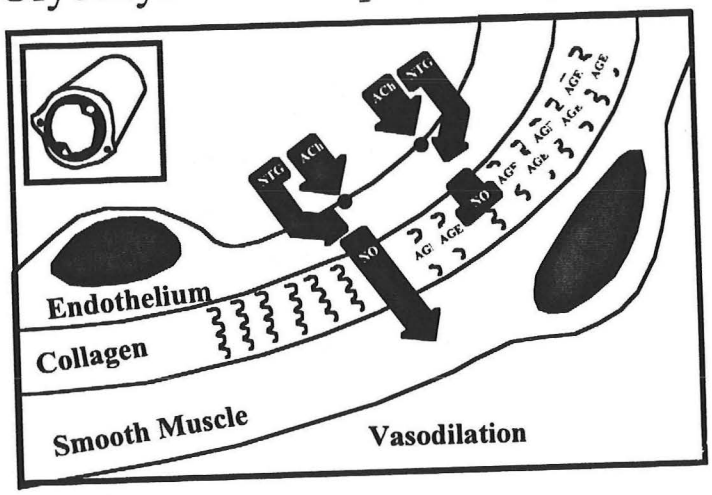


Fig. 7. *TNF-α release after incubation with glycated albumin and native albumin at different concentrations.* Results are expressed as the fold of basal values. Abbreviations are in the legend to Figure 1.

Therefore in early diabetes enhanced NO production could, in part, mediate the hyperfiltration, and aminoguanidine, in addition to its effect to prevent formation of AGEs, could also have a favorable outcome in diabetic nephropathy by inhibiting iNOS and preventing the early hyperfiltration in diabetes.

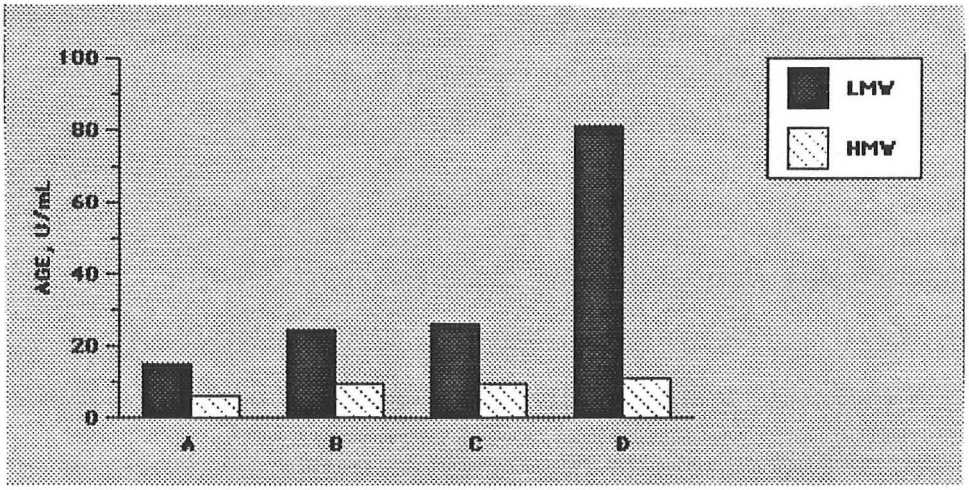
Interestingly, although Amadori products (early glycosylation products or glycated albumin) enhance NO synthesis, advanced glycosylation end products (AGEs) can react with and inactivate NO via a direct chemical reaction (Bucala et al 1991). As will be discussed in more detail later, the inactivation of NO by AGEs may play a role in the defective vasodilatory responses that occur in established diabetes mellitus. Furthermore, in the vascular smooth muscle cell and the renal mesangial cell, NO has an antiproliferative effect. The quenching of NO by subendothelial cells interferes with the antiproliferative acitivity of NO, resulting in myointimal and mesangial proliferation, which is linked to accelerated vasculopathy and glomerulopathy in long-standing diabetes mellitis (Hogan et al 1992).

Model Relating the Inactivation of Nitric Oxide (NO) by Subendothelial Advanced Glycosylation Endproducts (AGEs)



VII) AGEs IN END STAGE RENAL DISEASE (ESRD)

The kidney plays an important role in the clearance of circulating AGE from the bloodstream. A recent study has shown that AGE peptides are easily filtered and actively reabsorbed by the proximal convoluted tubule. The AGE peptides are then sequentially taken up by the early endosomes, late endosomes, and eventually in the lysosomes. The clearance of AGE peptides are impaired in subjects with renal failure, resulting in high plasma AGE levels in subjects with ESRD, even of non-diabetic origin. AGE levels are, however, further increased in diabetic subjects with ESRD (Makita et al 1991, Makita et al 1994, Papanastasiov et al 1994, Vlassara 1996).



Increased AGE levels in diabetic patients with renal disease
Plasma levels of low and high molecular weight (LMW and HMWO advanced glycosylation end products in four groups of patients: A = normal individuals; B = diabetic patients with normal renal function; C = nondiabetic patients with end-stage renal disease; and D = diabetic patients with end-stage renal disease. There was a marked increase in plasma levels of LMW AGEs in the last group; in addition, diabetics with normal renal function had a 60 percent increase above normal controls (group B versus group A). (Adapted from Vlassara, H, Blood Purif 1994; 12:54.)

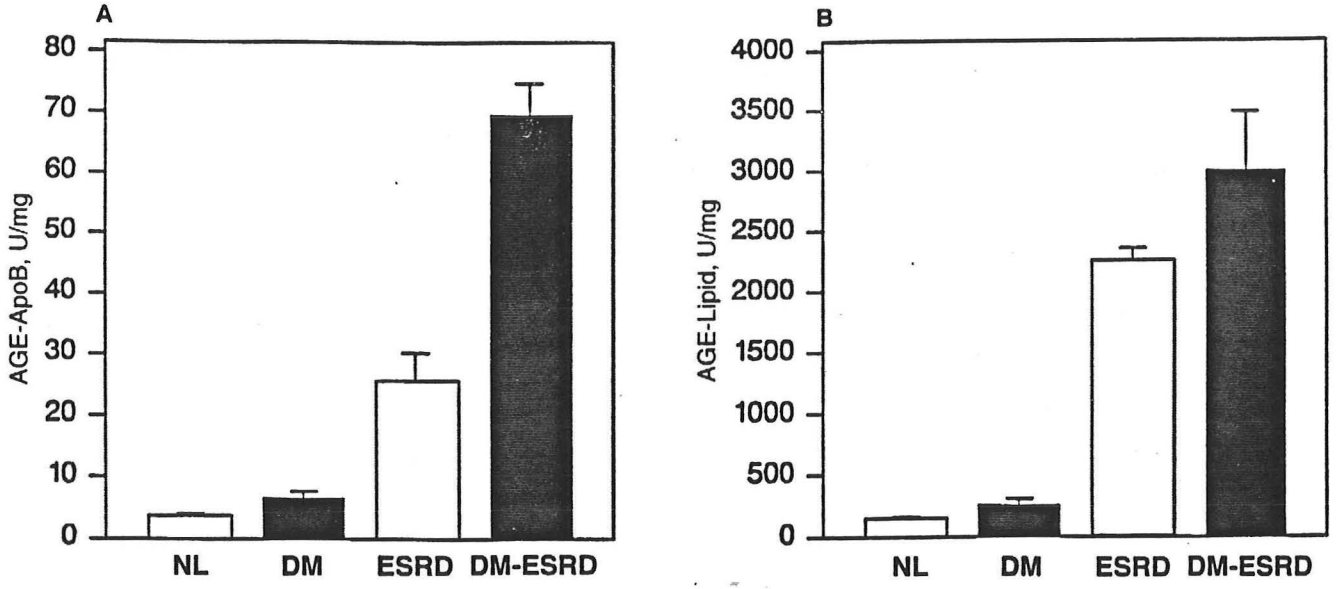
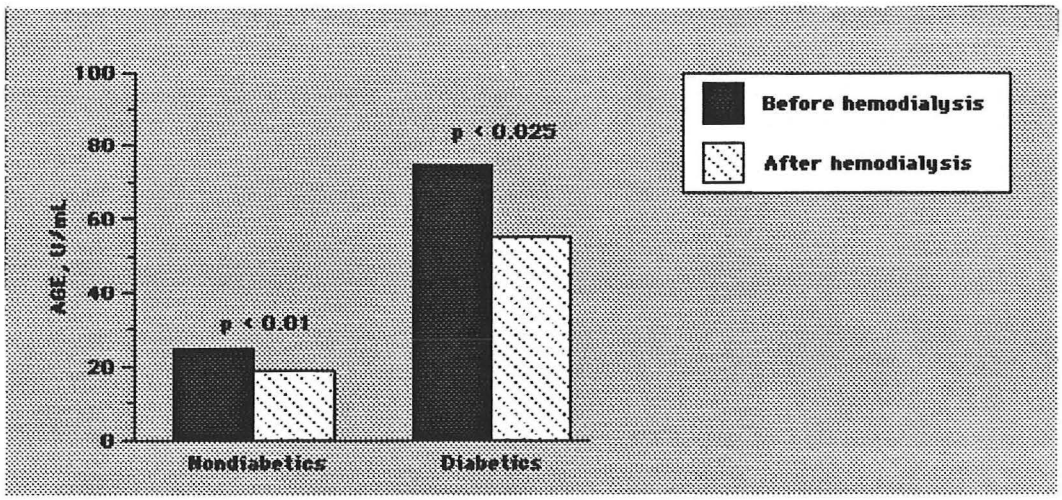


Fig. 2. *Serum AGE-ApoB* (**A**) *and AGE-lipid* (**B**) *levels in normal patients (NL), diabetic patients with normal renal function (DM), nondiabetic patients with end-stage renal disease (ESRD), and diabetics with renal failure (DM-ESRD).* $P < 0.001$ (Student's t test) for each patient group with ESRD versus control group with and without diabetes [12].

Dialysis has been shown to partially lower AGE levels and ongoing studies are now evaluating the role of high-flux hemodialyzers on long-term regulation of AGE levels, as illustrated by the third patient presented in the Case Presentations.



Dialysis partially lowers AGE levels Plasma levels of advanced glycosylation end products in nondiabetics (left) and diabetics (right) with end-stage renal disease studied before and after hemodialysis. AGE levels were markedly increased in the diabetic patients and only partially reduced by hemodialysis. (Data from Vlassara, H, Blood Purif 1994; 12:54)

The increased AGE levels in patients with diabetic and nondiabetic ESRD play an important role in a number of processes including:

a) Vascular dysfunction and enhanced atherosclerosis, as will be discussed in more detail in the next section;

b) Dialysis-related amyloidosis, which is a serious complication in patients maintained on long-term dialysis. Carpal tunnel syndrome, cystic lesions of long bones, particularly in the femoral and humeral heads, destructive spondyloarthropathy, and diffuse arthritis and periarthritis of the scapulohumeral region are seen with increasing frequency as a consequence of amyloid deposit in these patients.

The major component of amyloid has been demonstrated to be β_2-microglobulin (β_{2m}) (Miyata and Maeda 1995). β_{2m} isolated from the amyloid deposits in patients with DRA has been demonstrated to be modified with AGEs (Miyazaki et al 1995, Miyata 1996, Miyala et al 1996, Dolhofer-Bliesenger et al 1996, Miyata et al 1995, Niwa et al 1997). AGE-β_{zm} may be involved in the pathogenesis of DRA by stimulating the chemotaxis of monocytes, the secretion of IL-1β and TNF-α from macrophages, and the subsequent synthesis of collagenase in synovial cells. In addition AGE-β_{2m} also interact with osteoclasts and osteoblasts and induces bone reabsorption. Altogether these processes may result in bone and joint destruction as clinically encountered in dialysis-related amyloidosis.

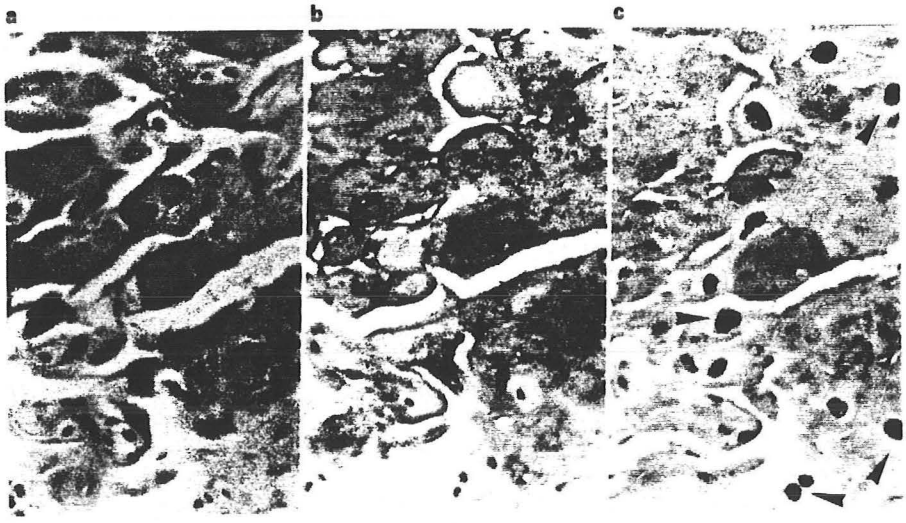


Fig. 1. Serial sections of the synovial amyloid tissue from long-term hemodialysis patients with carpal tunnel syndrome were stained with Congo red (**a**), anti-β_2-M antibody (**b**) and anti-AGE (AG-10) antibody (**c**). Note the similarity in distribution of AGE antigen and β_2-M amyloid. Arrowheads indicate AGE-antigen-positive cells around AGE-positive amyloid (**c**). × 80.

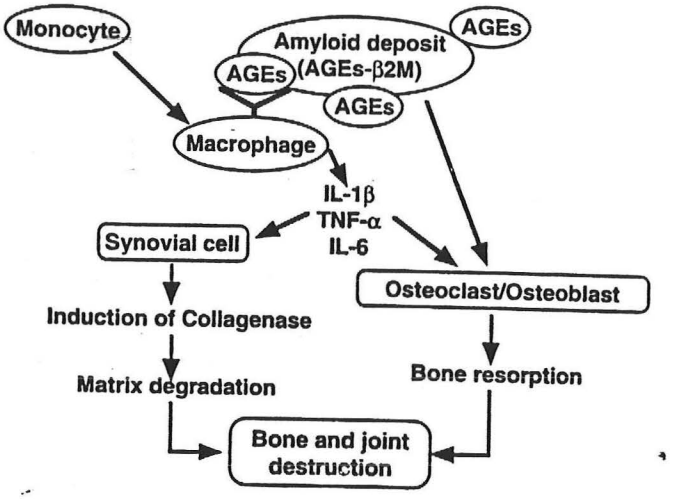


Fig. 2. *Pathological involvement of AGE-modified β2-microglobulin in bone and joint destruction of dialysis-related amyloidosis (hypothesis).* AGE-modified β2M is present in long-lived amyloid fibrils [10, 12, 15]. AGE-modified β2M might stimulate monocyte chemotaxis [16, 26, 34], macrophage secretion of cytokines leading to bone resorption and matrix destruction [16, 26, 27, 34], and osteoclast-induced bone resorption [35]. Thus, AGE-mediated tissue destruction might be the combined result of excessive accumulation of AGEs in long-lived amyloid deposits linked to a heightened cellular response to these deposits.

c) AGE accumulation in the peritoneum, dominantly in the vascular wall, especially in patients who have been on CAPD for more than 3 years (Nakayama et al 1997). By using the peritoneal equilibration test (PET), the accumulations of AGEs are functionally associated with increased permeability of the peritoneal membrane for glucose, creatinine, β_2-microglobulin, and albumin. These functional abnormalities result in impaired ultrafiltration and increased protein loss in the dialysate fluid.

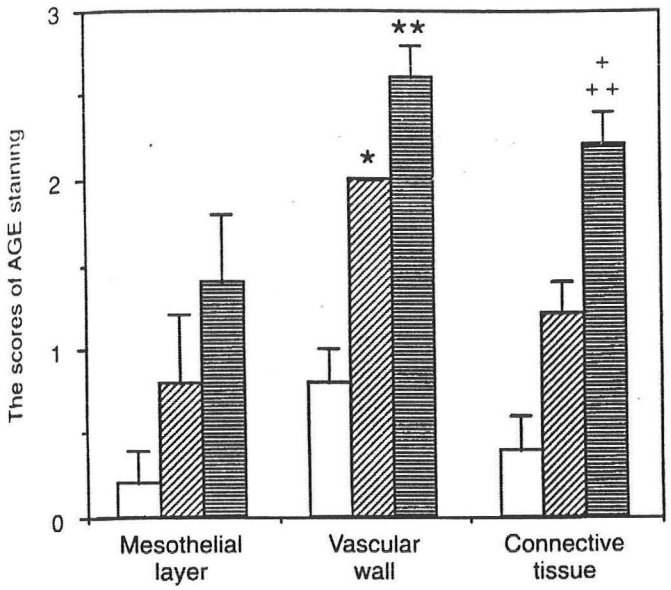


Fig. 7. *The scores of AGE stainings in the respective groups.* Data are presented as mean ± SE. Symbols are: (□) group I: (▨) group II: (▤) group III. In vascular wall: Groups II vs. I. $^{*}P = 0.0006$: Groups III vs. I. $^{**}P < 0.0001$, respectively. In connective tissue: Groups III vs. I. $^{+}P < 0.0001$; Groups II vs. III. $^{++}P = 0.0067$.

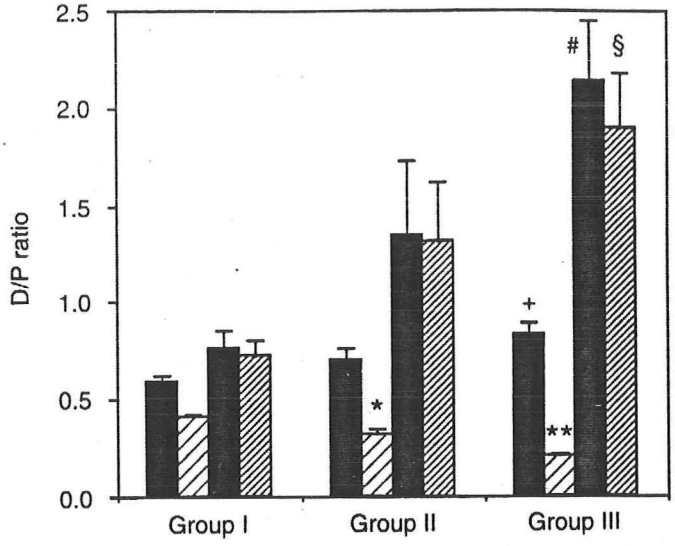


Fig. 8. *The D/D_0-glucose and D/P ratios in the respective groups (4-hour PET).* Data are presented as mean ± SE. Symbols are: (■) D/P-Cr; (▨) D/D_0-glucose: (■) DP-β_2m (×10); (▨) D/P-Alb ($\times 10^2$). The D/D_0-glucose: Groups II vs. I, $^{*}P = 0.0078$; Group III vs. I, $^{**}P < 0.0001$. The D/P-Cr: Groups I vs. III, $^{+}P = 0.0078$. The D/P-β_2m: Groups III and I, $^{\#}P = 0.0056$. The D/P-Alb: Groups III and I $^{\S}P = 0.0045$.

VIII) ROLE OF AGEs IN VASCULAR DYSFUNCTION AND ATHEROSCLEROSIS

There is increasing evidence that AGEs play an important-role in the vascular dysfunction in diabetic subjects and in the pathogenesis of atherosclerosis, and may thus play a major role in the increased cardiovascular disease in patients with diabetes, and diabetic and non-diabetic patients with ESRD.

a) Vascular Dysfunction in diabetes:

In a study in 29 patients with type 2 diabetes (NIDDM) and in 21 control subjects, forearm blood flow responses to increases doses of acetylcholine and glyceryl trinitrate were significantly impaired in diabetic subjects, which indicates the presence of impaired endothelium-dependent and independent vasodilatation (McVeigh et al 1992).

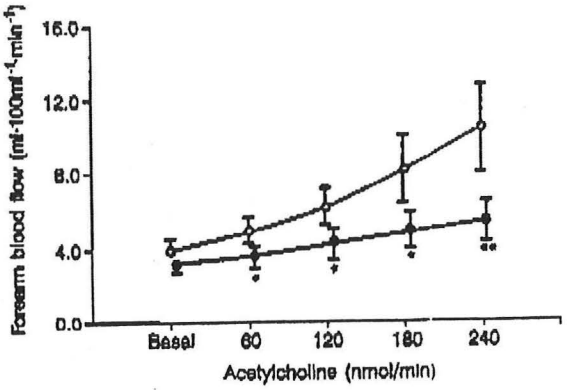


Fig. 2. Forearm blood flow response to the incremental intra-arterial infusion of acetylcholine in 21 control (○) and 29 diabetic (●) subjects, mean and 95% confidence intervals. $*p < 0.01$, $**p < 0.001$

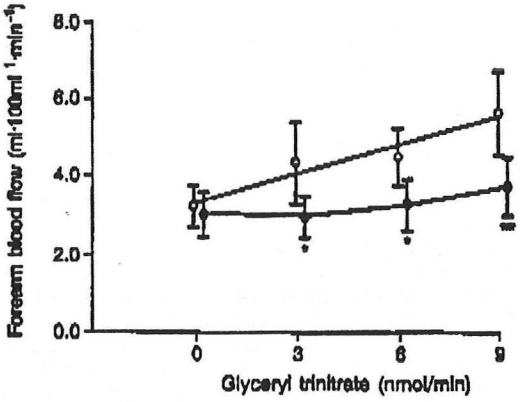


Fig. 5. Forearm blood flow response to the incremental intra-arterial infusion of glyceryl trinitrate in 21 control (○) and 29 diabetic (●) subjects, mean and 95% confidence intervals. $*p < 0.05$, $**p < 0.01$

A similar defect is also seen in the streptozotocin-induced diabetes (IDDM) in the rat (Bucala et al 1991). The same defects can be induced by administration of AGE-BSA to normal rabbits and rats. In these animals AGE administration was associated with a significant increase in vascular permeability. This alteration was absent in animals that received aminoguanidine in addition to AGE (Vlassara et al 1992, Corbett et al 1992). Blood pressure studies of AGE-treated rats and rabbits revealed markedly defective vasodilatory responses to acetylcholine and nitroglycerin compared to controls, and aminoguanidine treatment significantly prevented this defect.

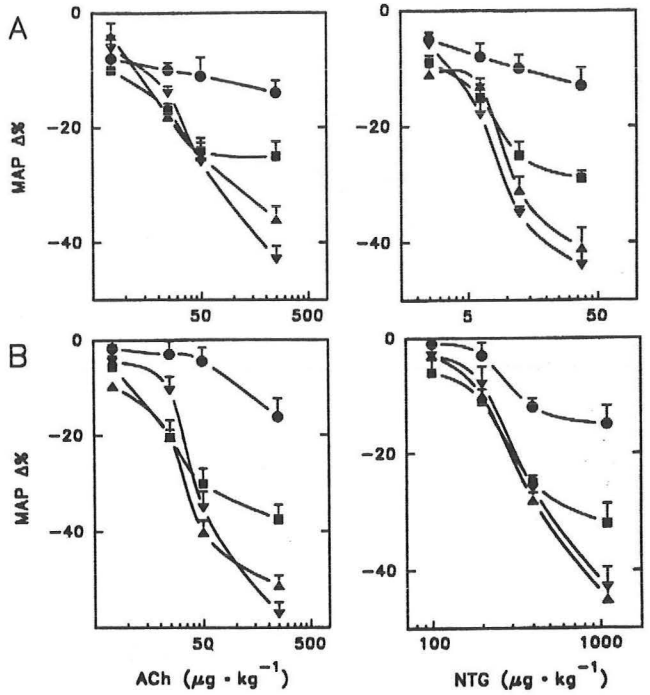


FIG. 4. Vasodilatory impairment in healthy rats (*A*) and rabbits (*B*) following administration of AGE-modified albumin. After a period of daily injections with species-matched AGE-albumin (circles), AGE-albumin plus aminoguanidine (squares), or unmodified albumin (triangles), vasodilatory responses to increasing doses of ACh or NTG were compared to untreated controls (inverted triangles). MAP, mean arterial pressure. Data represent the means ± SEM obtained from eight rats (*A*) and four rabbits (*B*). Significant *P* values: AGE-treated vs. untreated control animals, <0.001; AGE vs. AGE plus aminoguanidine, <0.001 for either *A* or *B* and for either drug, ACh or NTG.

These studies therefore indicate an important role for AGEs in the vascular functional abnormalities seen in diabetes. Further studies have shown that vascular endothelial cells have specific receptors for AGE (RAGE) (Schmidt et al 1994) and incubation of cultured endothelial cells with AGEs or administration of AGEs to normal animals induce expression of vascular cell adhesion molecular-1 (VCAM-1) (Schmidt et al 1995, Vlassara et al 1995). The increase in VCAM-1 expression is a result of AGE binding to the AGE receptor (RAGE), and is mediated by increased cellular oxidant stress and activation of the transcription factor NF-κB. Antibodies against RAGE or the anti-oxidant N-acetylcysteine each blocked the increase in endothelial cell VCAM-1 expression and their adhesivity (Schmidt et al 1995). *In vivo* increased expression of VCAM –1 has been thought to enhance targeting of mononuclear phagocytes to the vascular cells.

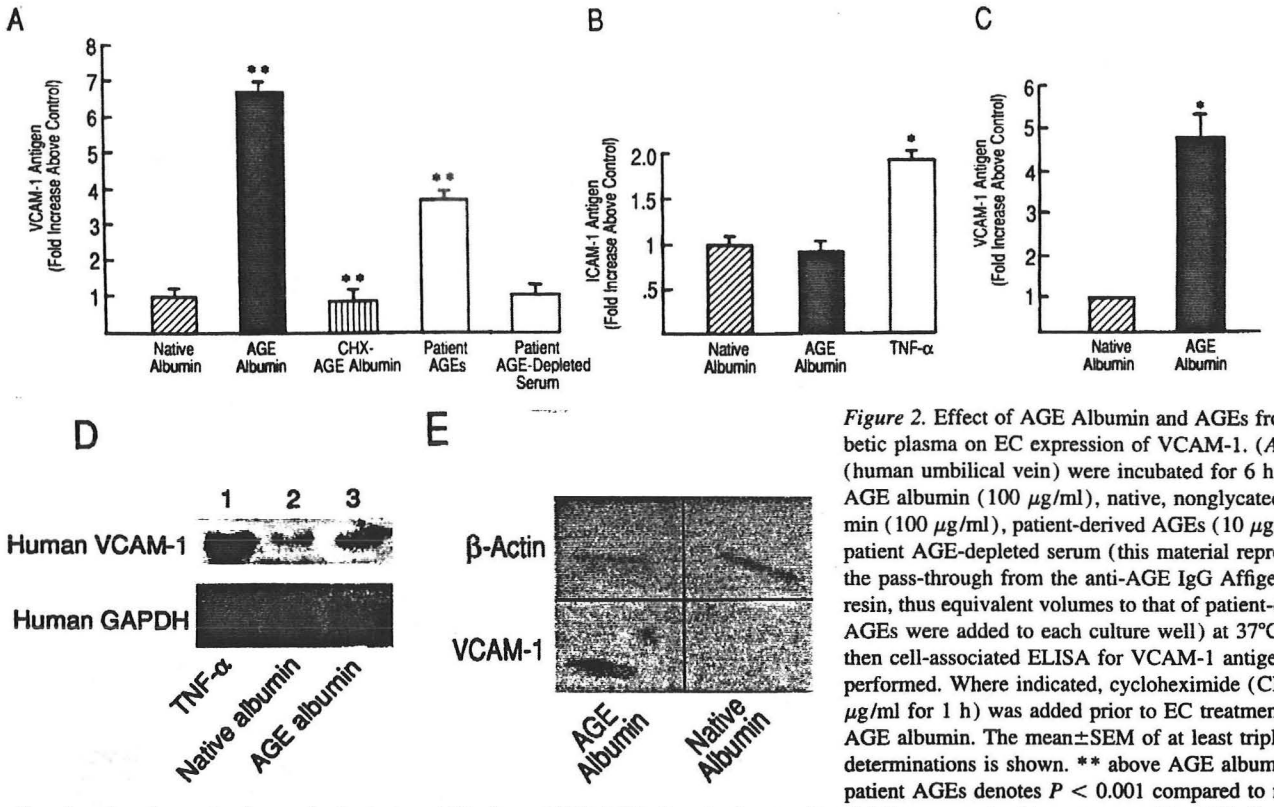


Figure 2. Effect of AGE Albumin and AGEs from diabetic plasma on EC expression of VCAM-1. (*A*) ECs (human umbilical vein) were incubated for 6 h with AGE albumin (100 μg/ml), native, nonglycated albumin (100 μg/ml), patient-derived AGEs (10 μg/ml) or patient AGE-depleted serum (this material represented the pass-through from the anti-AGE IgG Affigel 10 resin, thus equivalent volumes to that of patient-derived AGEs were added to each culture well) at 37°C, and then cell-associated ELISA for VCAM-1 antigen was performed. Where indicated, cycloheximide (CHX; 50 μg/ml for 1 h) was added prior to EC treatment with AGE albumin. The mean±SEM of at least triplicate determinations is shown. ** above AGE albumin and patient AGEs denotes $P < 0.001$ compared to native albumin using the student's unpaired *t* test, and ** above CHX-AGE albumin denotes $P < 0.001$ compared with treatment with AGE albumin alone using the student's unpaired *t* test. (*B*) ECs (human umbilical vein) were incubated for 6 h at 37°C with AGE albumin (100 μg/ml) or native albumin (100 μg/ml) and cellular ELISA for ICAM-1 antigen was performed. As indicated, TNF (10 nM) was incubated for 6 h at 37°C with ECs as a positive control. The mean±SEM of at least triplicate determinations is shown. * denotes $P < 0.01$ compared with native albumin alone using the student's unpaired *t* test. (*C*) Human aortic endothelial cells were incubated for 6 h with AGE albumin (100 μg/ml) or native, nonglycated albumin at 37°C, and then cell-associated ELISA for VCAM-1 antigen was performed. The mean±SEM of at least triplicate determinations is shown. ** above AGE albumin indicates $P < 0.001$ compared to native albumin using the student's unpaired *t* test. (*D*) Northern analysis of EC RNA for VCAM-1 transcripts: effect of AGE albumin. ECs were incubated with TNF-α (10 nM) (lane *1*) or native nonglycated albumin (100 μg/ml) (lane *2*) or AGE albumin (100 μg/ml) (lane *3*) as indicated for 6 h at 37°C, RNA was harvested, subjected to electrophoresis, blotted onto nitrocellulose and hybridization with ^{32}P-labeled cDNA probes for VCAM-1 or GAPDH performed as indicated. (*E*) Nuclear run-on transcription assay: ECs were treated with AGE albumin (100 μg/ml) or native albumin (100 μg/ml) for 6 h at 37°C, nuclei isolated and ^{32}P-labeled nuclear run on products hybridized to denatured human VCAM-1 gene DNA or β-actin gene DNA slot-blotted on nylon filters, as described above.

AGEs have also been implicated to play a role in the vascular hypertrophy. In the streptozotocin-induced diabetic rats, diabetes was associated with **a)** an increase in mesenteric vascular weight and an increase in media wall/lumen area, **b)** an increase in TGF-β_1 gene expression, and **c)** an increase in α_1 (IV) collagen gene expression (Rumble et al 1997). AGEs and extracellular matrix were present in abundance in diabetic but not in control vessels. Treatment of diabetic rats with aminoguanidine resulted in significant amelioration of the pathological changes, including a decrease in the vascular hypertrophy and over expression of TGF-β_1 and α_1 (IV) collagen.

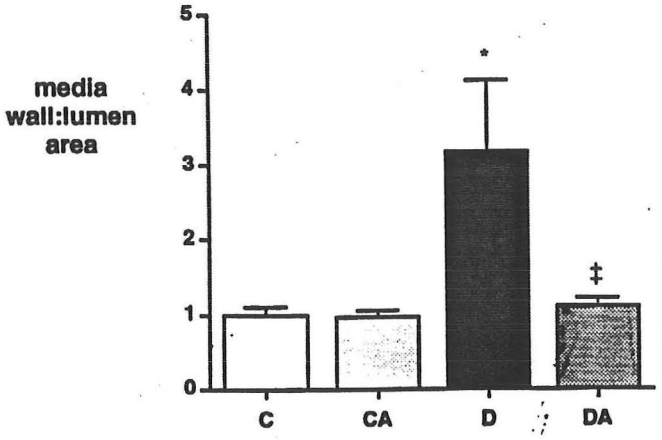


Figure 2. Mesenteric media wall/lumen area at 3 wk, expressed as mean±SE, relative to control values designated an arbitrary value of 1. *$P < 0.001$ vs. C; ‡$P < 0.001$ vs. D. C, control; D, diabetic; A, aminoguanidine.

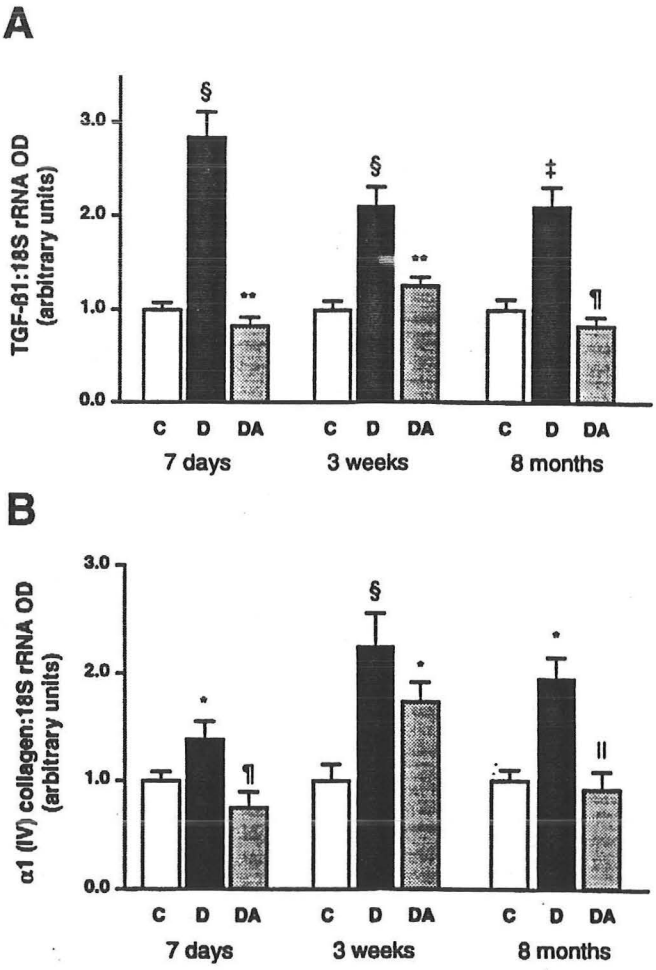


Figure 3. Quantitation of mesenteric TGF-β1 (*A*) and α1 (IV) collagen (*B*) mRNA. Data are shown as mean±SE of the ratio of optical density (OD) of specific mRNA to that of 18S rRNA, relative to control animals (designated an arbitrary value of 1) at 7 d, 3 wk, and 8 mo. *$P < 0.05$, ‡$P < 0.01$, §$P < 0.001$ vs. C; ‖$P < 0.05$, ¶$P < 0.01$, **$P < 0.001$ vs. D. C, control; D, diabetic; A, aminoguanidine.

b) Atherosclerosis:

Advanced glycosylation end products (AGEs) and the receptors for AGEs (RAGE) have been noted in **a)** atherosclerotic lesions of human aorta and coronary arteries (Kumet et al 1995), **b)** in arteriosclerotic lesions of euglycemic LDL receptor-deficient rabbits, but not in normal aortic tissues (Palinski et al 1995), and **c)** in arterial endothelium and endothelium of vasa vasorum of non-diabetic patients with ESRD (Greten et al 1996).

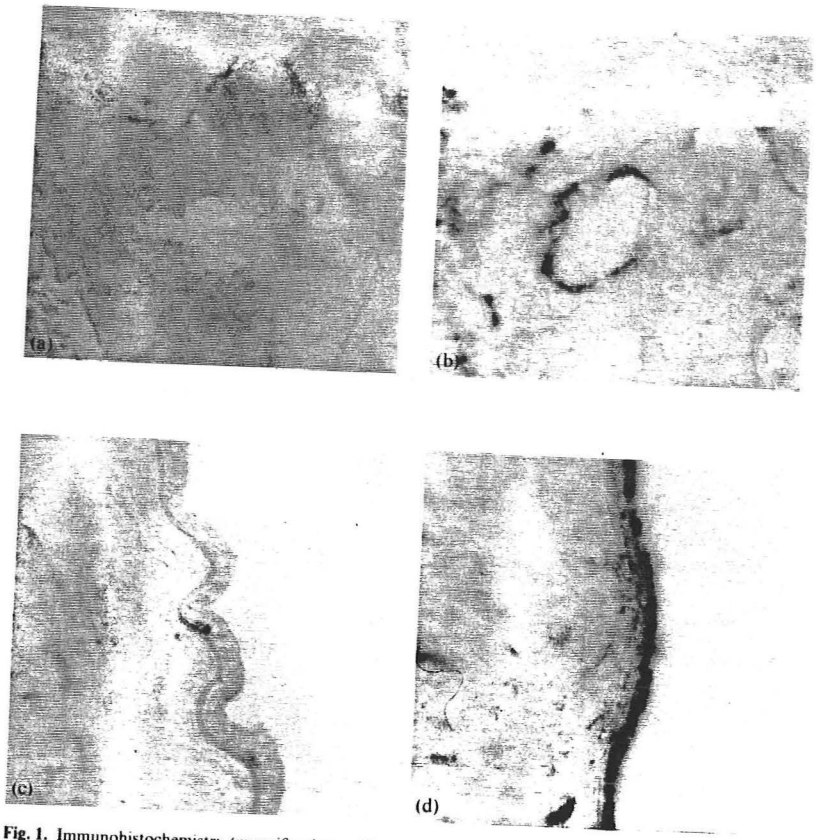


Fig. 1. Immunohistochemistry (magnification × 340).
A. Vasa vasorum of normal vessels exhibit little or no RAGE-like immunoreactivity in endothelial cells.
B. Immunoreactivity for RAGE in uraemic individuals is strongly positive in vasa vasorum.
C. Macrovascular endothelium is largely negative for RAGE in normal individuals.
D. Uraemic patients show strong immunopositivity for RAGE in macrovascular endothelial cells.

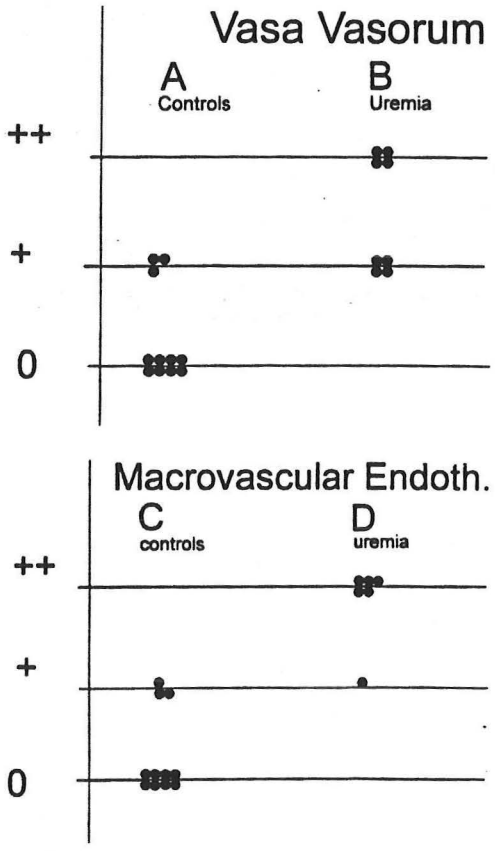


Fig. 2. Evaluation of staining intensity. Each dot represents a patient and the intensity of staining as blindly scored by three independent investigators.
Microvessels.
A. Control microvessels (vasa vasorum), indicating that microvessels were largely negative (8 of 11).
B. Microvessels in uraemic patients, indicating that there were no immunonegative sections with regards to microvessels. Half of the sections were strongly positive (+ +), the other half positive (+).
Large vessels.
C. Macrovascular endothelium of normal vessels, which were largely negative (8 of 11).
D. This however, indicates that all macrovascular endothelium evaluable in uraemic vessels was positive for RAGE, five of six even strongly positive.

Recent studies have provided important insights into the mechanisms by which AGEs may promote atherosclerosis. Phospholipids with primary amino groups, such as phosphatidylethanolamine or phosphatidylcholine, react directly with glucose to form AGEs that then initiate lipid oxidation. Aminoguanidine inhibit both the lipid advanced glycosylation and oxidative modification (Bucala et al 1993). The AGE modification of LDL interferes significantly with its normal, receptor-mediated uptake, as shown by fractional clearance studies performed in transgenic mice expressing the human LDL receptor (Bucala et al 1994). LDL-AGE also shows diminished recognition and uptake by human fibroblast LDL receptors (Bucala et al 1995).

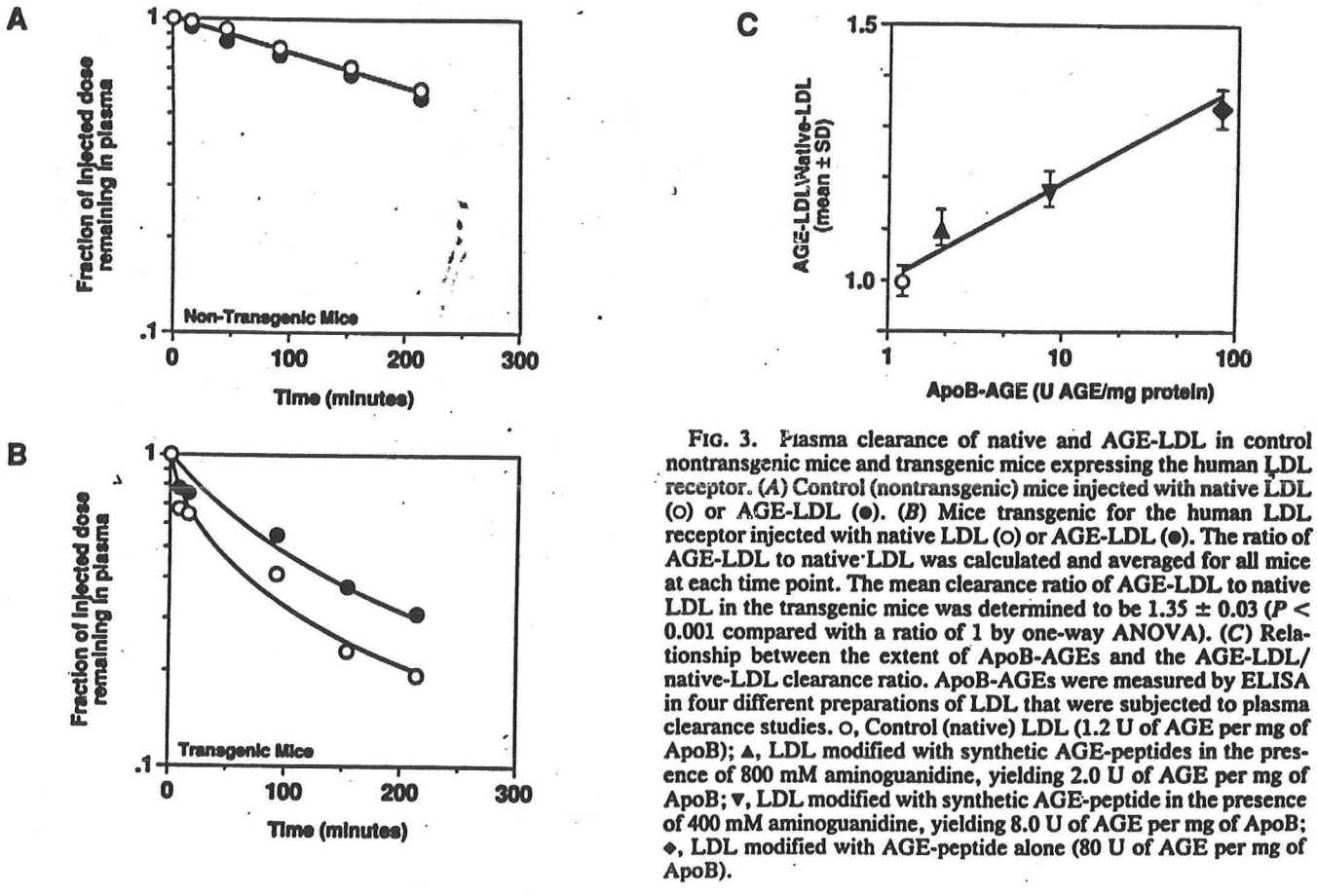


FIG. 3. Plasma clearance of native and AGE-LDL in control nontransgenic mice and transgenic mice expressing the human LDL receptor. (*A*) Control (nontransgenic) mice injected with native LDL (○) or AGE-LDL (●). (*B*) Mice transgenic for the human LDL receptor injected with native LDL (○) or AGE-LDL (●). The ratio of AGE-LDL to native LDL was calculated and averaged for all mice at each time point. The mean clearance ratio of AGE-LDL to native LDL in the transgenic mice was determined to be 1.35 ± 0.03 ($P < 0.001$ compared with a ratio of 1 by one-way ANOVA). (*C*) Relationship between the extent of ApoB-AGEs and the AGE-LDL/native-LDL clearance ratio. ApoB-AGEs were measured by ELISA in four different preparations of LDL that were subjected to plasma clearance studies. ○, Control (native) LDL (1.2 U of AGE per mg of ApoB); ▲, LDL modified with synthetic AGE-peptides in the presence of 800 mM aminoguanidine, yielding 2.0 U of AGE per mg of ApoB; ▼, LDL modified with synthetic AGE-peptide in the presence of 400 mM aminoguanidine, yielding 8.0 U of AGE per mg of ApoB; ◆, LDL modified with AGE-peptide alone (80 U of AGE per mg of ApoB).

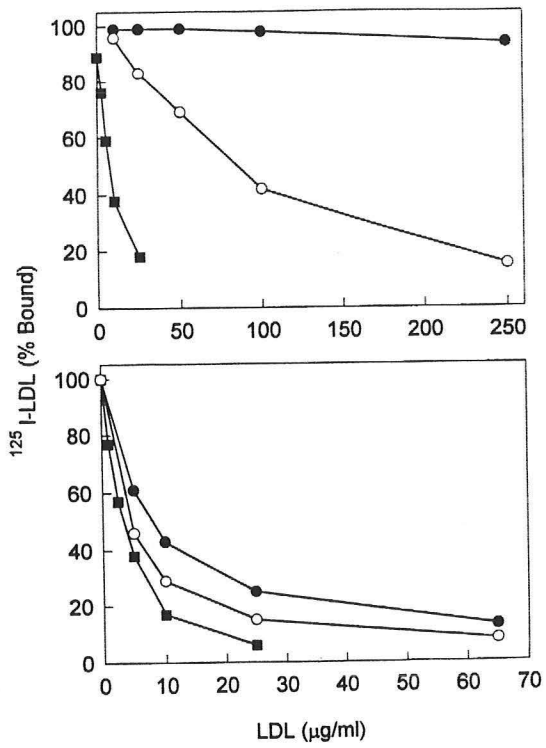
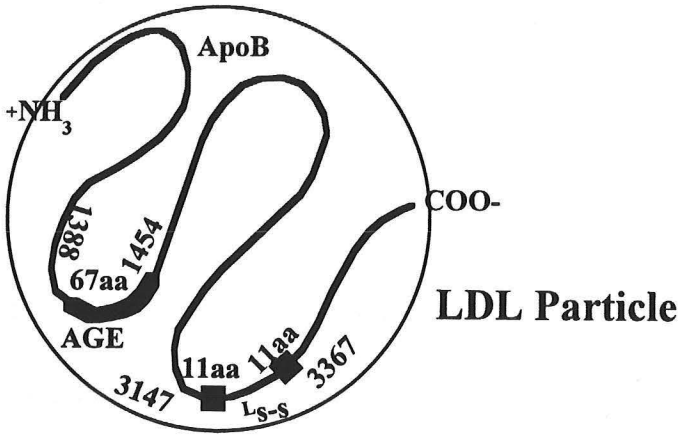


FIG. 1. **Inhibition of LDL receptor binding of ^{125}I-LDL by control, native LDL and AGE-LDL.** *Upper panel*, control LDL (■), AGE-LDL (80 units of AGE/mg of apoB) (●), and AGE-LDL prepared in the presence of aminoguanidine (20 units of AGE/mg of apoB) (○). *Lower panel*, control LDL (■), AGE-LDL (9 units of AGE/mg of apoB) (●), and AGE-LDL prepared in the presence of aminoguanidine (5 units of AGE/mg of apoB) (○). Advanced glycosylation reactions were performed by incubating control LDL (2.5 mg/ml, ~2 units of AGE/mg of apoB) with glucose (200 mM for 14 (*upper panel*) or 4 days (*lower panel*)) in aminoguanidine (300 mM, where indicated) in 0.2 M $NaPO_4$ buffer containing 1 mM EDTA and 20 μM BHT. Human foreskin fibroblasts were incubated at 4 °C with ^{125}I-labeled control LDL together with increasing amounts of unlabeled competitor LDL preparations, and the ^{125}I-LDL binding was measured as described under "Materials and Methods." The data points were calculated from the means of duplicate wells and displayed a variation of <10%.

Scheme Showing the Relative Positions of the Advanced Glycosylation Endproduct (AGE)-Reactive and Low-Density Lipoprotein (LDL)-Receptor Binding Domains in ApoB



AGE ELISA analysis of LDL specimens isolated from diabetic individuals have revealed increased levels of both apoprotein-and lipid—linked AGEs when compared to specimens obtained from normal, nor diabetic controls. In addition, circulating levels of oxidized LDL were elevated in diabetic patients and correlated significantly with lipid AGE levels (Bucala et al 1993, Bucala 1997).

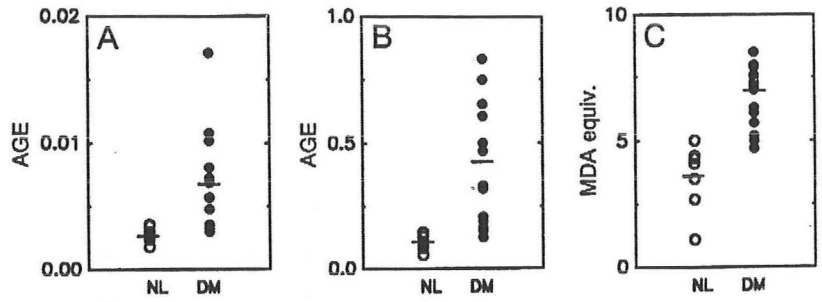


FIG. 5. Measurement of AGE and oxidative modification of LDL from normoglycemic, nondiabetic individuals (NL) and patients with diabetes mellitus (DM). (*A*) AGE modification of LDL apoprotein (unit of AGE per μg of apoprotein). (*B*) AGE modification of LDL lipid (unit of AGE per μg of lipid). (*C*) Oxidative modification of LDL (pmol of MDA equivalents per μg of LDL).

Importantly, treatment of diabetic patients with aminoguanidine results in significant decreases in LDL (Bucala et al 1994). Thus, aminoguanidine may be of dual benefit in inhibiting atherosclerosis, both by inhibiting the formation of AGEs and by inhibiting the modification of LDL apo B that result in its impaired clearance.

Biochemical Analysis of Blood Specimens from Diabetic Patients Who Received Aminoguanidine (n=18) or placebo control (n=8) for 28 days

	Treatment	
	Aminoguanidine	**Placebo**
Cholesterol	81.3 ± 7.2*	97.4 ± 5.4
Triglyceride	81.0 ± 6.2*	89.8 ± 5.8
VLDL	68.5 ± 28.7	96.4 ± 7.1
LDL	71.9 ± 9.9*	100.7 ± 11.2
HDL	104.7 ± 10.9	96.7 ± 16.4
Hb-AGE	72.7 ± 7.5*	90.8 ± 6.7
HbA_{1c}	89.7 ± 4.2	100.0 ± 4.5

Values are expressed as percent (mean ± SEM) of baseline value for each patient group [(day 28 value/day 0 value) X 100]. *, $p < 0.05$.

From: Bucala et al, Proc. Natl. Acad. Sci. USA , 91: 9444, 1994

IX) TREATMENT STRATEGIES FOR THE PREVENTION OF DIABETIC CARDIOVASCULAR AND RENAL DISEASE, INCLUDING AGE-INDUCED COMPLICATIONS

There is solid evidence to indicate that our overall treatment strategies to prevent or at least slow the progression of diabetic cardiovascular and renal disease need to include **1)** glycemic control, **2)** blood pressure control (the present data favors the use of angiotensin converting enzyme inhibitors and perhaps angiotensin II receptor antagonists), **3)** normalization of abnormal cholesterol and triglyceride levels, and **4)** normalization of abnormal AGE levels.

Long –term complications of non-insulin-dependent Diabetes mellitus (NIDDM)

Complication	**Cumulative life-time** Prevalence (%)
Retinopathy	
All cases	50-80
Macular edema	20
Proliferative retinopathy	20
Blindness	3-8
Nephropathy	
All cases	15-35
Microalbuminuria	20-35
Clinical grade Albuminuria	15-25
End-stage renal disease	6-20
Neuropathy	(50
	Relative risk
Macrovascular	2-7 fold

From: Nathan Clin Invest Med 48:332-339, 1995.

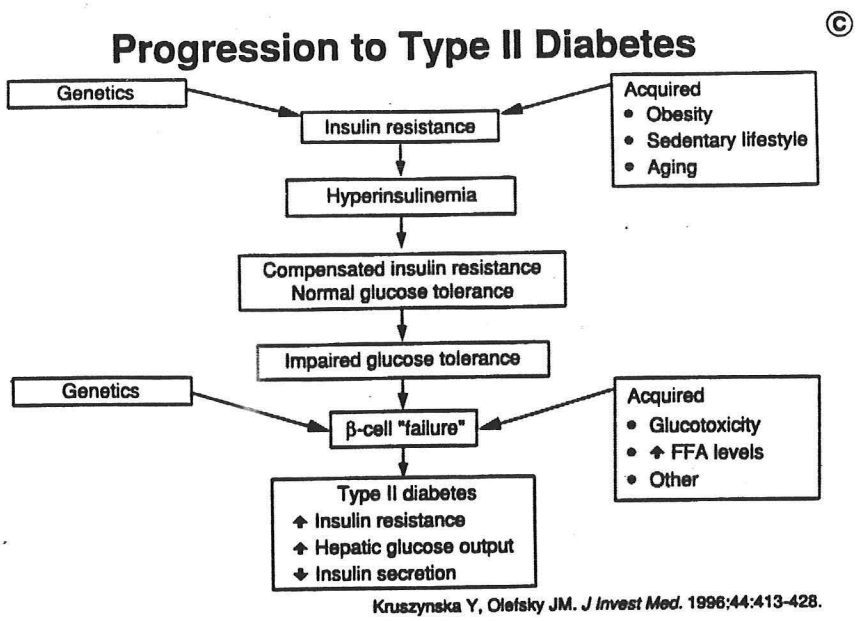
Major risk factors for diabetic nephropathy

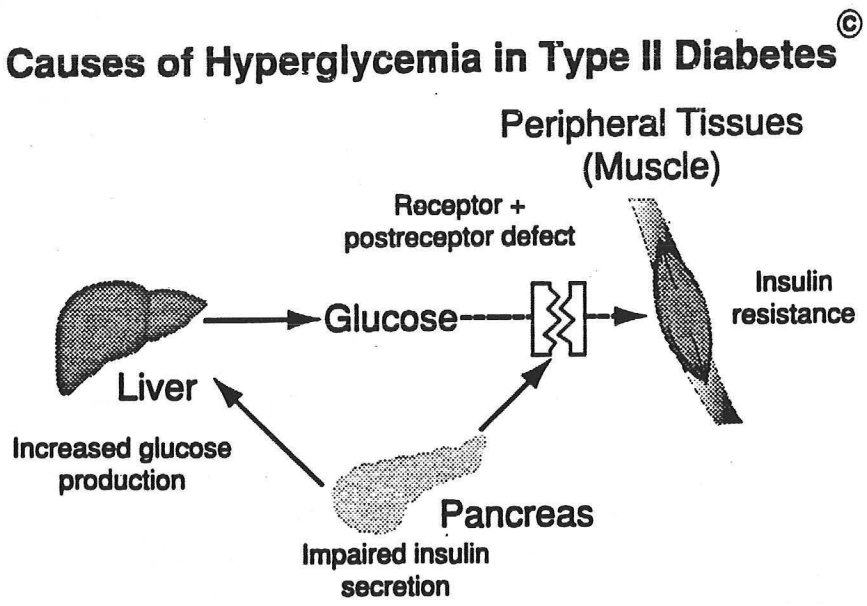
- Genetic susceptibility as evidenced by diabetic nephropathy in a sibling
- Hypertension or high-normal blood pressure
- Microalbuminuria
- Worse glycemic control
- Being African-American, Mexican-American, or Pima Indian

Major innovations in diabetic management

1. Capillary blood (finger stick) glucose testing using portable meter.
2. Repetitive measurements of glycosylated hemoglobin.
3. Normalization of hypertensive blood pressure
4. Recognition of the predictive value of screening for microalbuminuria.
5. Reduction of proteinuria with angiotensin converting enzyme (ACE) inhibitors.
6. Aggressive ophthalmologic preventive medicine: panretinal photocoagulation, vitrectomy, lens replacement, retinal reattachment.
7. Lower limb preservation by podiatric collaboration plus vascular surgical revascularization.
8. Recognition and treatment of autonomic neuropathic complications: gastroparesis, obstipation, diarrhea, and orthostatic hypotension.

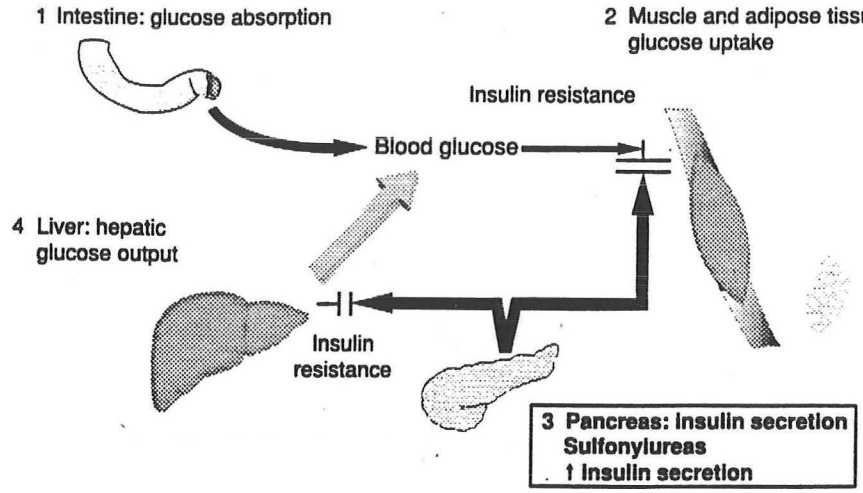
At least until recently the importance of glycemic control has been a somewhat controversial issue. However, recent data, especially the DCCT (Diabetes Control and Complications Trial Research Group) study in type I diabetics IDDM has convincingly shown the positive outcomes related to intensive glycemic control with insulin on nephropathy, retinopathy, and neuropathy. Glycemic control however, has been a more challenging issue in type II diabetic (NIDDM), especially because the presence of insulin resistance and the controversy about using very high doses of insulin, as these patients typically require.





Recent advances in the better understanding of the pathophysiology of type II diabetes and the introduction of new classes of pharmacological agents now brings at least some level of optimism that it may well indeed be possible to achieve excellent glycemic control in type II diabetics. Now, in addition to insulin, we also have several oral hypoglycemic agents, which if need be, can be used in combination to achieve the desired level of blood glucose control and of course hemoglobin A_1C. These include **1) Sulfonylureas**, which act by increasing insulin secretion, **2) Metformin**, which mainly acts by decreasing hepatic glucose output and also to some extent increasing glucose utilization, **3) α-Glucosidase Inhibitor**, which acts by decreasing glucose absorption secondary to decreasing digestion of carbohydrates, and **4) Thiazolidinediones**, which act mainly by decreasing insulin resistance and increasing glucose uptake, and also to some extent by decreasing hepatic glucose output.

Effects of Sulfonylureas on Type II Diabetes



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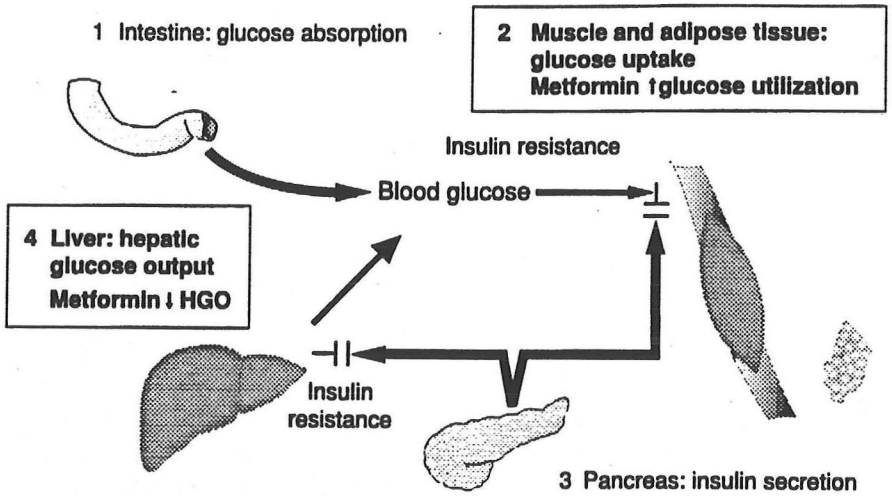
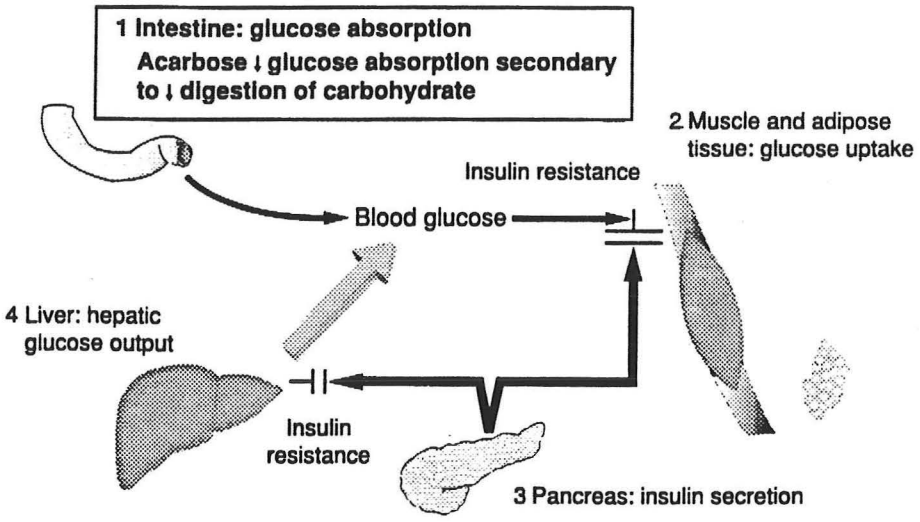
Metformin: Mechanism of Action



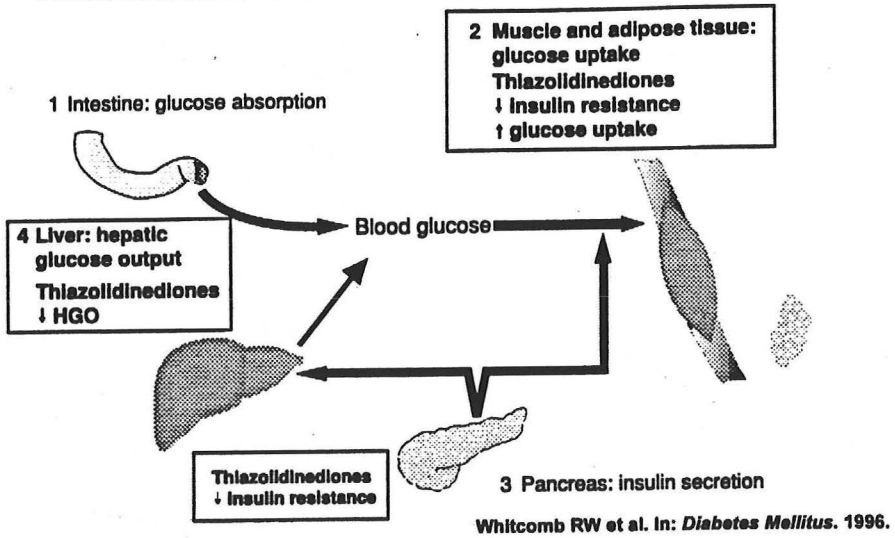
DeFronzo RA et al. *J Clin Endocrinol Metab.* 1991;73:1294-1301.

α-Glucosidase Inhibitor (Acarbose): Mechanism of Action



Amatruda JM. In: *Diabetes Mellitus*. 1996.

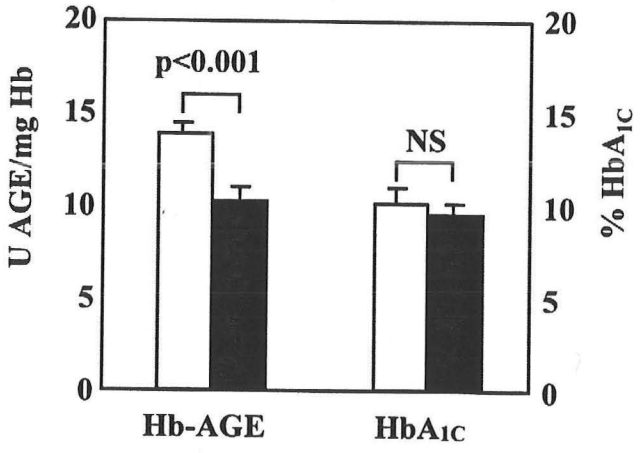
Thiazolidinediones: Mechanism of Action



Whitcomb RW et al. In: *Diabetes Mellitus*. 1996.

In spite of excellent glycemic control, however, if there is still evidence of increased levels of advanced glycosylation end products, including AGE-modified proteins, such as AGE-Hgb, and AGE-modified lipids, such as AGE-LDL, then it will be necessary to use Aminoguanidine to prevent further crosslinking of proteins and lipids. There is convincing data in experimental animals and some preliminary data in humans to indicate that aminoguanidine is very effective in preventing the formation of AGEs. Ongoing studies in type I and type II diabetes are now examining the long term effects of this drug in preventing or ameliorating target organ damage in diabetics.

Aminoguanidine Treatment Lowers the Levels of Hb-AGE in Diabetic Patients



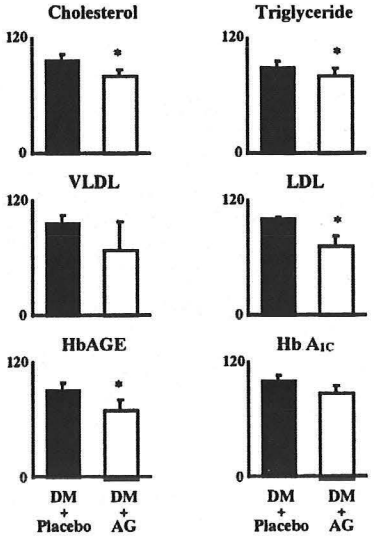
Biochemical Analysis of Blood Specimens from Diabetic Patients Who Received Aminoguanidine (n=18) or placebo control (n=8) for 28 days

	Treatment	
	Aminoguanidine	**Placebo**
Cholesterol	81.3 ± 7.2*	97.4 ± 5.4
Triglyceride	81.0 ± 6.2*	89.8 ± 5.8
VLDL	68.5 ± 28.7	96.4 ± 7.1
LDL	71.9 ± 9.9*	100.7 ± 11.2
HDL	104.7 ± 10.9	96.7 ± 16.4
Hb-AGE	72.7 ± 7.5*	90.8 ± 6.7
HbA_{1c}	89.7 ± 4.2	100.0 ± 4.5

Values are expressed as percent (mean ± SEM) of baseline value for each patient group [(day 28 value/day 0 value) X 100]. *, $p < 0.05$.

From: Bucala et al, Proc. Natl. Acad. Sci. USA , 91: 9444, 1994

Biochemical Parameters After 28 Days of Aminoguanidine Treatment in Diabetics (% of Baseline Value)



Aminoguanidien can prevent the formation crosslinks, however cannot undo the crosslinks once they are formed. Recently, a new agent has been found, a prototypic AGE crosslinks "breaker", N-phenacythiazolium bromide (PTB), which reacts with and cleaves covalent, AGE-derived protein crosslinks (Vasan et al 1996).

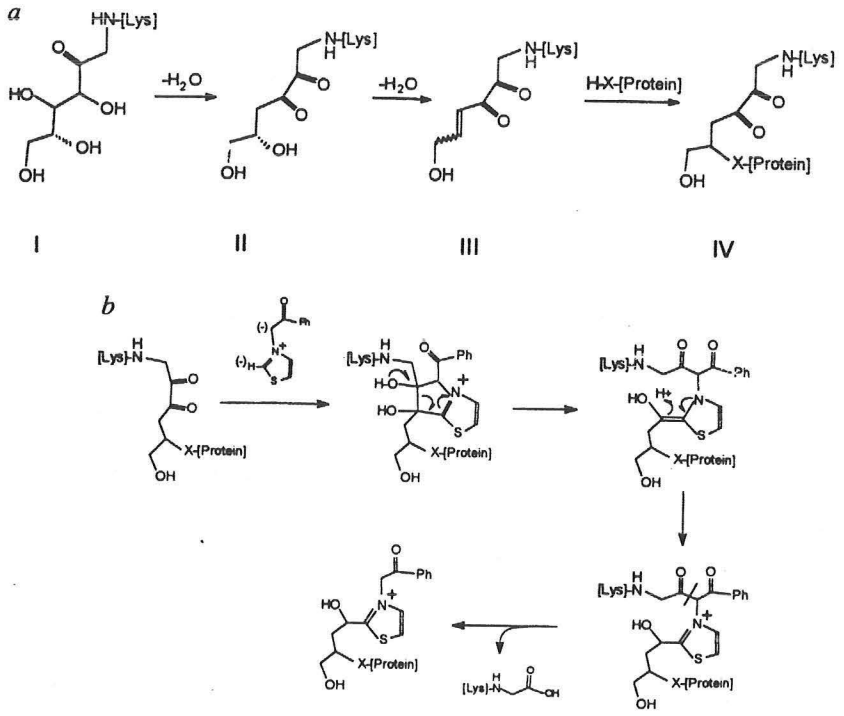
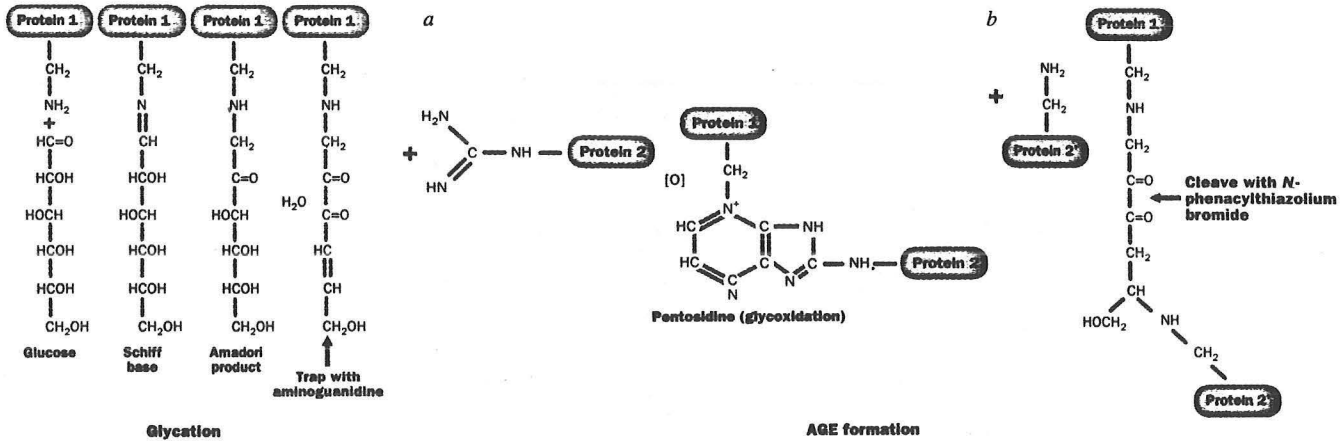


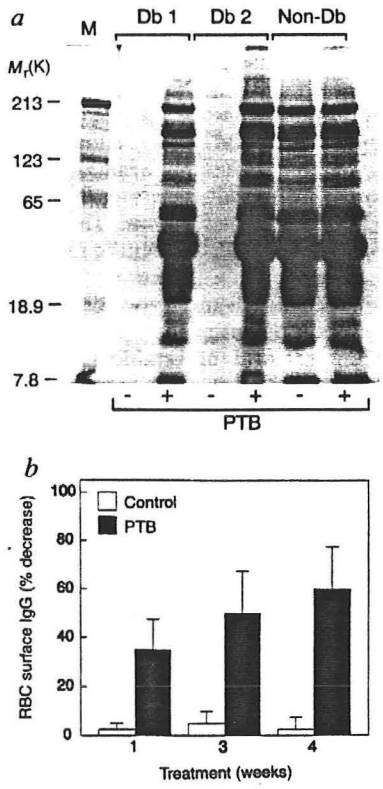
FIG. 1 Scheme for the formation of glucose-derived protein crosslinks from Amadori products and cleavage by a thiazolium-based AGE breaker. *a*, Successive dehydration by β-elimination of protein-bound (lysine) Amadori products (I) to AP-dione (II), AP-ene-dione (III), and reaction with a protein nucleophile (X-[Protein]) to form a stable protein–protein crosslink (IV). Molecule I exists predominantly in a pyranose form, and II, IV and the *cis* form of III may also prefer pyranose- or furanose-like cyclic hemiacetal structures[17]. *b*, Proposed reaction scheme for the cleavage of an AP-ene-dione-derived, protein–protein crosslink by *N*-phenacylthiazolium bromide (PTB).



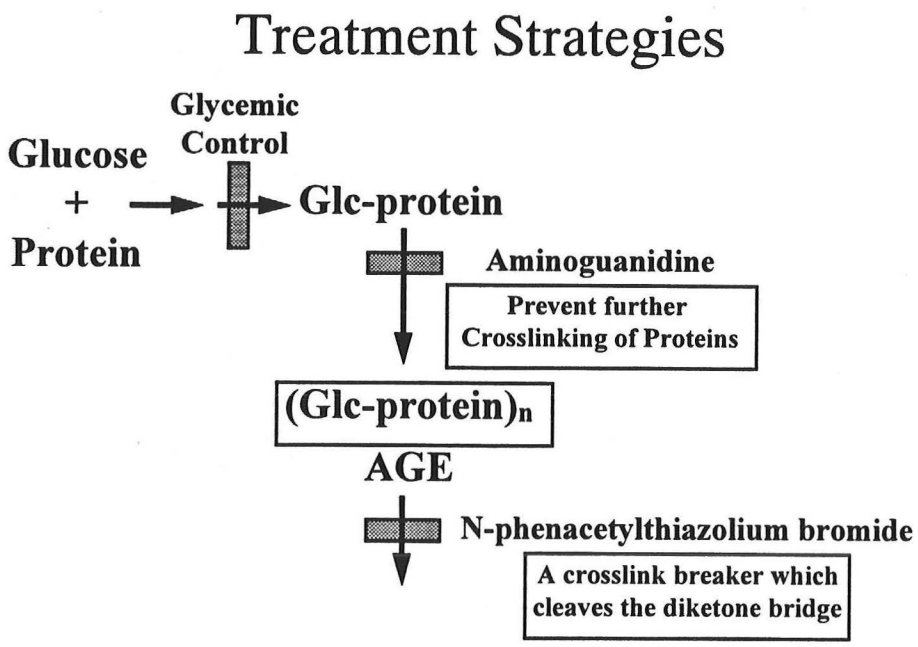
Chemical steps that may result in protein–protein crosslinking following glycation. One pathway (*b*) is postulated to follow directly from reaction of the diketone intermediate with amino groups of other proteins[1,5]. For simplicity, ε-amino groups of lysine side chains are shown in the first and second steps, although α-amino groups might participate in the first step and a variety of protein side chains could be involved in the final addition reaction. Alternative mechanisms of crosslink formation (*a*), involving condensation with arginine side chains and oxidation (glycoxidation) to form pentosidine, have been proposed[6,7]. In both cases, much of the chemistry underlying the formation of crosslinks remains hypothetical. Points at which the formation of AGEs (advanced-glycation end-products) can be disrupted are indicated in grey.

FIG. 3 PTB cleaves AGE crosslinks that form *in vivo*. *a*, PTB treatment *in vitro* decreases AGE crosslinking in diabetic, rat tail-tendon collagen. Data are representative of collagen from two diabetic animals (Db1, Db2) and one non-diabetic control (non-Db). Lane M, relative molecular mass markers. *b*, PTB treatment decreases IgG crosslinked to the red-blood-cell (RBC) surface.

METHODS. *a*, Diabetes was induced in male Lewis rats (150–175 g) by i.p. injection of steptozotocin (65 mg kg^{-1}) and confirmed after one week by plasma glucose measurement ($\geq$250 mg dl^{-1}). Thirty-two weeks later, the rats were killed and collagen isolated from their tail-tendon fibres using a standard protocol[25]. The insoluble collagen then was treated with cyanogen bromide[26] and the hydroxyproline content measured[27]. Aliquots containing 1 μg equivalent of hydroxyproline were run on SDS–PAGE under reducing conditions and stained with Coomassie blue. *b*, IgC crosslinked to the RBC surface was determined in an anti-IgG ELISA adapted for use with cellulose-ester-membrane-sealed 96-well microtitre plates (Multiscreen-HA, Millipore). Heparinized blood was washed three times with PBS; the packed RBC were diluted 1:250–1:500 in PBS. Membrane-containing wells were first blocked with 0.3 ml Superblock (Pierce), then washed with 0.3 ml PBS/0.05% Tween, followed by 0.1 ml PBS. RBCs were gently vortexed and 50-μl aliquots pipetted into wells. Cells were then washed and 50 μl of a polyclonal rabbit anti-rat IgG (Sigma, diluted 1:25,000) was added. After incubation at room temperature for 2 h, the cells were washed 3 times with PBS, once with Tris-buffered saline, and 0.1 ml *p*-nitrophenyl phosphate substrate was added (1 mg ml^{-1} in 0.1 M diethanolamine buffer, pH 9.5). By this technique, the A_{410} of non-diabetic red cells was 0.10 ± 0.02 and the A_{410} of diabetic red cells was 0.57 ± 0.06 ($n = 4$; $P < 0.0002$). The ability of PTB to reduce RBC-surface IgG *in vitro* was evaluated as follows. RBCs from diabetic rats were washed and 0.1-ml aliquots incubated overnight at 37 °C with 1 ml PTB in PBS. Control incubations contained RBCs and PBS alone. At the end of the reaction, RBCs were assayed for RBC-IgG; per cent decrease was calculated as $100 \times ((A_{410}, \text{PBS control}) - (A_{410}, \text{PTB}))/(A_{410}, \text{PBS control})$. Diabetic rats were treated for up to 4 weeks with PTB (10 mg kg^{-1} q.d. by oral gavage) or saline as control ($n = 4$–6 rats per group). At intervals, blood was collected from tail veins into heparinized tubes, washed 3 times with 10 vol PBS, and assayed for surface IgG. Values are means ±s.d. of the per cent decrease with respect to day 0. *P* values were calculated by the Student's *t*-test, independent variable.

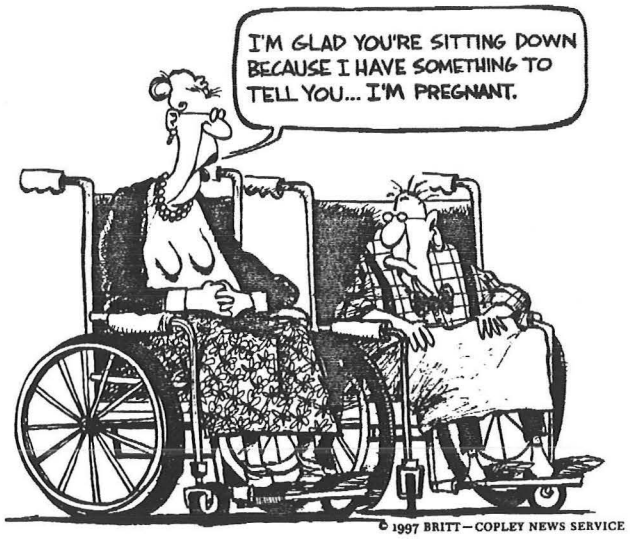


The ability of PTB to break AGE crosslinks *in vivo* now offers a potential therapeutic approach for the removal of established AGE crosslinks as well as the prevention of the formation of crosslinks, which is accomplished by initially achieving intensive glycemic control and then using aminoguanidine.



SUMMARY

I hope that I have been able to provide convincing evidence that AGEs play an important role in diabetic cardiovascular and renal disease. Although I have not discussed it in these Grand Rounds, AGEs are also implicated in the pathology of the ageing process and Alzheimer's disease. Hopefully, if progress continues in understanding the mechanisms in AGE crosslinks formation and hence removal of established crosslinks, then perhaps one day the facts presented in the cartoon below occur in a more favorable setting!



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