Developing New Therapies for Vascular Complications:

New Investigative Tools in Nephropathy:

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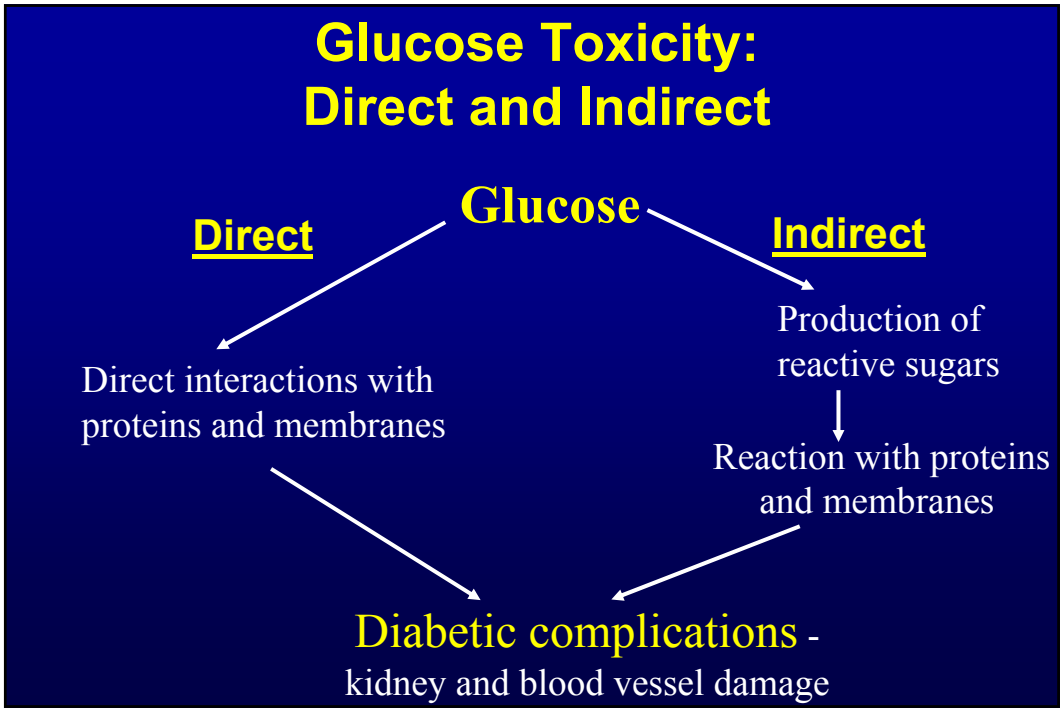
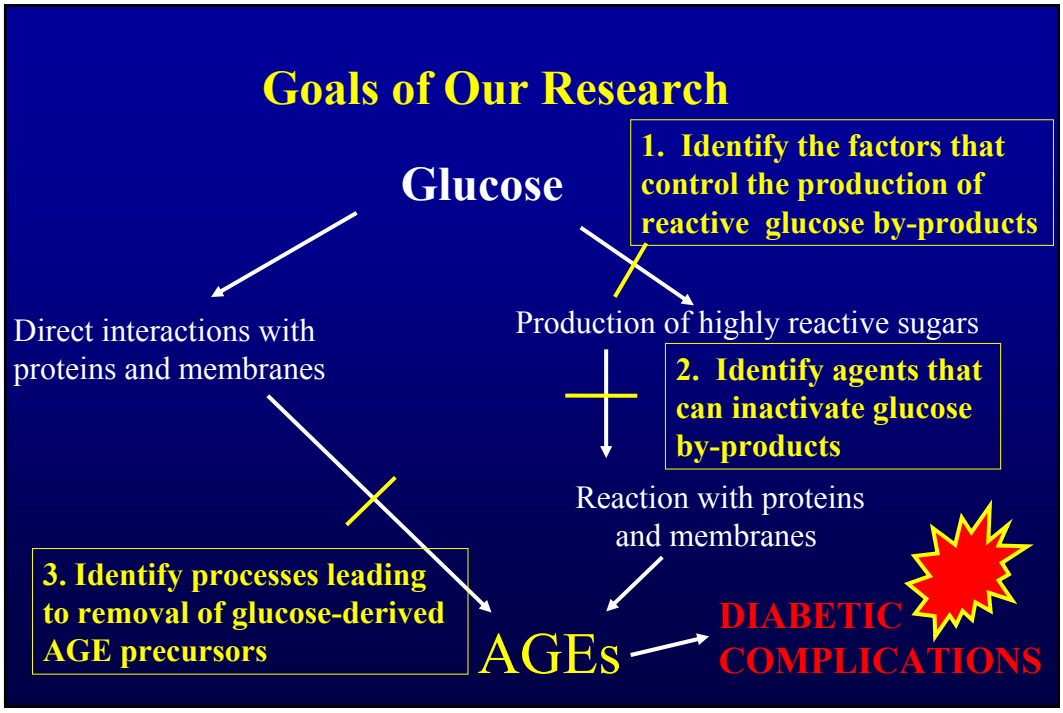
Protective Mechanisms against Glycation

The fundamental concept underpinning our work is the idea that humans have mechanisms to control the damage caused by unavoidable nonenzymatic glycation.

- These protective mechanisms are determined by genetically encoded enzymes which determine the levels of glycating agents.
- In diabetes these mechanisms are important, due to increased glycemic stress.
- These protective mechanisms are further impaired by metabolic perturbations produced by the diabetic state.

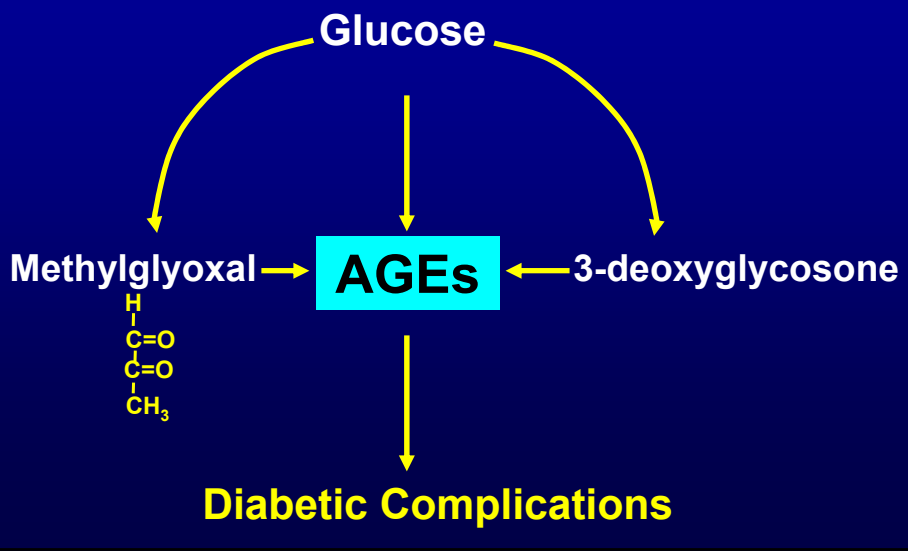

Glucose Toxicity:
Direct and Indirect
Glucose
Direct
Indirect
Direct interactions with proteins and membranes
Production of reactive sugars
Reaction with proteins and membranes
Diabetic complications -
kidney and blood vessel damage


Goals of Our Research
Glucose
1. Identify the factors that control the production of reactive glucose by-products
Direct interactions with proteins and membranes
Production of highly reactive sugars
2. Identify agents that can inactivate glucose by-products
Reaction with proteins and membranes
3. Identify processes leading to removal of glucose-derived AGE precursors
AGEs
DIABETIC COMPLICATIONS

First Area of Study

Identify the factors that control the production of reactive glucose by-products, particularly ∝ Dicarbonyls.

Formation of α-Dicarbonyls from Glucose



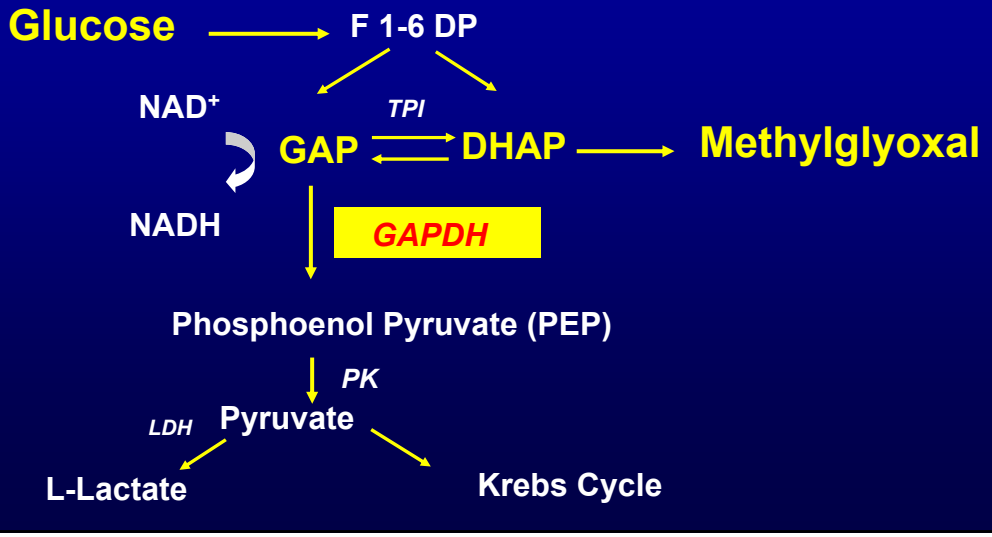
Toxicity of Carbonyls

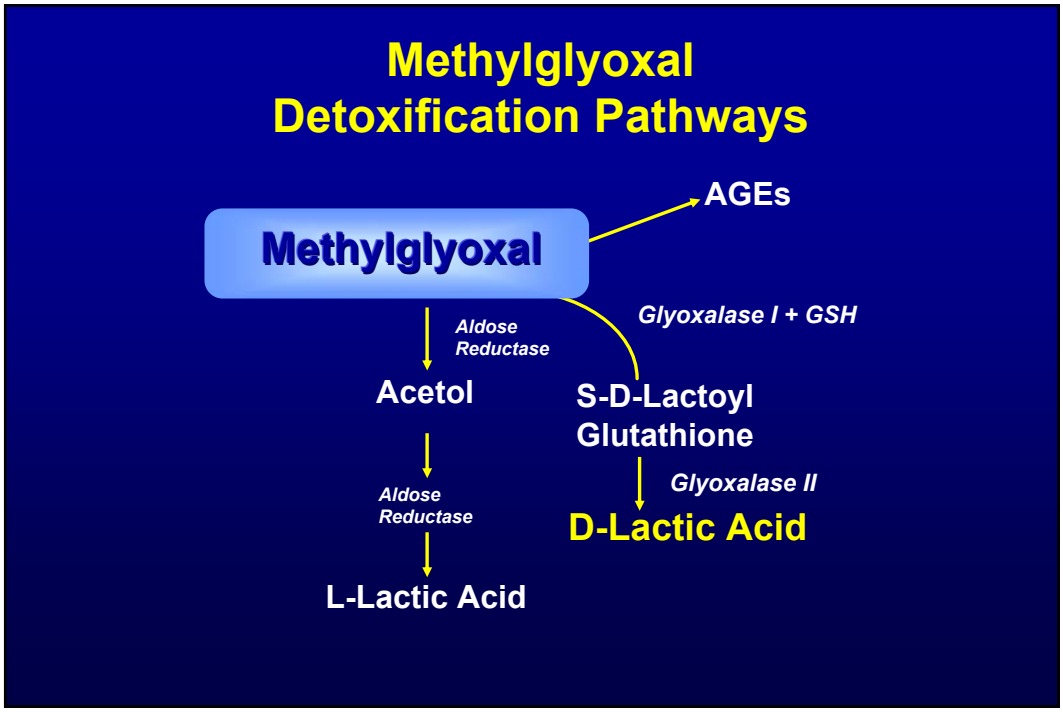
- **Methylglyoxal, 3-Deoxyglucosone, Glyoxal**

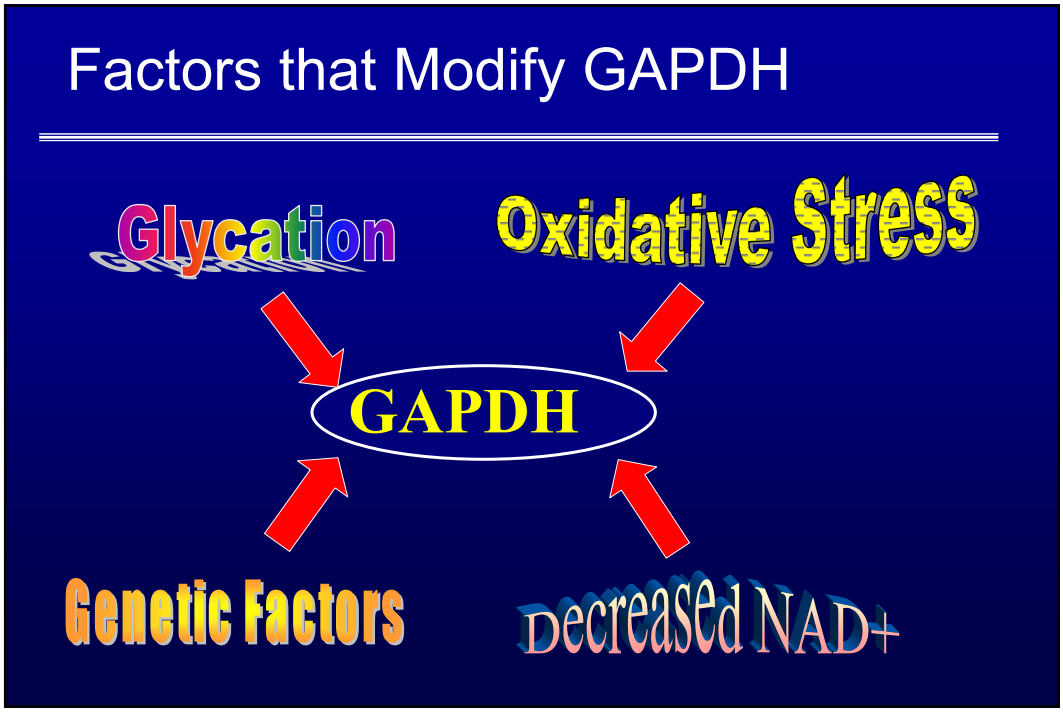
Up to 10,000 X more chemically reactive than glucose

- **Inhibit cell growth**
- **Inhibit DNA synthesis and mutagenic**
- **Inhibit enzymatic activity**
- **Produce protein cross-linking and fragmentation**
- **Produce protein Precursors for AGE formation**

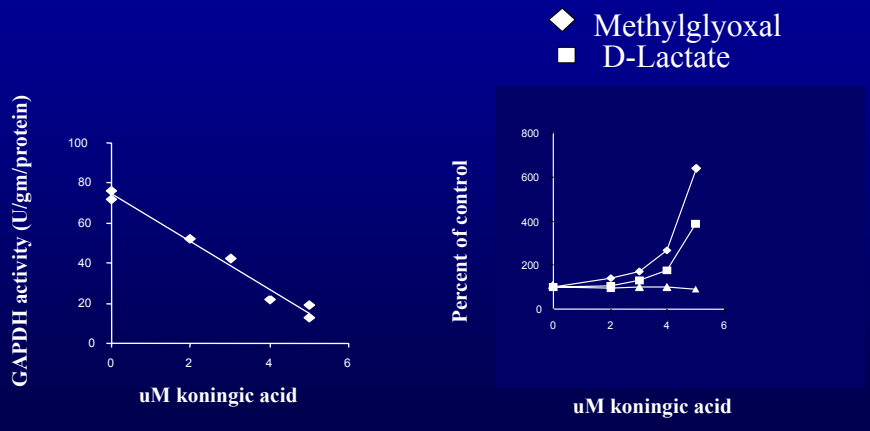
Methylglyoxal Production Pathways




Methylglyoxal
Detoxification Pathways
AGEs
Methylglyoxal
Aldose Reductase
Glyoxalase I + GSH
Acetol
S-D-Lactoyl Glutathione
Aldose Reductase
Glyoxalase II
D-Lactic Acid
L-Lactic Acid

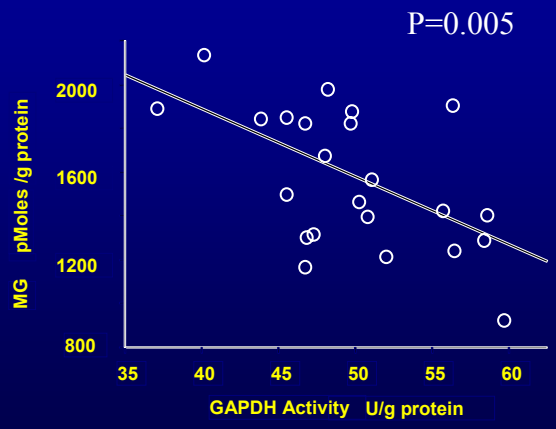

Factors that Modify GAPDH
Glycation
Oxidative Stress
GAPDH
Genetic Factors
Decreased NAD+

Inhibition of GAPDH by Koningic Acid and MG Production



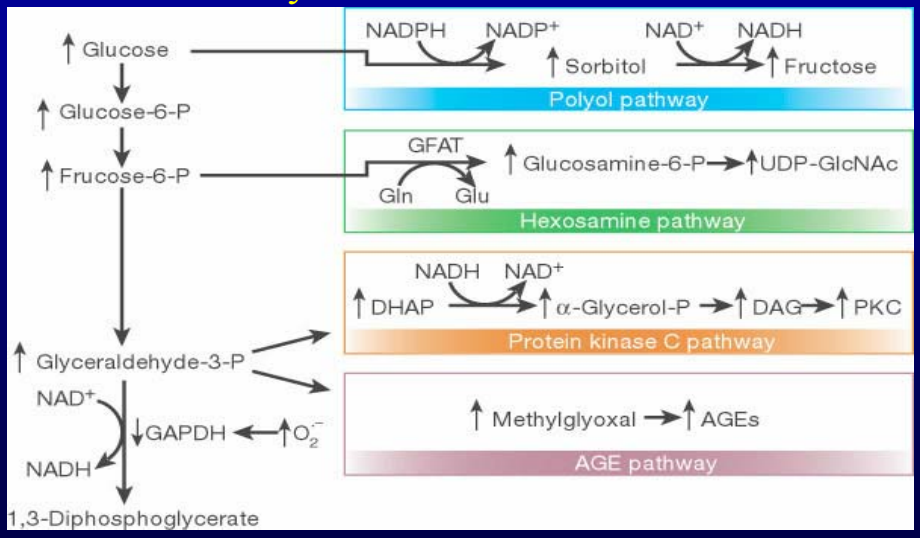
Beisswenger et al, Biochem. Biophys, Acta, 1637, 98-106, 2003

Cellular GAPDH Activity and Methylglyoxal Production



Beisswenger et al, Biochem. Biophys, Acta, 1637, 98-106, 2003

Activation of four pathways of hyperglycemic damage By GAPDH Inhibition



Brownlee, M, Nature, 414, 813-820, 2001

Susceptibility to Diabetic Kidney Disease is Largely Determined by Genetic Predisposition

- **If you have a diabetic sibling with kidney disease, your risk is much greater. (Seaquist et al, NEJM,320, 1161-65, 1989)**
- **Higher rates of kidney disease are seen if your close relative has rapid progression (DCCT Study Group, Diabetes, 46, 1829-39, 1997)**
- **Diabetic kidney disease clusters in families among the Pima Indians (Kunzelman and Knowler, Kidney Int., 35, 681-87, 1989)**
- **Diabetic siblings show similar pathological patterns of kidney damage. (Fioretto and Mauer, Diabetes, 48, 865-69, 1999)**

Hypothesis 1

We hypothesize that increased susceptibility to diabetic kidney disease is closely related to the increase in methylglyoxal generated on exposure to high glucose levels, while lower MG production infers protection.

Study One:
Group with Accelerated Nephropathy

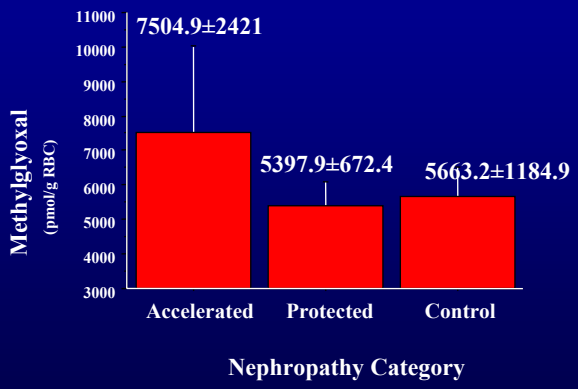
Complication-resistant cohort: **(Protected)**

- Greater than 25 yrs duration.
- Retinal grade of 20 or less based on modified Arlie House criteria.
- AER< 20 mg/24 hrs.

Complication-susceptible cohort: **(Accelerated)**

- Duration ≤ 15 years at onset of complications.
- Retinal grade of ≥ 30 (severe background, pre-proliferative or proliferative retinopathy)
- Persistent AER ≥ 40 mg/24 hrs.

Methylglyoxal Production by RBCs from Subjects with Accelerated Nephropathy

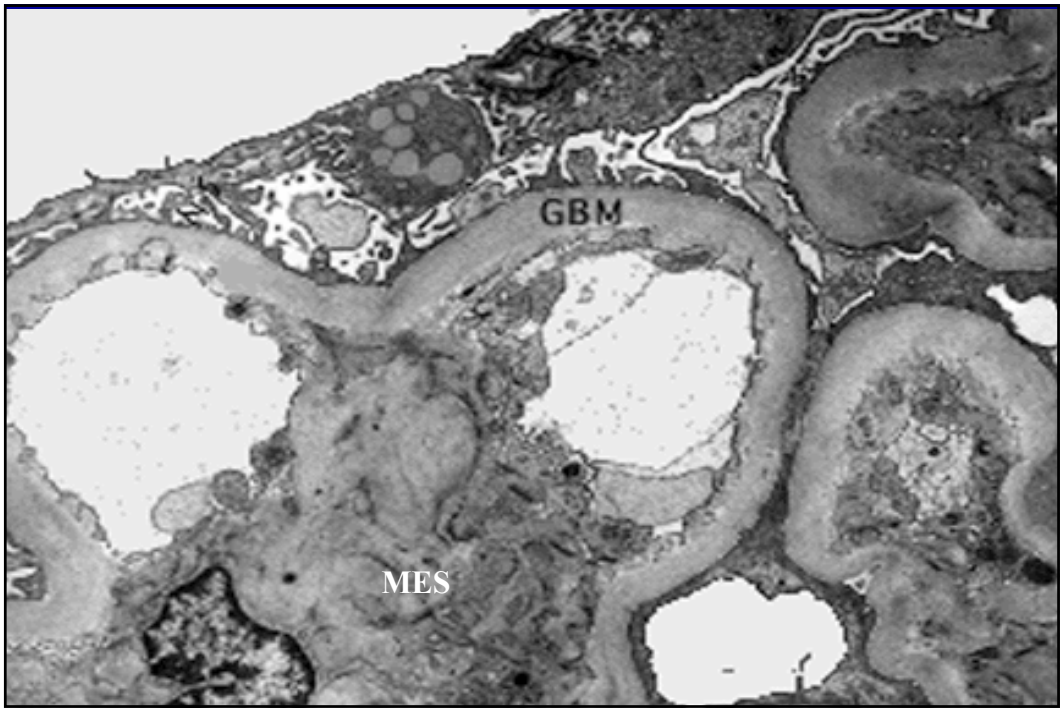


Study Two: Natural History of Nephropathy Study

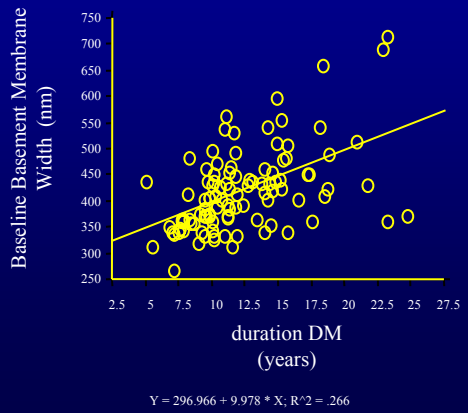
- Consists of 110 subjects with type 1 diabetes of mean age 22.3 ± 7 years and duration 12.3 ± 4.1 years participating in "The Natural History of Diabetic Nephropathy Study" *

- All subjects had normal renal function with mean GFR of 131 ± 26 ml/min and Mean Urinary Albumin Excretion of 13.5 ± 26 mg/24 hrs

* Mauer, M. and K. Drummond, *The early natural history of nephropathy in type 1 diabetes: I. Study design and baseline characteristics of the study participants.* Diabetes., 2002. **51**(5): p. 1572-9.

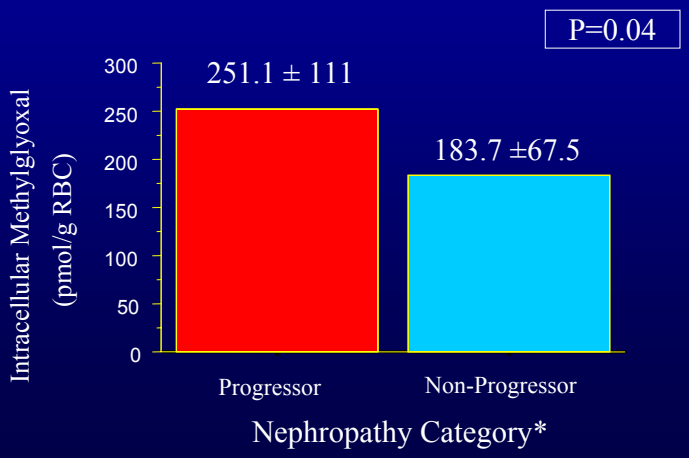


Basement Membrane Width Increases With Duration of Diabetes



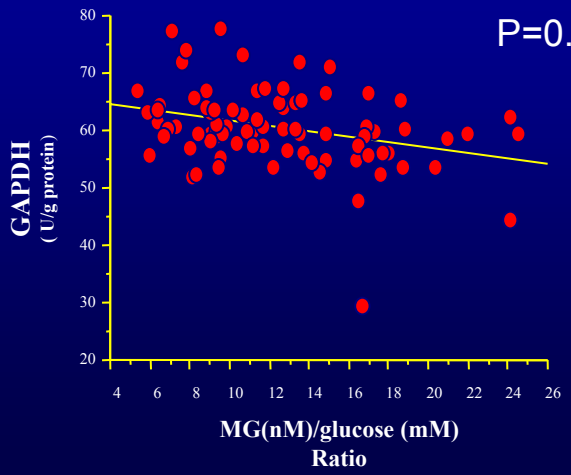
R=0.52
P=<0.0001

Cellular Production of Methylglyoxal from Nephropathy Progressors and Non-Progressors

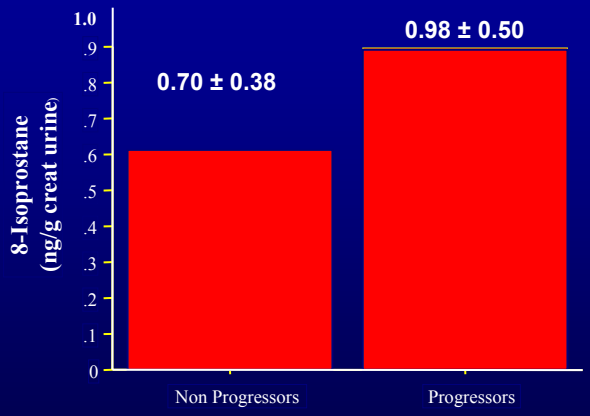


* Based on upper and lower quintiles of GBM thickening over 5 years

GAPDH activity was also found to be inversely related to the MG/glucose ratio.



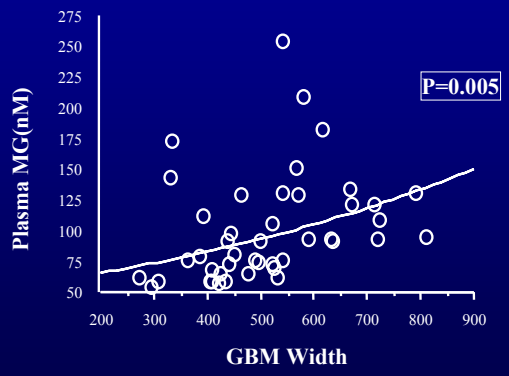
8-Isoprostanes in Nephropathy Progressors And Non-Progressors



P=0.028

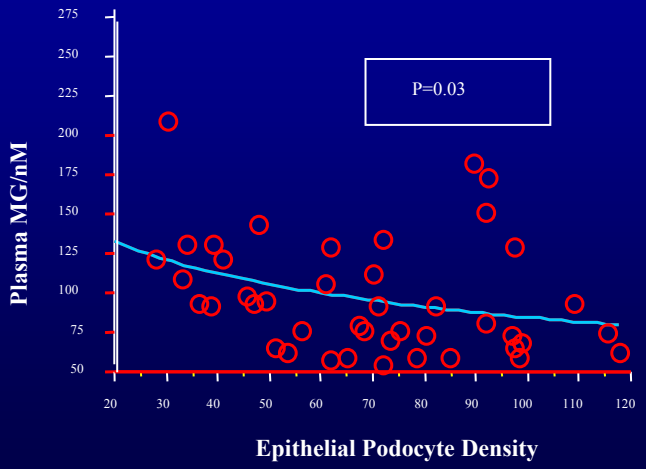
(Progressor defined as Upper quartile)

Relationship Between GBM Width and Methylglyoxal Levels in Pimas



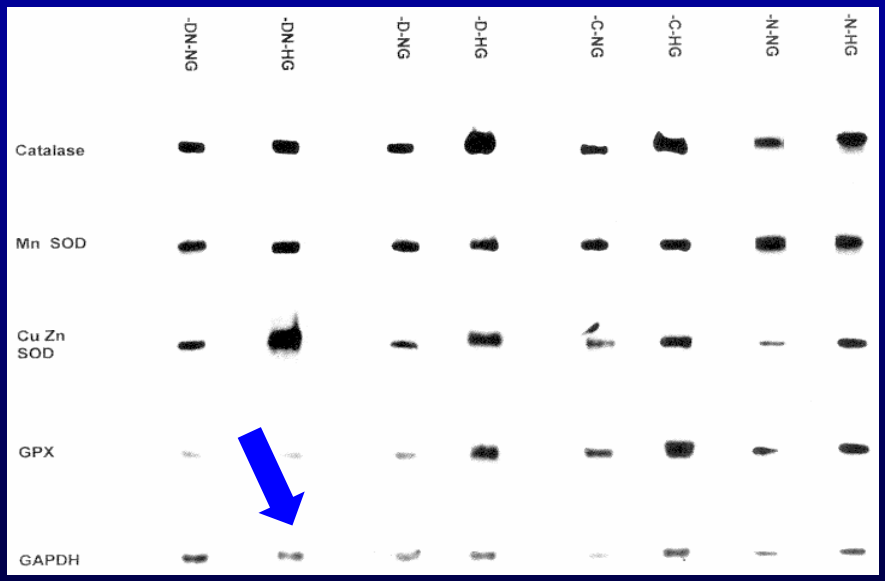
Beisswenger, P, and R. Nelson, Presented at the ADA Scientific Meetings, 2002

Epithelial Podocyte Density and Methylglyoxal Levels in Pimas



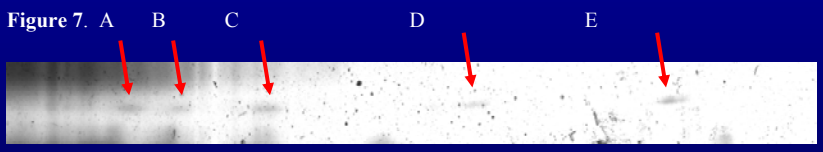
Beisswenger, P, and R. Nelson, Presented at the ADA Scientific Meetings, 2002

Northern Blots of Fibroblasts Exposed to 5 and 22 mM Glucose



Ceriello, A, Diabetes 49, 2170-2177, 2000

Isoforms of GAPDH



AGEs in Plasma and Hemoglobin in Type 1 Diabetes

		CEL	MG-HI	MOLD
Plasma protein (µmol/molLys or Arg.)	Diabetic	58 ± 42*.	1040 ± 9*	10.4 ± 8.6*
	Control	12 ± 5.0	31 ± 20	1.1 ± .7
Hemoglobin (%)	Diabetic	0.30 ± 0.06^{++}	4.35 ± 1.59$^{\#}$	0.027 ± 0.026^{ns}
	Control	0.23 ± 0.10	2.83 ± 1.59	0.023 ± 0.024

* P <0.001, # P< 0.01, ++ P<0.05

Thornalley and Beisswenger 2003

Conclusions

- MG levels are significantly elevated in subjects with type 1 diabetes who show more rapid progression of kidney damage.
- Red blood cells from rapid progressors produce more MG when exposed to high glucose levels.
- Increased MG levels are related to the degree of reduction in GAPDH activity

Second area of Study:

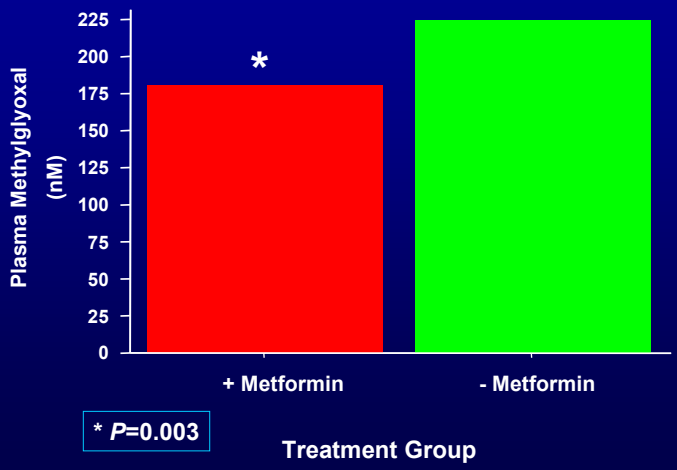
Identify agents that can inactivate glucose by-products.

Guanidino Compounds that Bind Dicarbonyls

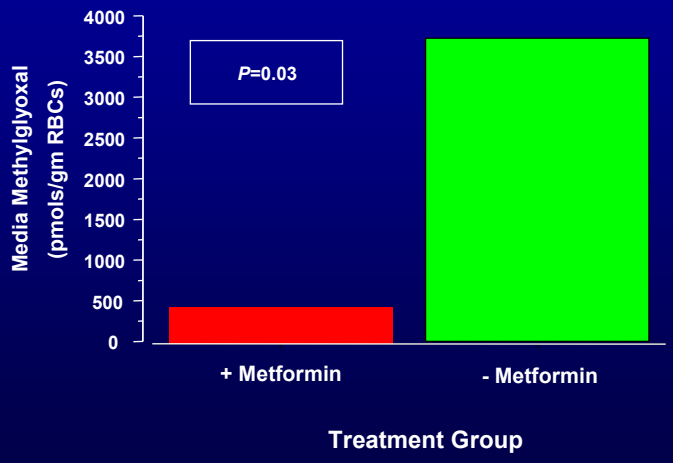
Aminoguanidine

Metformin
(Dimethylbiguanide)

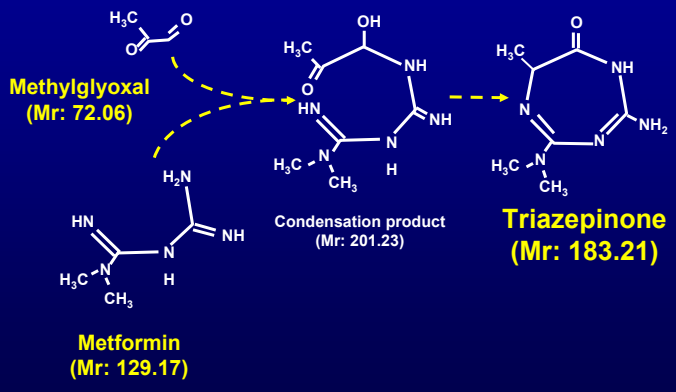
Plasma Methylglyoxal With and Without Metformin



Media levels of Methylglyoxal With or Without Metformin

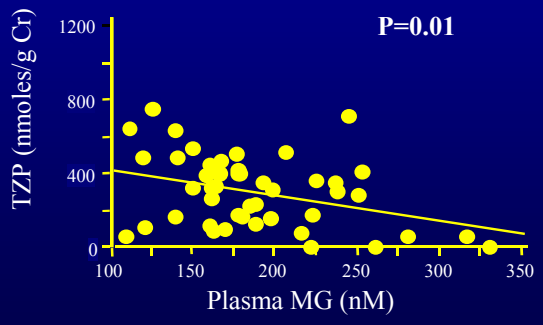


Formation of Triazepinone

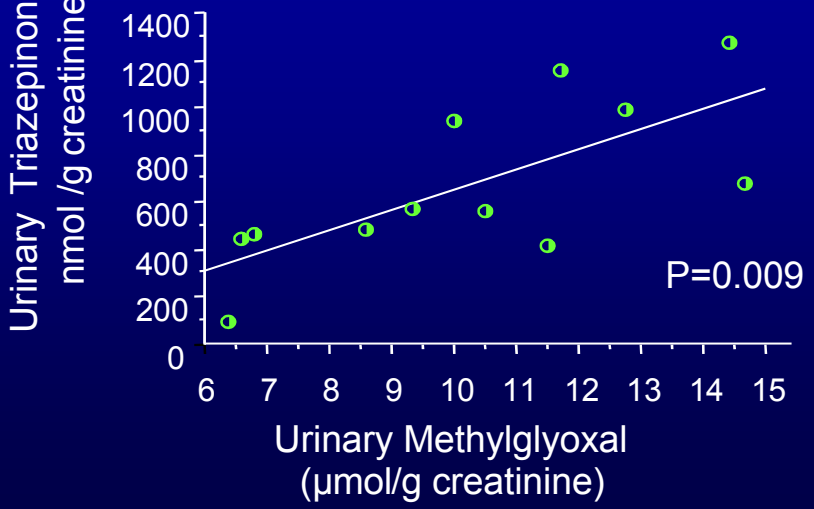


Ruggiero - Lopez et al. *Bio Pharm*. 1999;58:1765-1773.

Relationship between Methylglyoxal And Triazepinone in Type 2 Diabetes



Urinary Methylglyoxal and Triazepinone During Metformin Therapy

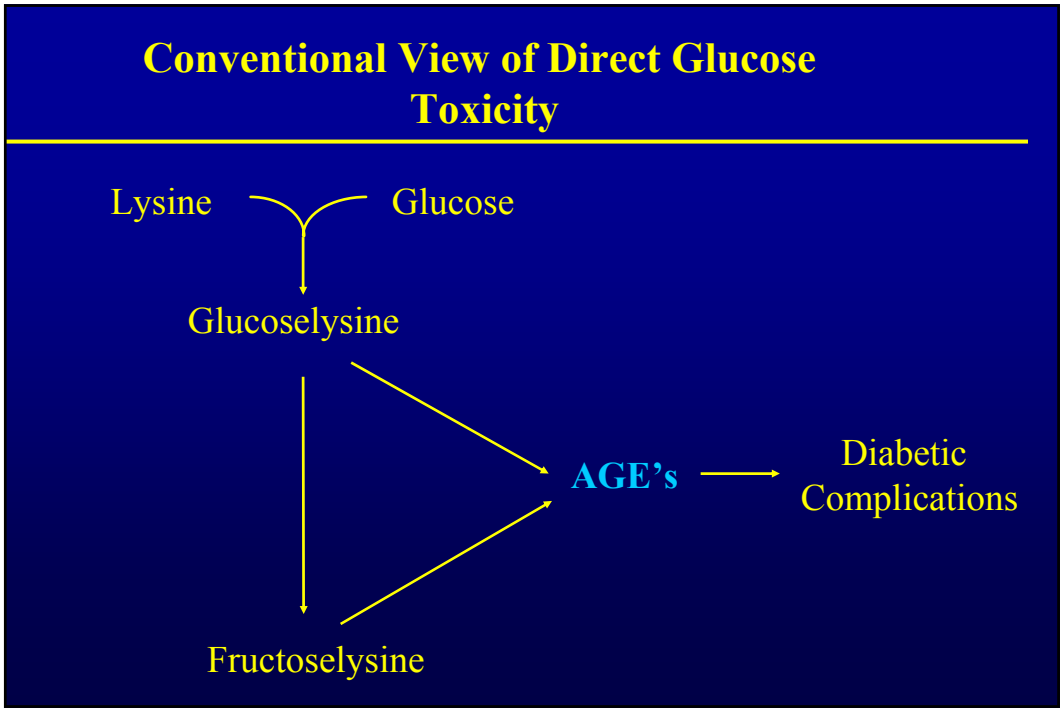


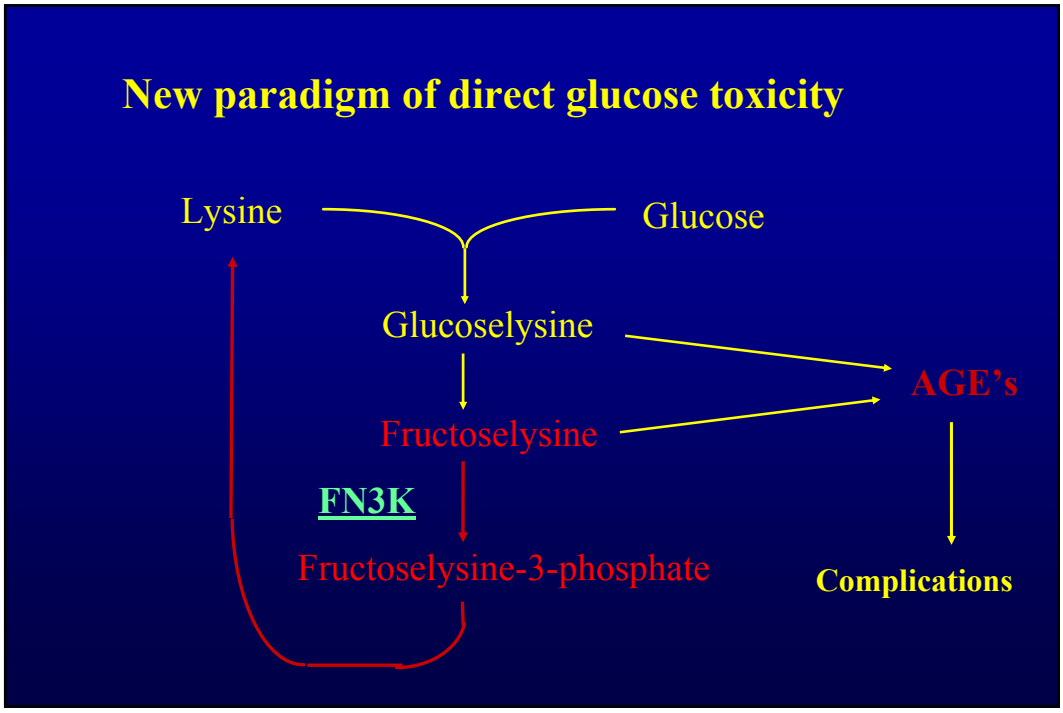
Third Area of Investigation

Identify processes leading to removal of glucose-derived AGE precursors

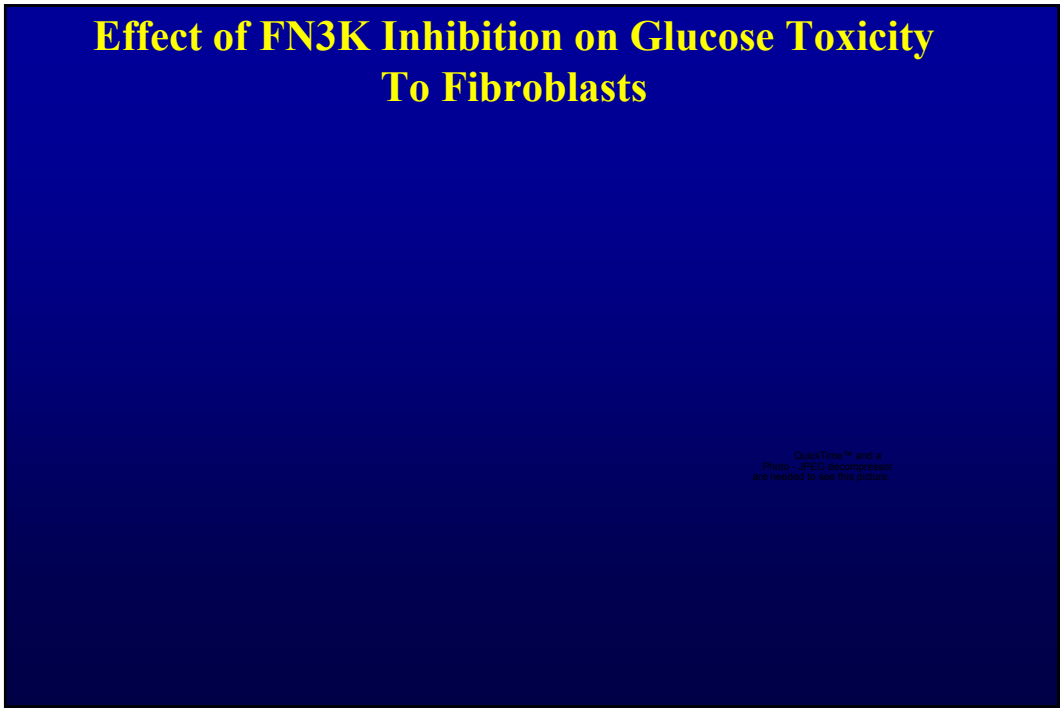
Therapeutic Implications

- Degree of glycemic control required to prevent complications may differ among individuals
- Inhibitors of AGE formation may work though blocking ∝ dicarbonyl toxicity
  - Aminoguanidine
  - Pyridoxamine
  - Metformin
- Antioxidants may reduce glycation stress and PKC activity partially through increased GAPDH activity
- ARIs may reduce glycation as well as polyol pathway activity


Conventional View of Direct Glucose Toxicity
Lysine
Glucose
Glucoselysine
AGE's
Diabetic Complications
Fructoselysine


New paradigm of direct glucose toxicity
Lysine
Glucose
Glucoselysine
AGE's
Fructoselysine
FN3K
Fructoselysine-3-phosphate
Complications

Effect of FN3K Inhibition on Glucose Toxicity To Fibroblasts



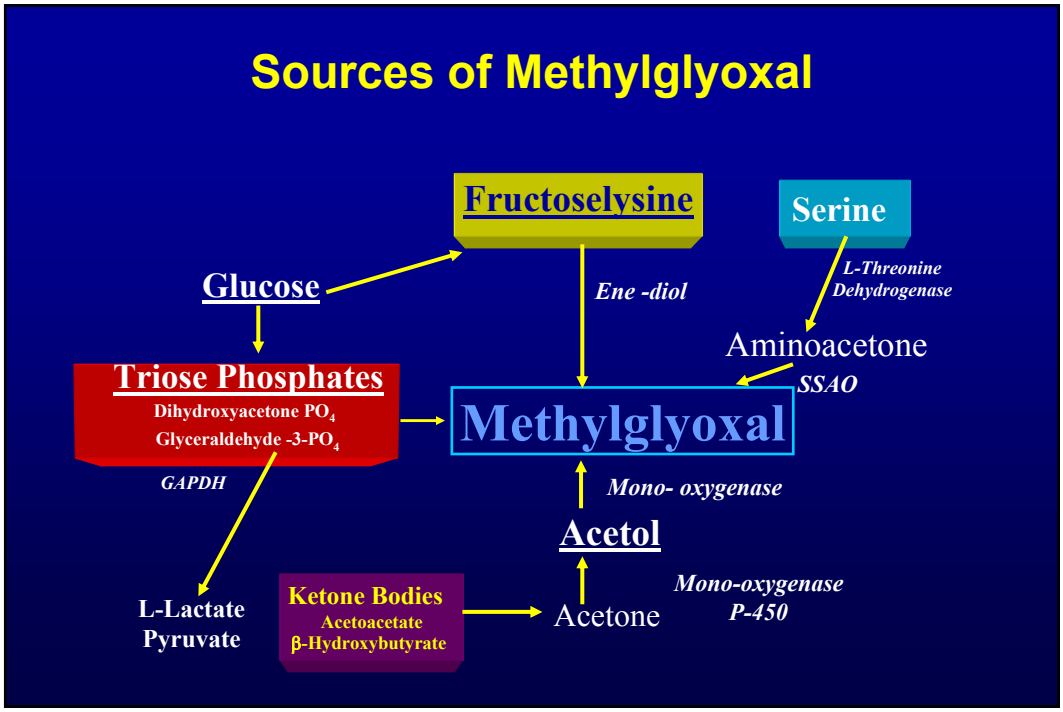
Disease Burden of Diabetes

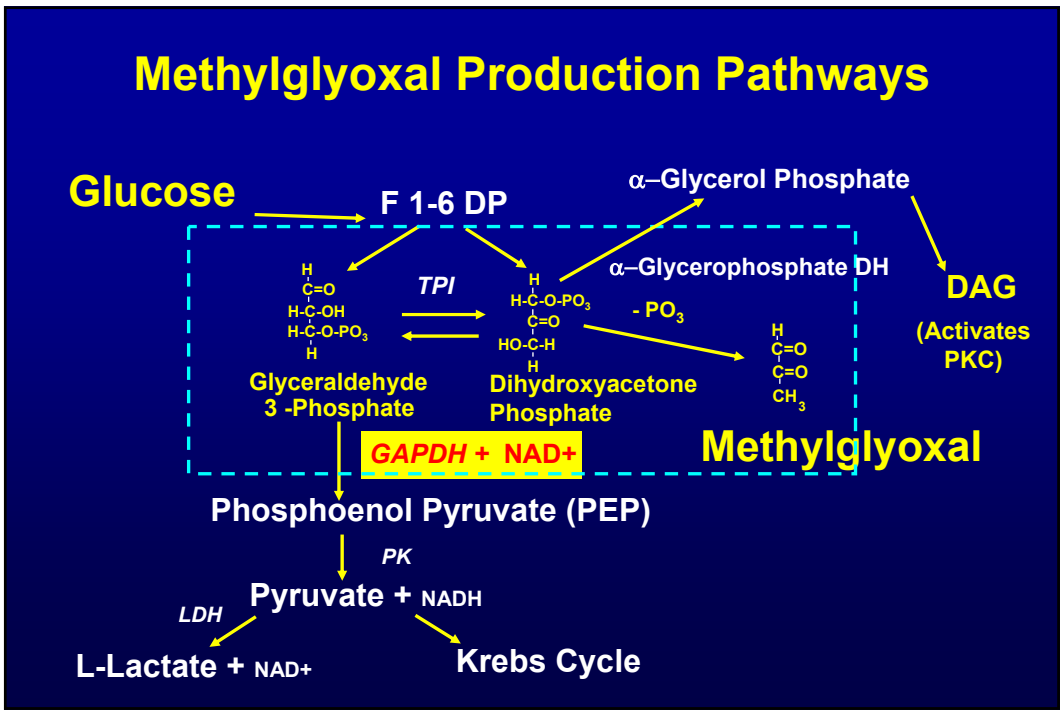
- Macrovascular disease
  - 2- to 4-fold more likely to have heart disease or stroke
  - 2- to 8-fold more likely to have heart failure
  - Accounts for 60% to 70% of all diabetes-related deaths
  - Lower extremity amputations
- Microvascular disease
  - Up to 24,000 new cases of blindness annually
  - Leading cause of end-stage renal disease
  - Neuropathy (including erectile dysfunction)

Centers for Disease Control and Prevention. *National Diabetes Fact Sheet*. 1998.
American Heart Association. *2001 Heart and Stroke Statistical Update*.
National Heart, Lung, and Blood Institute. *Facts about heart failure*. 1997, online edition.

Premises of our research

1. Blood sugar (glucose) is inherently toxic, either directly or indirectly by some products of it's metabolism
2. Cells have defense mechanisms to protect themselves against this toxicity
3. Individuals vary in the effectiveness of these defenses
4. In nondiabetic individuals these mechanisms function efficiently, while in diabetes they are often overwhelmed and result in damage to cells and organs

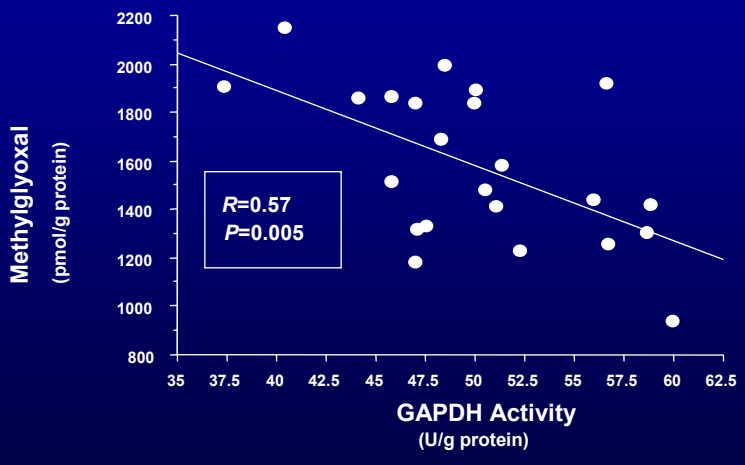

Sources of Methylglyoxal
Fructoselysine
Serine
Glucose
Ene -diol
L-Threonine Dehydrogenase
Aminoacetone
SSAO
Triose Phosphates
Dihydroxyacetone PO4
Glyceraldehyde -3-PO4
Methylglyoxal
GAPDH
Mono- oxygenase
Acetol
L-Lactate
Pyruvate
Ketone Bodies
Acetoacetate
β-Hydroxybutyrate
Acetone
Mono-oxygenase
P-450


Methylglyoxal Production Pathways
Glucose
F 1-6 DP
α–Glycerol Phosphate
α–Glycerophosphate DH
DAG
(Activates PKC)
TPI
- PO3
Glyceraldehyde 3 -Phosphate
Dihydroxyacetone Phosphate
Methylglyoxal
GAPDH + NAD+
Phosphoenol Pyruvate (PEP)
PK
Pyruvate + NADH
LDH
L-Lactate + NAD+
Krebs Cycle

Study population and Methods:

- To test this hypothesis we have studied 110 subjects with type 1 diabetes who have had their degree of kidney damage measured on kidney biopsies at the University of Minnesota.
- In each person we determined methylglyoxal production and GAPDH activity by their blood cells in response to high glucose incubation systems.
- Subjects with rapid and slow progression of kidney damage were identified and studied.

GAPDH Activity Determines Methylglyoxal Levels

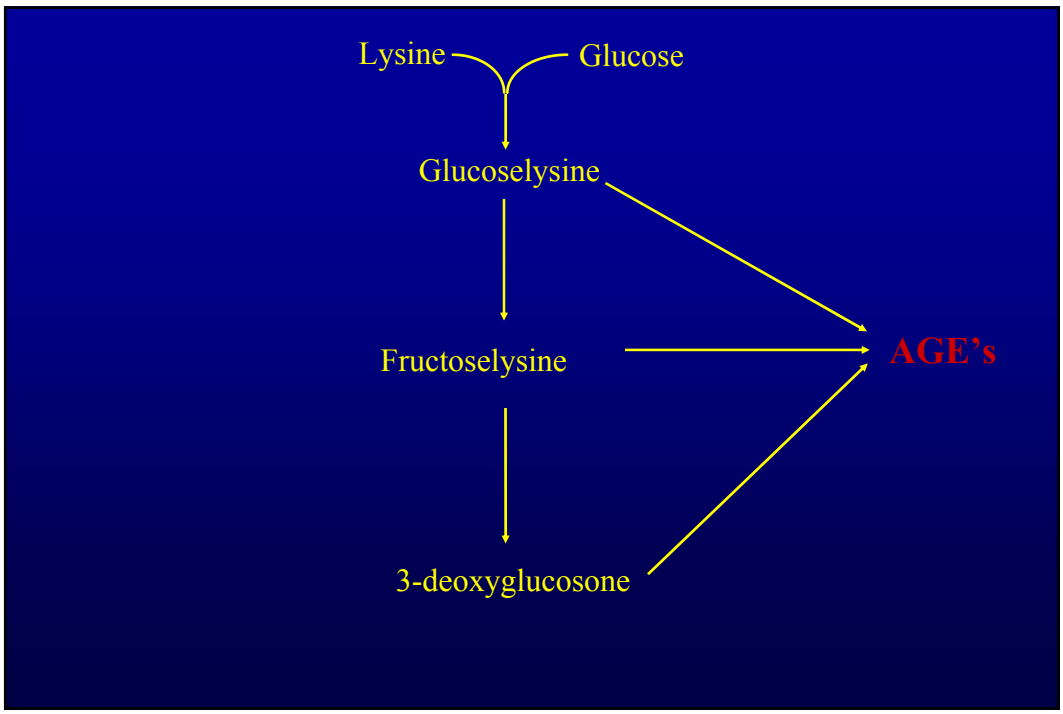


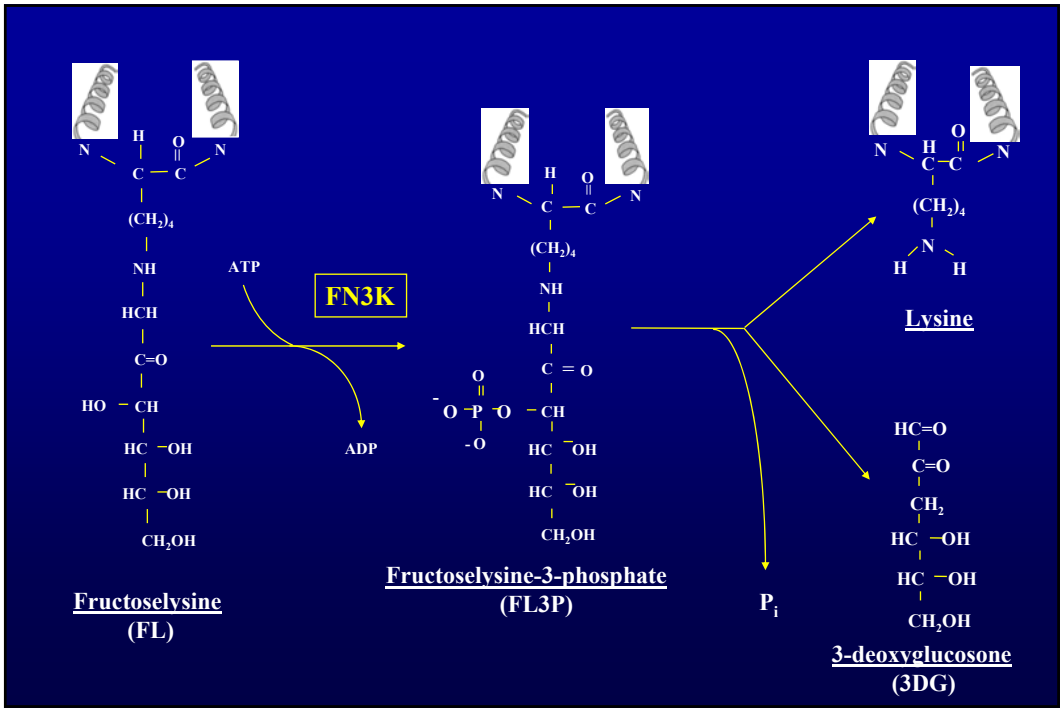
Future Directions for Research

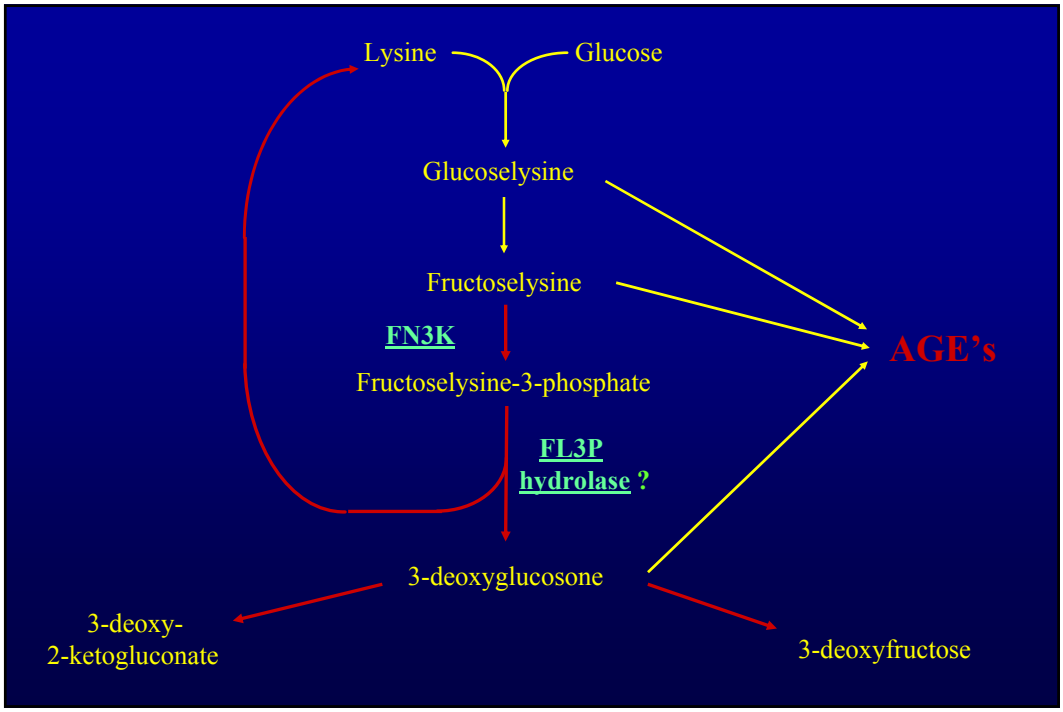
•Since the increase in methylglyoxal stress could be due to an inherited abnormality in GAPDH or other enzymes, we will perform genetic studies on kidney and other cells in larger study populations to discover the factors responsible for the observed susceptibility to kidney damage.

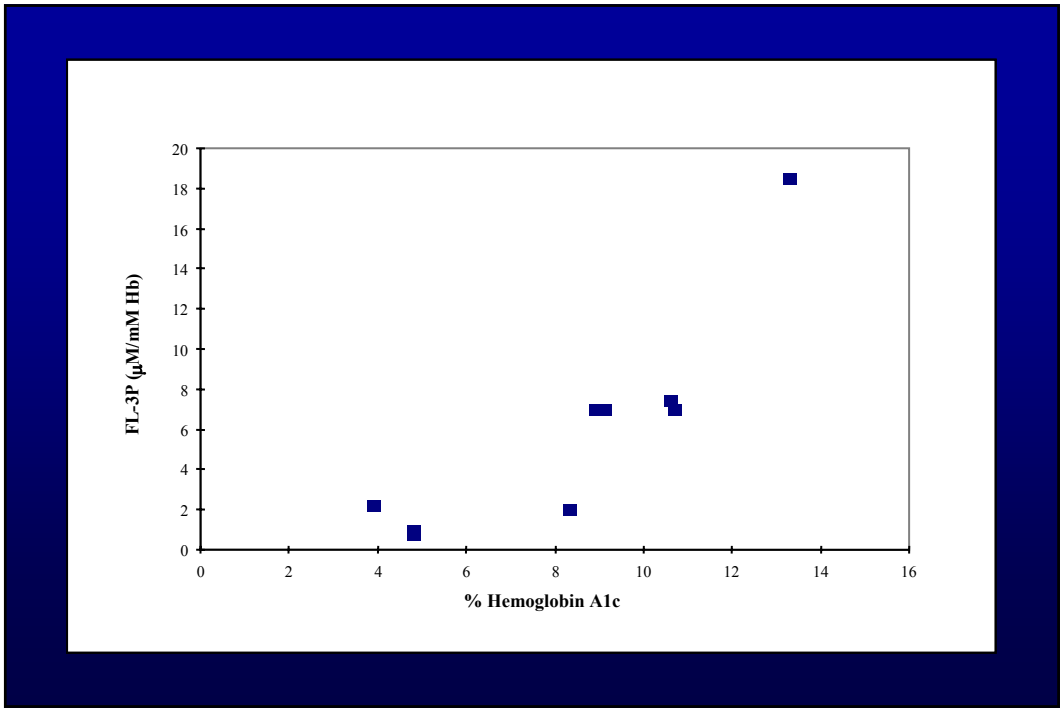
• We will continue to develop markers that can be used to determine those at greatest risk for kidney disease in the clinical setting

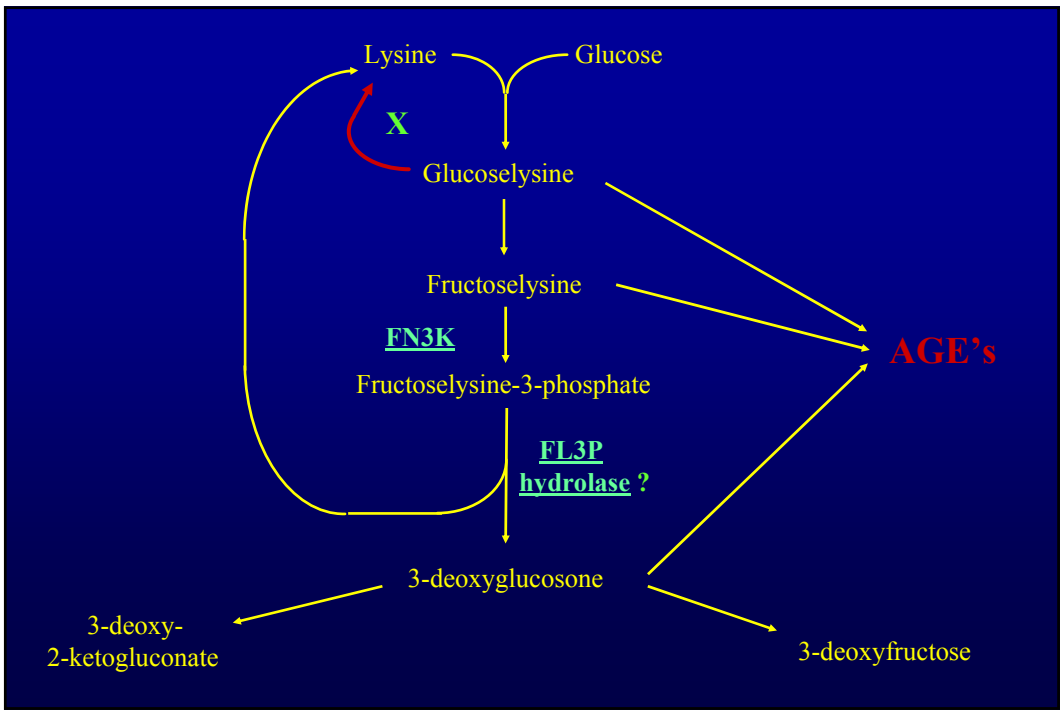
• We will look for other products formed by methylglyoxal and potential binding drugs, and apply these tools to large study populations with type 2 diabetes, where coronary disease outcomes are being studied.




ATP
FN3K
ADP
Fructoselysine
(FL)
Fructoselysine-3-phosphate
(FL3P)
P_i
Lysine
3-deoxyglucosone
(3DG)


Lysine
Glucose
Glucoselysine
Fructoselysine
FN3K
Fructoselysine-3-phosphate
FL3P
hydrolase ?
AGE's
3-deoxyglucosone
3-deoxy-
2-ketogluconate
3-deoxyfructose


FL-3P (μM/mM Hb)
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
% Hemoglobin A1c


Lysine
Glucose
X
Glucoselysine
Fructoselysine
FN3K
Fructoselysine-3-phosphate
AGE's
FL3P
hydrolase ?
3-deoxyglucosone
3-deoxy-
2-ketogluconate
3-deoxyfructose

Current Studies in Our Laboratory

1. Identify the factors that control the production of toxic glucose by-products

CONTROL OF PRODUCTION OF GLUCOSE BY-PRODUCTS (METHYLGLYOXAL) AND DIABETIC KIDNEY DISEASE. (Paul Beisswenger)

2. Identify agents that can inactivate glucose by-products

STUDIES OF THE EFFECT OF THE DRUG, METFORMIN, ON METHYLGLYOXAL LEVELS. (Paul Beisswenger)

3. Identify processes which control direct glucose toxicity

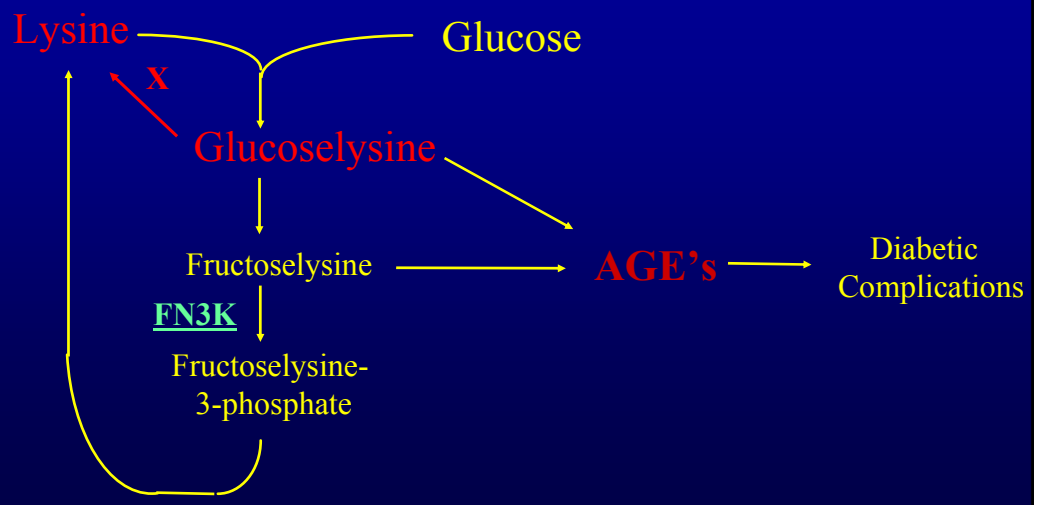
STUDIES OF FN3K, THE FIRST ENZYME SHOWN TO REMOVE TOXIC GLUCOSE PRODUCTS (Benjamin Szwergold)

Structural Renal Studies

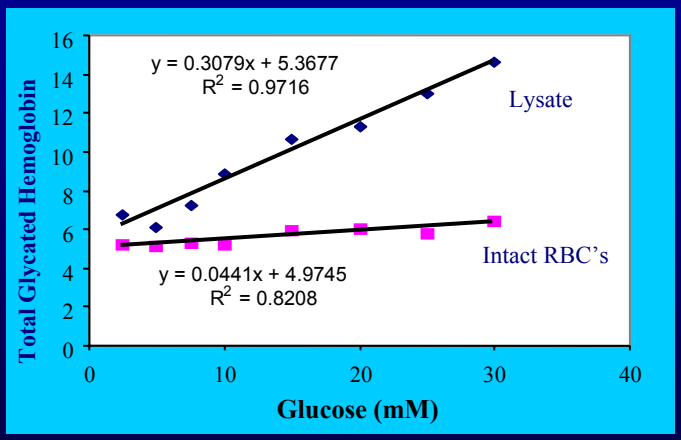
- Each subject underwent a renal biopsy at entry and after an interval of five years
- Glomerular basement membrane width (GBM) and volume fraction of mesangium per glomerulus Vv(Mes/glom) were measured on electron microscopic images.
- Change in these parameters was determined by subtracting the baseline from the 5 year value.

* Mauer, M. and K. Drummond, *The early natural history of nephropathy in type 1 diabetes: I. Study design and baseline characteristics of the study participants.* Diabetes., 2002. **51**(5): p. 1572-9.

New paradigm of direct glucose toxicity - version 2



Glycation of Hemoglobin in Intact Or Disrupted Cells



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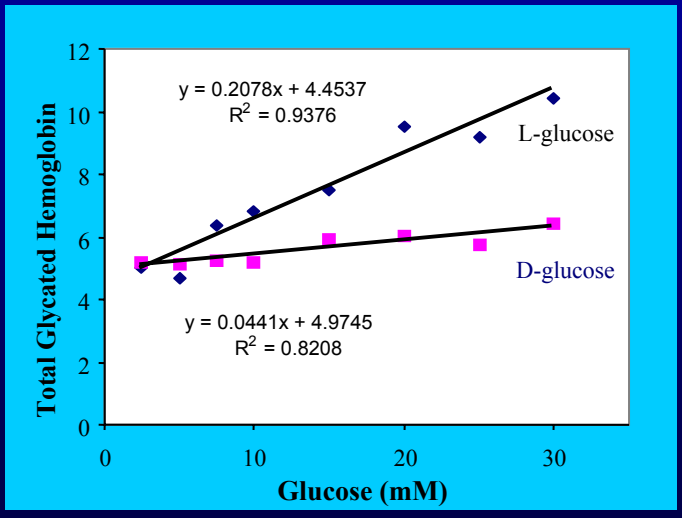
Hemoglobin Glycation by L and D Glucose In Intact Cells



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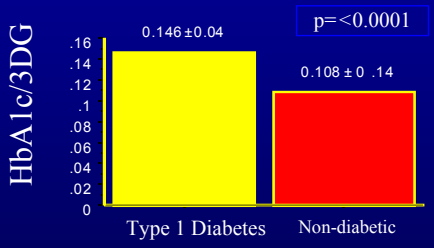
FN3K Activity in Diabetes and in Nephropathy Progressors



IN NEPHROPATHY PROGRESSORS: The HbA_{1c}/3DG was also higher in those with greater mesangial expansion over 5 years in the "Natural History" population suggesting that lower FN3K activity was associated with greater progression of nephropathy in this population.

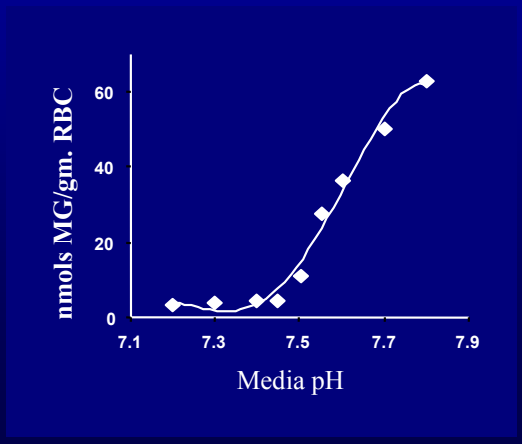
Study Three: Pima Study Population and Degree of Nephropathy

- Pima subjects (n=45) underwent a renal biopsy from which Glomerular basement membrane width (GBM-W), fractional mesangial volume (FMV),and Epithelial Podocyte number (EPN) were determined on electron microscopic images.

- Glomerular filtration Rate: All subjects had normal renal function with mean GFR (iothalamate clearance) of 156.2±52.8 ml/min (range 70-270ml/min)

- Urinary Albumin Excretion: Subjects had a spectrum of renal involvement with 16 having normal (<30), 17 micro (30-300), and 12 macroalbuminuria (>300mg/g).

Methylglyoxal Production and pH



Beisswenger et al, Biochem. Biophys, Acta, 1637, 98-106, 2003