Developing New Therapies for Vascular Complications

Animal Models of Diabetic Complications

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Animal Models of Diabetic Complications

How well do diabetes-induced complications that develop in animal models reproduce those which develop in humans?

Available animal models of diabetes develop the early, but not late, stages of retinopathy

	Human	Dog	Rat	Mouse
BACKGROUND				
Microaneurysms	+	+	**0?**	**0***
Acellular, nonperfused capillaries	+	+	+	+
Pericyte loss	+	+	+	+
IRMA	+	+	+	**0***
Hemorrhages	+	+	**0**	**0**
BM thickening	+	+	+	+
PRE-RETINAL NEOVASCULARIZATION	+	**0**	**0**	**0**

* Observed in galactose-feeding

ELEVATED BLOOD HEXOSE

↓

METABOLIC ABNORMALITIES

↓

NONPROLIFERATIVE RETINOPATHY

(especially capillary nonperfusion)

↓

RETINAL ISCHEMIA

Release of vasoproliferative factors

↓

PROLIFERATIVE RETINOPATHY

Available animal models of diabetes develop early, but not late, stages of kidney disease

	Human	Dog	Rat	Mouse
BACKGROUND				
Glom & tubular hypertrophy	+	+	+	±
Mesangial expansion	+	+	+?	+?
↑ capillary filter surface area	+	+	+	?
Microalbuminuria	+	+	+	+
ADVANCED RENAL DISEASE				
↓ capillary filter surface area	+	0	0	0
Declining GFR	+	0	0	0
↑ plasma creatinine	+	0	0	?
Severe glomeruluosclerosis	+	+	0	0
Macroproteinuria	+	+	0	0

1. **Critical events in the pathogenesis of complications**
    1. Role of hyperglycemia (diabetes vs experimental galactosemia)
    2. Role of specific cell types and of specific biochemical abnormalities
        1. Knockouts of ICAM-1 and CD-18
    3. Importance of apoptosis
        1. Over-expression of *bcl-2* in endothelium
    4. Gene alterations in diabetic complications
2. **Unanticipated results**
    1. Resistance of retinopathy to arrest after re-institution of normal glycemic control
    2. Mechanism of beneficial effect of ACE inhibitors on retinopathy
3. **Comparison between tissues**

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Experimental "hyperglycemia" in nondiabetic animals

- Nondiabetic animals fed galactose-rich diet (dogs, rats, mice)
- Morphologic lesions of retinopathy are identical to those seen in diabetes. This remains the strongest evidence that elevation of hexose level per se is sufficient to initiate development of retinopathy in diabetes

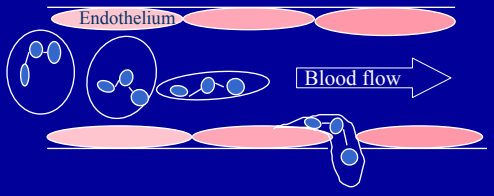
Differences compared to diabetes:

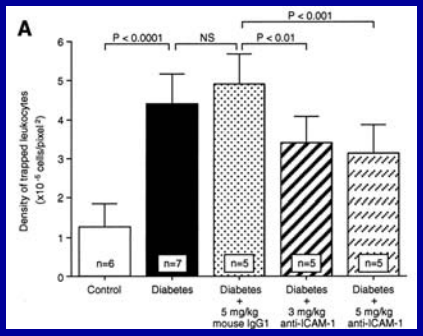
–Lesions of retinopathy are inhibited by aminoguanidine in diabetic animals, but not in galactosemic animals

–Unlike retina, kidney developed relatively less pathology in galactose-feeding compared to diabetes

Diabetes increases adherence of leukocytes to retinal vessels via the adhesion molecule, ICAM

Rolling → Adhesion → Transmigration



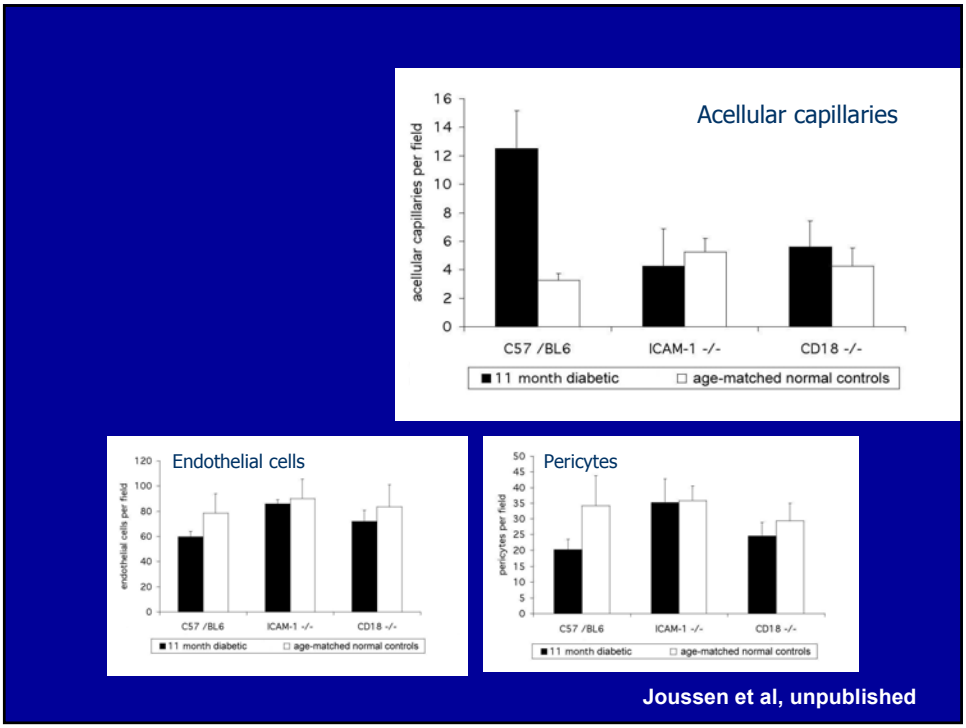


Miyamoto et al, *PNAS* 96:10836-41, 1999

Mice in whom ICAM or its ligand, CD18, had been knocked out were made diabetic (11 months) or experimentally galactosemic (up to 22 months).

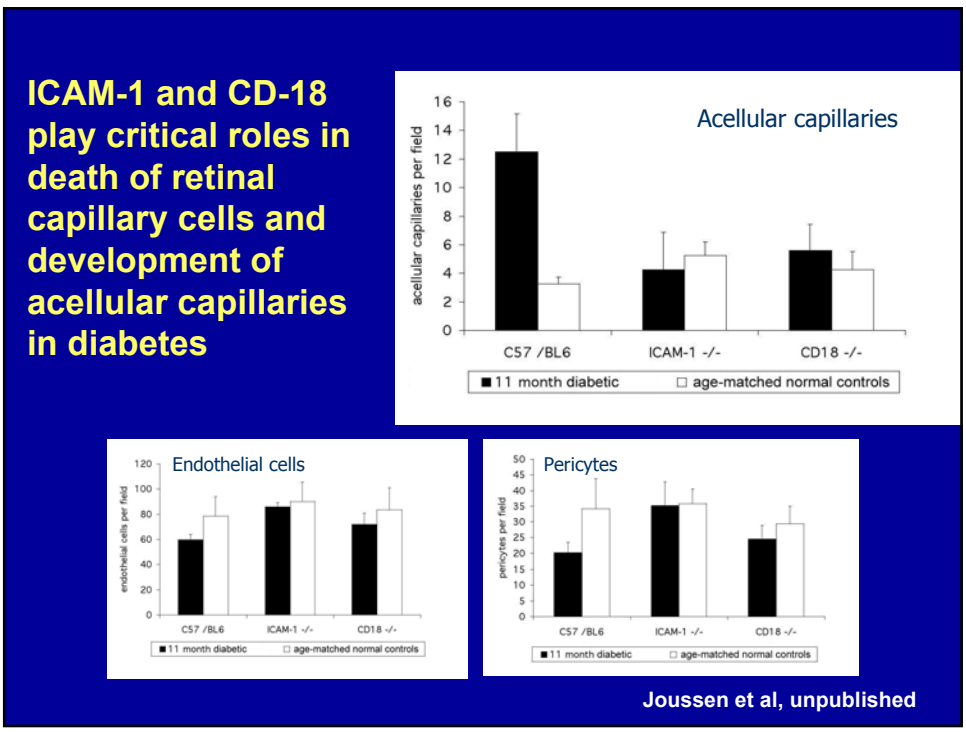
Leukostasis, vascular permeability, cell death and development of lesions of retinopathy were assessed.

Joussen et al, unpublished



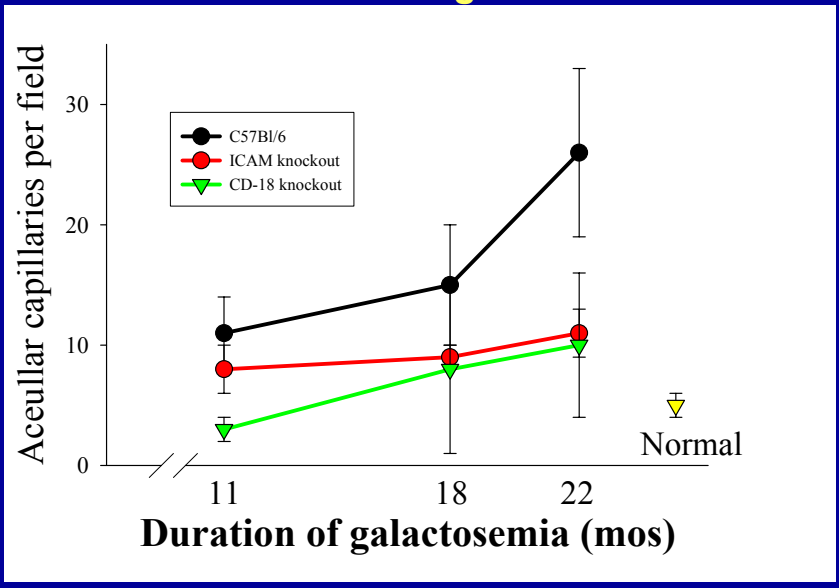
Joussen et al, unpublished

ICAM-1 and CD-18 play critical roles in death of retinal capillary cells and development of acellular capillaries in diabetes



Joussen et al, unpublished

ICAM and CD-18 contribute to acellular capillary formation also in galactosemia



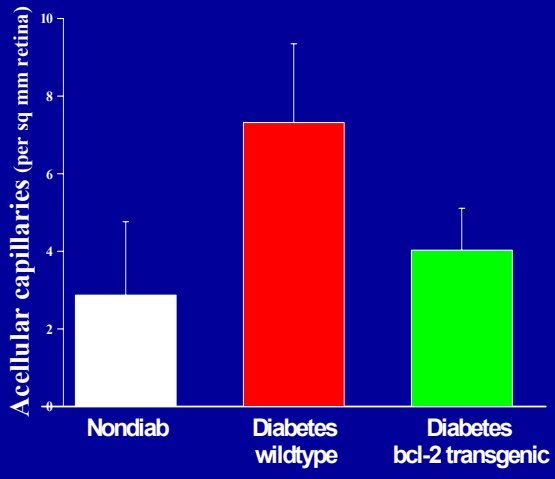
Joussen et al, unpublished

Accelerated death of retinal cells in diabetes; apoptosis

1. Occurs prior to appearance of retinal histopathogy
   1. Vascular endothelial cells and pericytes (Mizutani, Kern and Lorenzi, 1996; Kern *et al*, 2000)
   2. Neuronal/glial cells (Barber *et al*, 1998, Asnaghi *et al*, 2003)
2. Strongly associated with development of important lesions characteristic of diabetic retinopathy. Therapies that significantly inhibit development of acellular capillaries in retina of diabetic animals likewise inhibit capillary cell apoptosis at an earlier time in those animals.
   1. Aminoguanidine
   2. Vitamin E
   3. Nerve growth factor

Over-expression of *bcl-2* in the endothelium inhibits formation of acellular capillaries in retinas of mice diabetic 8 months

bcl-2 is expressed under the control of the pre-proendothelin promoter



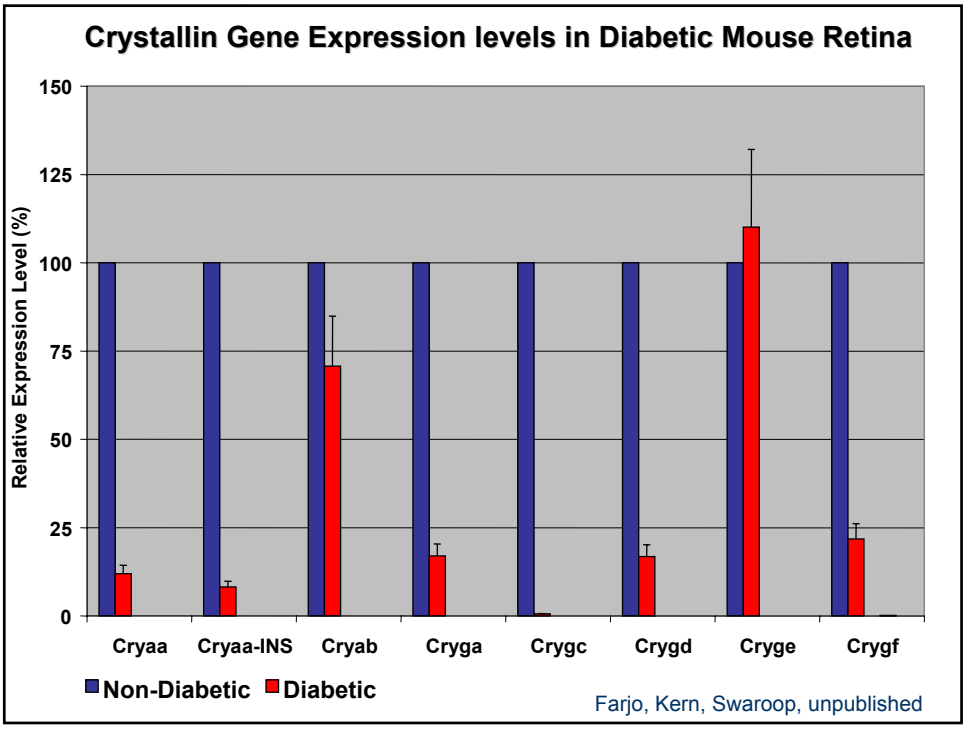
Levin and Kern, unpublished

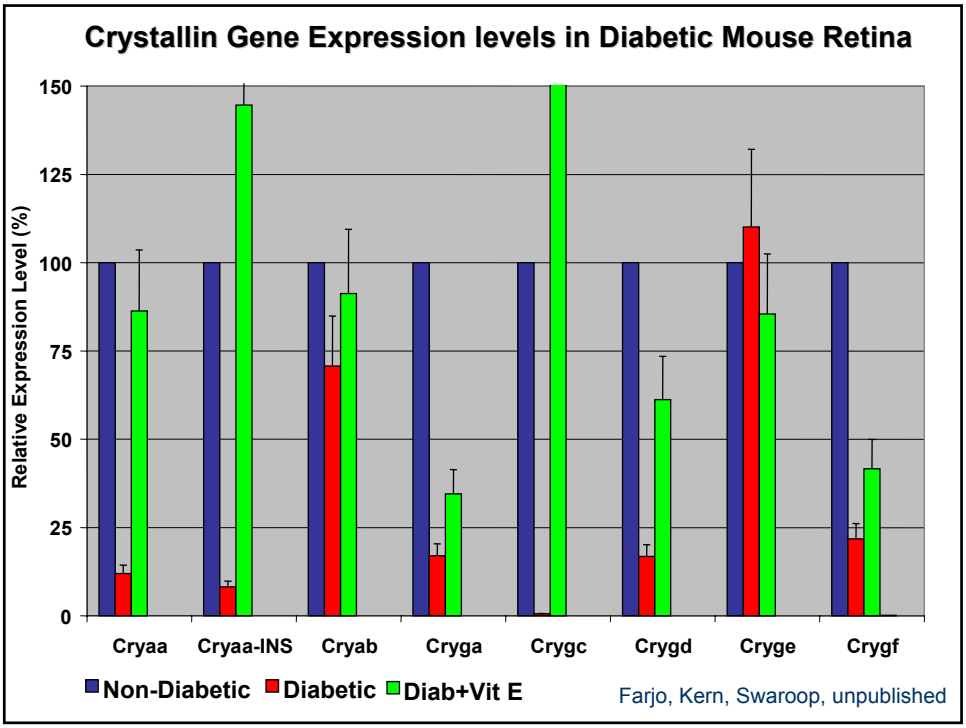
Experimental inhibition of retinopathy in diabetic animals

- Aminoguanidine
- Aspirin (high dose)
- Antioxidant mix
- α-Tocopherol
- Nerve growth factor
- *bcl-2* overexpression
- Pyridoxamine
- Aldose reductase inhibitor?
- Benfotiamine

Effect of therapies which inhibit diabetic retinopathy on gene expression in retinal tissue

- Microarray analysis of mouse retinas using custom chips prepared to contain eye genes (I-Gene microarrays)
  - Nondiabetic Controls
  - Streptozotocin Diabetic (2 months)
  - Diabetic treated with aminoguanidine, aspirin, or vitamin E (α tocopherol)
- 471 spots across both slides identified as differentially expressed (>2-fold change). 96% of these spots show lower expression in diabetes, and 285 spots correspond to crystallin genes.





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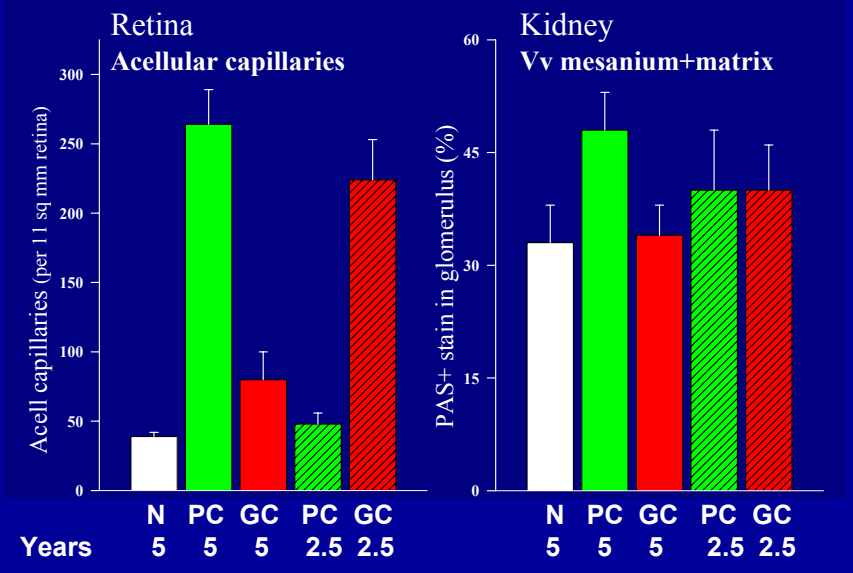
Retinal lesions continue to progress after intervention with good glycemic control; kidney lesions do not progress



5 year diabetic dog studies; Engerman and Kern

	n	Duration (months)	GHb
Normal (N)	7	13	4.2 ± 0.5
Poor Control (PC)	6	13	11.6 ± 0.9
Good Control (GC)	6	13	5.1 ± 2.3
Poor Control		2	11.6 ± 1.3
↓ (P2 → G7)	6	↓ 9	↓
Good Control		7	5.3 ± 0.4
Poor Control		6	11.4 ± 0.7
↓ (P6 → G7)	7	↓ 13	↓
Good Control		7	4.9 ± 0.4

Kowluru, Diabetes 52:818-23, 2003

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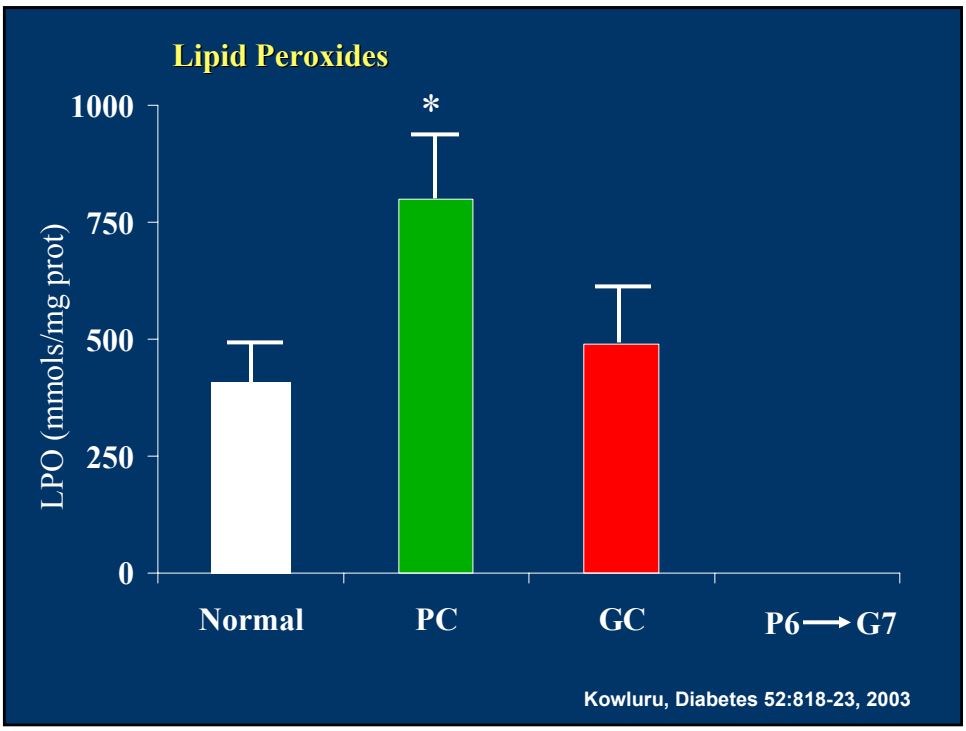
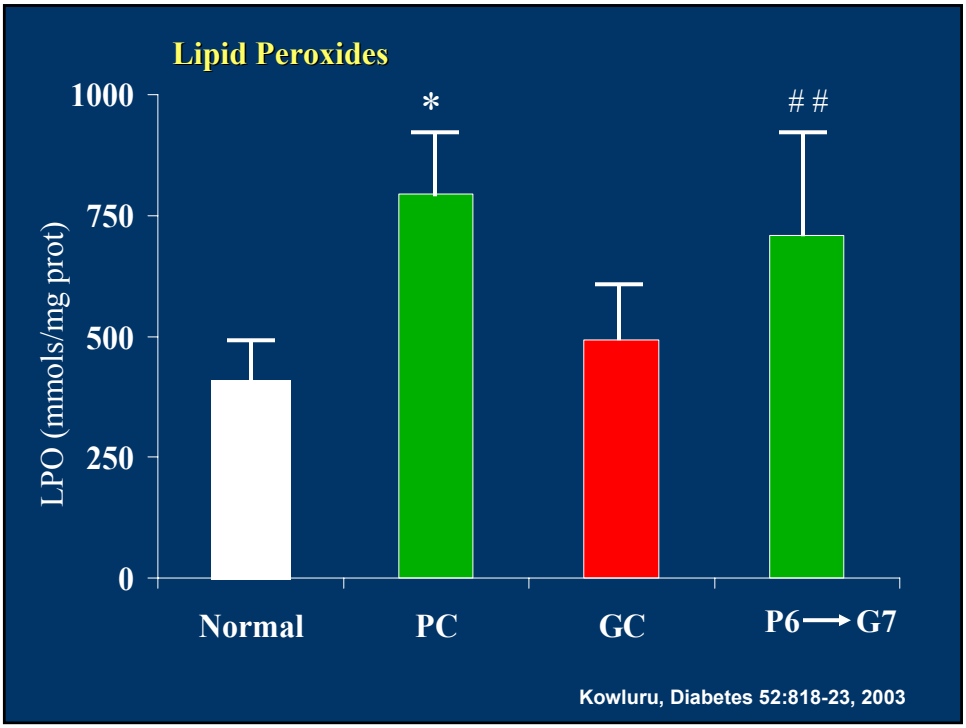
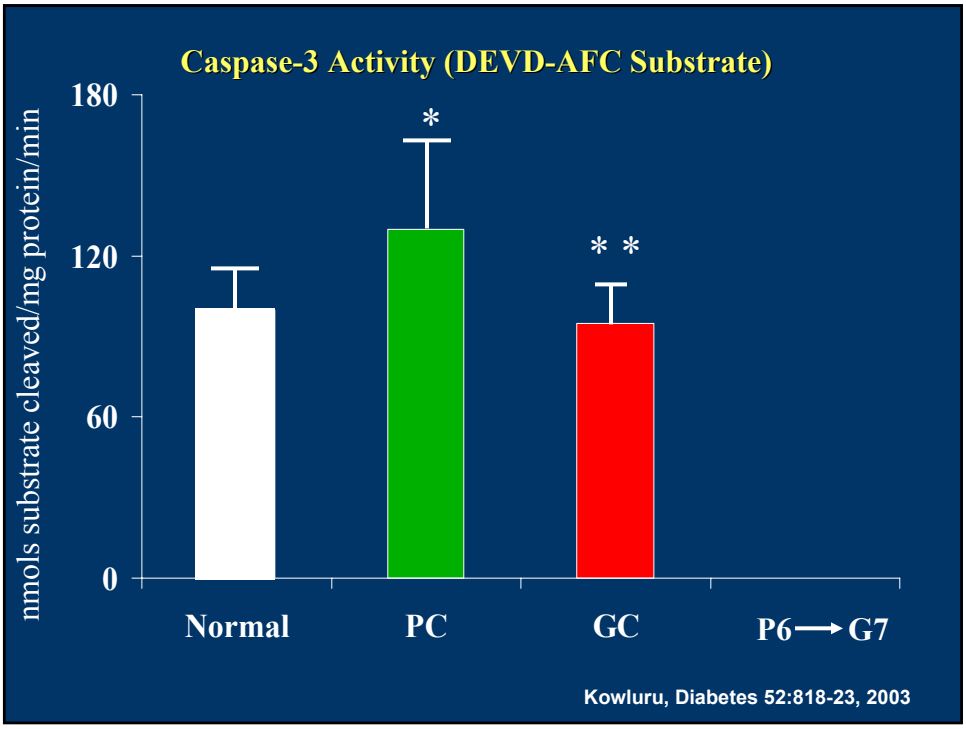
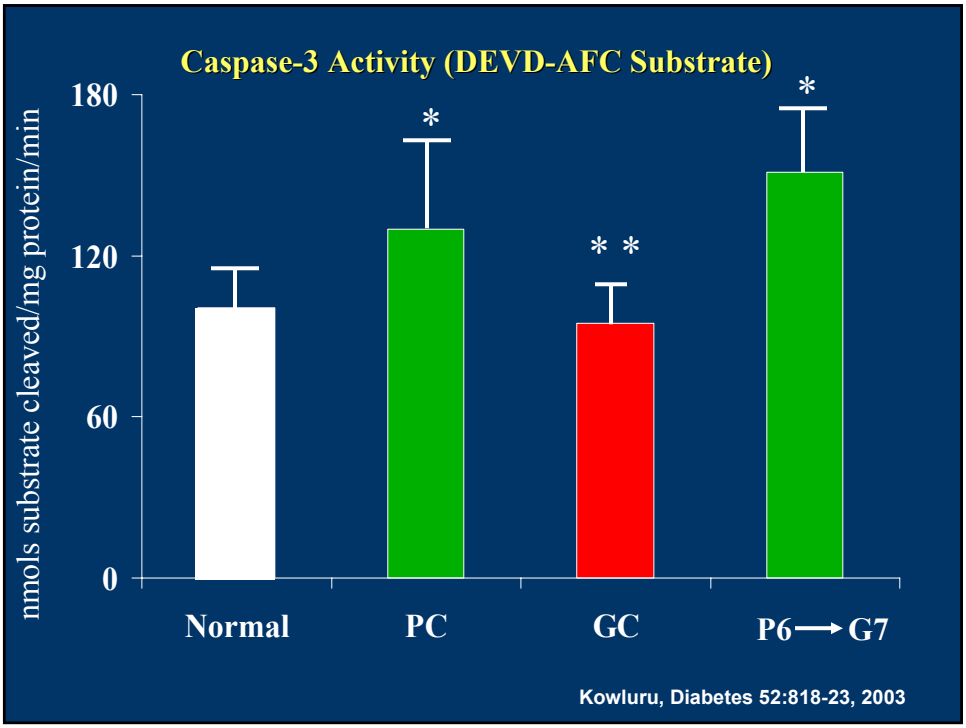
Kowluru, Diabetes 52:818-23, 2003



Kowluru, Diabetes 52:818-23, 2003


Lipid Peroxides
1000
750
500
250
0
LPO (mmols/mg prot)
*
# #
Normal
PC
GC
P6 → G7
Kowluru, Diabetes 52:818-23, 2003


Caspase-3 Activity (DEVD-AFC Substrate)
180
120
60
0
nmols substrate cleaved/mg protein/min
*
* *
Normal
PC
GC
P6 → G7
Kowluru, Diabetes 52:818-23, 2003

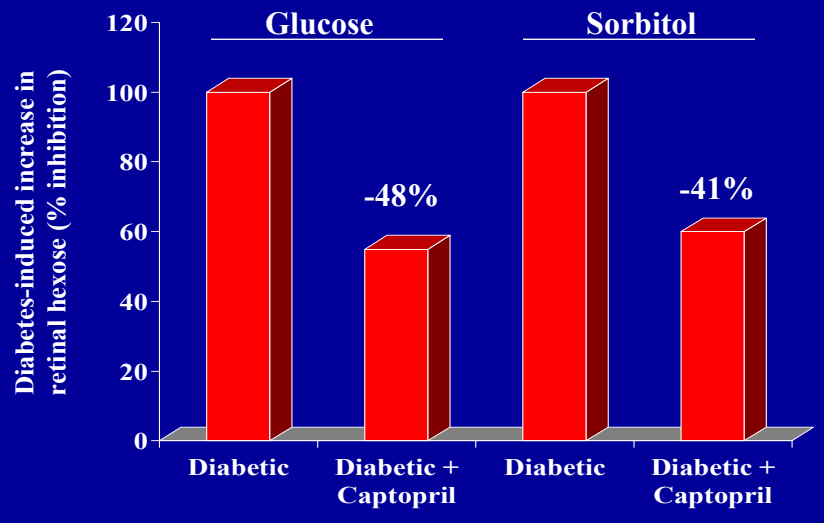


How do ACE inhibitors inhibit the development of diabetic retinopathy?

Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications of type 2 diabetes (UKPDS)
BMJ 317:703, 1998

Effect of lisinopril on progression of retinopathy in normotensive people with type 1 diabetes (Euclid Study)
Lancet 351:28, 1998

Captopril inhibits the diabetes-induced accumulation of glucose and its metabolites in the retina



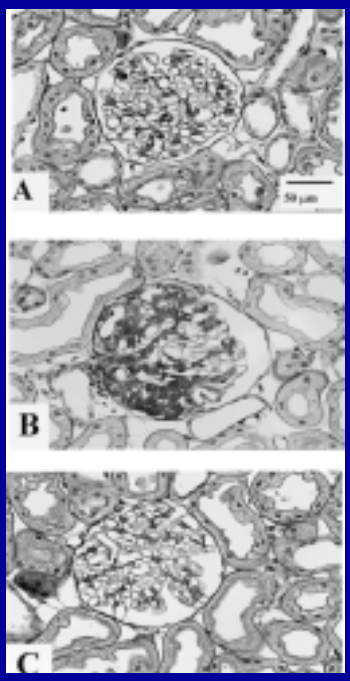
Zhang and Kern, unpublished

If glucose does not accumulate intracellularly, the biochemical abnormalities postulated to cause diabetic complications will not develop

- Activation of protein kinase C
- Intracellular nonenzymatic glycation
- Oxidative stress
- Aldose reductase (sorbitol) pathway

Is diabetic retinopathy a "chronic" inflammatory process?

- Inhibition of retinopathy by high dose aspirin in diabetic dogs
- Excessive platelet aggregation within retinal capillaries
- Occlusion of retinal capillaries by leukocytes which is inhibited with ICAM inhibitors or TNFα receptor antagonists
- Increased permeability of retinal vasculature
- Activation of pro-inflammatory caspase (caspase 1)
- Increased retinal levels of :
  - Cyclo-oxygenase 2 and prostaglandins
  - NF-kB
  - nitric oxide and iNOS
  - cytokines (TNFα, Il-1β)

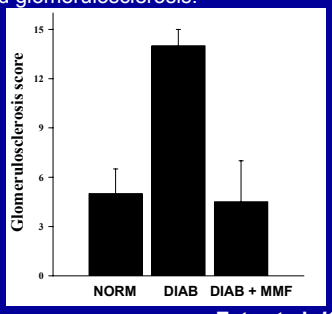


Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes (Kid Int 63: 209,2003)

MMF is an anti-lymphocyte drug with immunosuppressive/anti-inflammatory properties

Uninephrectomized Munich-Wistar rats; hemodynamic studies at 6 to 8 weeks; morphological studies at 8 months

Treatment with MMF had no effect on blood pressure, glomerular dynamics or blood glucose levels, but did inhibit albuminuria, glomerular macrophage infiltration and glomerulosclerosis.



Zatz et al, *Kid Int* 63: 209,2003

Available models provide much information about the early stages in the pathogenesis of diabetic complications, the stages which precede, and apparently contribute to development of, the later stages of the disease.

Animal Models of Diabetic Complications Consortium (AMDCC) is attempting to develop and characterize new animal models that provide further insight into the pathogenesis of these complications.

http://www.amdcc.org

Collaborators

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