# The “Metabolic Memory”: The New Challenge in The Therapy of Diabetes.

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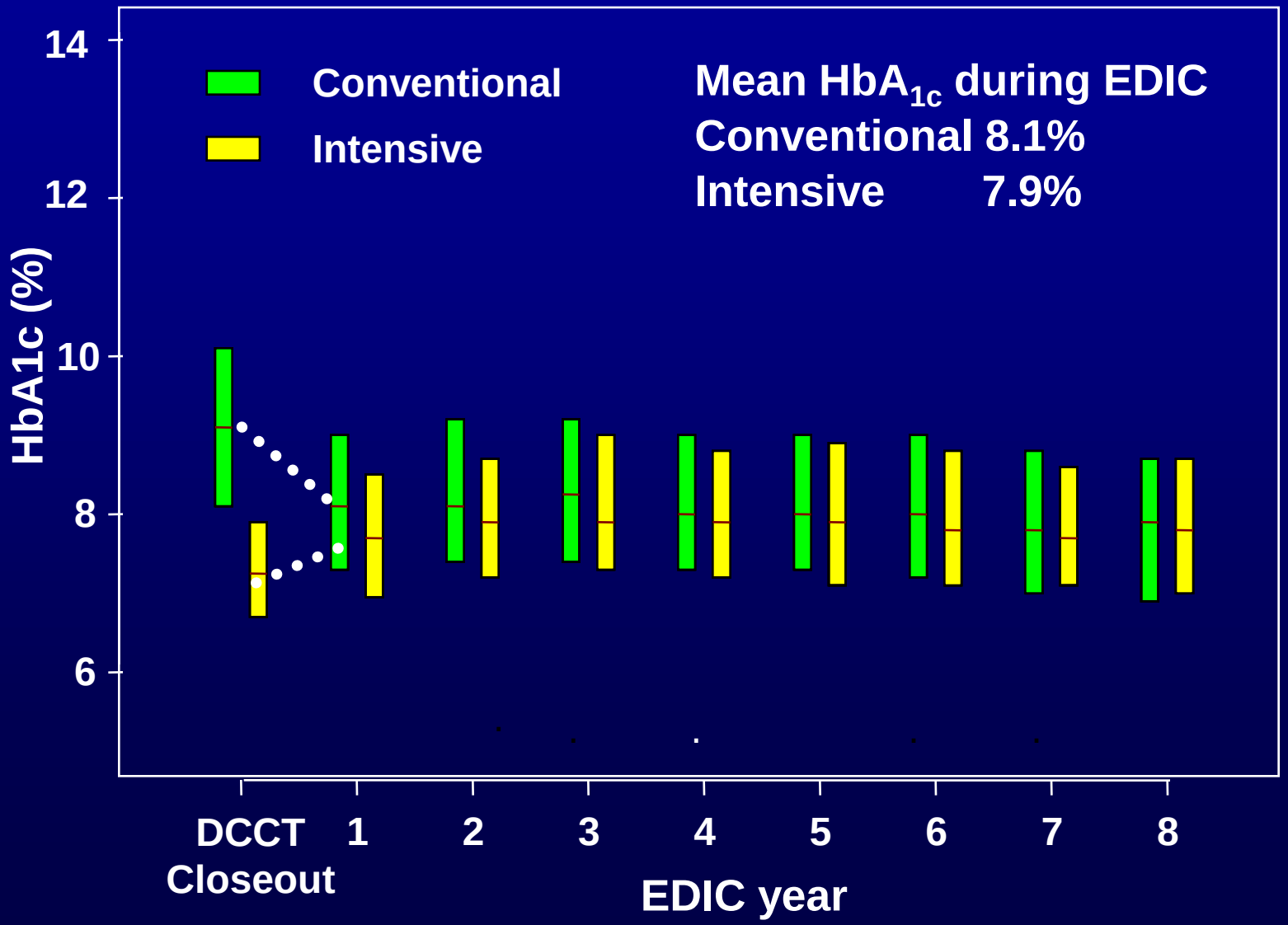
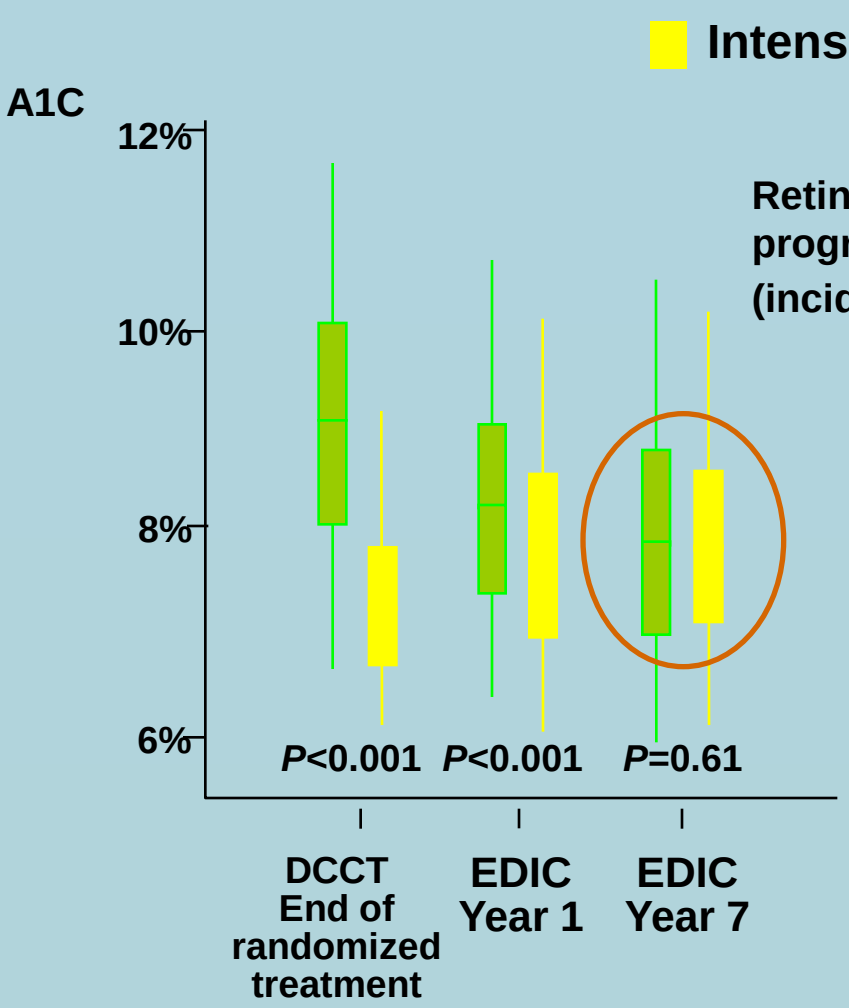
# EDIC - AIMS

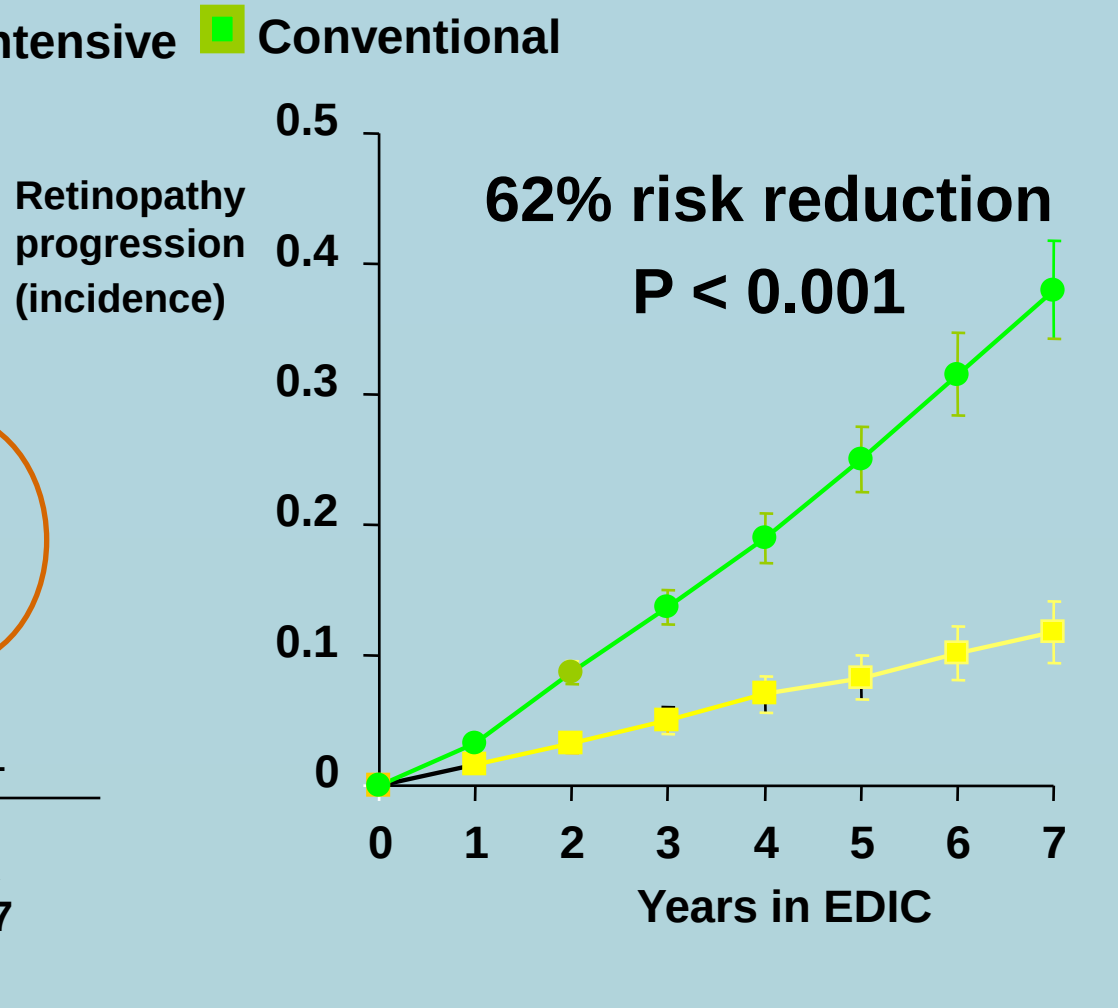
**Examine the longer-term effects of the original DCCT interventions, such as cardiovascular and more advanced stages of retinal and renal disease as well as cognitive function.**

# Distribution of HbA1c in the Former DCCT Intensive and Conventional Groups During EDIC



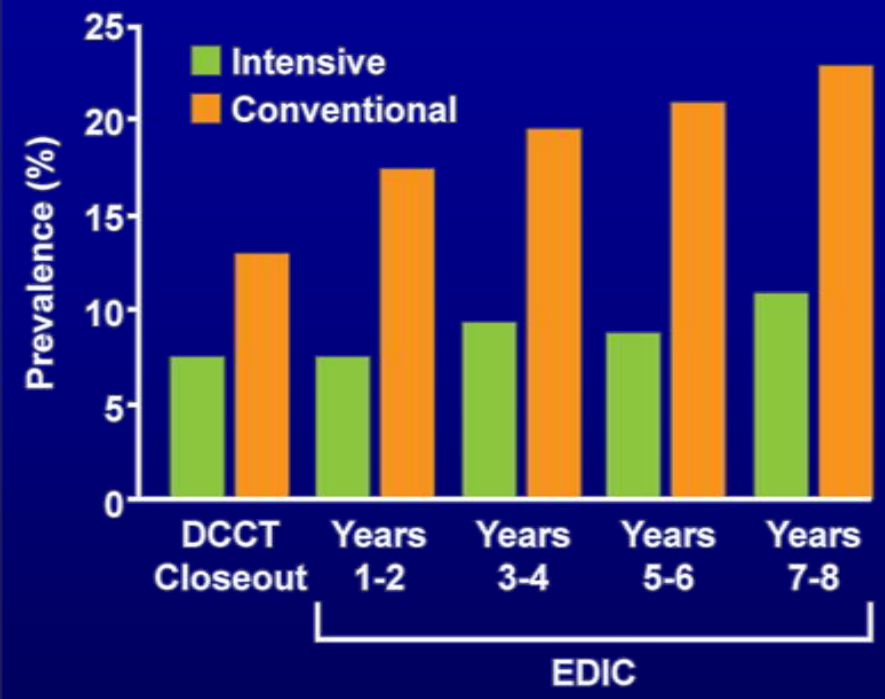
# DCCT/EDIC - Long-term Microvascular Risk Reduction in Type 1 Diabetes

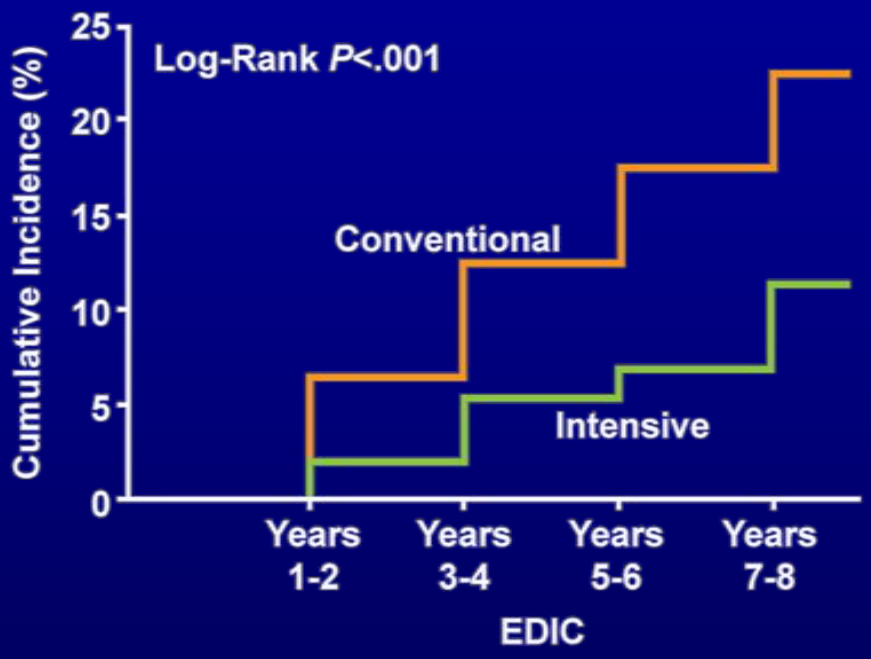



DCCT/EDIC Research Group. *JAMA*. 2002;287:2563-2569

# EDIC Results: Nephropathy—Microalbuminuria




No. at Risk

| | | | | |
|---|---|---|---|---|
| Conventional | 586 | 545 | 509 | 480 |
| Intensive | 626 | 609 | 586 | 576 |

**At DCCT closeout, cumulative incidences of microalbuminuria were 22% in the intensive cohort and 34% in the conventional cohort**

**Writing Team for the DCCT/ EDIC Research Group. *JAMA*. 2003;290:2159-2167.**

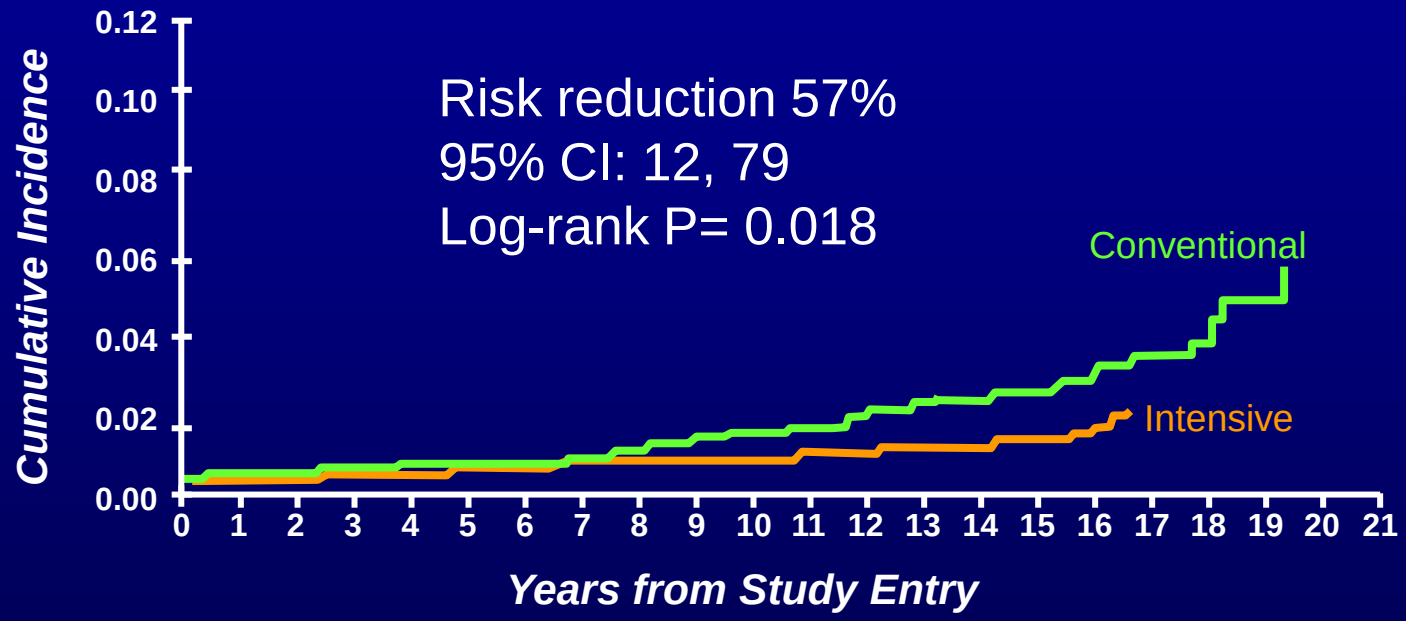
# Intensive Diabetes Treatment and Cardiovascular Disease in Patients with Type 1 Diabetes

DCC/EDIC Research Group

*N Engl J Med, 2005*

# Cardiovascular Events

*Non-Fatal MI, Stroke or CVD Death*




| *Number at Risk* | | | | |
|---|---|---|---|---|
| Intensive | 705 | 686 | 640 | 118 |
| Conventional | 721 | 694 | 637 | 96 |

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

# 10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes

Rury R. Holman, F.R.C.P., Sanjoy K. Paul, Ph.D., M. Angelyn Bethel, M.D.,
David R. Matthews, F.R.C.P., and H. Andrew W. Neil, F.R.C.P.

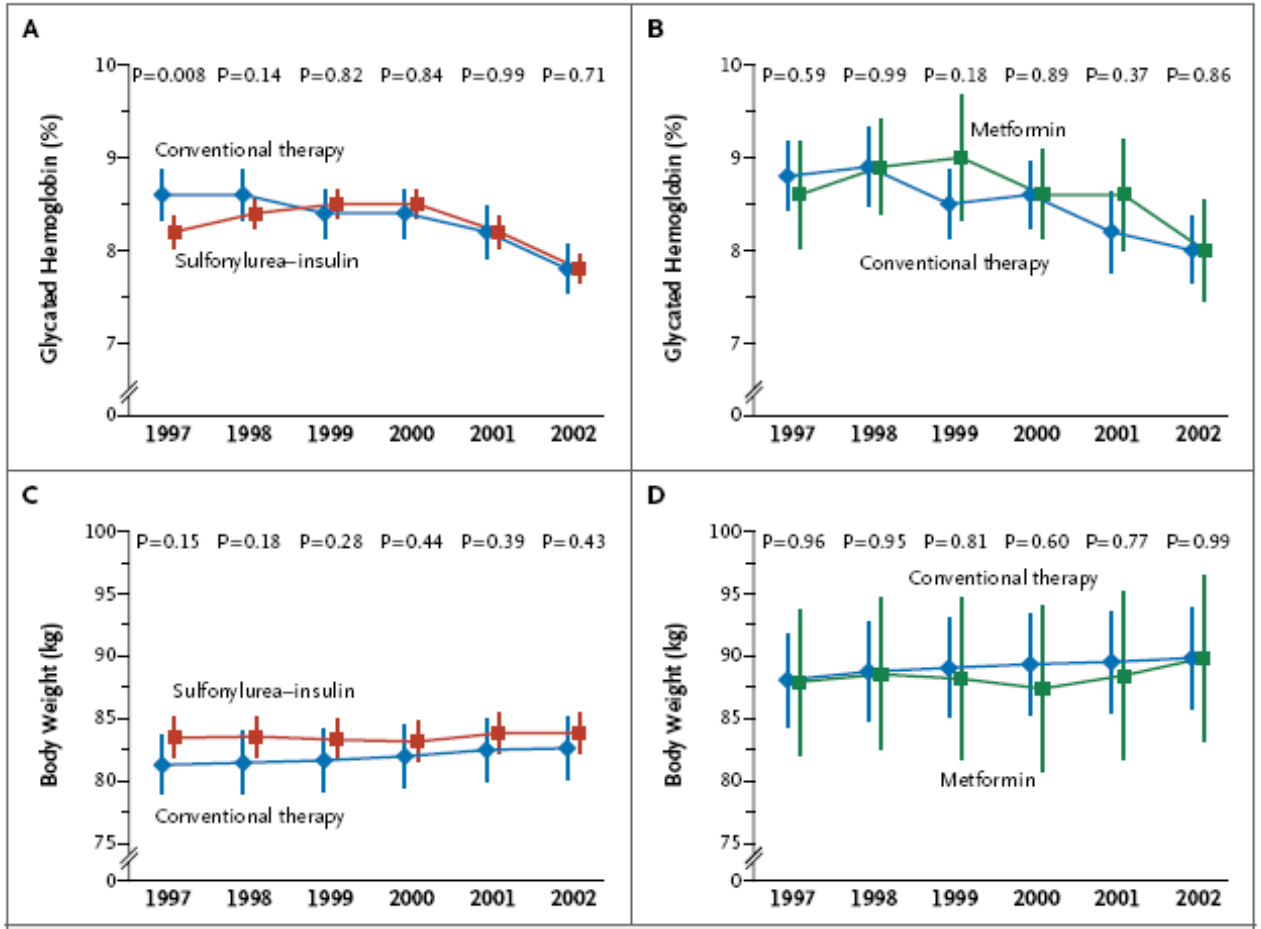
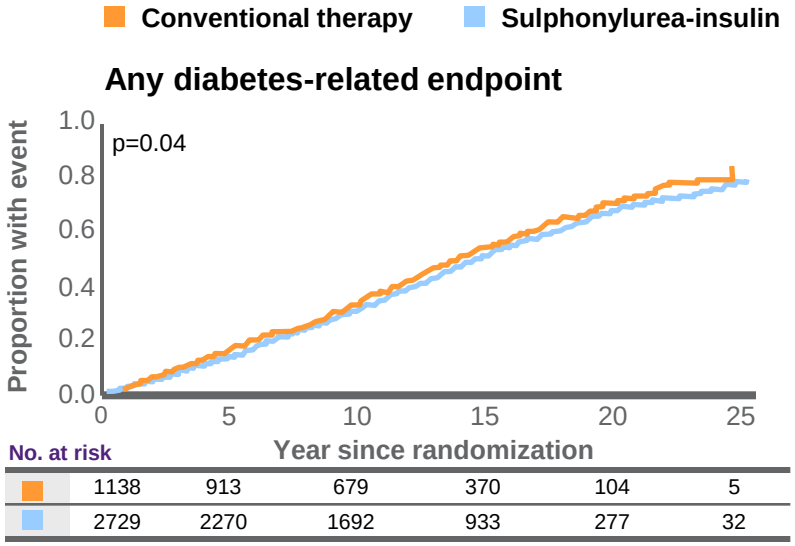
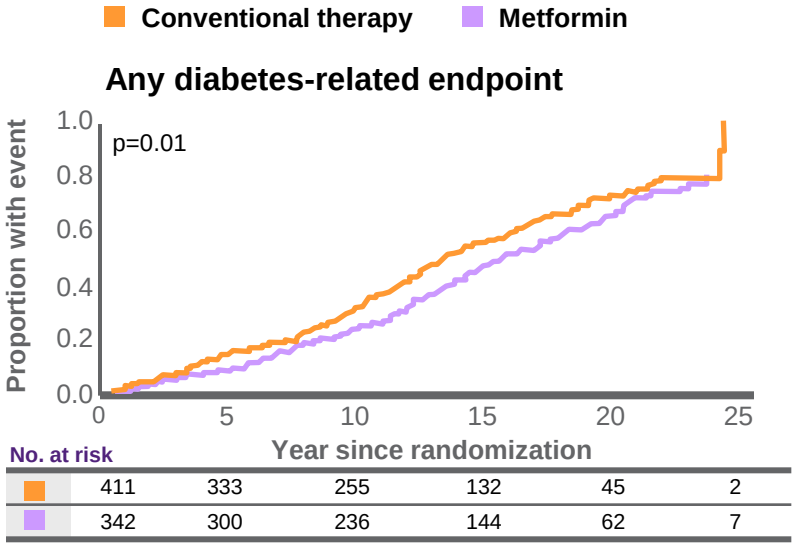


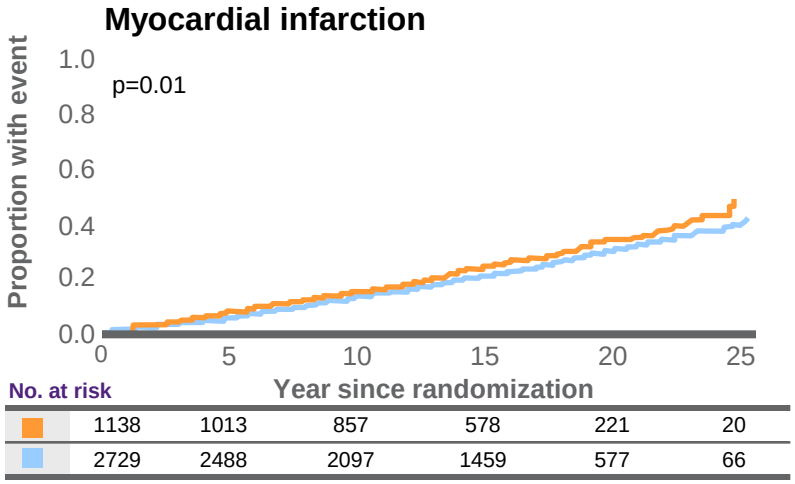

**Figure 2. Mean Glycated Hemoglobin Levels and Body Weight.**

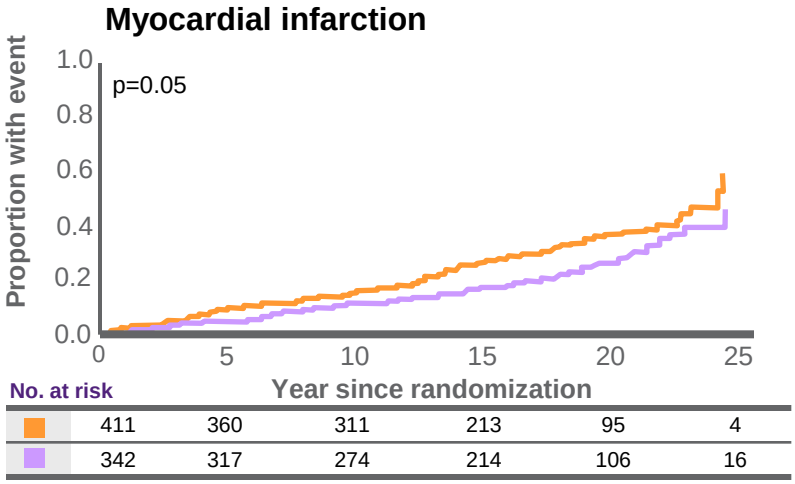
Glycated hemoglobin levels for patients who were originally assigned to receive either sulfonylurea–insulin or conventional therapy (Panel A) or metformin or conventional therapy (Panel B) are shown. Panels C and D show the corresponding mean body weights in the two groups. Clinical data were not available in years 6 through 10, when questionnaires were used. The vertical bars represent 95% confidence intervals.

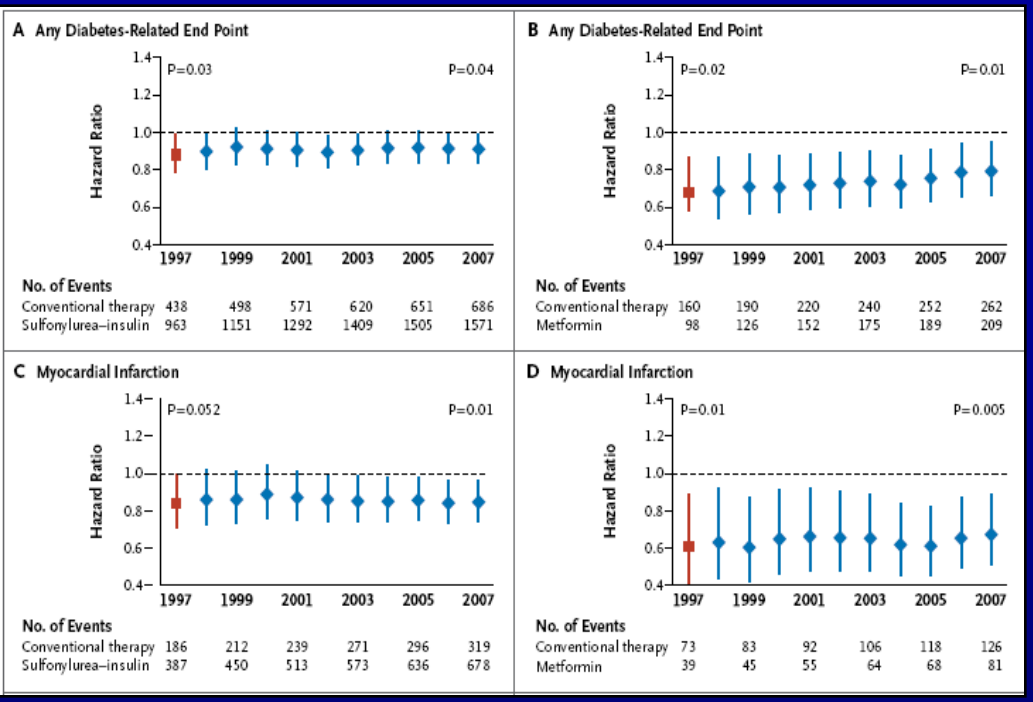
# 10-Year follow-up of intensive glucose control in type 2 diabetes

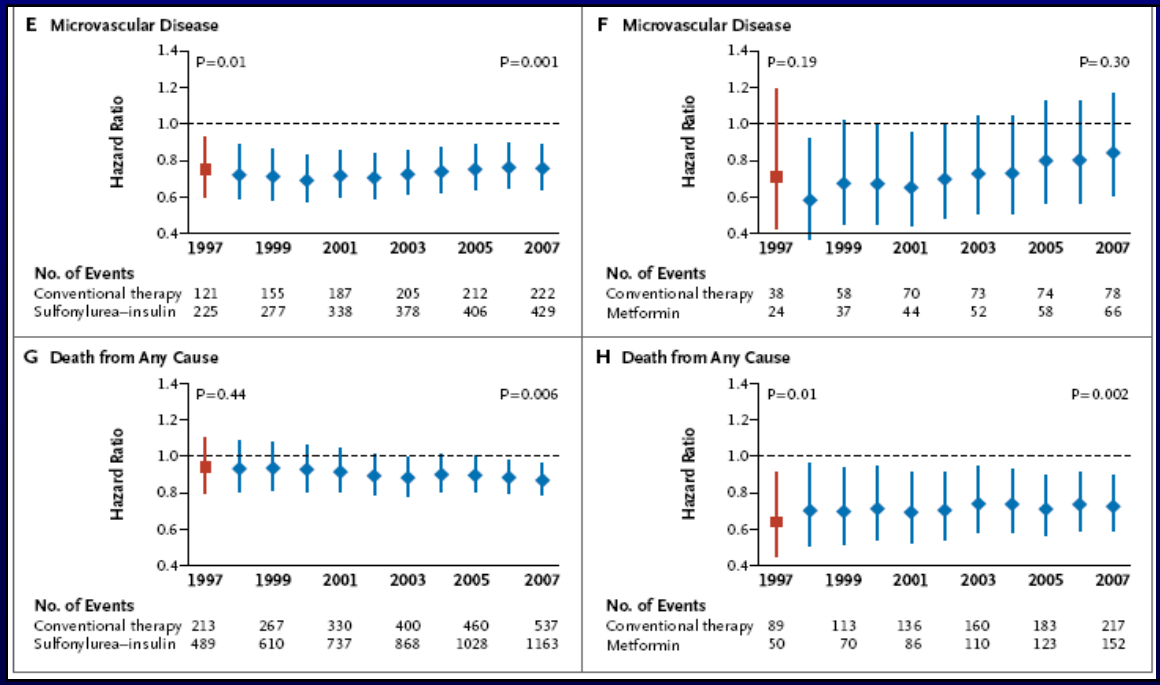



A Any Diabetes-Related End Point
P=0.03
P=0.04
Hazard Ratio
1.4
1.2
1.0
0.8
0.6
0.4
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 438 498 571 620 651 686
Sulfonylurea–insulin 963 1151 1292 1409 1505 1571
B Any Diabetes-Related End Point
P=0.02
P=0.01
Hazard Ratio
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 160 190 220 240 252 262
Metformin 98 126 152 175 189 209
C Myocardial Infarction
P=0.052
P=0.01
Hazard Ratio
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 186 212 239 271 296 319
Sulfonylurea–insulin 387 450 513 573 636 678
D Myocardial Infarction
P=0.01
P=0.005
Hazard Ratio
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 73 83 92 106 118 126
Metformin 39 45 55 64 68 81


E Microvascular Disease
P=0.01
P=0.001
Hazard Ratio
1.4
1.2
1.0
0.8
0.6
0.4
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 121 155 187 205 212 222
Sulfonylurea–insulin 225 277 338 378 406 429
F Microvascular Disease
P=0.19
P=0.30
Hazard Ratio
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 38 58 70 73 74 78
Metformin 24 37 44 52 58 66
G Death from Any Cause
P=0.44
P=0.006
Hazard Ratio
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 213 267 330 400 460 537
Sulfonylurea–insulin 489 610 737 868 1028 1163
H Death from Any Cause
P=0.01
P=0.002
Hazard Ratio
1997 1999 2001 2003 2005 2007
No. of Events
Conventional therapy 89 113 136 160 183 217
Metformin 50 70 86 110 123 152

# “The Metabolic Memory”

## *Evidence for a long-term persistence of hyperglycaemia-induced damage*

DCC/EDIC Research Group

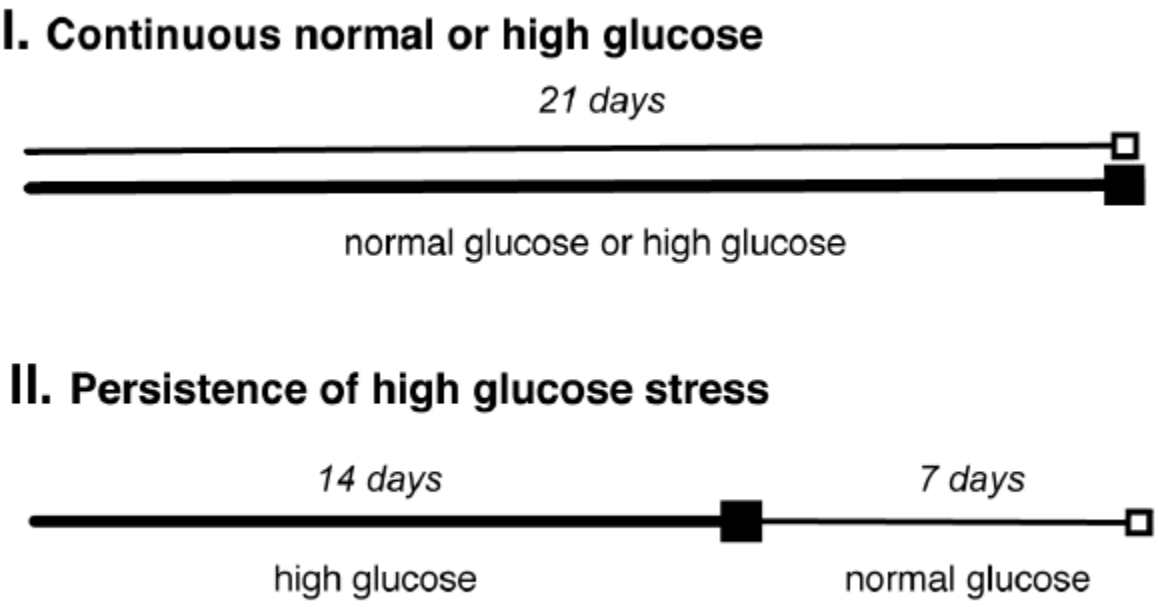
*N Engl J Med, 2005*

# Overexpression of fibronectin induced by diabetes or high glucose: Phenomenon with a memory

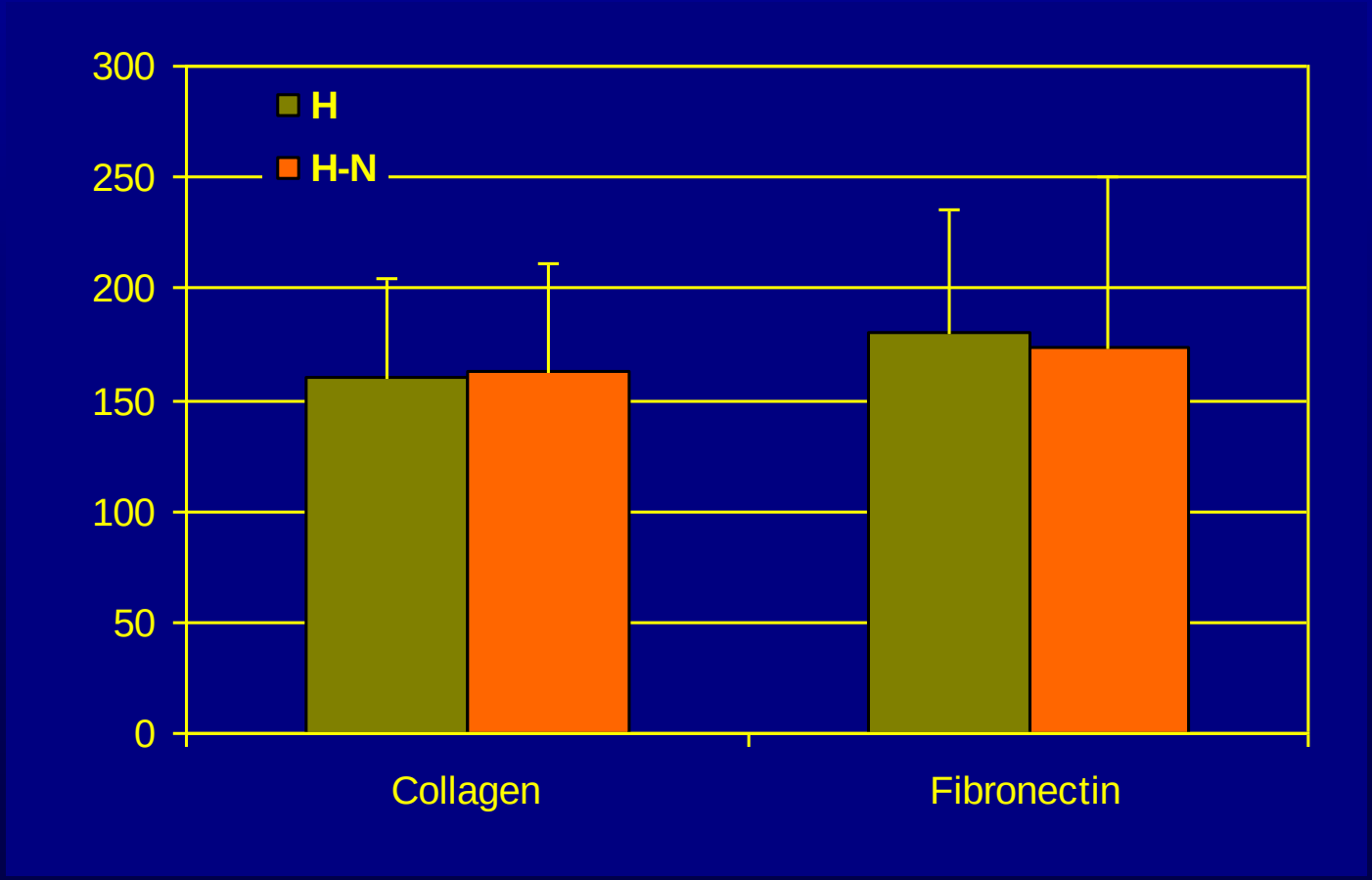
S. ROY, R. SALA, E. CAGLIERO, M. LORENZI

*Proc. Natl. Acad. Sci. U.S.A.. 87: 404-408, 1990*

a


I. Continuous normal or high glucose
21 days
normal glucose or high glucose
II. Persistence of high glucose stress
14 days
7 days
high glucose
normal glucose

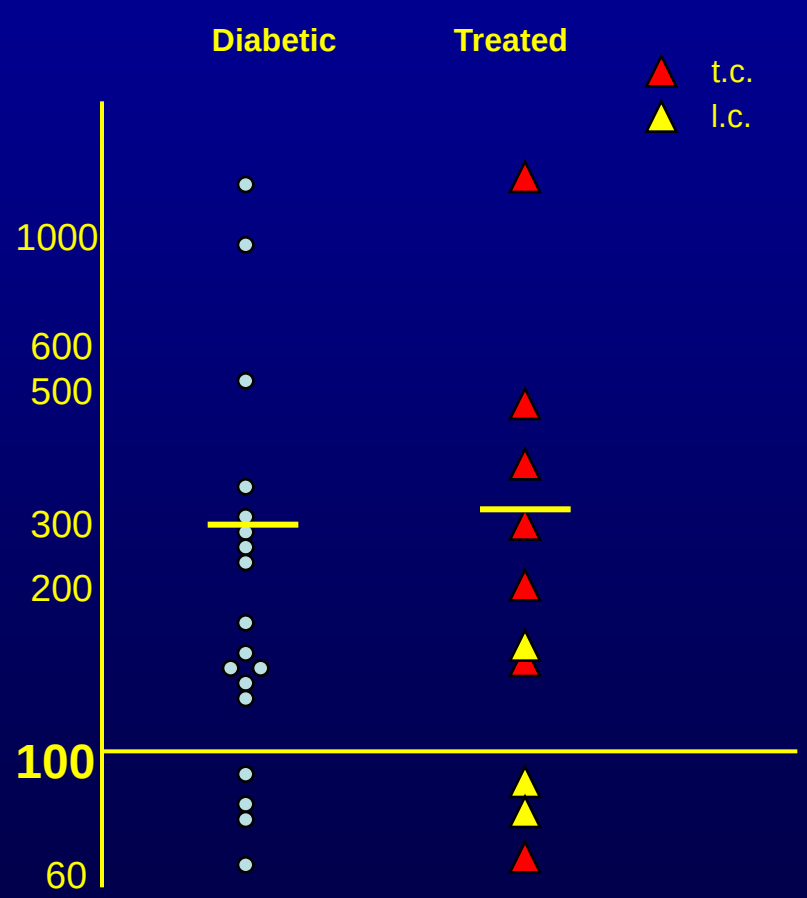
# Densitometric quantitation of mRNA levels in human endothelial cells obtained from umbilical veins.



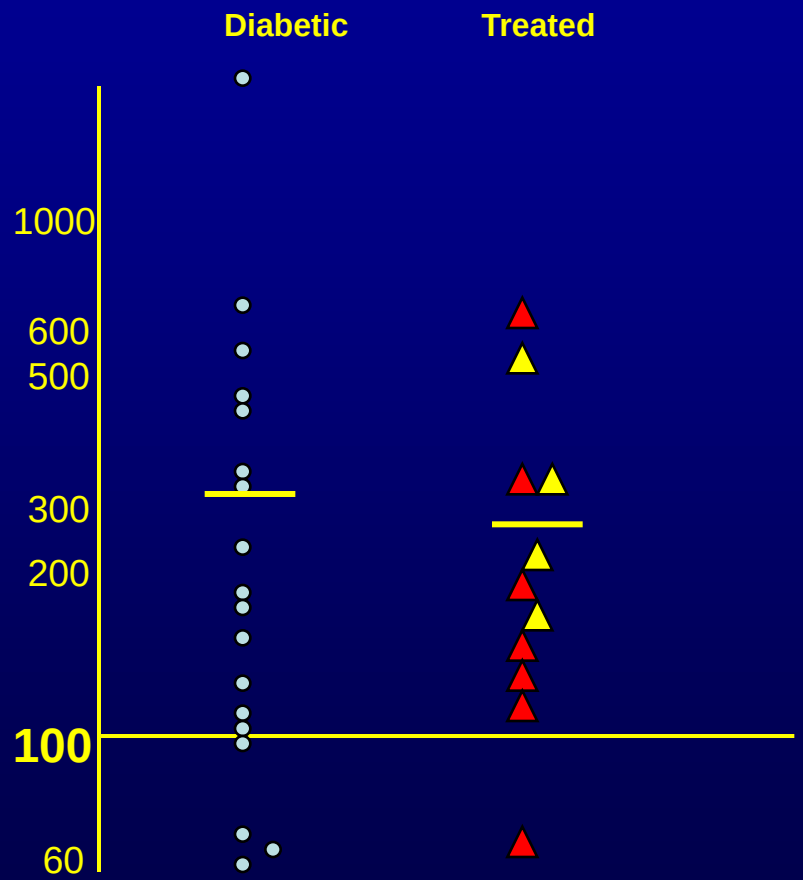

*Proc. Natl. Acad. Sci. U.SA.. 87: 404-408, 1990*

*Proc. Natl. Acad. Sci. U.SA.. 87: 404-408, 1990*

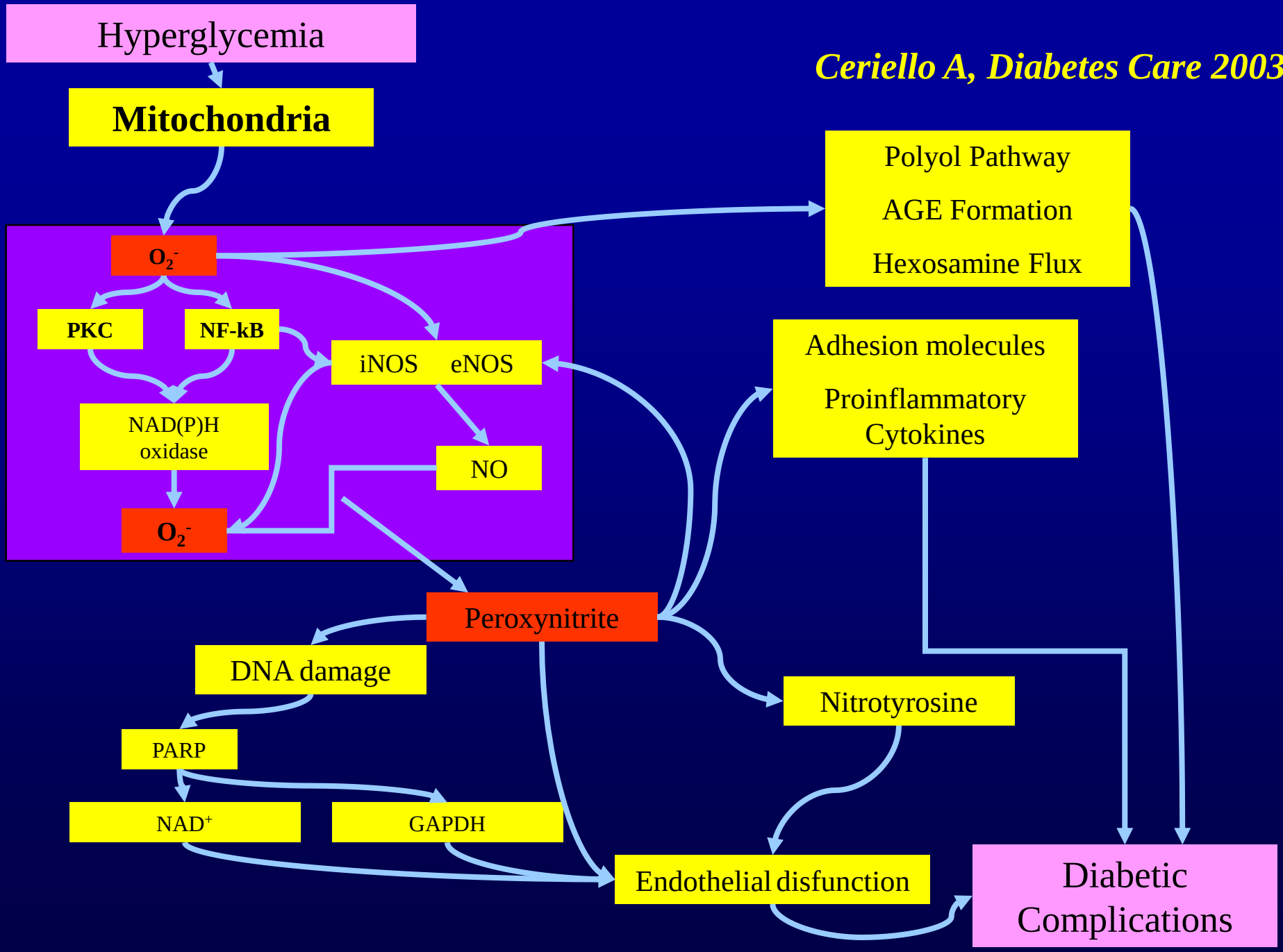
# Densitometric quantitation of fibronectin in individual rats



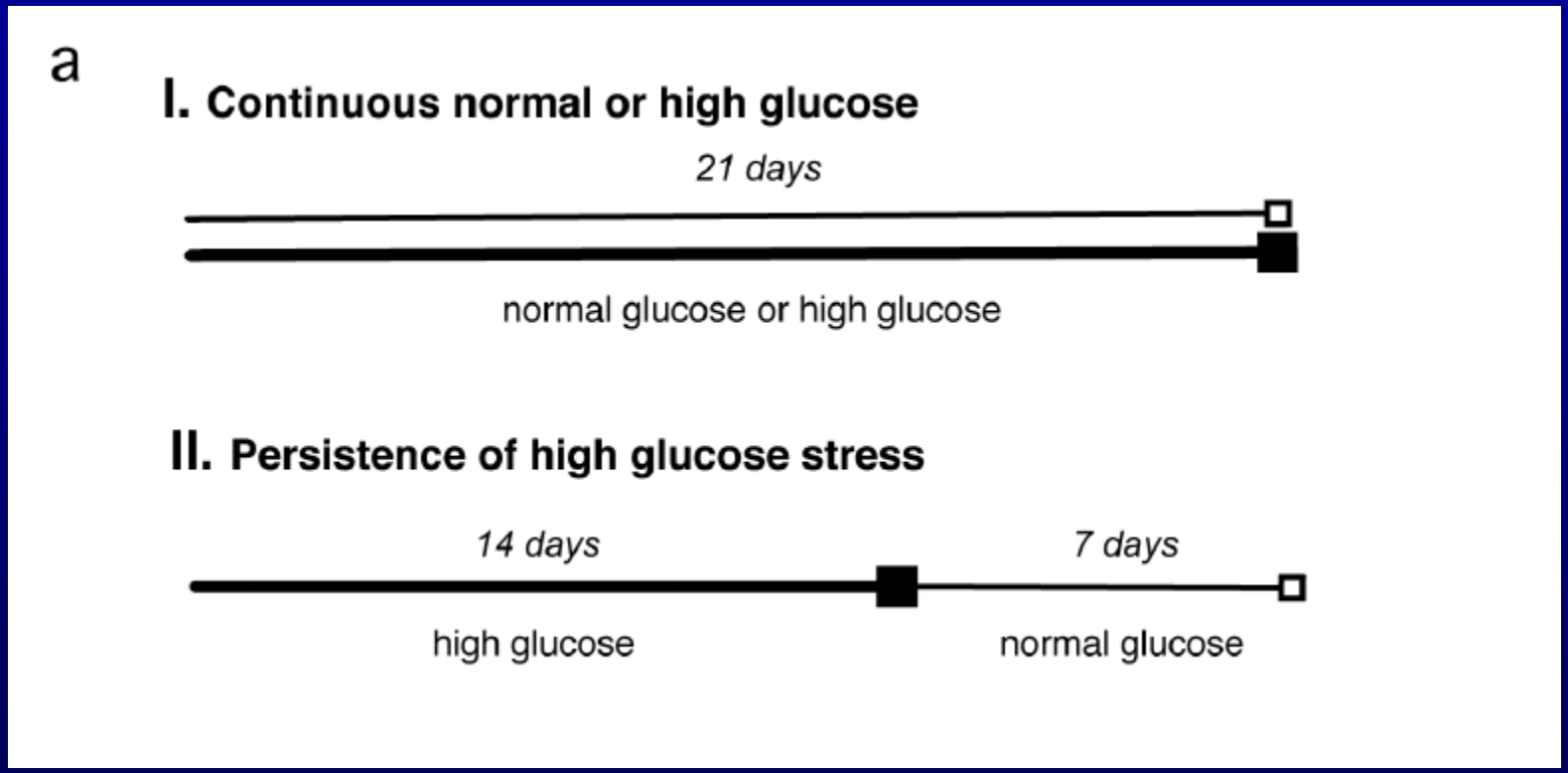

cardiac fibronectin




kidney fibronectin

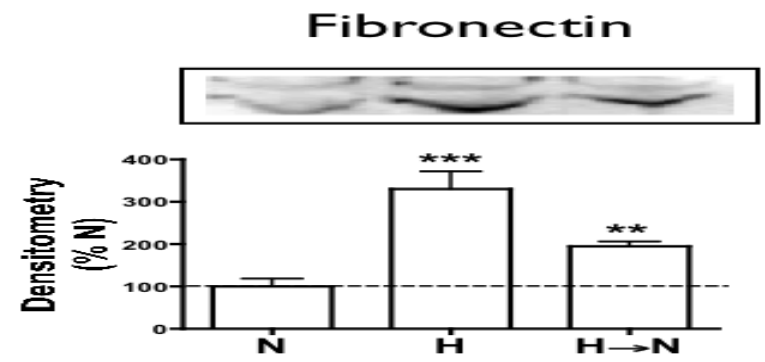

Hyperglycemia
Mitochondria
Ceriello A, Diabetes Care 2003
Polyol Pathway
AGE Formation
Hexosamine Flux
$O_2^-$
PKC
NF-kB
iNOS
eNOS
NAD(P)H oxidase
NO
$O_2^-$
Adhesion molecules
Proinflammatory Cytokines
Peroxynitrite
DNA damage
Nitrotyrosine
PARP
$NAD^+$
GAPDH
Endothelial disfunction
Diabetic Complications

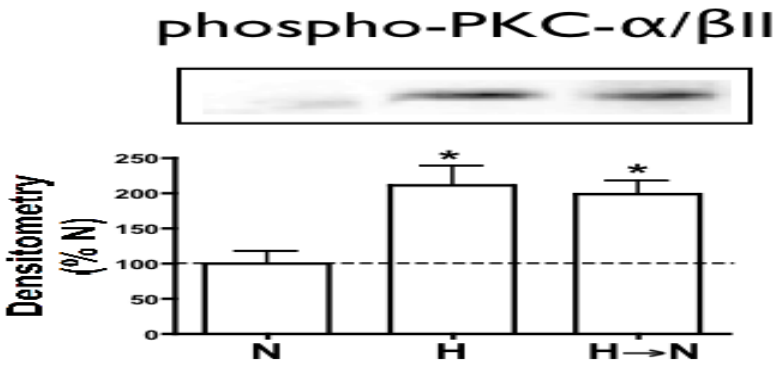
# The “Metabolic Memory: the role of oxidative stress

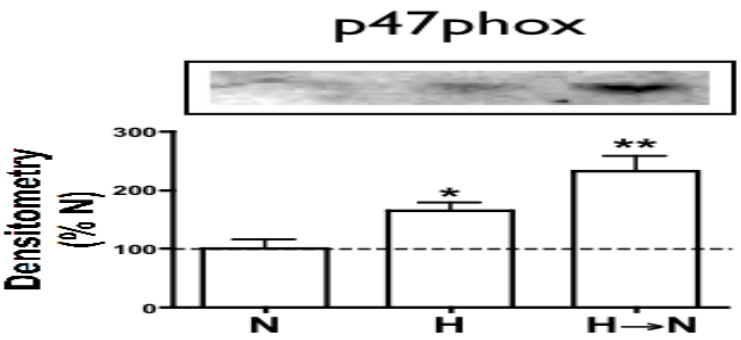


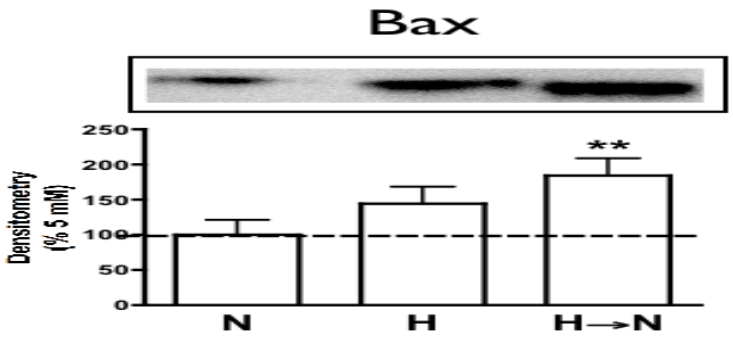

**Ihnat M et al, Diabetologia 2007**

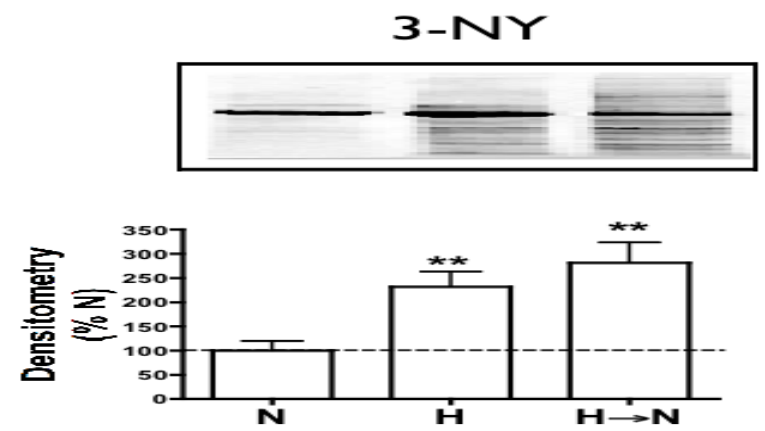
# Effects of high glucose for three weeks or for two weeks plus one week of normal glucose in HUVECs

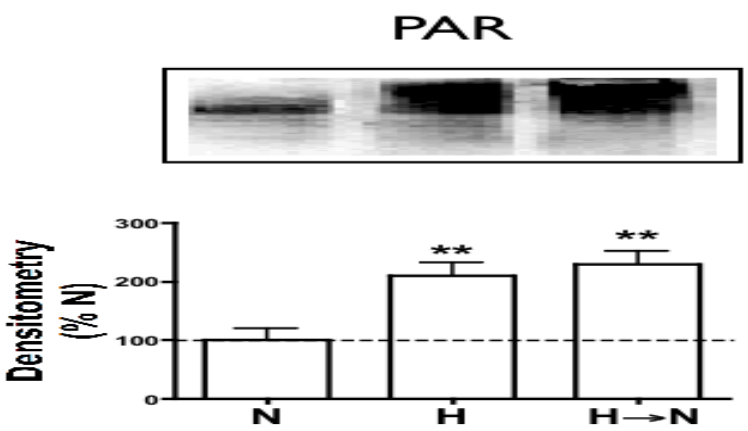


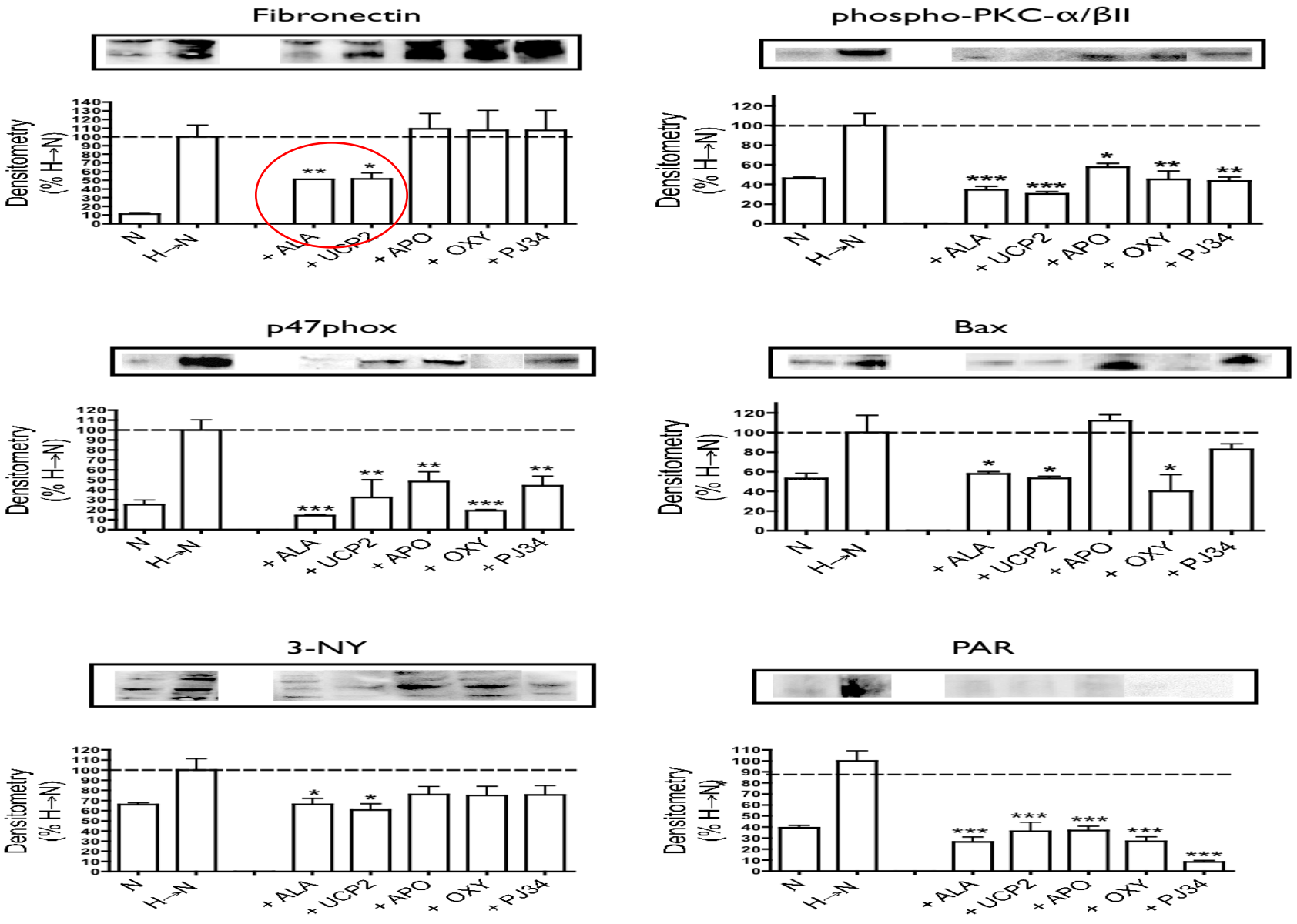



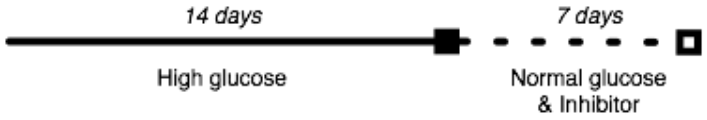



# Effects of various antioxidant treatments on the hyperglycemia-induced “Memory”



# Free nuclear and mitochondrial ROS in HUVECs




**H→N &**

| | **N** | **H** | **-** | **α-LA** | **UCP2** | **APO** |
|---|---|---|---|---|---|---|
| **Nuclear RS** (DHE) | | | | | | |
| **Mito'l RS** (MitoSOX) | | | | | | |

# Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia

Assam El-Osta , Daniella Brasacchio , Dachun Yao , Alessandro Pocai ,
Peter L. Jones , Robert G. Roeder , Mark E. Cooper ,and Michael Brownlee

JEM VOL. 205, September 29, 2008

Abstract

The current goal of diabetes therapy is to reduce time-averaged mean levels of glycemia, measured as HbA1c, to prevent diabetic complications. However, HbA1c only explains < 25% of the variation in risk of developing complications.

Because HbA1c does not correlate with glycemic variability when adjusted for mean blood glucose, we hypothesized that transient spikes of hyperglycemia may be an HbA1c – independent risk factor for diabetic complications.

We show that transient hyperglycemia induces long-lasting activating epigenetic changes in the promoter of the nuclear factor B (NF- B) subunit p65 in aortic endothelial cells both in vitro and in nondiabetic mice, which cause increased p65 gene expression.

Abstract

Both the epigenetic changes and the gene expression changes persist for at least 6 d of subsequent normal glycemia, as do NF- B – induced increases in monocyte chemoattractant protein 1 and vascular cell adhesion molecule 1 expression.

Hyperglycemia- induced epigenetic changes and increased p65 expression are prevented by reducing mitochondrial superoxide production or superoxide-induced - oxoaldehydes.

These results highlight the dramatic and long-lasting effects that short-term hyperglycemic spikes can have on vascular cells and suggest that transient spikes of hyperglycemia may be an HbA1c – independent risk factor for diabetic complications.

# Previous studies have shown that in type 1 diabetic patients even when glycemia is normalized endothelial dysfunction still persists.

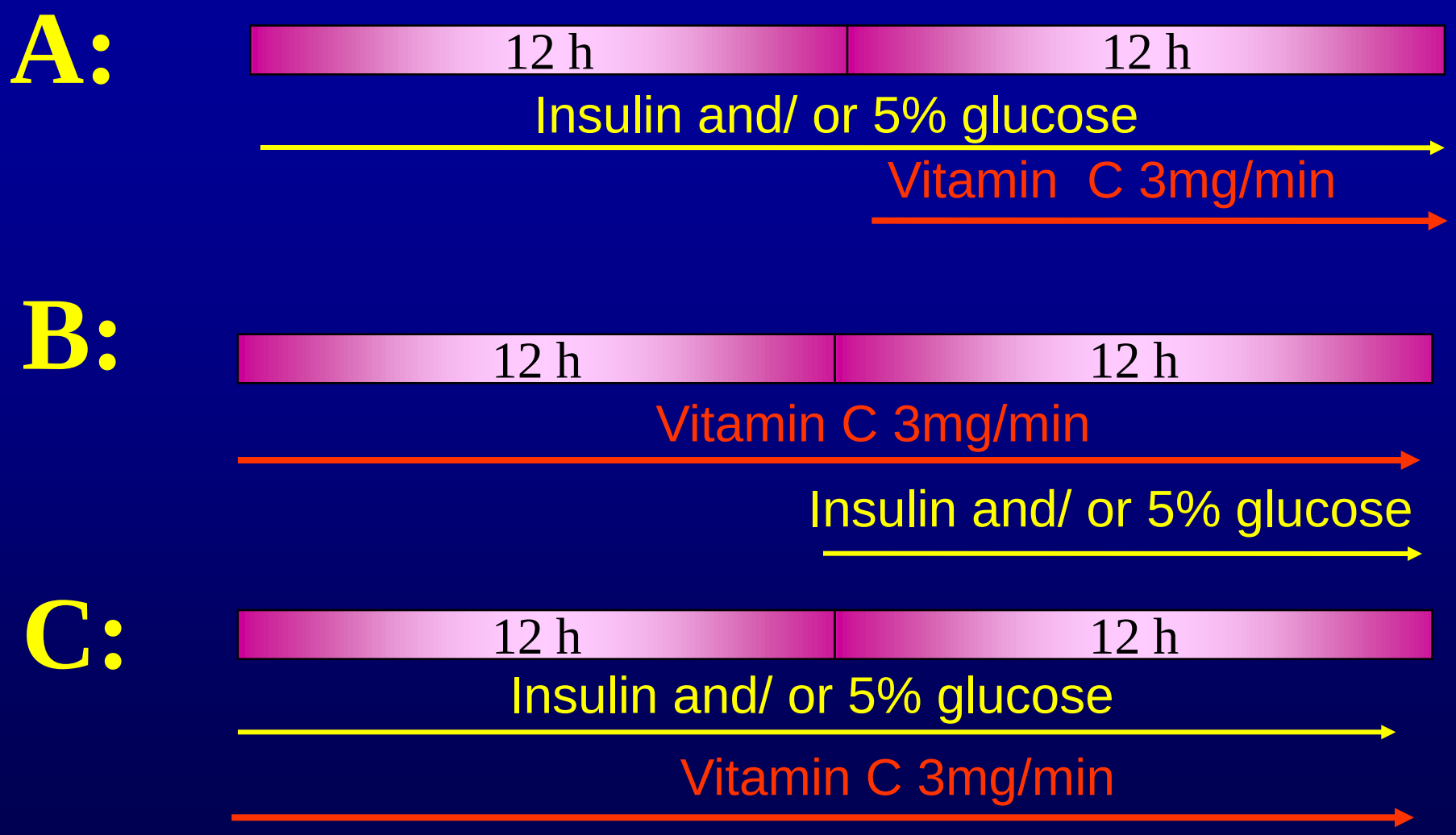

1. Huvers FC, De Leeuw PW, Houben AJ, et al. Endothelium-dependent vasodilatation, plasma markers of endothelial function, and adrenergic vasoconstrictor responses in type 1 diabetes under near-normoglycemic conditions. *Diabetes.* 1999; 48: 1300-1307.

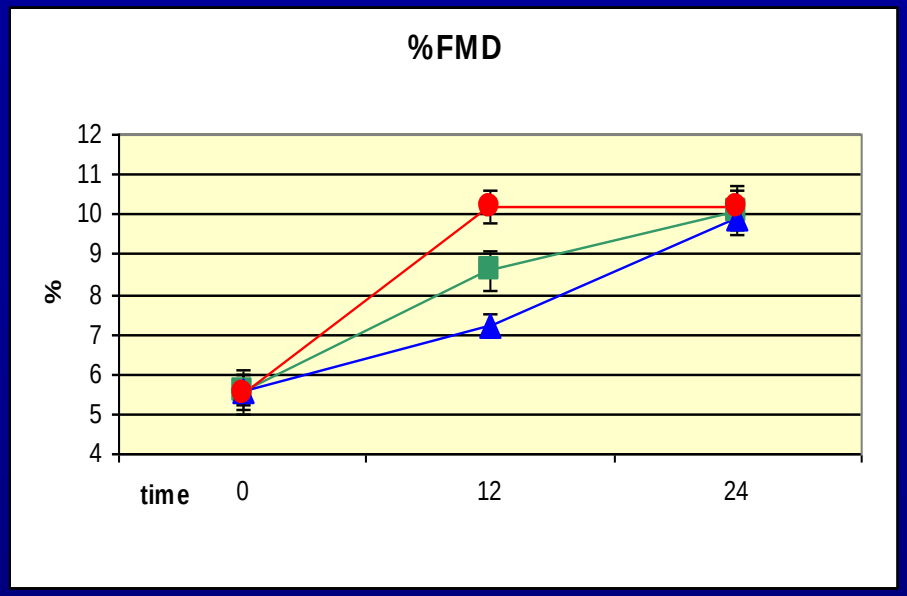
2. Dogra G, Rich L, Stanton K, Watts GF.Endothelium-dependent and independent vasodilation studies at normoglycaemia in type I diabetes mellitus with and without microalbuminuria. *Diabetologia.* 2001 ;44: 593-601.

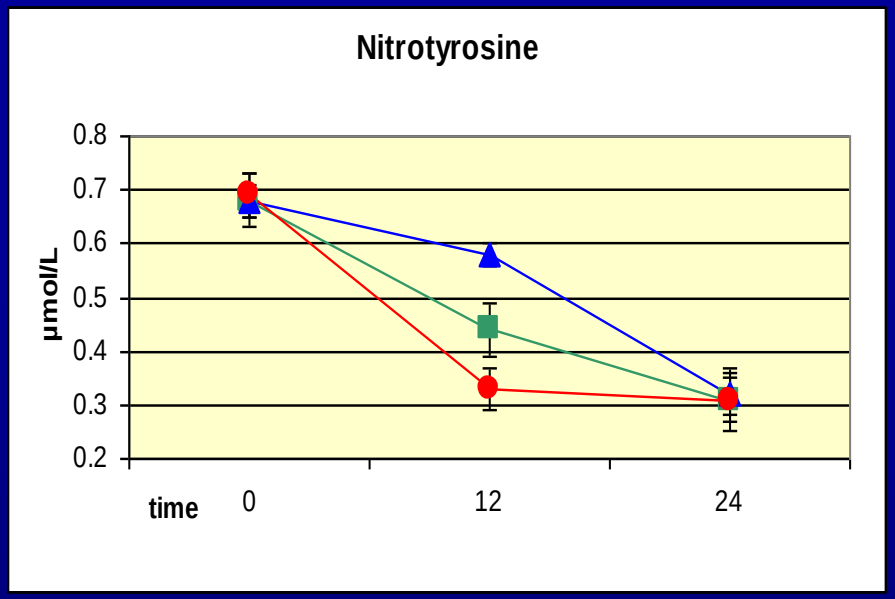
| | Controls | Protocol A | Protocol B | Protocol C |
|---|---|---|---|---|
| Sex | 6M 6F | 7M 5F | 5M 7M | 7M 5F |
| Age (years) | 22.3±1.2 | 22.6±1.7 | 21.3±1.5 | 23.3±1.4 |
| Duration of the disease (years) | | 7.1±2.2 | 7.7±2.6 | 7.5±2.5 |
| BMI (Kg/m2) | 23.8±2.2 | 22.7±2.1 | 23.5±2.2 | 22.4±2.3 |
| Fasting glucose (mmol/l) | 4.3±0.3 | 8.2±2.3* | 8.1±3.2* | 7.8±2.4* |
| HbA1c (%) | | 8.3±0.3 | 8.2±0.5 | 8.3±0.5 |
| Resting systolic blood pressure (mm | 119.1±3. | 120.4±2.5 | 125.3±2.1 | 123.2±1.5 |
| Resting diastolic blood pressure (mm | 80.4±2.1 | 81.2±3.1 | 82.6±2.1 | 82.4±3.7 |
| Total cholesterol (mmol/l) | 4.8±0.6 | 4.9±0.8 | 4.9±0.9 | 5.0 ±0.6 |
| Triglycerides (mmol/l) | 1.1±0.5 | 1.3±0.4 | 1.3±0.5 | 1.2±0.4 |
| HDL-C (mmol/l) | 1.4±0.3 | 1.4±0.3 | 1.4±0.4 | 1.4±0.2 |
| LDL-C (mmol/l) | 2.6±0.5 | 2.5±0.4 | 2.5±0.4 | 2.6±0.6 |
| FMD (%) | 10.8±0.8 | 5.5±0.5* | 5.6±0.7* | 5.6±0.8* |
| Nitrotyrosine (µmol/l) | 0.35±0.3 | 0.73±0.2* | 0.71±0.4* | 0.72±0.3* |

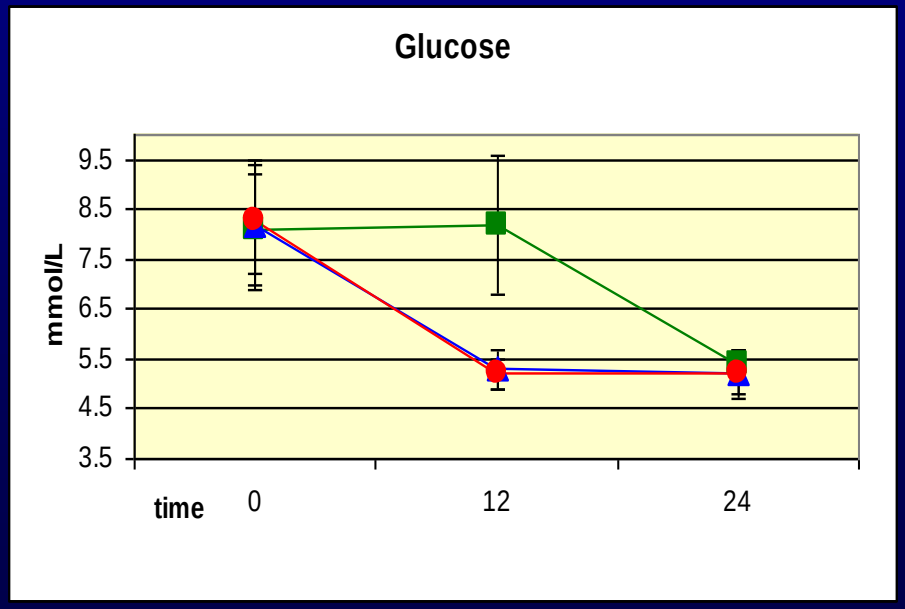
Ceriello A. et al., *Diabetes Care 2007*

* p< 0.001 vs controls


A:
12 h
12 h
Insulin and/ or 5% glucose
Vitamin C 3mg/min
B:
12 h
12 h
Vitamin C 3mg/min
Insulin and/ or 5% glucose
C:
12 h
12 h
Insulin and/ or 5% glucose
Vitamin C 3mg/min




Insulin and Vit. C 24 h

Vit. C 24 h + Insulin 12 h

Insulin 24 + Vit. C 12 h

# PATIENTS: No Complications

- Subgroup 1: Patients enrolled within 1 month from the diagnosis.

- Subgroup 2: Patients had 4.8-5.2 years of diagnosis and included in these subgroup because the mean HbA1c over the last 5 years was ≤ 7%.

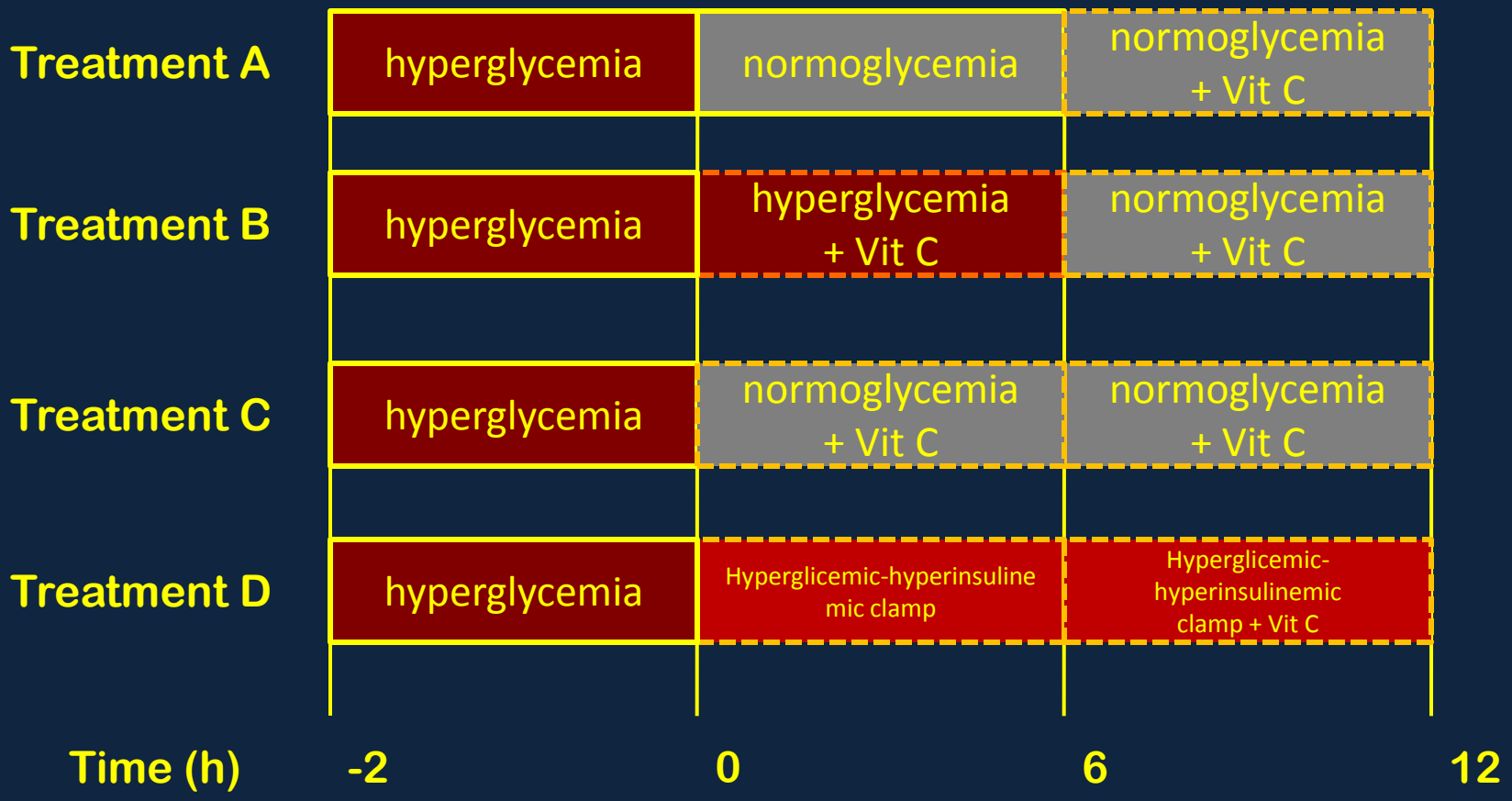
- Subgroup 3: Patients had 4.6-5.4 years of diagnosis and included in these subgroup because the mean HbA1c over the last 5 years was ≥ 7%.

# Baseline characteristics of controls and subgroups of type 1 diabetic patients

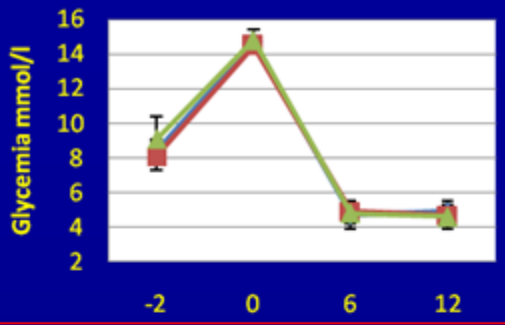
| | Controls | Subgroup 1 | Subgroup2 | Subgroup3 |
|---|---|---|---|---|
| **Sex** | 12M 8F | 11M 9F | 12M 9M | 12M 12F |
| **Age years** | 23.4±2.2 | 19.8±3.2 | 22.7±4.1 | 23.6±4.5 |
| **BMI Kg/m2** | 23.5±2.4 | 23.7±2.5 | 23.5±3.2 | 22.7±3.3 |
| **Fasting glucose mmol/l** | 4.2±0.7 | 8.4±2.3* | 7.1±1.7* | 8.5±2.2* |
| **HbA1c % at the time of the study** | | 8.3±0.8 | 6.7±0. 2** | 8.9±0. 7 |
| **Mean HbA1c %of the last 5 years** | | | 6.4±0. 3*** | 8.7±0. 6 |
| **(range of the last 5 years)** | | | (5.5-6.9) | (8.4-10.5) |
| **Resting systolic blood pressure mm Hg** | 114.3±2.0 | 112.4±2.2 | 110.3±2.1 | 112.2±2.0 |
| **Resting diastolic blood pressure mm Hg** | 78.1±1.1 | 71.5±4.1 | 72.6±2.1 | 72.7±2.7 |
| **Total cholesterol mmol/l** | 4.3±0.5 | 4.4±0.6 | 4.6±0.5 | 4.8 ±0.3 |
| **Triglycerides mmol/l** | 1.2±0.5 | 1.2±0.2 | 1.4±0.4 | 1.2±0.5 |
| **HDL-C mmol/l** | 1.4±0.6 | 1.4±0.7 | 1.4±0.2 | 1.4±0.4 |
| **LDL-C mmol/l** | 2.3±0.3 | 2.4±0.4 | 2.5±0.6 | 2.5±0.9 |
| **FMD %** | 10.8±1.6 | 6.7±0.9* | 6.2±1.14* | 4.1±0.8*β |
| **Nitrotyrosine µmol/l** | 0.35±0.03 | 0.74±0.07* | 0.75±0.04* | 0.94±0.07*β |
| **8-iso-PGF2a (pg/ml)** | 32.6±4.6 | 64.4±4.2* | 63.4±3.8* | 83.4±5.3*β |
| **sICAM-1a (ng/ml)** | 124.2±17.5 | 170.5±12.5* | 165.5±12.8* | 190.9±18.4*β |
| **sVICAM-1 (ng/ml)** | 280.51±15.5 | 370.15±12.3* | 387.22±22.4* | 460.41±28.4*β |
| **IL-6 (pg/ml)** | 124.51±16.3 | 220.35±11.1* | 227.12±12.5* | 280.31±18.3*β |
| **IL-18 (pg/ml)** | 73.7±16.5 | 125.8±17.5* | 125.5±17.8* | 164.4±16.2*β |

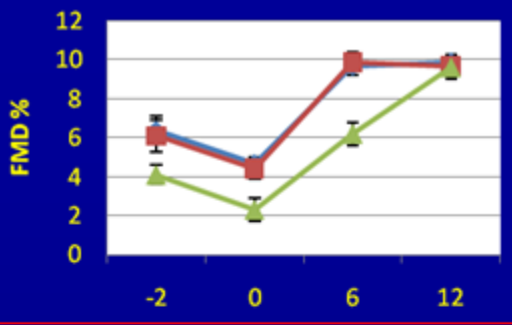
Data are expressed as mean±SD * p< 0.001 vs controls ** p< 0.001 vs sub1 and 3 *** p< 0.001 vs sub3 β p< 0.05 vs sub1 and 2

# Study design

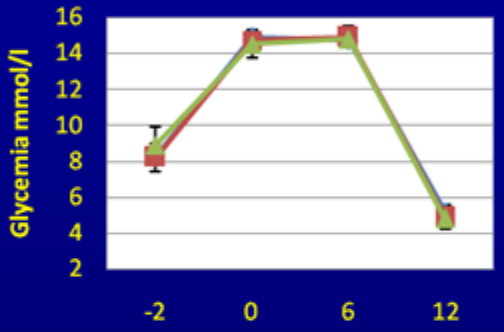


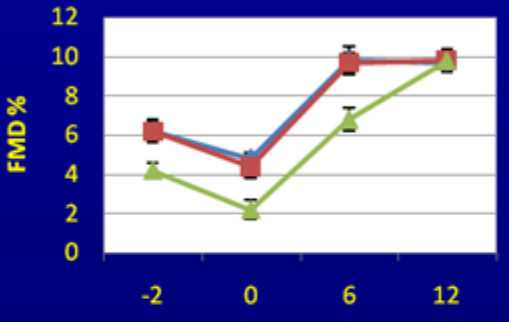
Treatment A



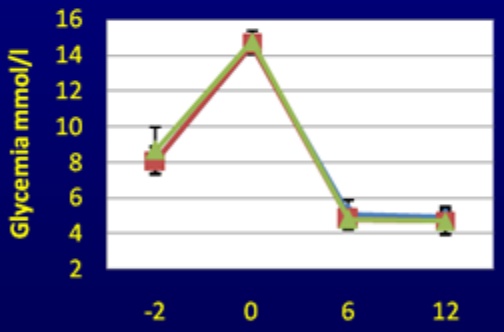



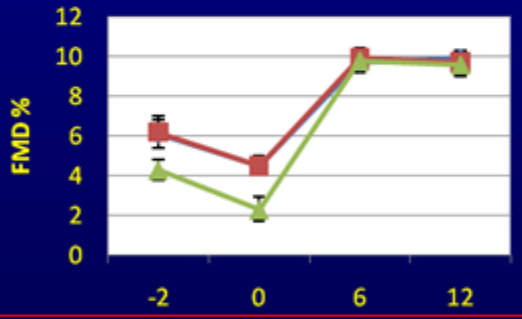

Treatment B



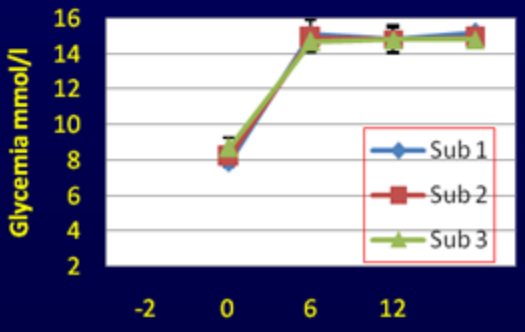



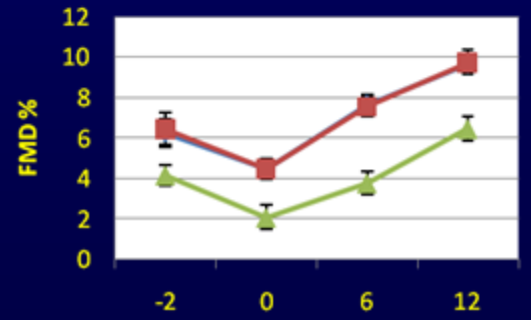

Treatment C




Treatment D



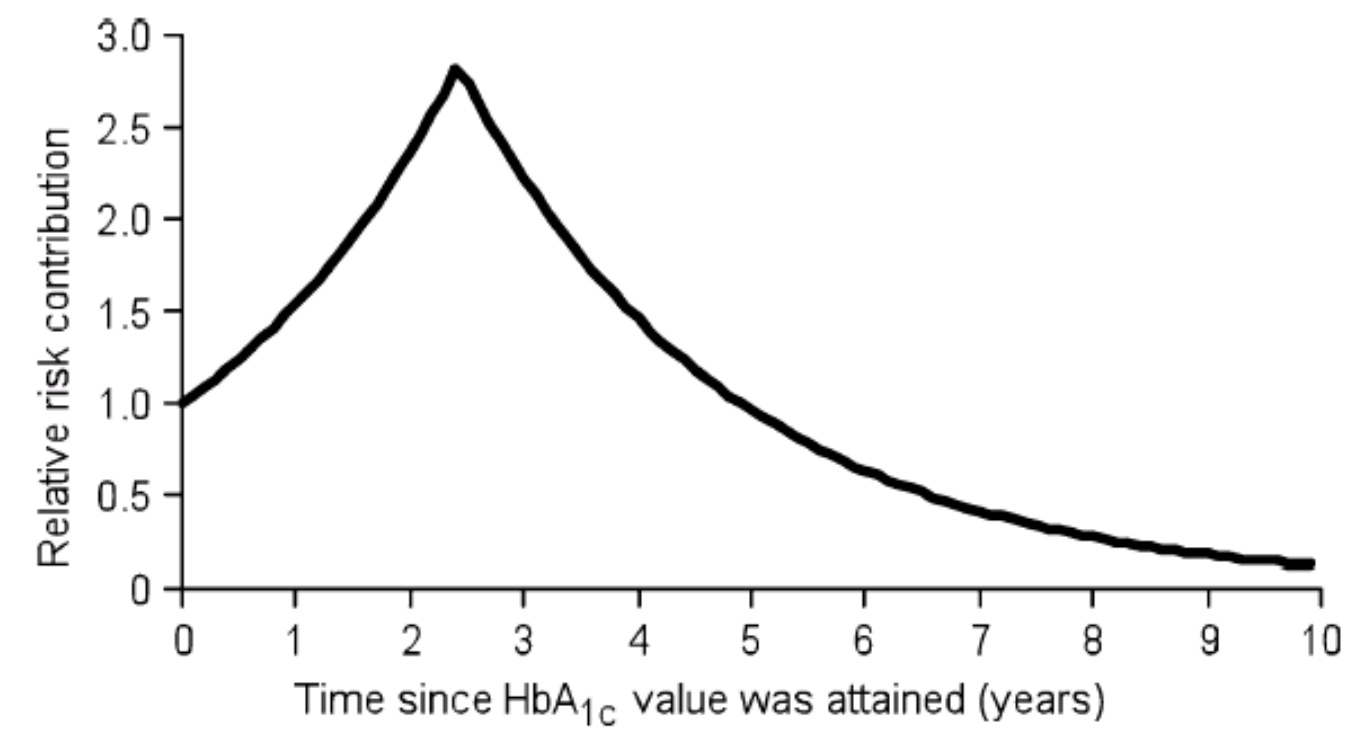



# The shape of the metabolic memory of HbA1c: re-analysing the DCCT with respect to time-dependent effects

M. Lind , A. Odén , M. Fahlén and B. Eliasson

*Diabetologia* (2010) 53:1093–1098

# The relative contribution of HbA1c values at different points in time in the past to risk of current retinopathy progression.

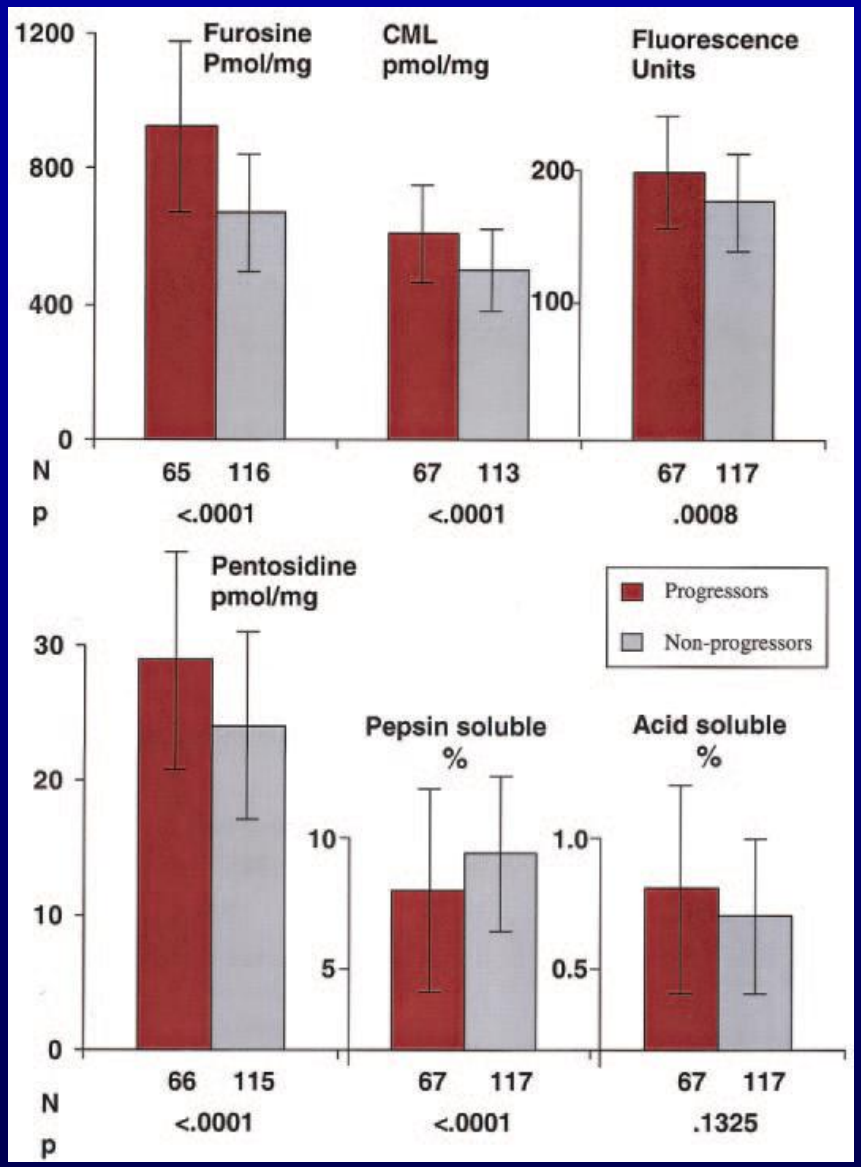



The relative contribution is largest from values 2.4 years ago, which is 2.8 times greater than the contribution from present values. The contribution is greater than that from present values for times up to 4.9 years ago. For values from 6.5 and 8.4 years ago, the contribution is 50% and 25% of present values, respectively.

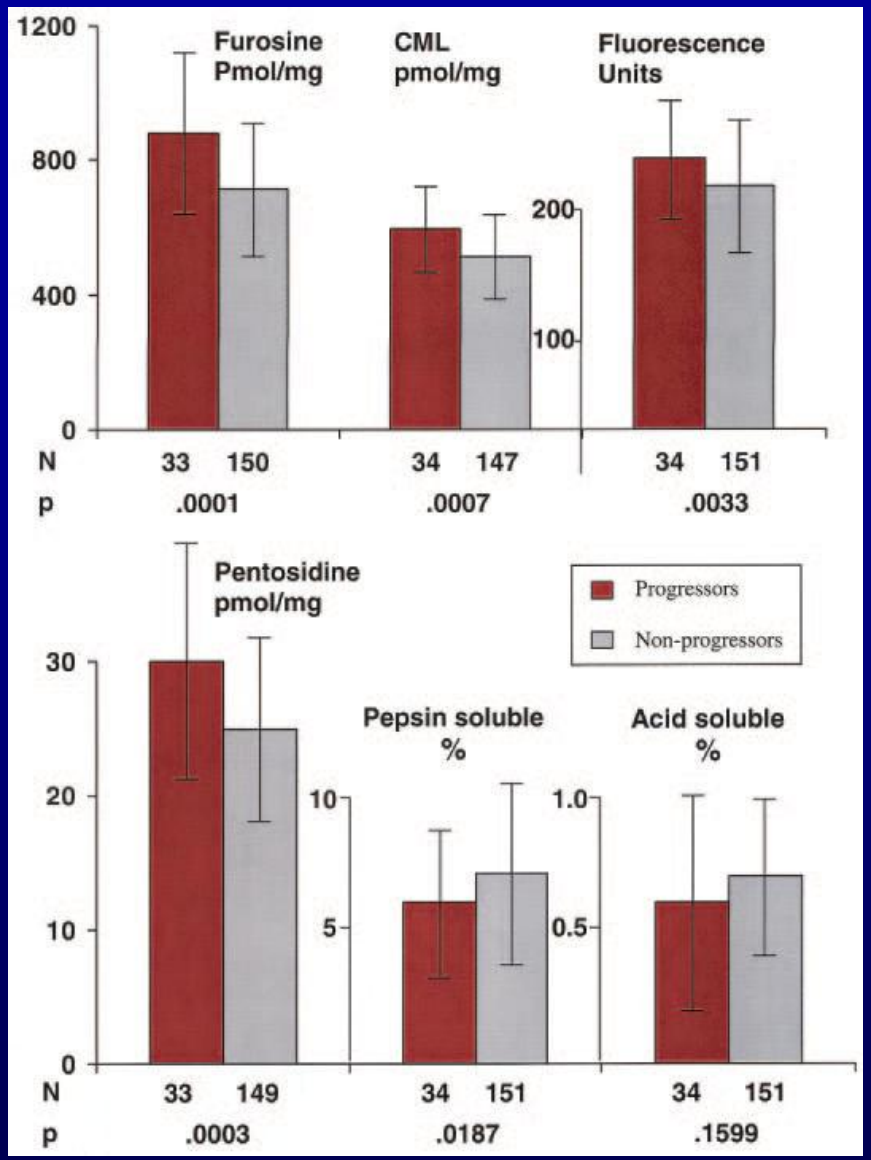
# Glycation and Carboxymethyllysine Levels in Skin Collagen Predict the Risk of Future 10-Year Progression of Diabetic Retinopathy and Nephropathy in the Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications Participants With Type 1 Diabetes

S. Genuth, W. Sun, P. Cleary, D. R. Sell, W. Dahms, J. Malone, W. Sivitz, and V.M. Monnier, for the **DCCT Skin Collagen Ancillary Study Group***

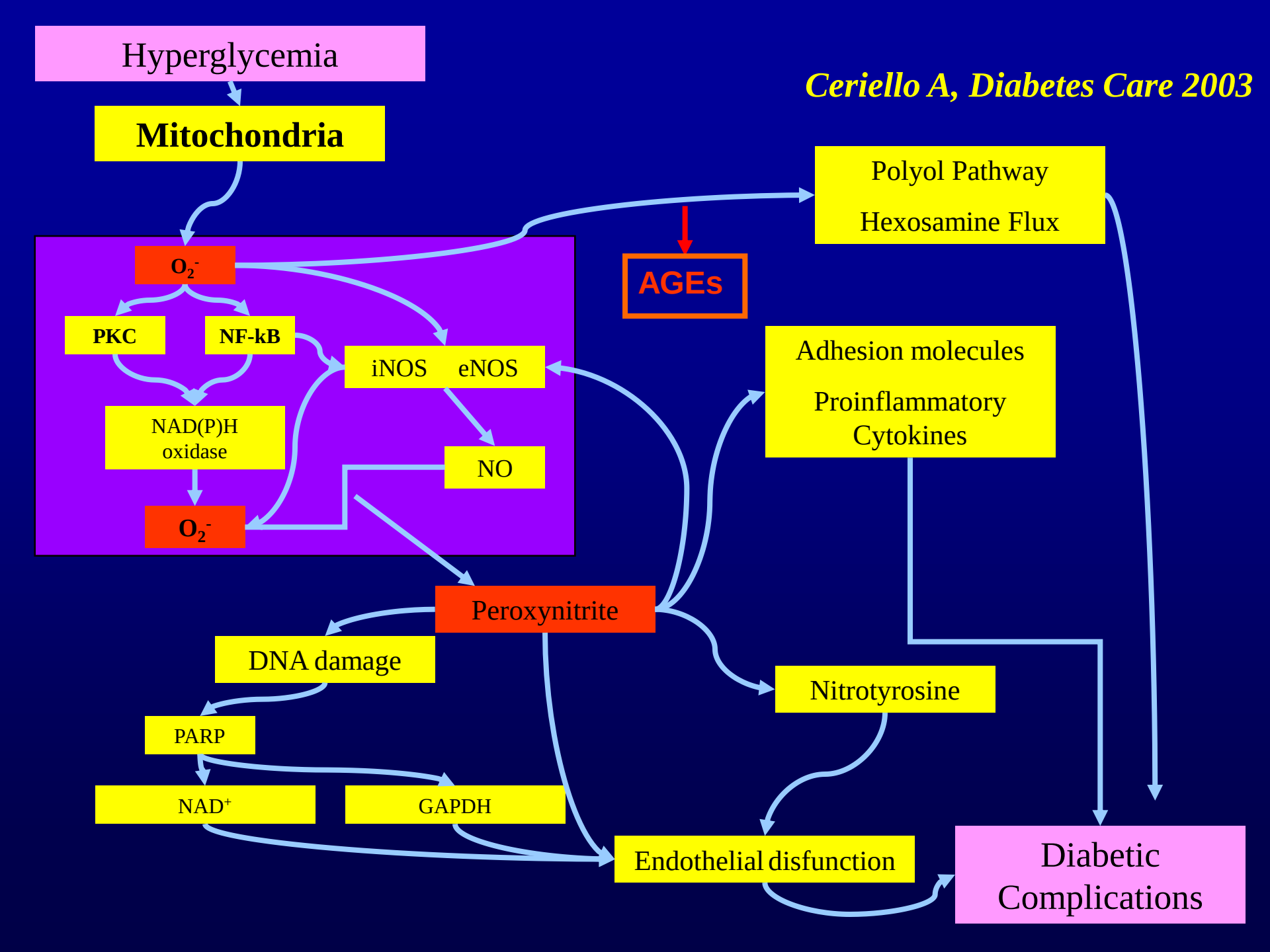
DIABETES, VOL. 54, NOVEMBER 2005

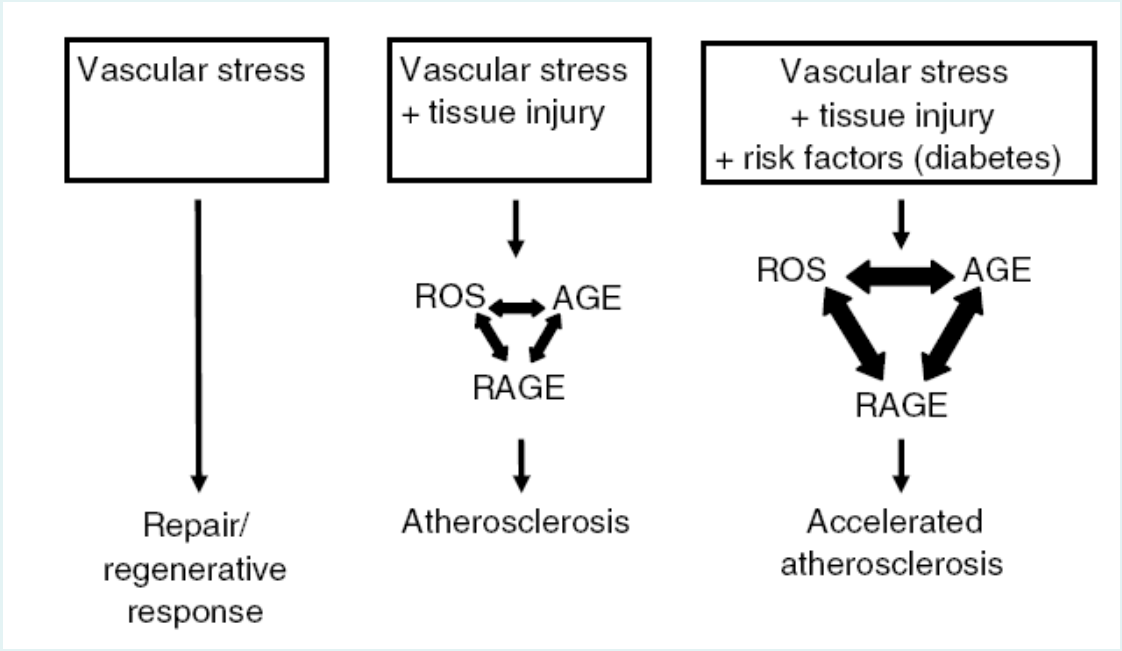



Distribution of skin collagens by ***retinopathy*** progression status.



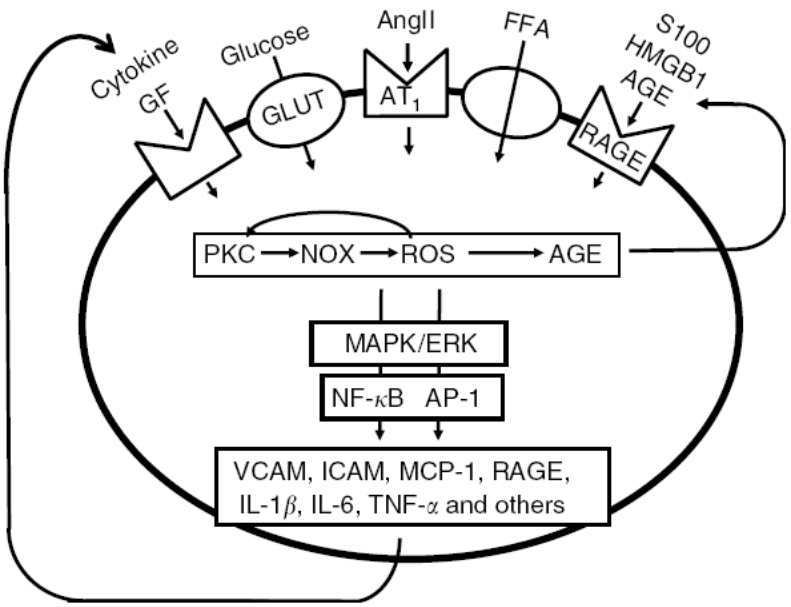

Distribution of skin collagens by ***nephropathy*** progression status.


Hyperglycemia
Mitochondria
Ceriello A, Diabetes Care 2003
Polyol Pathway
Hexosamine Flux
AGEs
$O_2^-$
PKC
NF-kB
iNOS
eNOS
NAD(P)H oxidase
NO
$O_2^-$
Adhesion molecules
Proinflammatory Cytokines
Peroxynitrite
DNA damage
Nitrotyrosine
PARP
$NAD^+$
GAPDH
Endothelial disfunction
Diabetic Complications



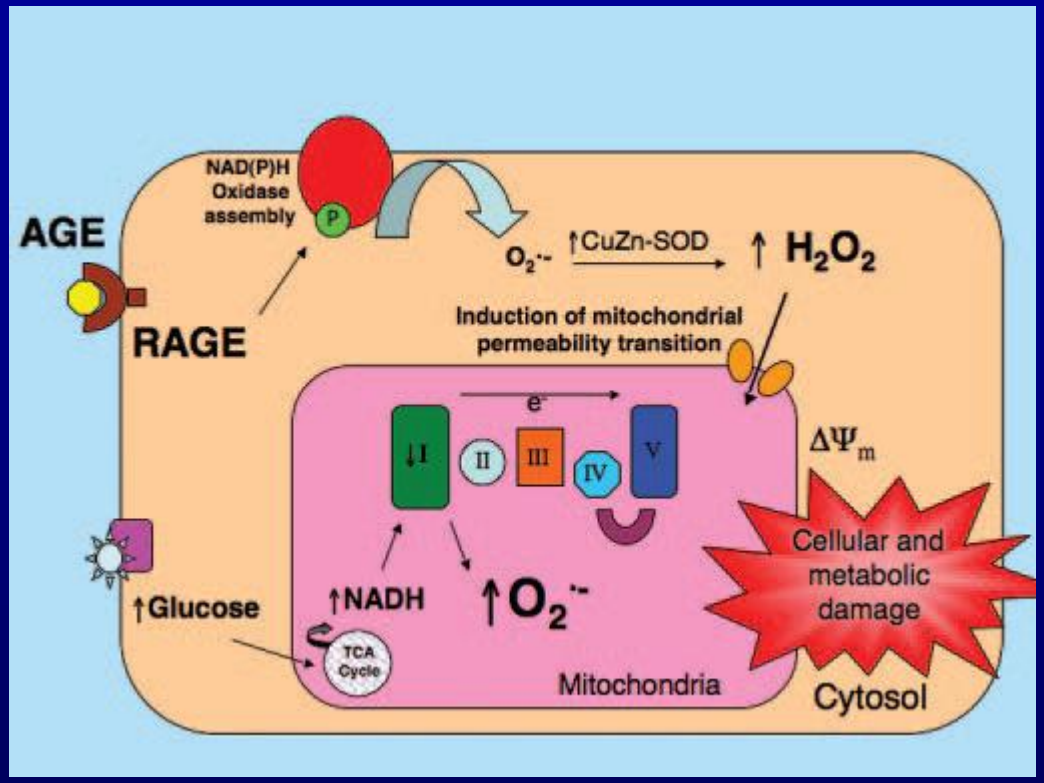

# Working hypothesis for the accelerated atherosclerosis in diabetes.

# Effects of ROS in vascular cells




E Schleicher and U Friess:Kidney International (2007) 72, S17–S26

# Schematic representation of the interplay between AGE and RAGE and high glucose in promoting mitochondrial superoxide production in the diabetic kidney.




AGEs binding to RAGE induce cytosolic H2O2 production. Cytosolic H2O2 facilitates induction of mPT, promoting a deficiency in complex I of the mitochondrial respiratory chain. Hyperglycemia provides increased mitochondrial NADH availability for OXPHOS, which, when coupled with a deficient complex I activity, amplifies mitochondrial superoxide generation. Both the AGE-RAGE interaction and hyperglycemia synergistically coordinate overproduction of mitochondrial superoxide and promote diabetic kidney disease.

M. T. Coughlan et al., J Am Soc Nephrol 20: 742–752, 2009

# Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation

M. Rosca, T. Mustata, M. Kinter, A. Ozdemir, T. Kern, L. Szweda, M.Brownlee,V. Monnier,M. Weiss

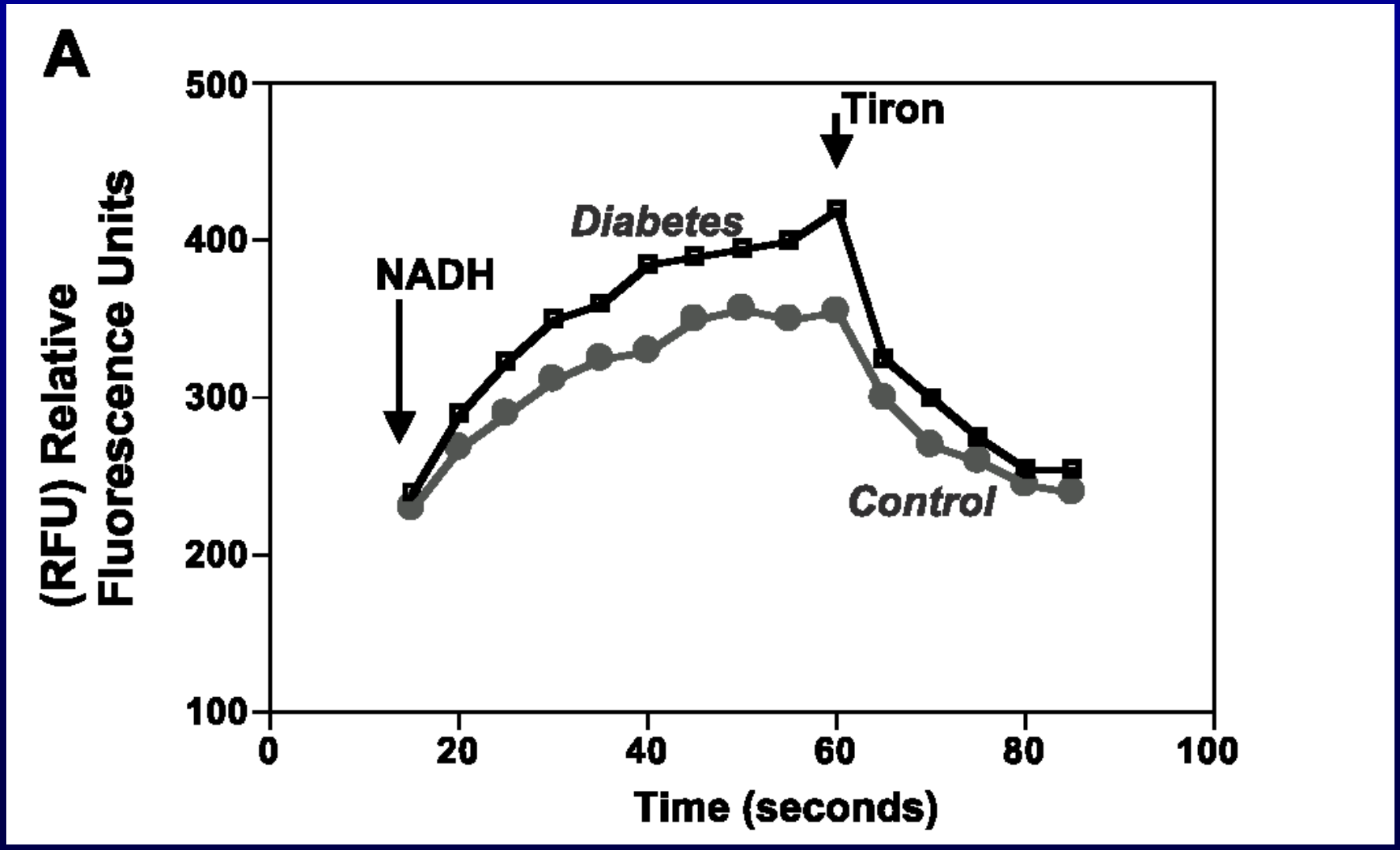
*Am J Physiol Renal Physiol* 289: F420–F430, 2005.

# Effect of 12 m diabetes on electron respiratory chain activity

| Complex | Control | Diabetes | *P* Value |
|---|---|---|---|
| *Complex I* | 177.5±33.4 | 168.4±36.9 | 0.63 |
| *Complex III* | 100.4±2.9 | 82.7±12.3 | 0.0006 |
| *Complex IV* | 209.3±43.5 | 215.5±51.6 | 0.81 |

Values are means ± SD; units are nmol·min$^{-1}$·mg mitochondrial protein$^{-1}$.

Cortical renal mitochondria isolated from 12 mo diabetic animals (squares) form higher levels of superoxide than their age-matched controls (circles).

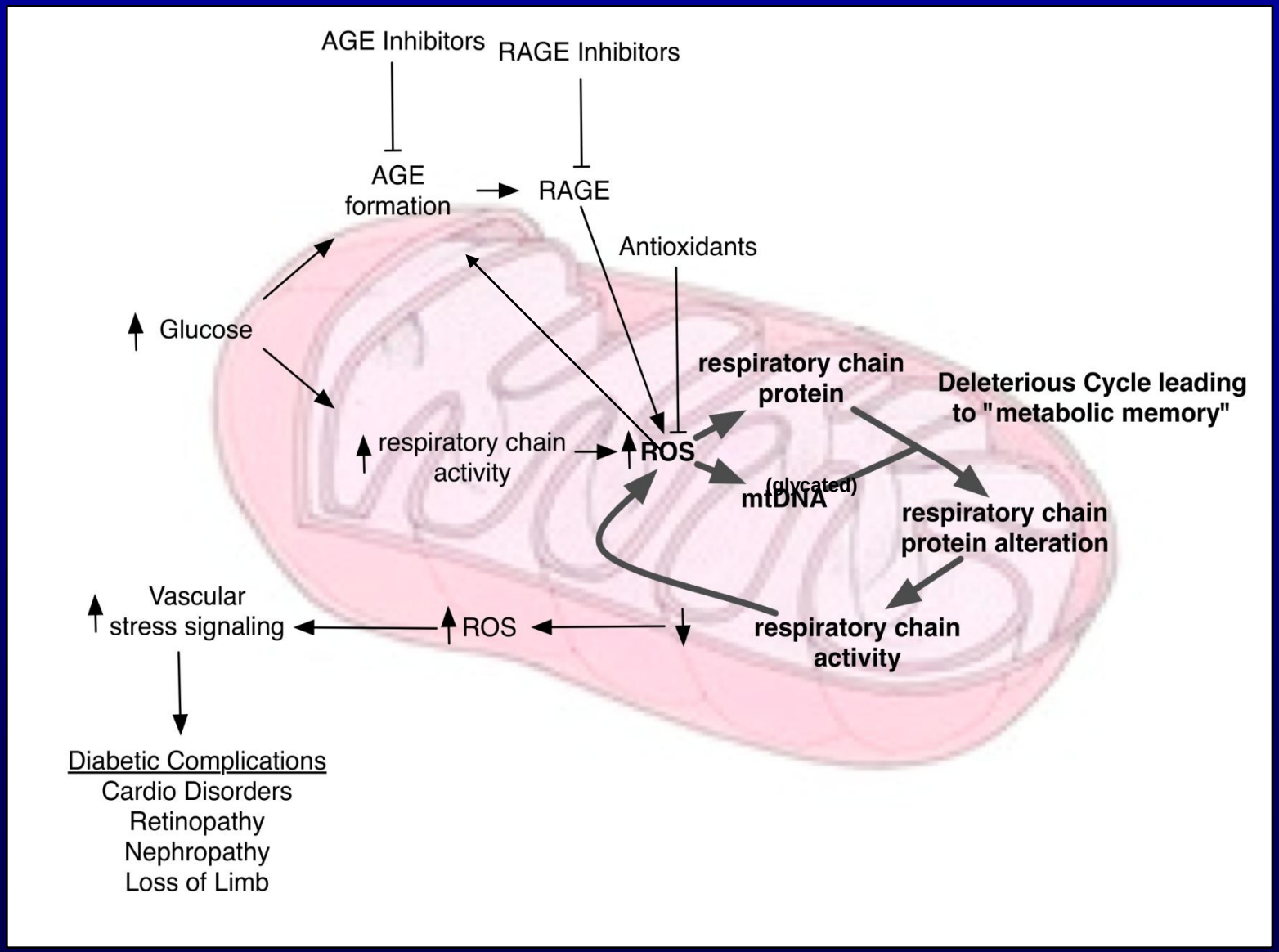


# “The “Metabolic Memory”: Is More than Just Tight Glucose Control Necessary to Prevent Diabetic Complications?.”

A. Ceriello, J. Thorpe, M. Ihnat

**J Clin Endocrinol Metabol, 2009;94:410-5**

# The vicious circle of the *"Metabolic Memory"*




A. Ceriello et al. J Clin Endocrinol Metab, 2009;94:410-5

# “The Metabolic Memory”

## *Evidence for a long-term persistence of hyperglycaemia-induced damage*

- Experiments in the cells, in the animals and in hmans confirm the exsistence of the *“Metabolic Memory”.*

-The unyfing hypothesis, suggesting hyperglycemia-induced free radicals overgeneration as the key event in the development of diabetic complications, seems to explain the perisistence of the *“Metabolic Memory”.*

Dinosaur tracks, Dinosaur State Park, Rocky Hill, CT

*“The take-home message is that*

good glucose control should be started as early as possible to delay or prevent serious diabetes-related complications,”

*said Alan D. Cherrington, PhD, president, American Diabetes Association.*
*ADA  S. Diego 2005*