Oxidative Stress and Diabetic Complications:

Results of Anti-oxidant Treatments

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Oxidative Stress in Diabetes

Is Oxidative Stress Increased in Diabetes ?

How Are the Oxidants Formed in Diabetes ?

Do the Oxidants Cause Specific Complications In Diabetes ?

Can Anti-Oxidants Prevent, Stop or Delay the Onset and the Progression of Diabetic Complications ?

Is Oxidative Stress increased in Diabetes ?

- PubMed search showed the following on the topic of Oxidative Stress and Diabetes:
- 1242 paper since 1982
- 315 Review papers since 1989
- Greater than 95% concluded that Oxidative Stress is significantly increased in Diabetes

Oxidative Stress in Diabetes

Is Oxidative Stress Increased in Diabetes ?

YES

Oxidative Stress in Diabetes

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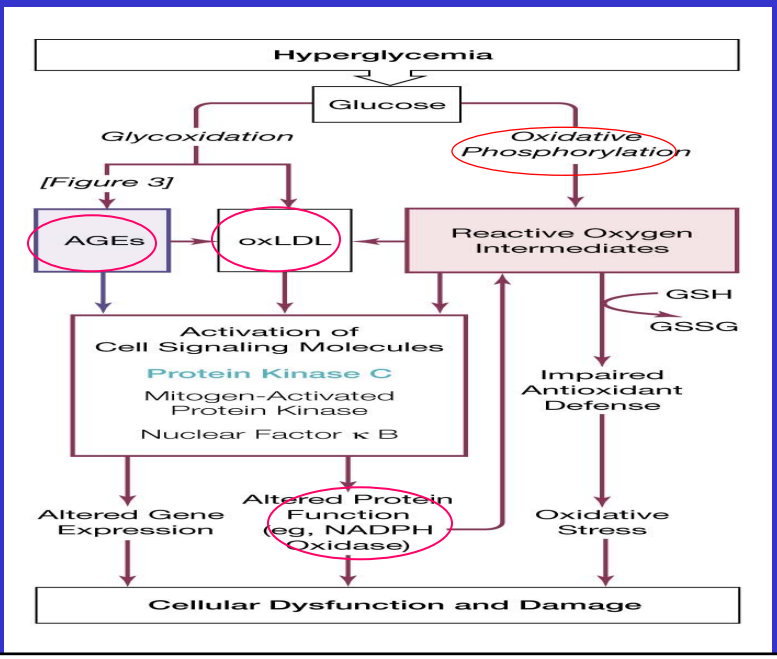
Possible Mechanisms of Hyperglycemia's Adverse Effects

- Sorbitol-myoinositol osmolarity changes (via aldose reductase pathway)
- Oxidative-redox stress
- Non-enzymatic glycation reactions(Advanced glycation products-AGE's)
- Activation of protein kinase C (PKC) - Diacylglycerol (DAG) Pathway
- Hexosamine Pathway

Oxidative Stress Theory



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Can Oxidants induce Vascular Pathologies Similar to Diabetes ?

Cultured cells or tissues : H_2O_2, AGE, and other Oxidants can mimic many abnormalities induced by high glucose levels

Animal Models – Transgenic animals with increased Oxidant Productions (SOD KO mice) do not develop vascular lesions without diabetes.

Indicators of Oxidative Stress which are increased in Diabetes

F(2)isoprostane, malondialdehyde, methylgloxyal, superioxide, 8-hydroxy-2deoxyguanosine, carboxymethyllysine, lipid hydroperoxides, ox-LDL, nitrosytyrosine, mitochondrial DNA mutations and many others.

Natural anti-oxidants :

Decreased levels of GSH, Ascorbate, and NO

Increased levels of SOD, GSH Reductase + others, catalase

Unclear : Vitamine E,

Inflammatory markers : NFκB, TNFα, IL6+18, p38 +JNK activations

Indicators of Oxidative Stress which are increased in Diabetes or Insulin Resistance

F(2)isoprostane, malondialdehyde, methylgloxyal, superioxide, 8-hydroxy-2deoxyguanosine, carboxymethyllysine, lipid hydroperoxides, ox-LDL, nitrosytyrosine, mitochondrial DNA mutations and many others.

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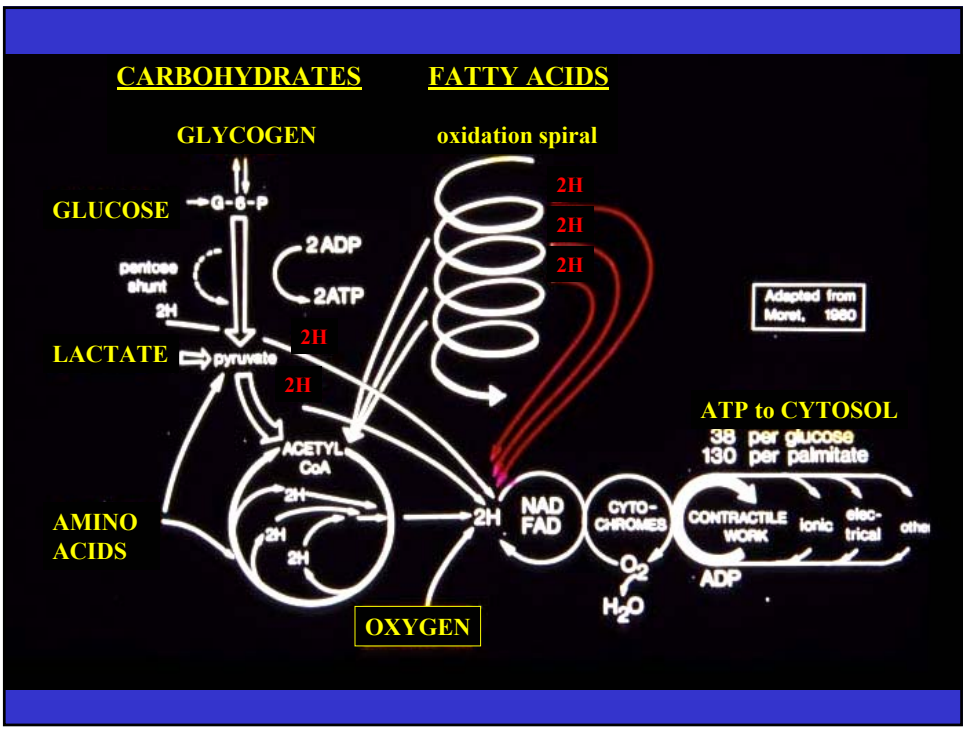
VERY SIMILAR BETWEEN DIABETES AND INSULINRESISTANCE

Reviewed by Evens et al Diabetes, Jan 2003



Known Risk Factors for Vascular Complications of Insulin Resistance

~~Retinopathy~~	~~Neuropathy~~
~~Hyperglycemia~~	~~Hyperglycemia~~
Cardiovascular	~~Nephropathy~~
Hyperglycemia	~~Hyperglycemia~~
Insulin Resistance	
Free Fatty Acidemia	
Hypertension	Angiotensin Action
Hyperlipidemia	

Known Risk Factors in Diabetic and Insulin Resistant Complications

Retinopathy	Neuropathy
Hyperglycemia	Hyperglycemia
Cardiovascular	Nephropathy
Hyperglycemia	Hyperglycemia
Insulin Resistance	
Free Fatty Acidemia	
Hypertension	Angiotensin Action
Hyperlipidemia	

Oxidative Stress in Diabetes

?

Do Oxidants Cause Specific Complications Of Diabetes ?

Since both FFA and hyperglycemia can increase oxidative stress, it is unlikely that the specific pathologies in the microvessels of diabetes are mainly due to or initiated by Oxidative Stress.

Oxidative Stress in Diabetes

Is Oxidative Stress Increased in Diabetes ?

How Are the Oxidants Formed in Diabetes ?

Do the Oxidants Cause Specific Complications In Diabetes ?

Can Anti-Oxidants Prevent, Stop or Delay the Onset and the Progression of Diabetic Complications ?

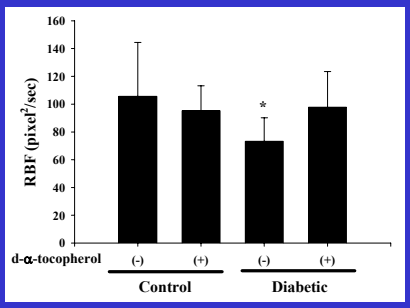
Some of the Anti-oxidants which have been tried.

Vit. E, Vit. C, Probucal, α-lipoic acid, N-acetyl cysteine, aminoguanindine, taurine, Co-enzyme Q, β-carotene, pyridoxamine, statins, selenium and many many others singularly or in combinations.

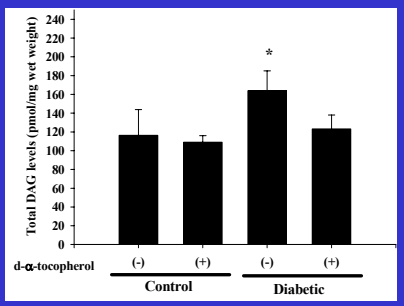
Results of the Non-clinical Anti-oxidant Trials for Diabetic Complications

	Results
Cultured cells exposed to high glucose levels	Many Positive Reports
Diabetic Animals :	
Retina	Positive
Glomeruli	Positive
Peripheral Nerves	Positive
Cardiovascular	No Report on late Pathology

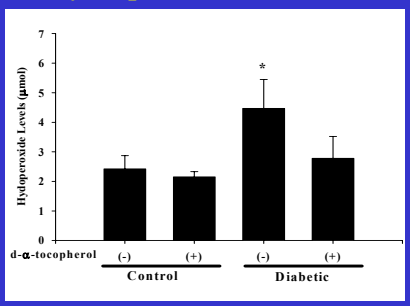
Retinal Blood Flow



Total DAG Levels



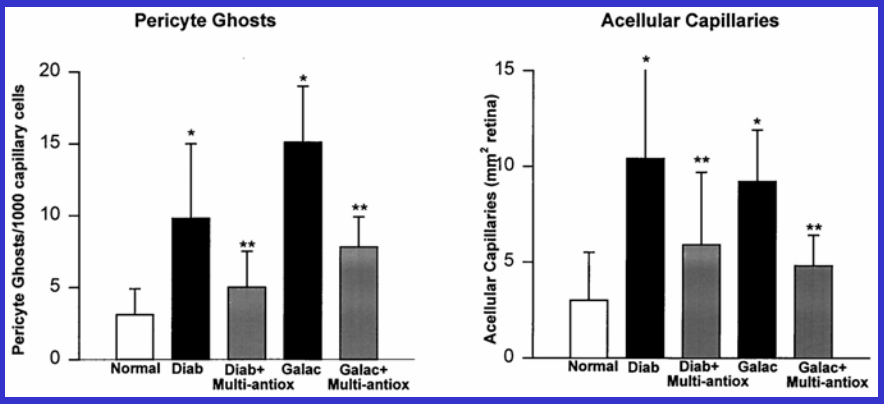
Hydroperoxide Levels



Retinal Blood Flow (RBF), Diacylglycerol (DAG) level, and oxidative stress level changes in 2 week duration diabetic and non-diabetic rat retinas with vitamin E (d-a-tocopherol) treatment (+) and placebo treatment (-). * p<0.05

Abiko et al , Diabetes, 2003

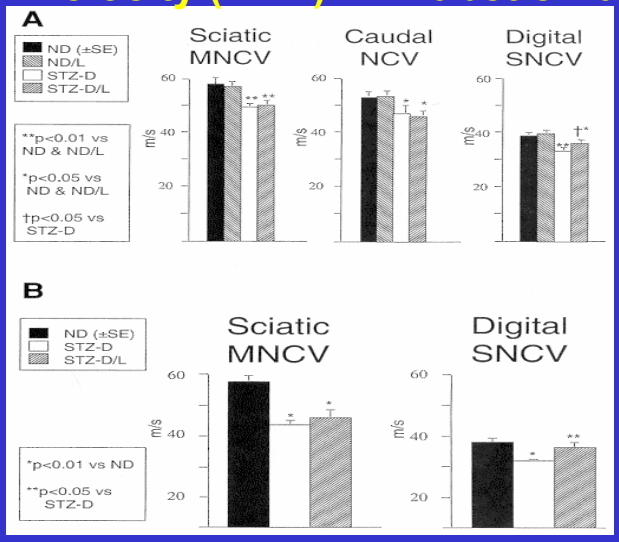
Effect of Multi-antioxidant Treatment on Retinal Pathologies In Diabetic and Hypergalactosemic Rats



12 months of Trolox, Vit. E+C, N-a cysteine, b-carotene +selenium.

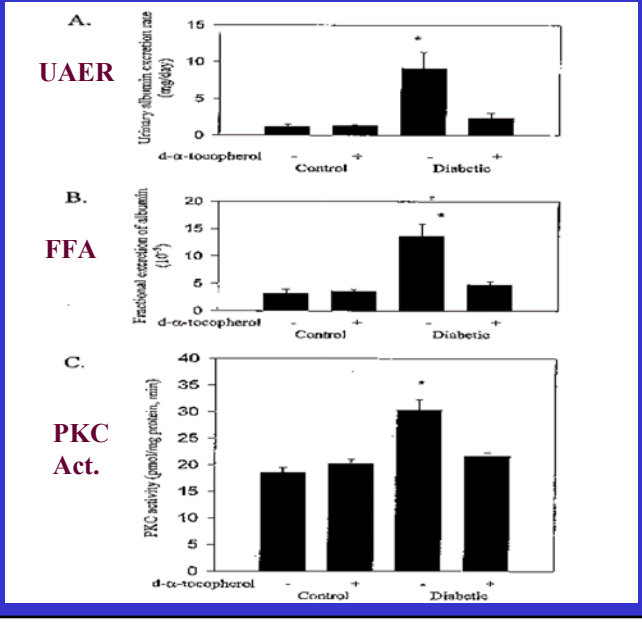
Kowluru, Tang + Kern, Diabetes, 2001

Effect of a-Lipoic Acid on Nerve Conduction Velocity (NCV) in Diabetic Rats



Stevens et al. Diabetes, 49:1006-1015, 2000

Effect of Vitamin E (High Dose) on Renal Functions In Diabetic Rats



Koya et Al, JASN, 1997

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Cultured cells exposed to high glucose levels	Many Positive Reports
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Peripheral Nerves	Positive
Cardiovascular	No Report on late Pathology

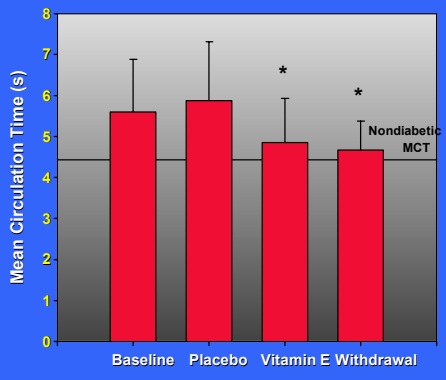
Results of Clinical Studies in Antioxidants on Diabetic Complications

Early Surrogate Endpoints :
Many studies have been positive for Retinopathy, Nephropathy, Neuropathy, and Cardiovascular Changes Induced by Diabetes

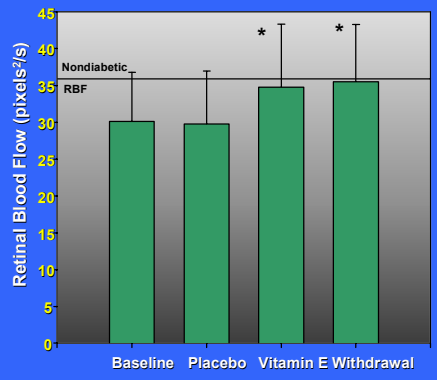
Vitamin E Study Design

- Double-Masked, Randomized, Placebo-Controlled Crossover Clinical Trial.
- Study duration of 8 months with crossover at 4 months.
- Randomization to either 1,800 IU/d (1350 mg/d) of Vitamin E (d-alpha tocopherol acetate in 50 mg vegetable oil) p.o. or placebo (450 mg soybean oil in 50 mg vegetable oil) for 4 months.
- Normalization of Retinal blood flow and mean circulation time as clinical end-points.

Mean Circulation Time and Retinal Blood Flow for Diabetic Subjects at Baseline,Vitamin E Therapy, Pre-Vitamin E Placebo, and Post-Vitamin E Withdrawal

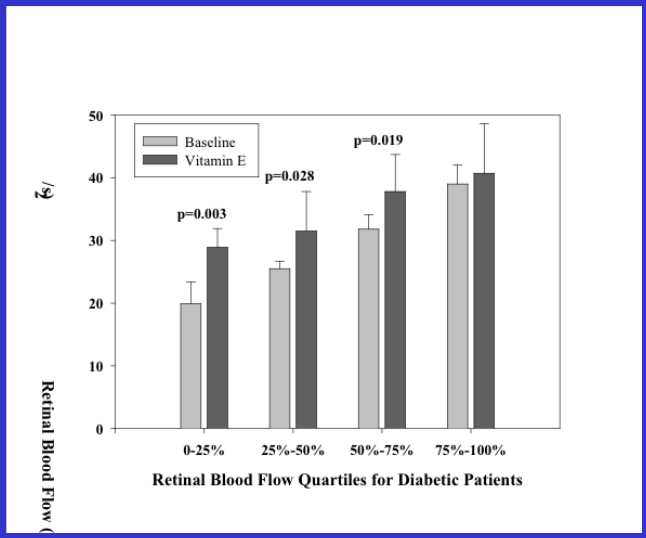


* $p < 0.002$ as compared to baseline by paired t-test

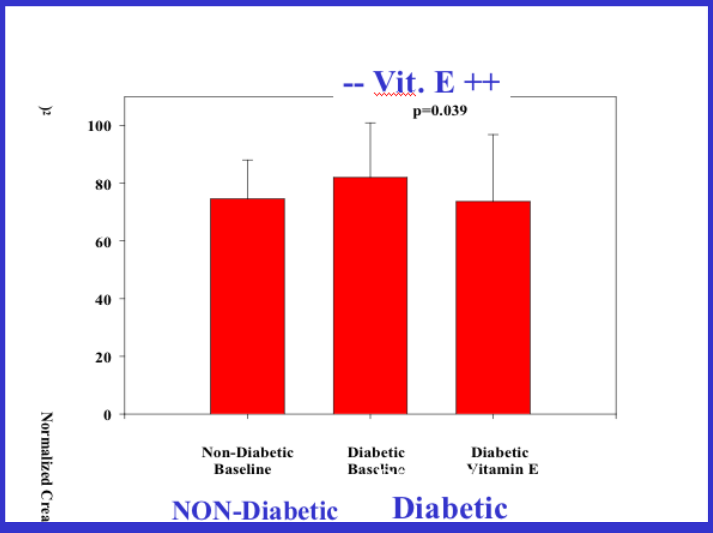


* $p < 0.005$ as compared to baseline by paired t-test

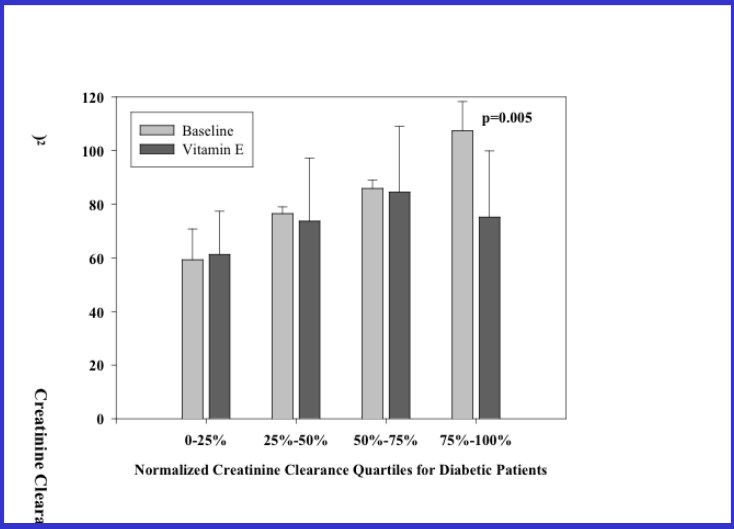
Retinal Blood Flow in Diabetic Patients (quartiles groups based on baseline retinal blood flow values. Lowest quartile represents lowest blood flows): Effect of Vitamin E treatment (paired t-test comparisons). HbA1c for lowest quartile =8.6±1.8% and for the highest quartile = 7.5±1.3%



Normalized Creatinine Clearance in Non-Diabetic and Diabetic Patients and the effect of Vitamin E Treatment in Diabetic Patients (Paired t-test comparison on diabetic patient groups



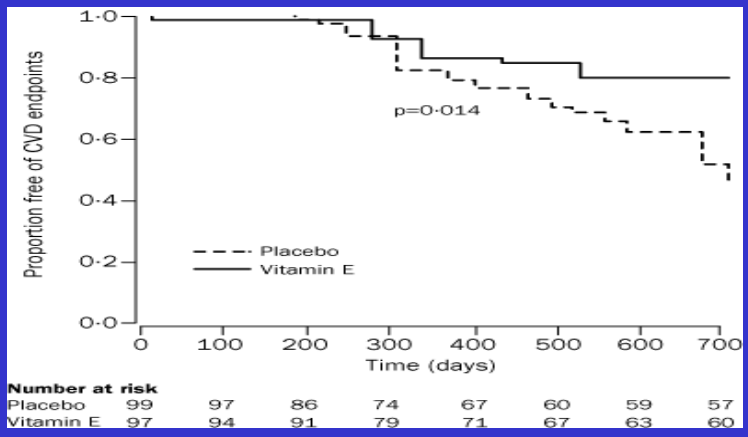
Normalized Creatinine Clearance in Diabetic Patients (quartile groups based on creatinine clearance values at baseline): Effect of Vitamin E Treatment (Paired t-test) HbA1c for lowest quartile =7.7±1.8% and for highest = 8.4±1.3%



Results of Clinical Anti-oxidant Trials for Diabetic Complications Measuring Hard Endpoints

	NO. OF PTS.	DRUG	DURATION	END PT.	RESULTS
Gaede et al (2001)	30	Vit. C (1250 mg) Vit. E (680 mg)	4 Weeks	Albuminuria	19% (P=0.04)
SPACE Trial ERD (2001)	196	Vit. E (800 IU)	519 Days	CV Events Death	↓Risk 0.46 No Difference
CHAOS Trial (1999) DM + NDM	2002	Vit. E (400-800 IU)	510 Days	Non Fatal AMI CV Death	↓ 0.0001 No Difference
HOPE (2000)	3654 (Type 2)	400 IU Vit. E	4.5 Yrs.	CV + Nephropathy	No Effect
SECURE (2001)	732 (DM + NDM)	400 IU Vit. E	4.5 Yrs.	IMT	No Effect

Results of SPACE Trial

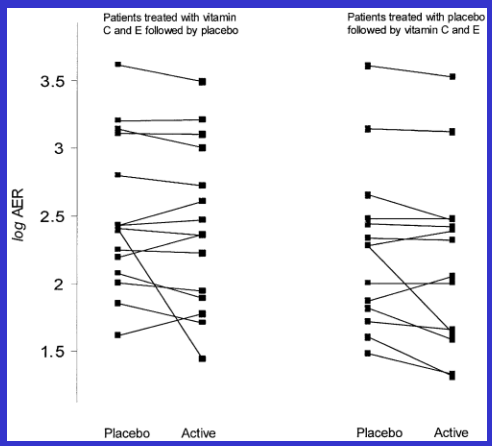


800 IU/day for >500 days

No difference in CV or total mortality

Boaz et al, Lancet, 2000

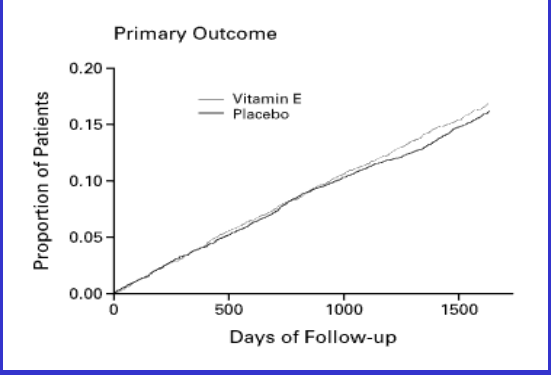
Effect of Vit. C+E on Albuminuria



Gaede et al Diabetic Medicine, 2001

Results of the HOPE Trial

(NEJM, 2000 and Diabetes, 2002)



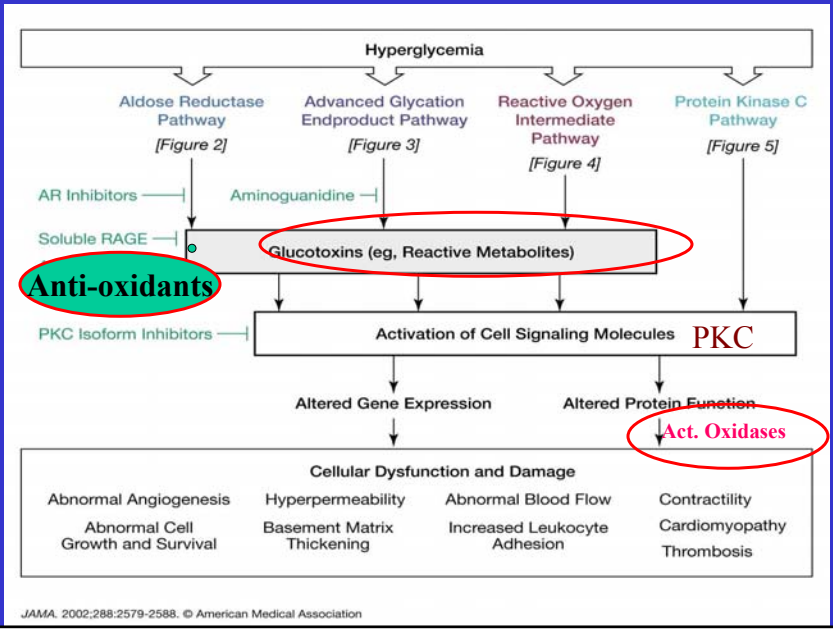
No differences CV, nephropathy or carotid thickness

Results of Clinical Studies in Antioxidants on Diabetic Complications

Large clinical trials with Pathological EndPoints have not been successful.

Why ?

Diagram of the Toxins and Cell Signaling Pathways of Hyperglycemia's Adverse Effects

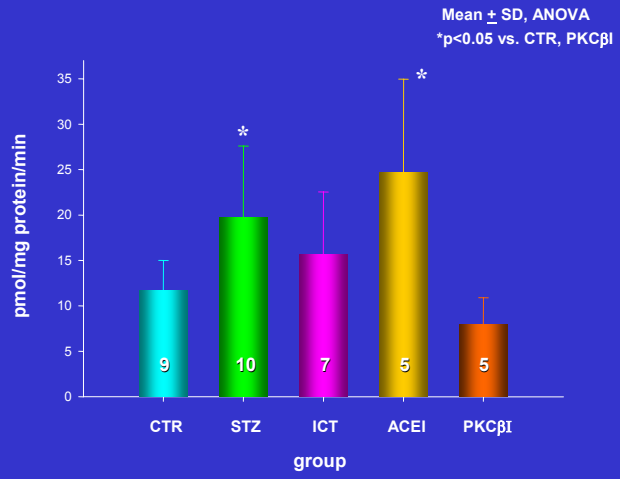


JAMA. 2002;288:2579-2588. © American Medical Association

Biochemical parameter PKC activity - glomeruli - (1-1)

PKC Activity in Glomeruli

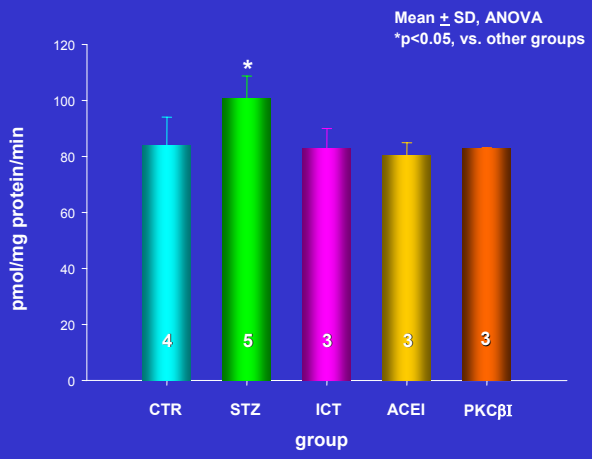
- in situ assay -



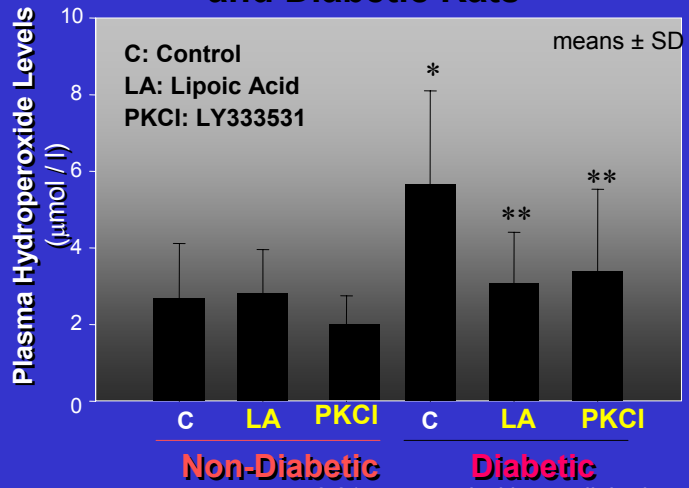
Biochemical parameter PKC activity - heart - (1-2)

PKC Activity in Heart Membrane Fraction

- classical assay -



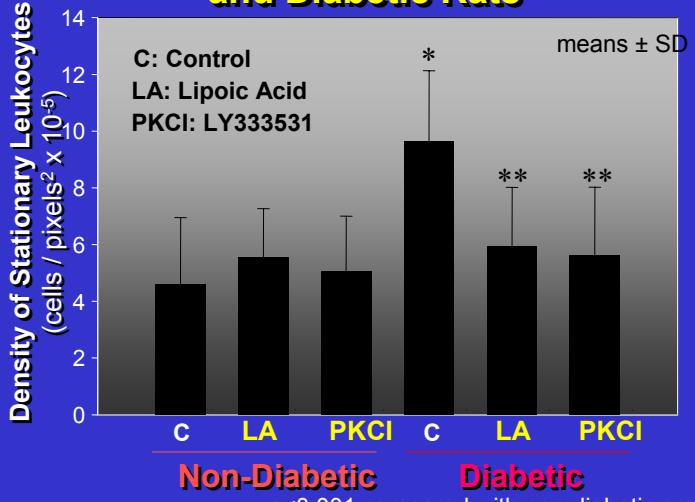
Plasma Hydroperoxide Levels after Lipoic Acid and LY333531(or Vit.E) Treatment in Non-Diabetic and Diabetic Rats



* p<0.01 compared with non diabetic groups

** p<0.05 compared with diabetic control

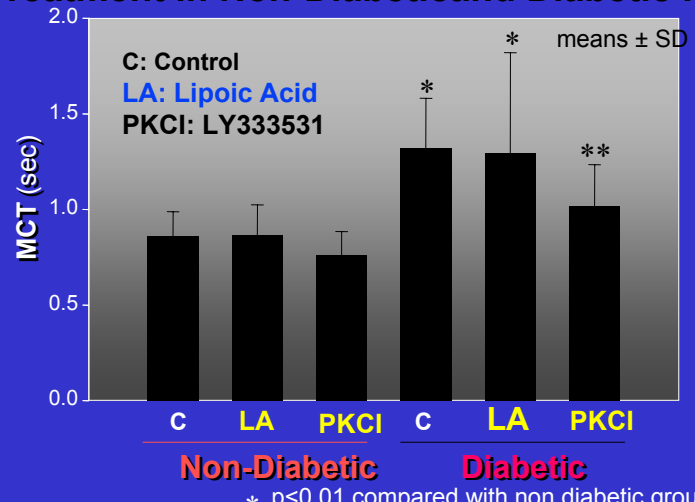
Retinal Leukostasis after Lipoic Acid and LY333531(or Vit.E) Treatment in Non-Diabetic and Diabetic Rats



* p<0.001 compared with non diabetic groups

** p<0.001 compared with diabetic control

MCT (Retinal Blood Flow) after Lipoic Acid and LY333531(vit. E) Treatment in Non-Diabeticand Diabetic Rats



* p<0.01 compared with non diabetic groups

** p<0.05 compared with diabetic control

Oxidative Stress in Diabetes

Is Oxidative Stress Increased in Diabetes ?

YES

However, specific tissue markers of oxidative stress are needed, especially for human studies

Oxidative Stress in Diabetes

Is Oxidative Stress Increased in Diabetes ?

Yes

How Are the Oxidants Formed in Diabetes ?

By multiple pathways involving non-enzymatic reactions, mitochondrial metabolisms, activations of oxidases and other means.

An important question is the relative importance of all these pathways in the development of the various diabetic complications.

Oxidative Stress in Diabetes ?

Do the Oxidants Cause Specific Complications Of Diabetes ?

Since FFA and hyperglycemia can both increase oxidative stress, it is unlikely that the specific pathologies in the microvessels of diabetes is mainly due to Oxidative Stress.

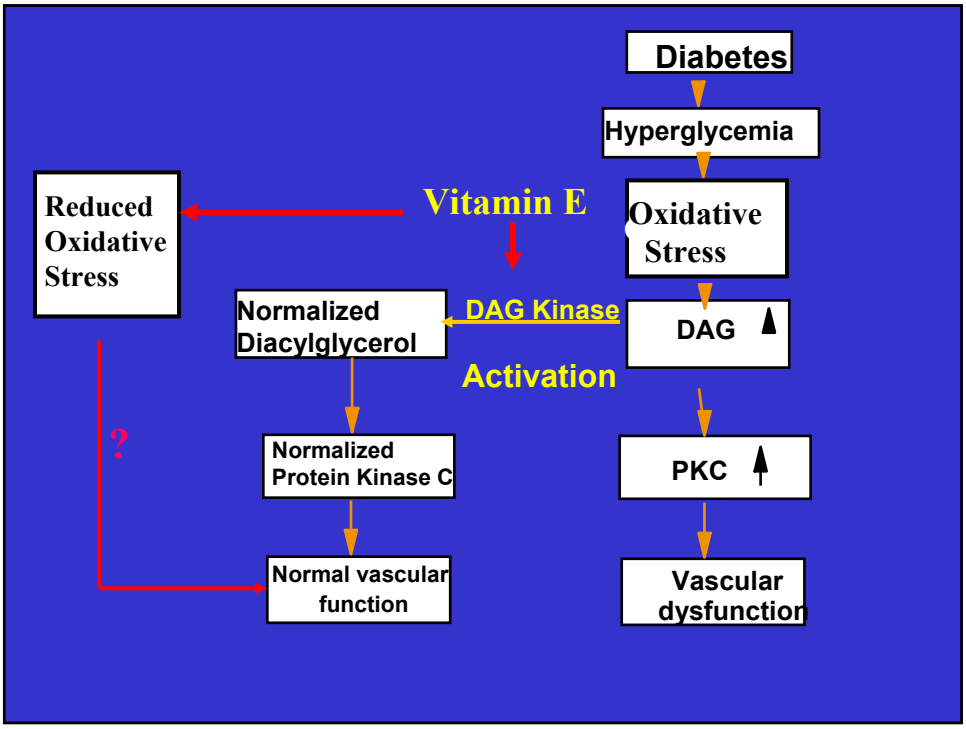
Results of Clinical Studies in Antioxidants on Diabetic Complications

Large clinical trials with Pathological EndPoints have failed.

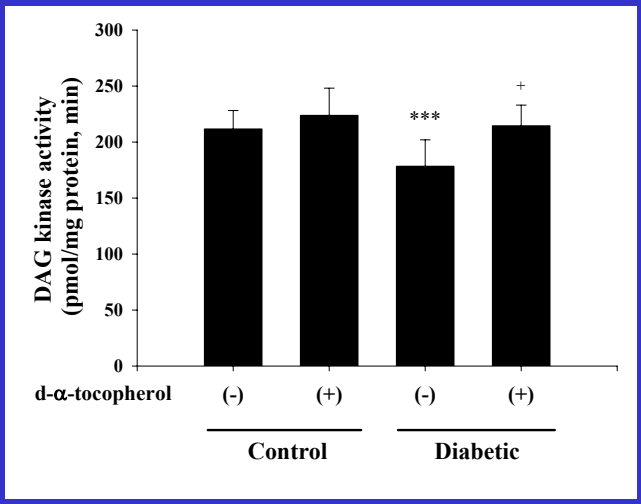
New multi-functional antioxidants are needed to inhibit several pathways used by hyperglycemia to mediate its adverse effects.

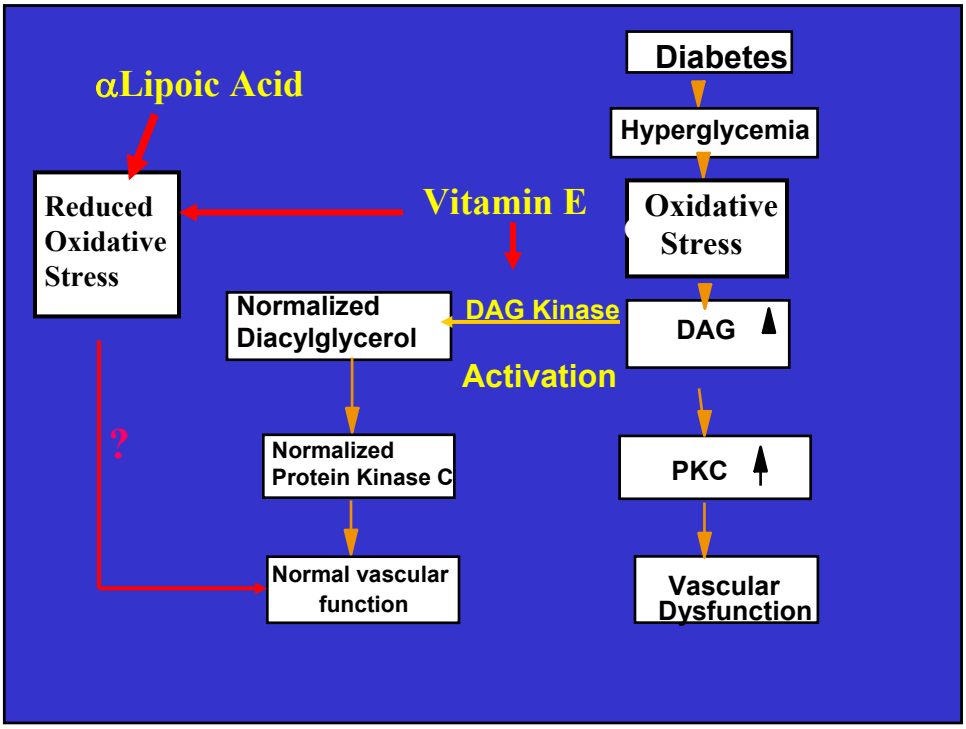
Vitamin E and PKC

- Hyperglycemia activates the DAG-PKC pathway in cultured retinal endothelial cells and in the retinas of diabetic rats
- Vitamin E inhibited the effects of hyperglycemia on DAG-PKC activation in cultured retinal endothelial cells
- Vitamin E at different concentrations incubated with purified PKC had no direct effect of PKC activation

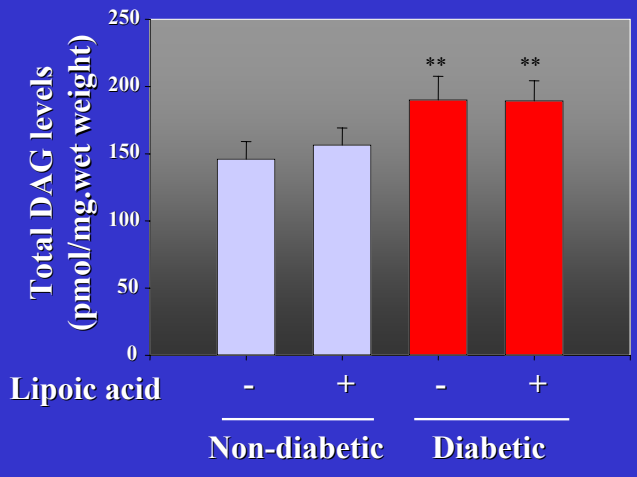


Retinal DAG Kinase activity in 2 week diabetic and non-diabetic rats



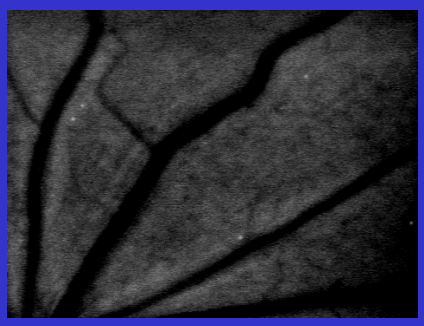


Retinal DAG Levels in Non-diabetic and Diabetic Rats after Lipoic Acid Treatment

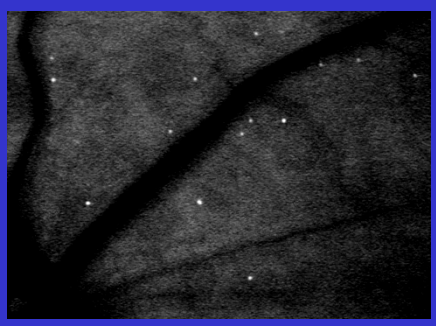


**P < 0.01 vs. untreated non-diabetic

The images of acridine orange leukocyte fluorography

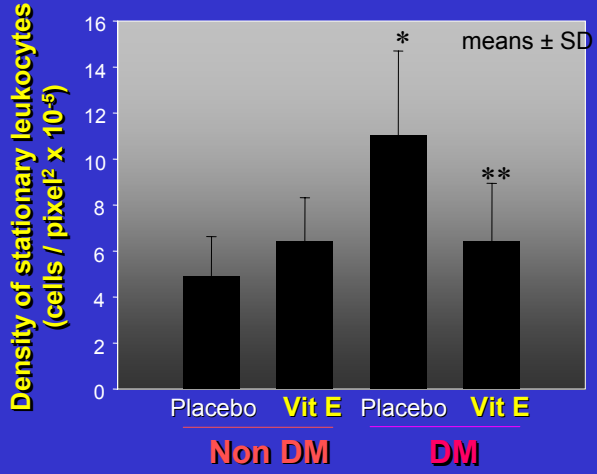


Non diabetic



Diabetic

Effect of Vitamin E Treatment on Retinal Leukostasis

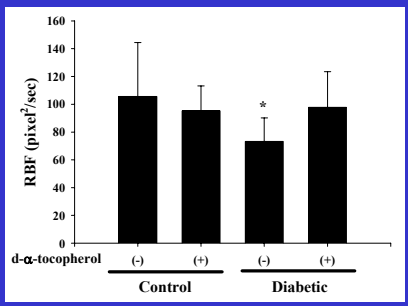


*; p< 0.05 compared with Non DM

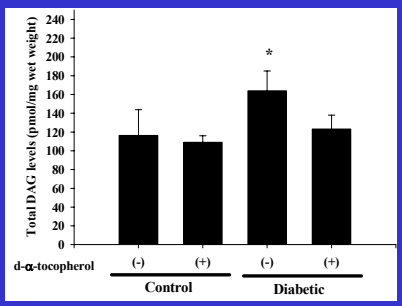
**groups

; p< 0.01 compared with DM-Control

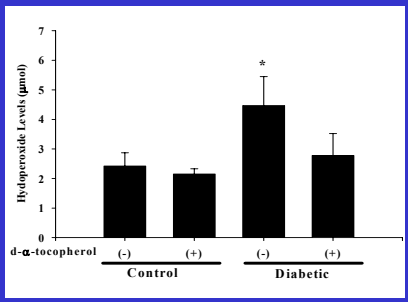
Retinal Blood Flow



Total DAG Levels



Hydroperoxide Levels



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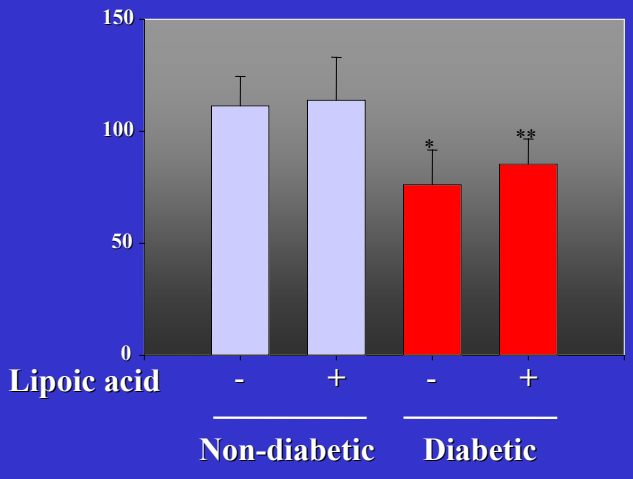
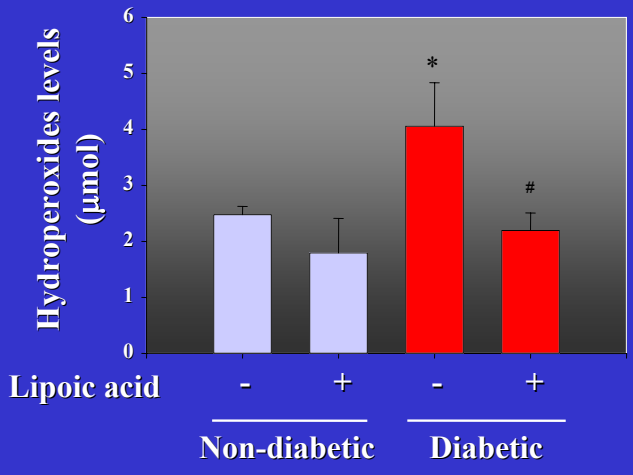
Retinal Blood Flow in Non-diabetic and Diabetic Rats after Lipoic Acid Treatment



*$P < 0.001$ and **$P < 0.01$ vs. untreated non-diabetic

Plasma Hydroperoxides Levels in Non-diabetic and Diabetic Rats after Lipoic Acid Treatment



*$P < 0.001$ vs. untreated non-diabetic; #$P < 0.001$ vs. untreated diabetic