

# **Antioxidant and Atherosclerosis**

**Oxidation of LDL and of lipids by free radicals is one of the most important factors for the initiation of atherosclerosis (Parthasarathy et al, 1992).**

- Significantly less progression of atherosclerosis in men but not in women in the Antioxidant Supplementation in Atherosclerosis Prevention study (Salonen et al 2003).**
- Inverse correlation between coronary events and vitamin C intake in the Nurse's Health study (Stampfer et al., 1993).**
- Beta carotene was associated with reduced coronary risk, vitamin E only in the smokers and no benefit of vitamin C in Health Professionals Follow-up Study (Ascherio et al., 1999).**
- No cardiovascular benefits of vitamin E and beta-carotene in Finnish Alpha-Tocopherol-Beta-Carotene prevention study (Virtamo et al., 1998) and in the Physicians Health study (Muntwyler et al., 1996).**

# **Antioxidants in Mouse Models of Atherosclerosis**

**Inhibitory effect of vitamin E (Cyrus et al, 2003, Pratico et al., 1998), Coenzyme Q(10) (Thomas et al., 2001), Tocotrienols (Black et al., 2000), N,N'-diphenyl-1,4-phenylenediamine (Tangirala et al., 1995), or a combination of vitamin C, E and beta carotene (Crawford et al., 1998).**

**No effects of vitamin E (Munday et al., 1998) or its combination with beta-carotene (Shaish et al., 1999).**

**Probucol has detrimental effects and increased plaque sizes (Zhang et al., 1997, Moghadasian et al., 1999).**

**Chronic vitamin C deficiency does not influence either the initiation or progression of atherosclerosis but significantly limits collagen deposition in vitamin C-dependent apoE<sup>-/-</sup> mice (Nakata et al, 2002).**

**Like other treatments, protective effect of antioxidants can be seen only when the treatments are started early.**

# **Accelerated Atherosclerosis in Diabetes**

**Excess generation of mitochondrial ROS due to hyperglycemia initiates a vicious circle of oxidative stress through increased polyol, AGE, PKC and hexosamine pathways (Brownlee, 2004).**

**Oxidation of LDL and of lipids by free radicals is one of the most important factors for the initiation of atherosclerosis (Parthasarathy et al, 1992).**

**Despite overwhelming evidence on the damaging consequences of oxidative stress, large scale clinical trials with classic antioxidants failed to demonstrate any benefit for diabetic patients.**

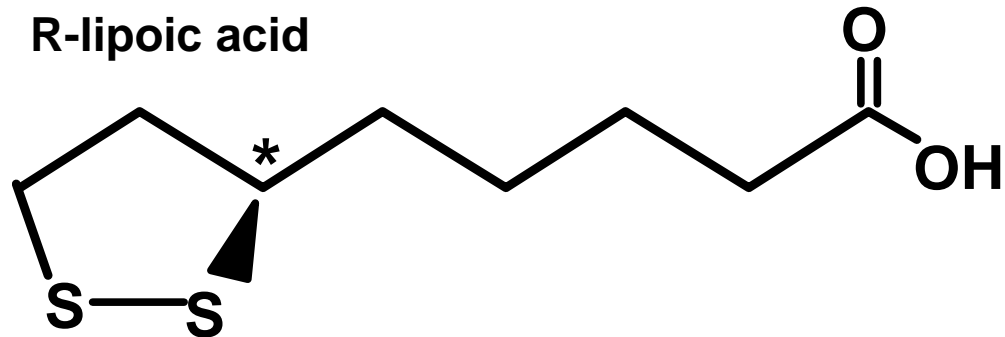
# Antioxidants and Atherosclerosis in Diabetic Mice

## Protective Role of Lipoic Acid

Xianwen Yi

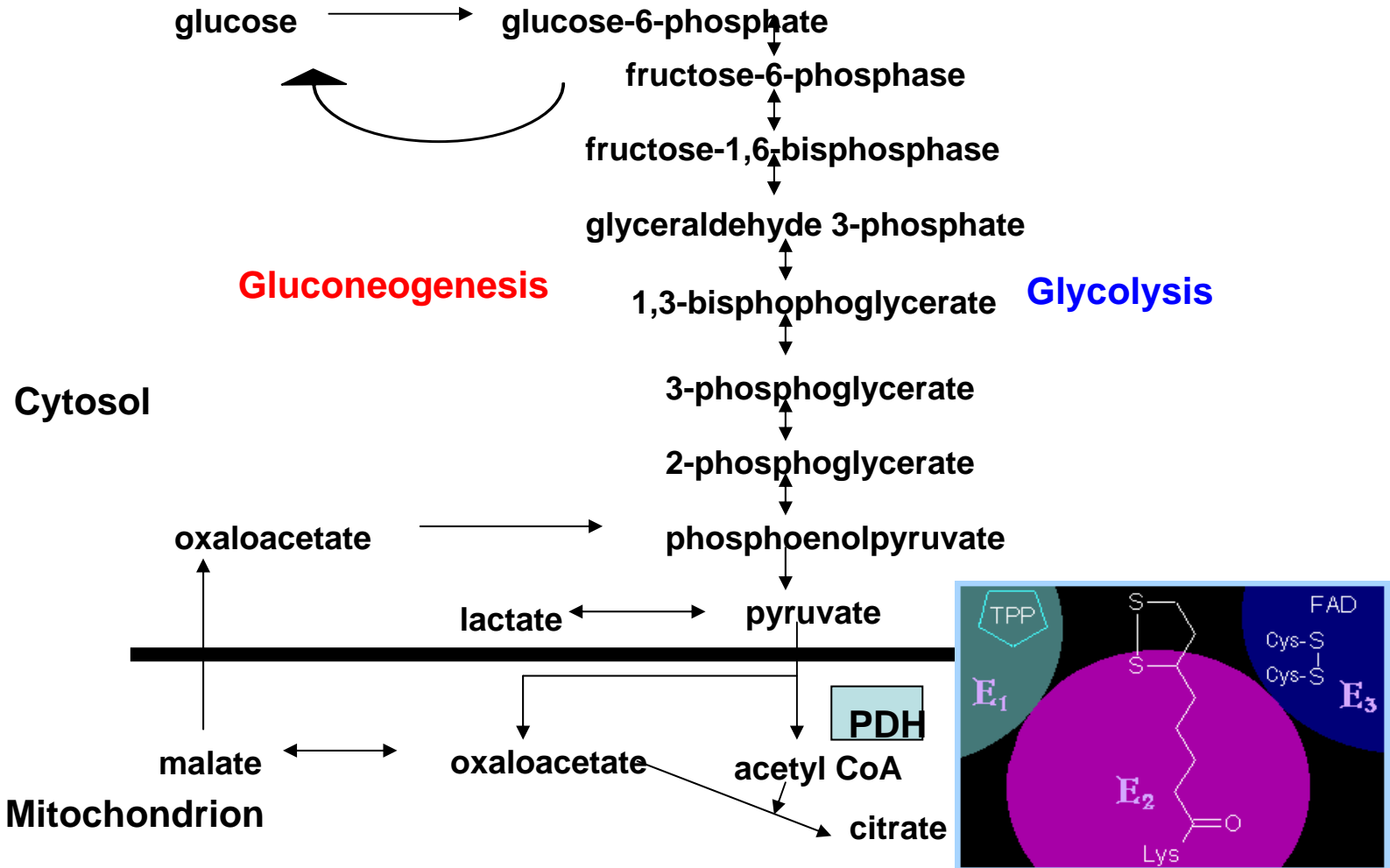


**Treatment with alpha-lipoic acid (600 mg/day i.v.) over 3 weeks is safe and significantly improves both positive neuropathic symptoms and deficits to a clinically meaningful degree in diabetic patients with symptomatic polyneuropathy: a meta-analysis (Ziegler et al., 2005).**

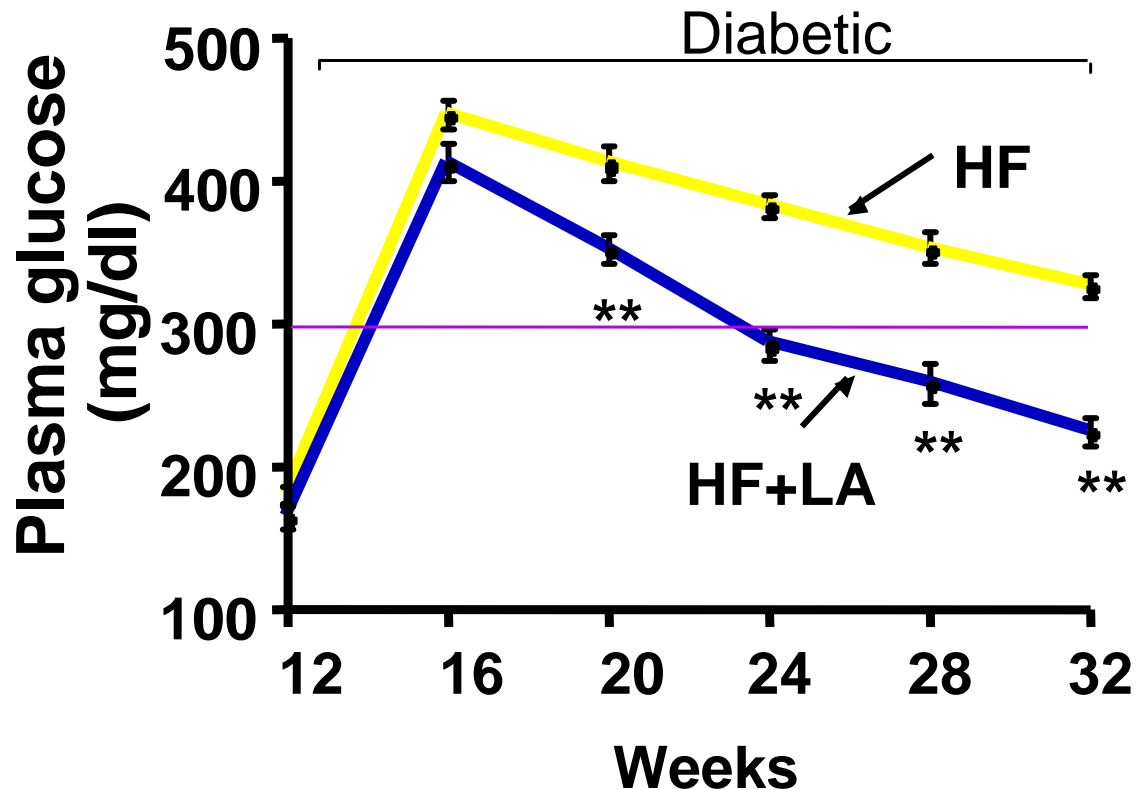
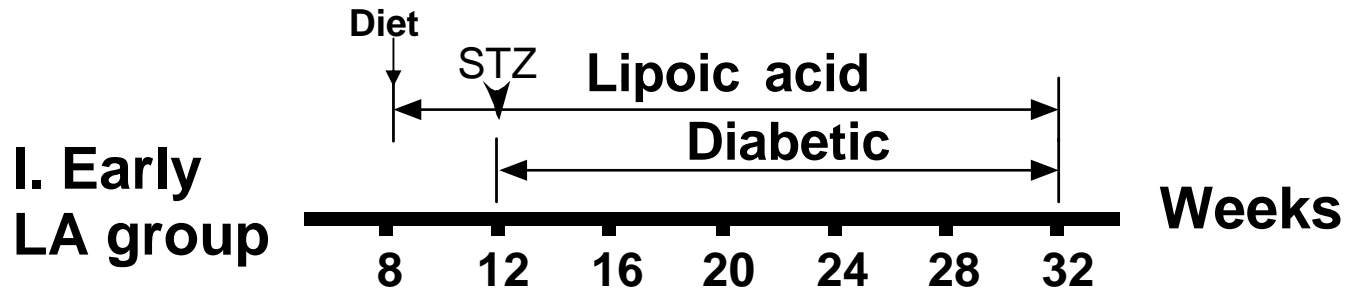


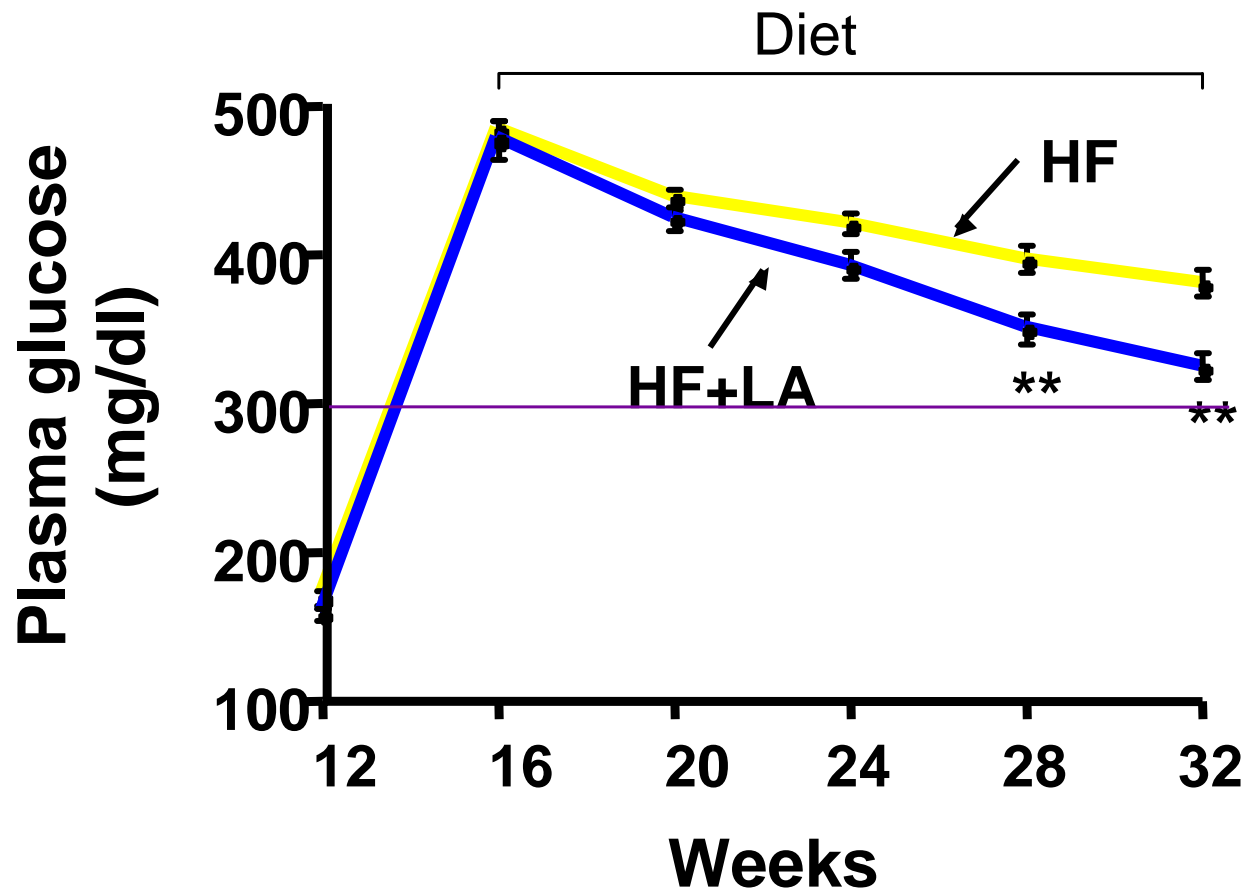
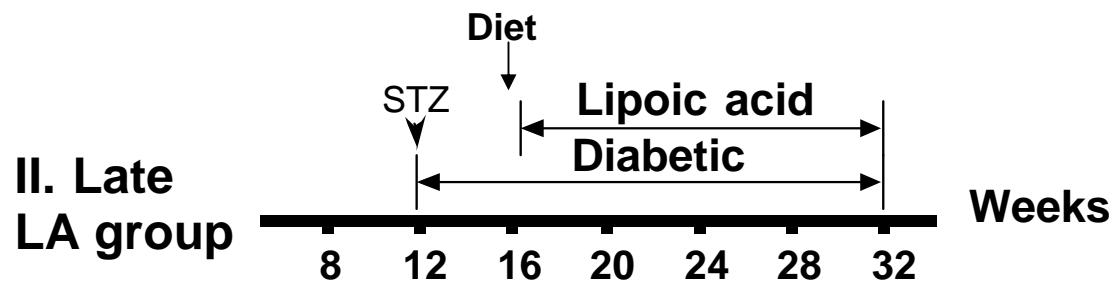
- LA is a water- and fat-soluble antioxidant that can easily cross cell membranes.
- Removes catalysts by metal-chelating  
e.g. Fenton reaction  
$$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \longrightarrow \text{OH}^* + \text{OH}^- + \text{Fe}^{3+}$$
- A strong reducing agent and recycles other antioxidants like reduced glutathione (GSH)

# LA is an essential cofactor for several key enzyme complexes in metabolism



# Effects of LA on STZ Treated ApoE-/- Mice

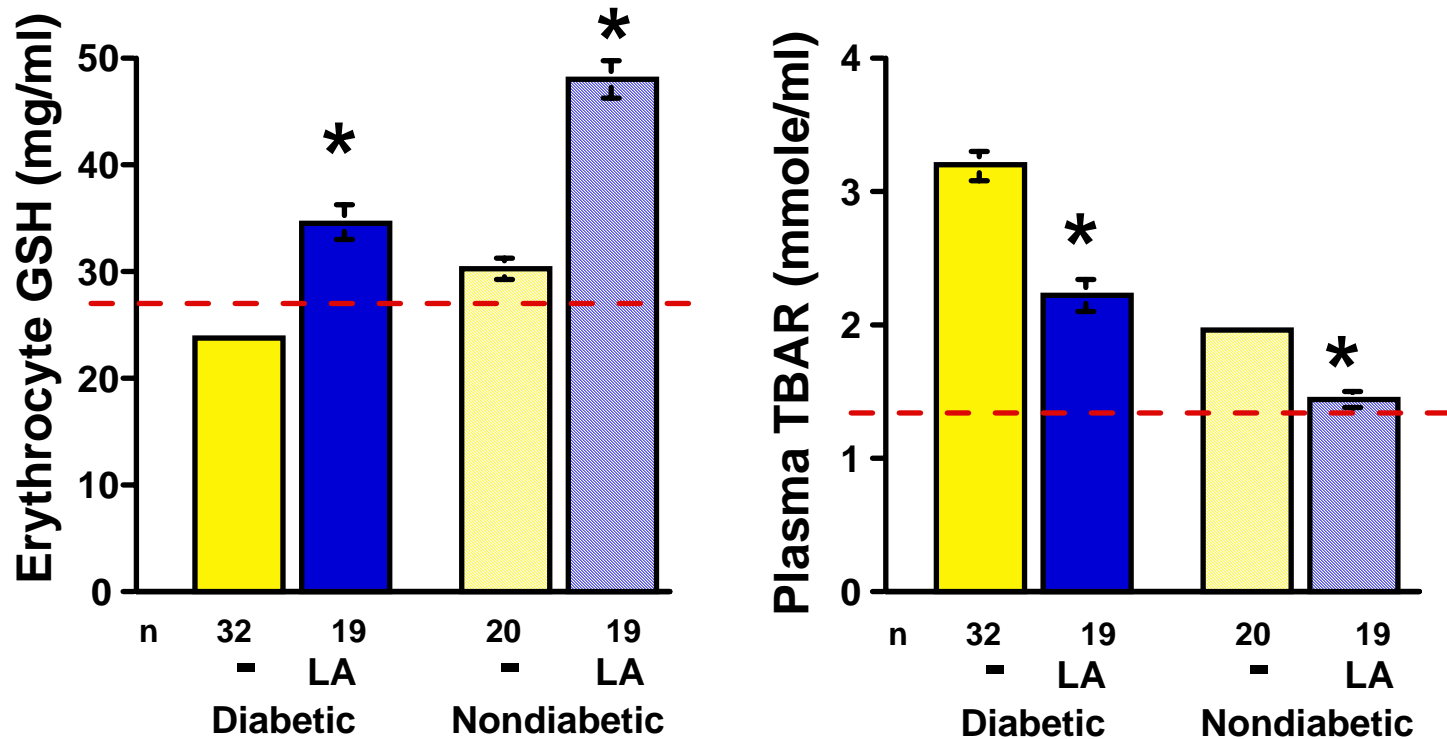






# Oxidative stress is increased in diabetic mice

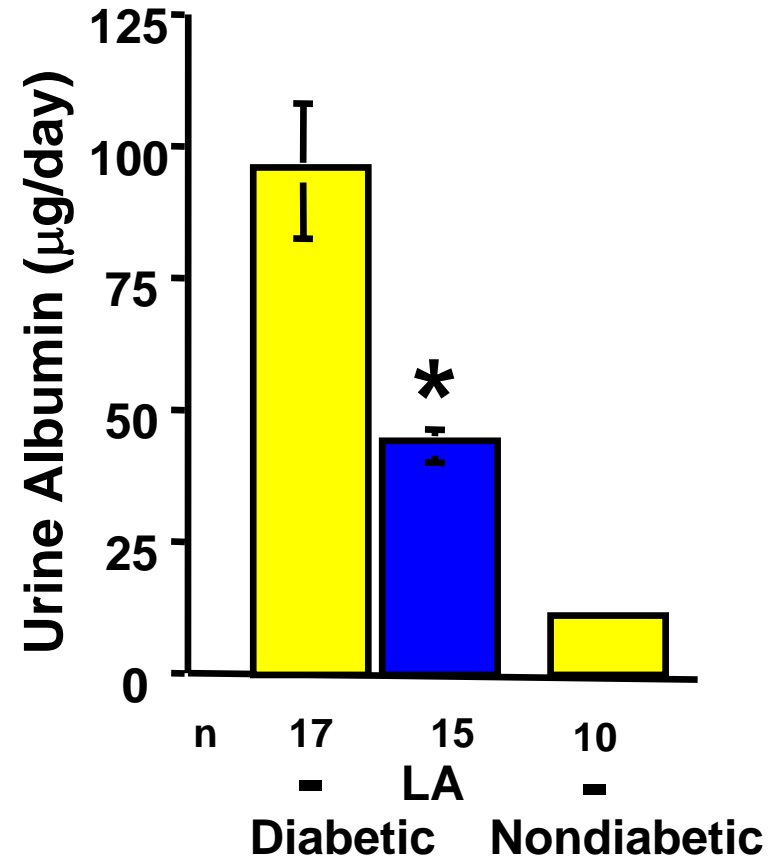
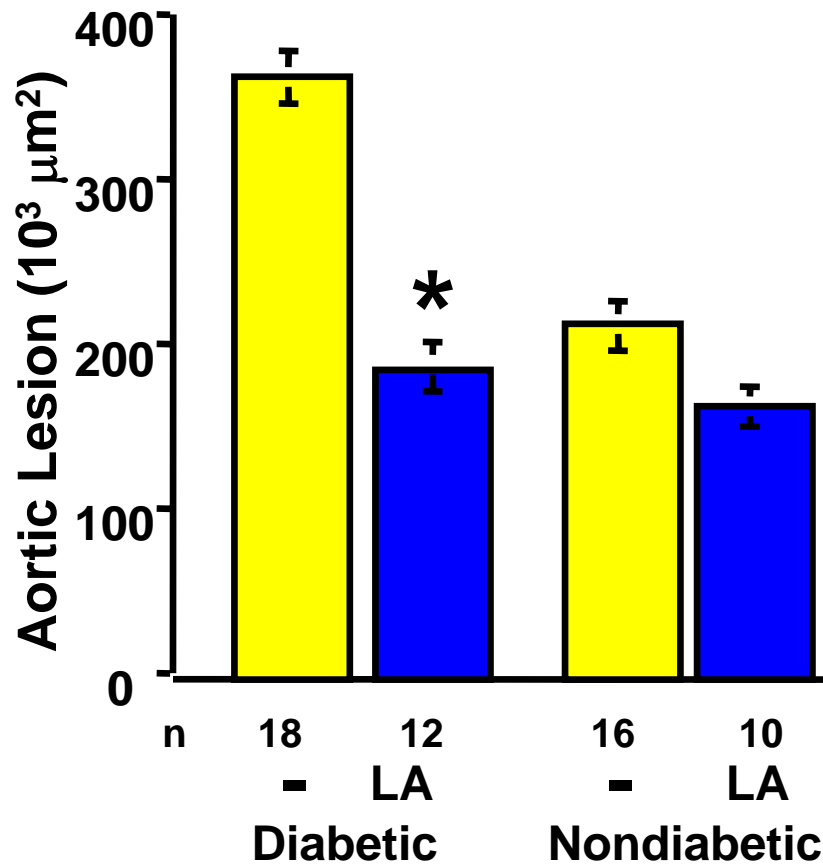
5mo post STZ treatment



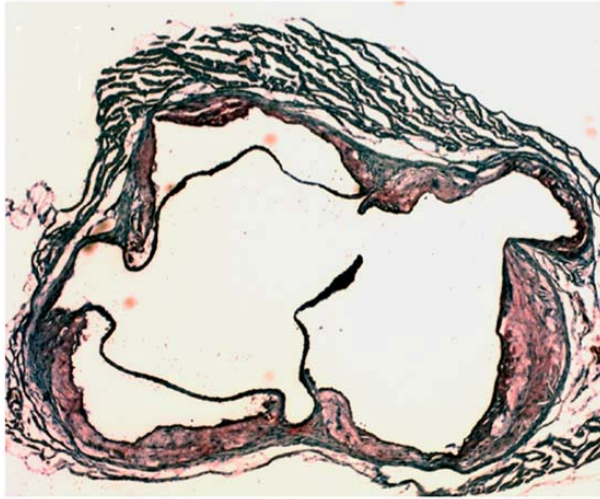
LA significantly improves antioxidant status

# LA in diet prevents enhanced atherosclerosis and kidney damage induced by diabetes in apoE-/- mice

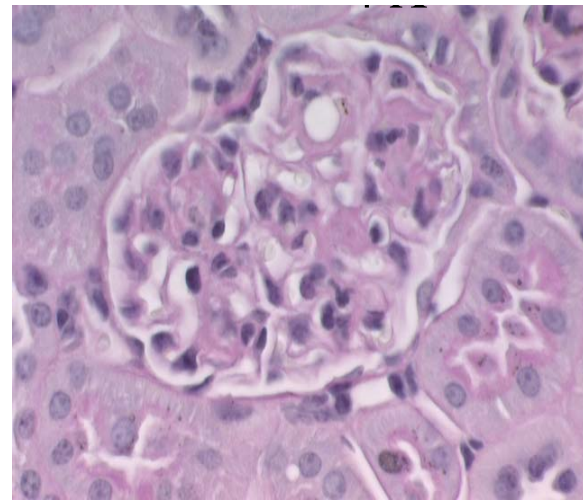
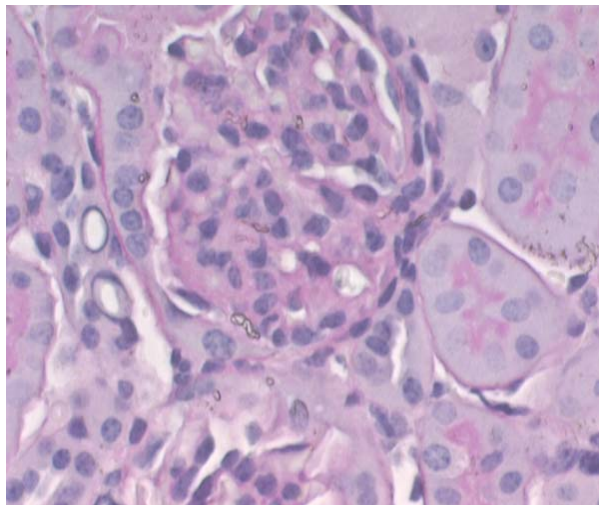
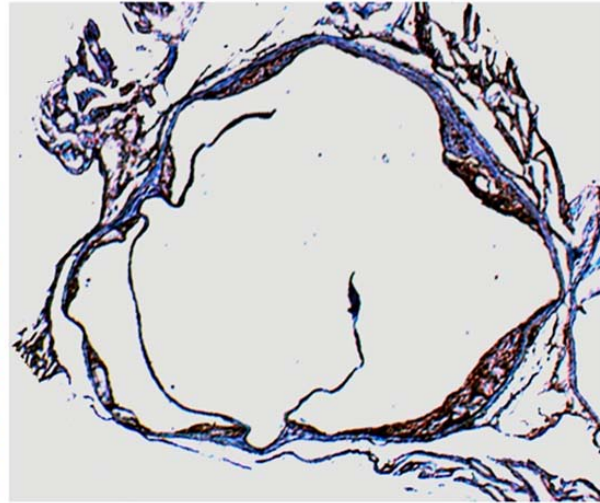
(5mo post STZ)

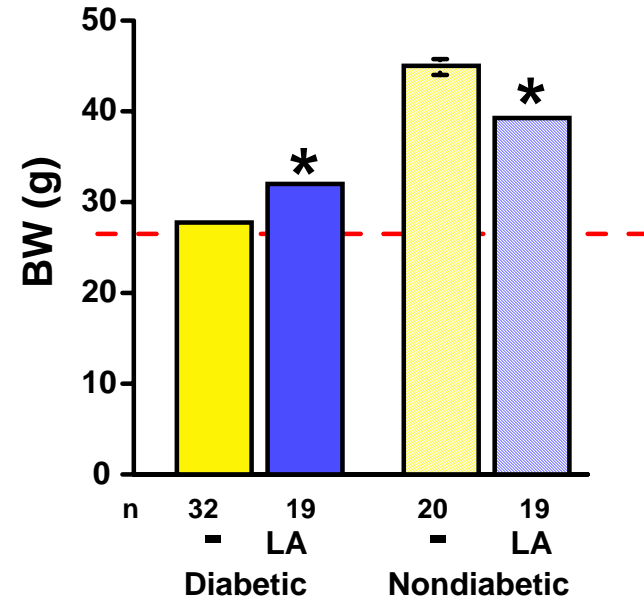
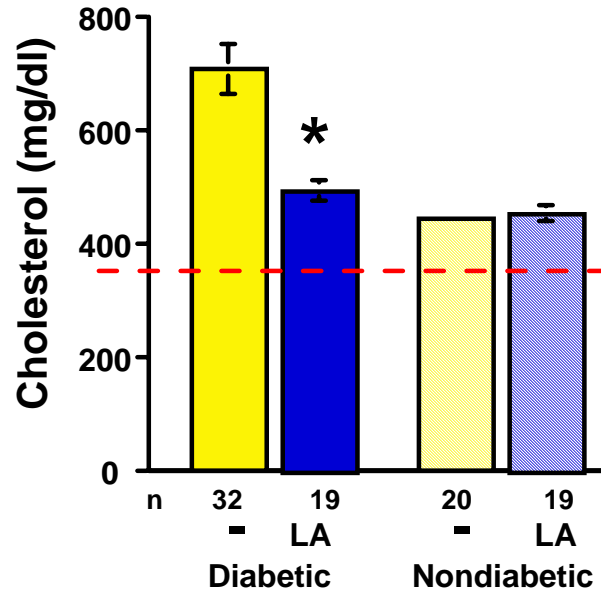
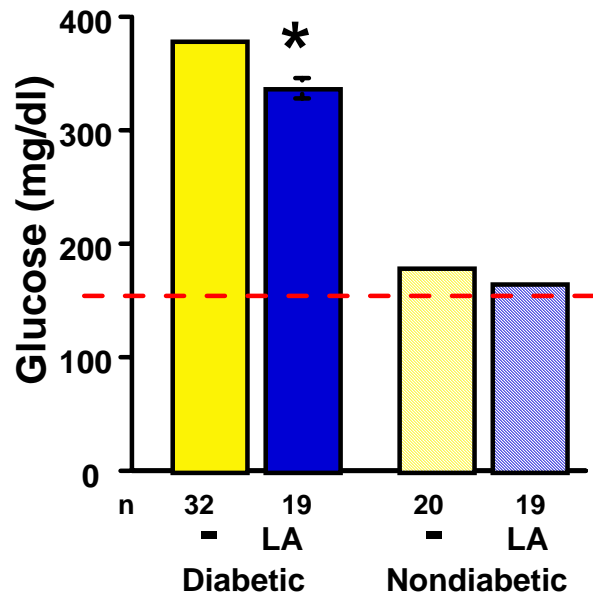


HF



HF+LA





**Plasma cholesterol was significantly elevated in diabetic mice with a control diet at 5 mo post STZ treatment.**

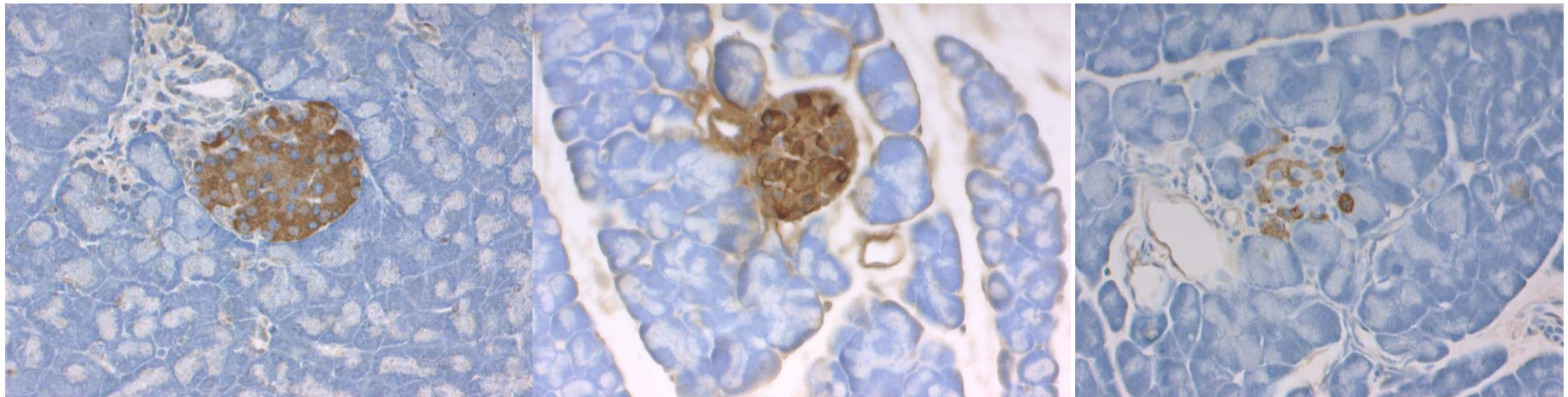
**Increased plasma TC may be the consequence of dehydration. It may be contributing to the increased atherosclerosis.**

# Protective effects of LA are partly due to protection from pancreatic $\beta$ cell damage by STZ and their regeneration over time

**Control**

**Diabetic/HF+LA**

**Diabetic/HF**

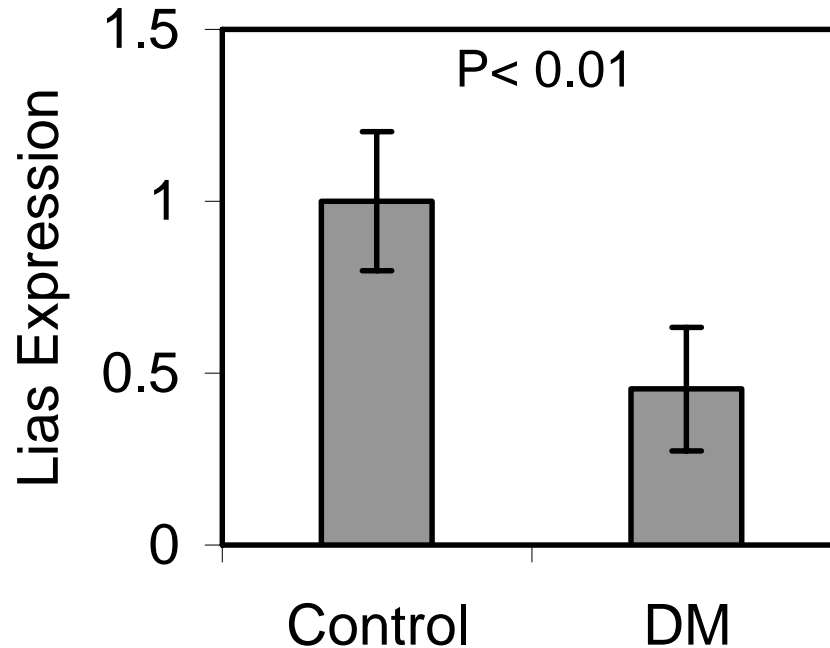


**X 10**

**(Immunostaining of beta cells with insulin)**

- **LA, as a natural antioxidant, is effective against atherosclerosis & kidney damage in STZ-induced diabetic apoE<sup>-/-</sup> mice.**
- **LA has robust protective and therapeutic functions partly through protection of beta cells.**
- **Other diabetic models?**

## Mammals can produce LA in all the cells



**Lipoic acid synthase (Lias) gene expression in the kidney of STZ induced DM C57BL6 mice is 50% normal**

**Endogenous production of LA is essential**

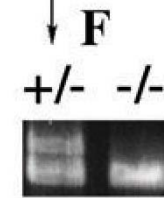
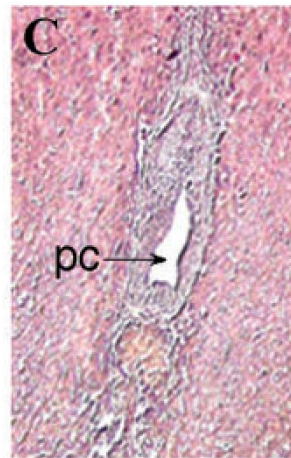
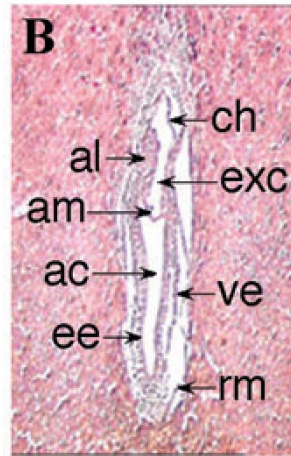
***Lias*<sup>-/-</sup> embryos are developmentally retarded and die immediately after implantation**



**7.5 dpc**

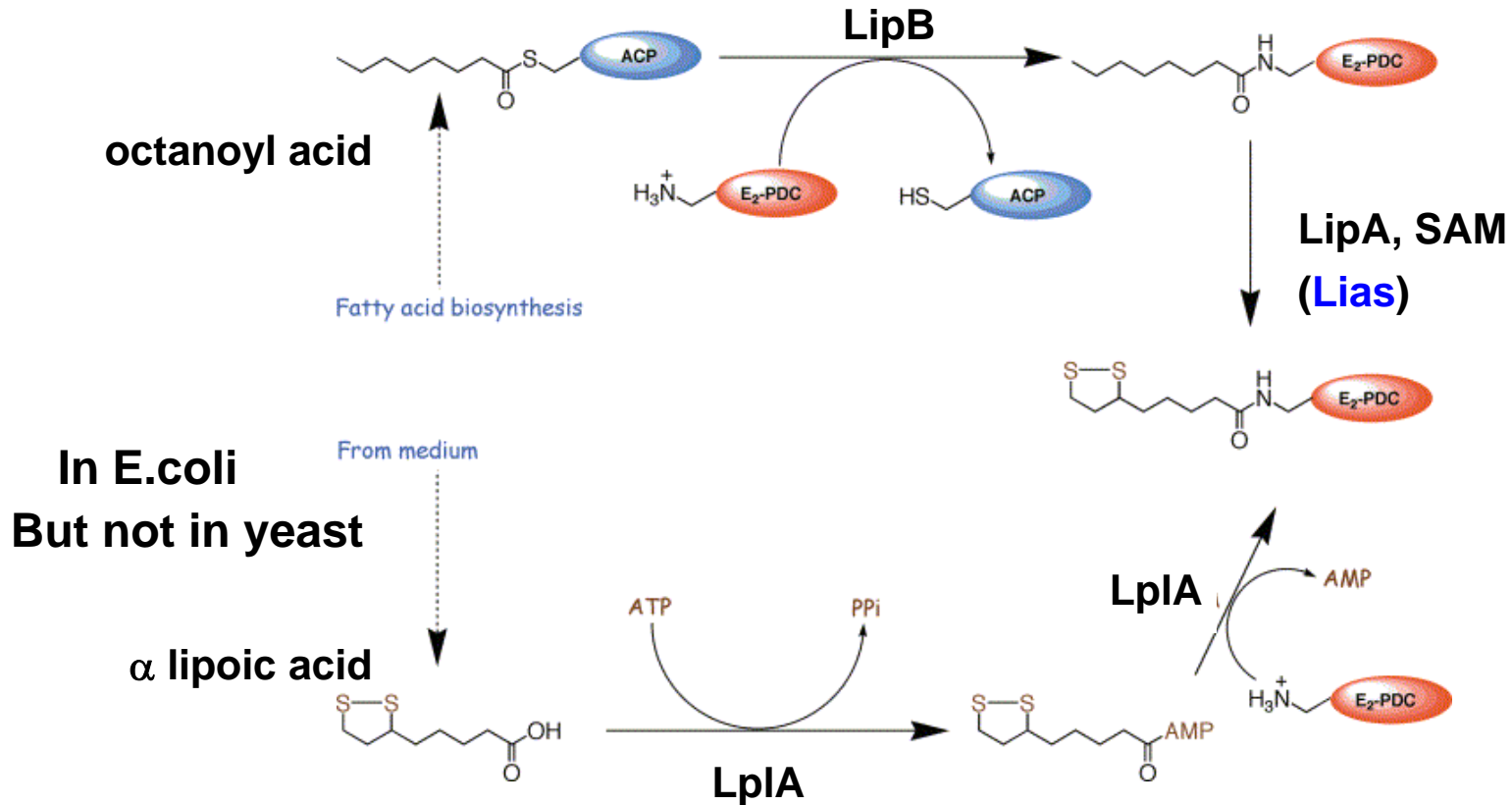
**Dietary LA supplement at 1.65 g/kg to *Lias*<sup>+/-</sup> mothers failed to rescue *Lias*<sup>-/-</sup> embryo**





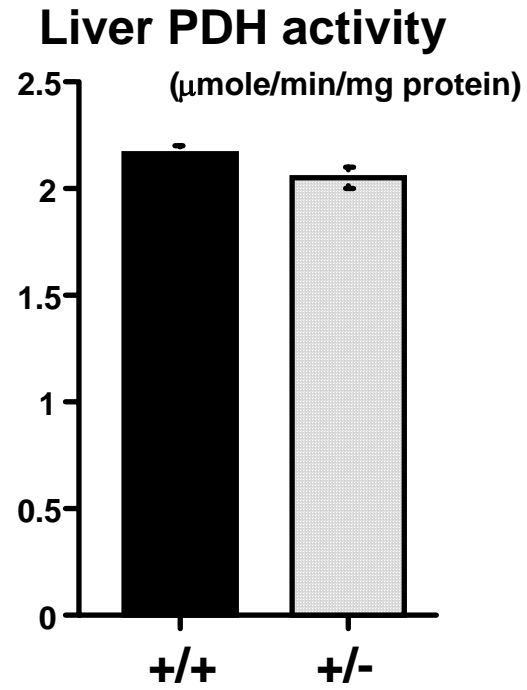
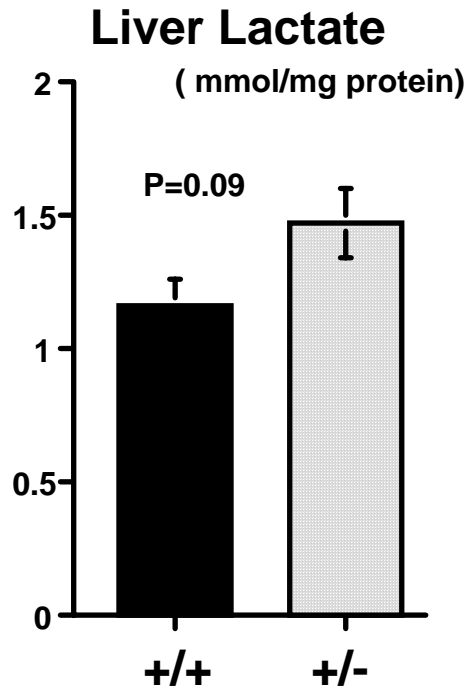
# Lipoic acid synthesis

From Booker, 2004

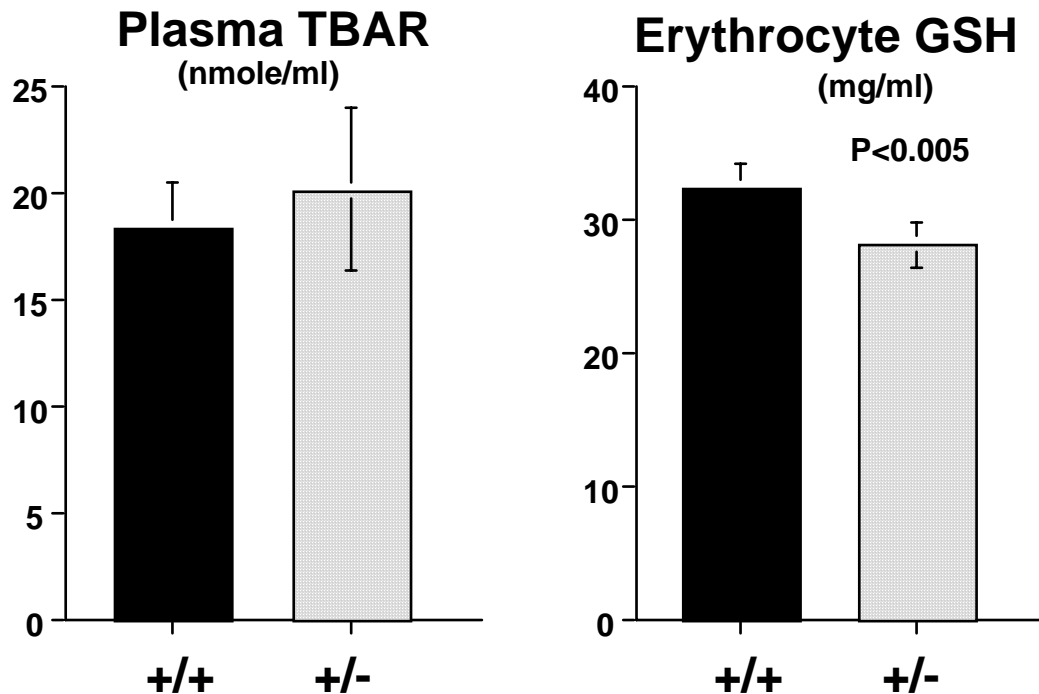


Growth of yeast mutants lacking Lias or ACP cannot be rescued by LA in media  
(Sulo and Martin, 1993)

# Lias +/- heterozygotes have a trend increased lactate



## Lias+/- heterozygotes have a trend reduced antioxidant capacity

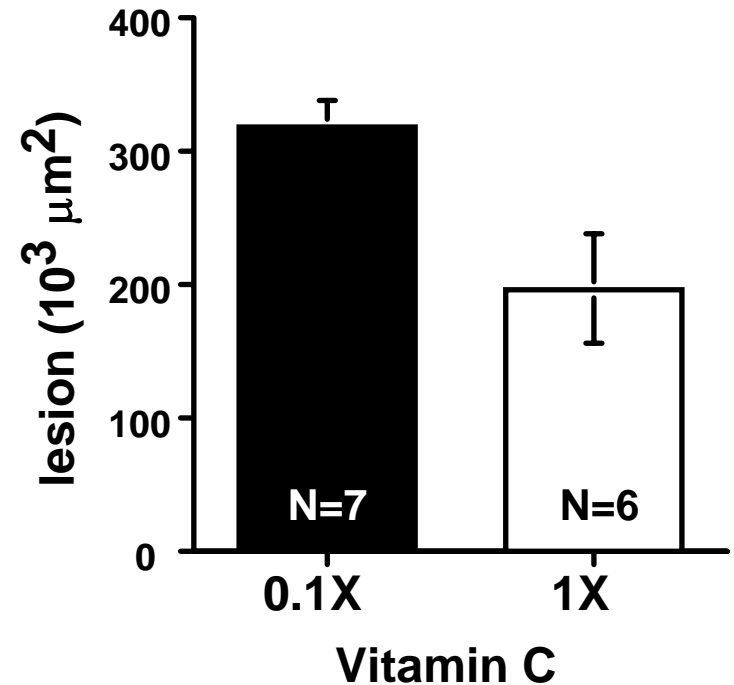


## Conclusion

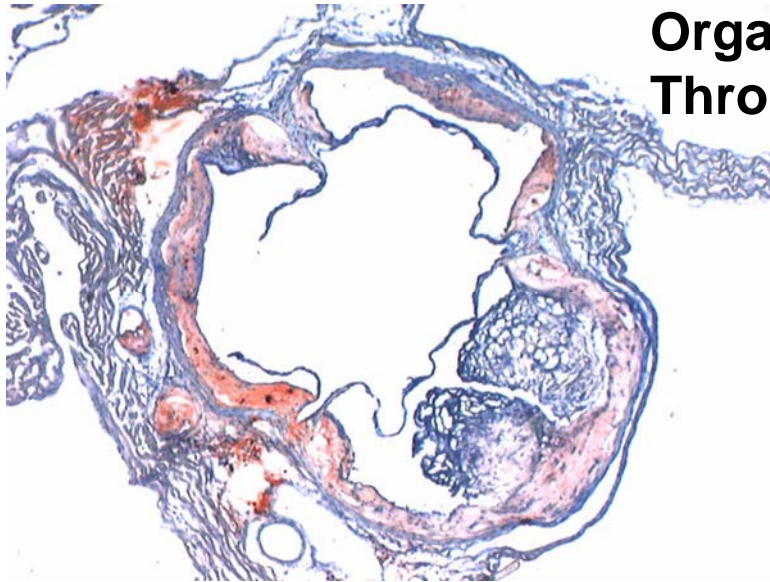
- Dietary supplement of LA, as a natural antioxidant, has athero-protective effects in STZ-induced diabetic apoE<sup>-/-</sup> mice.
- Endogenous production of LA is essential for development and cannot be replaced by exogenous LA.
- Heterozygotes with reduced Lias may be useful for studying the role of LA in disease.

# Vitamin C Deficiency and Atherosclerosis in Diabetic *Gulo*<sup>-/-</sup>*Apoe*<sup>-/-</sup> Mice

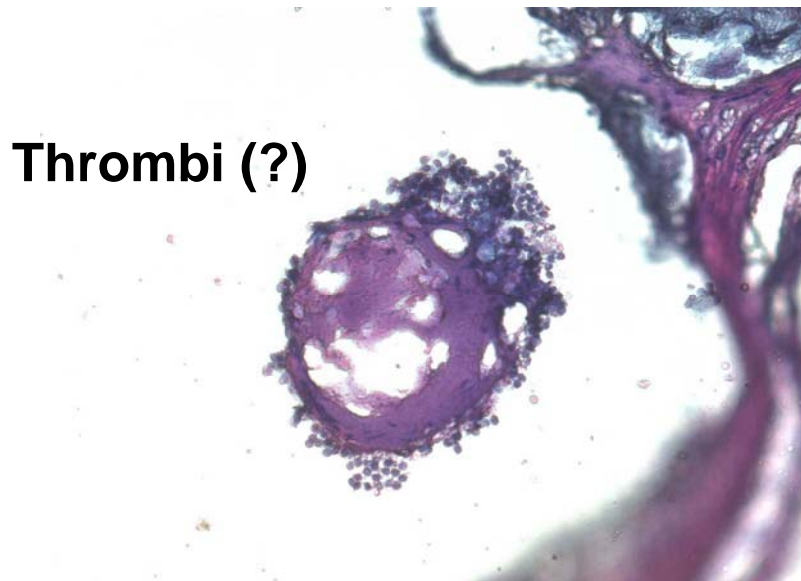
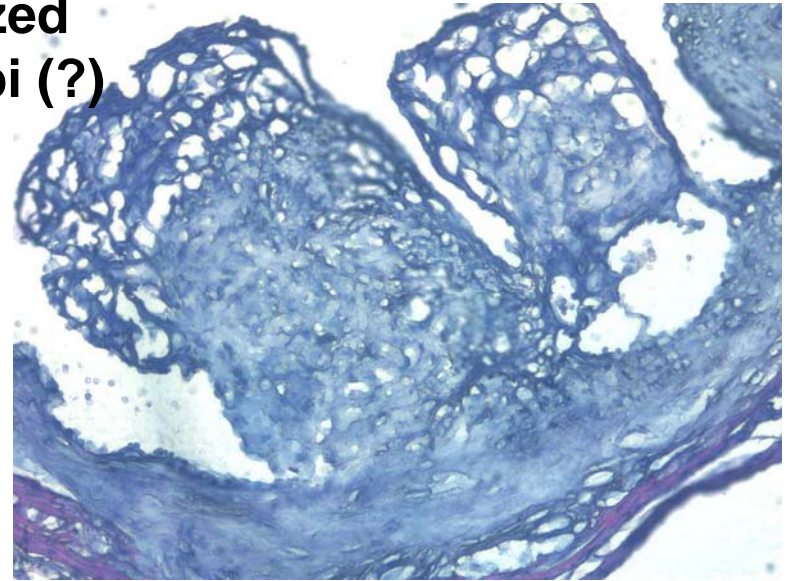
VitC in water at 0.33g/L	Liver ( $\mu\text{g/g}$ )	Plasma ( $\mu\text{g/ml}$ )
1X	150 $\pm$ 26	9.6 $\pm$ 3.7
0.1X	30 $\pm$ 13	3.0 $\pm$ 0.8



# Complex plaque morphologies in diabetic apoE<sup>-/-</sup> mice (129/SvEv background, chow diet)



Organized  
Thrombi (?)



Thrombi (?)

Inflammation

