Apolipoprotein E isoforms in diabetic dyslipidemia and atherosclerosis

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Diabetic Dyslipidemia

- Cluster of harmful changes to lipoprotein metabolism commonly noted in diabetic patients
- Characterized by high VLDL triglycerides, low HDL-cholesterol, and a increase in small, dense LDL particles
- Increases the risk of developing atherosclerosis

Apolipoprotein E Isoforms

	ApoE2	ApoE3	ApoE4
position 112	cys	cys	arg
position 158	cys	arg	arg
frequency	7.2%	78.3%	14.3%
LDLR affinity	<1%	100%	>100%
plasma TC	-6%	ref.	+6%
MI risk	reduced (type III)	ref.	Increased

Plasma lipoproteins

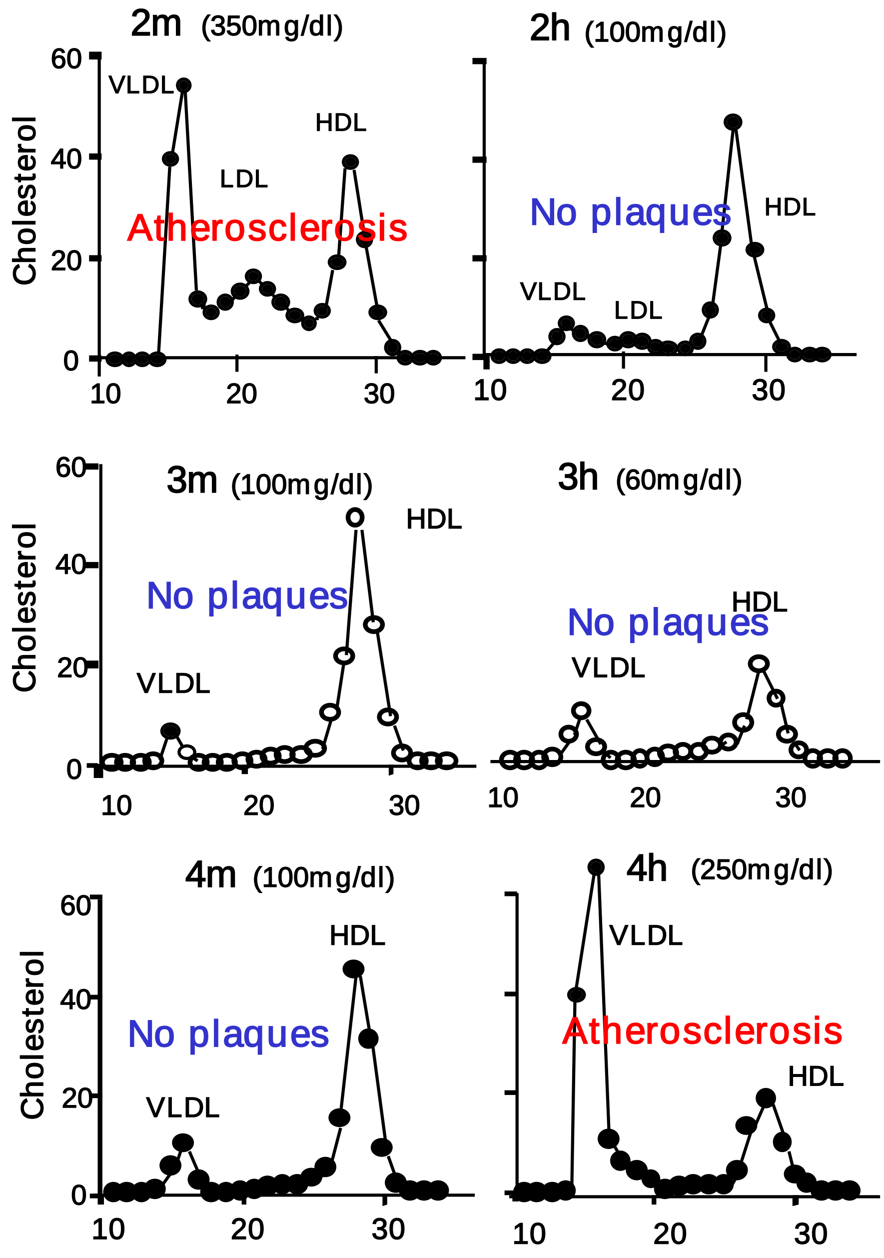
Normal LDLR | **Increased LDLR**

On a HFW diet

Mice with Human apoE2 (poor ligand)

Mice with Human apoE3 (reference)

Mice with Human apoE4 (better ligand)



ApoE -Trapping by LDL receptor

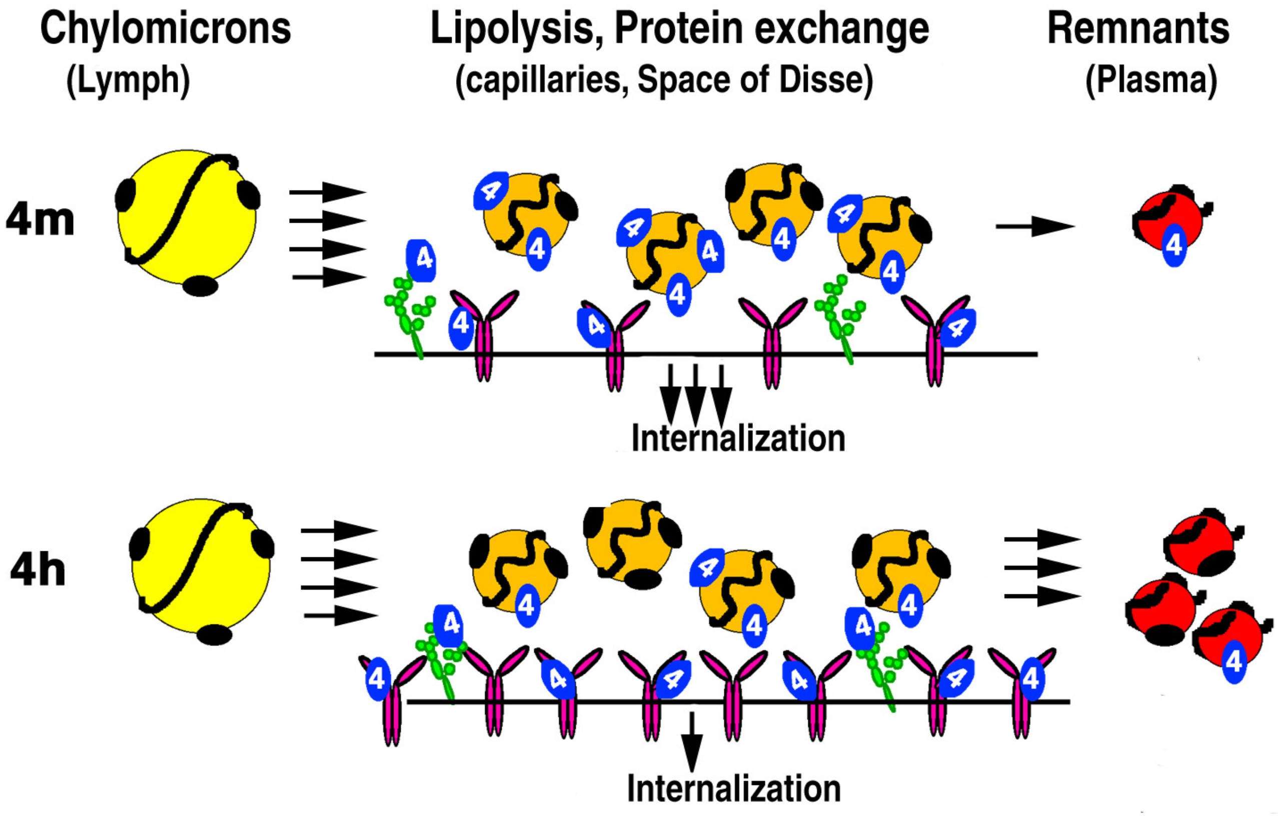
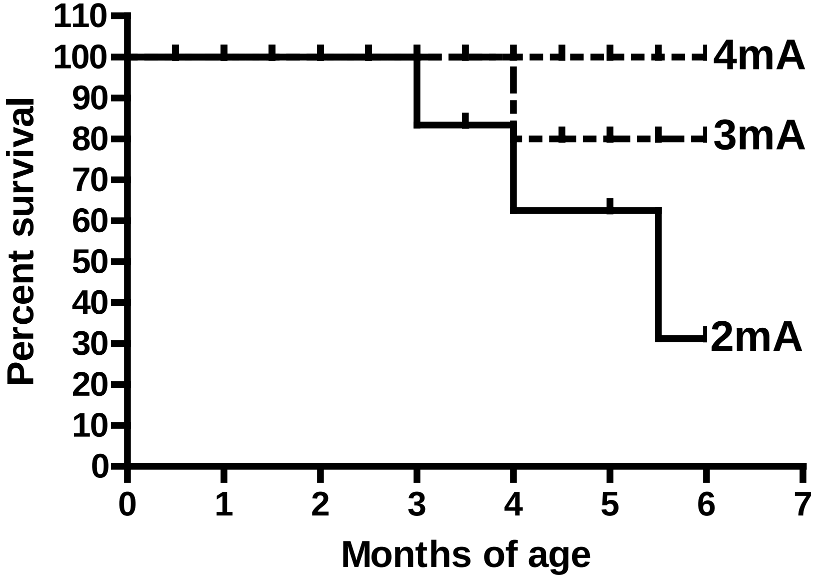


Figure 2 ApoE trapping. Efficient transfer of apoE4 facilitates internalization of large lipoproteins in 4m mice. In 4h mice, the efficiency of transfer is reduced because apoE4 are trapped by the increased LDLR.

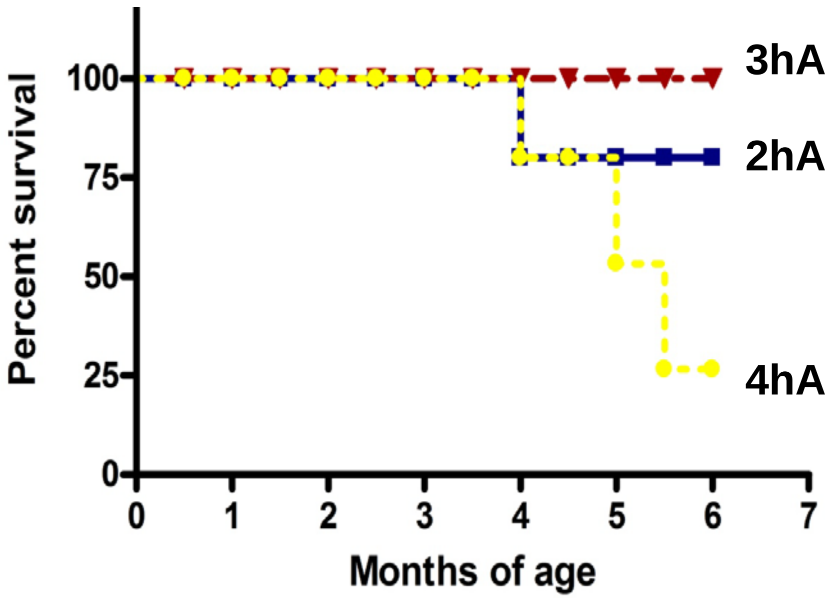
Akita-diabetic mice with human apoE isoforms

Survival

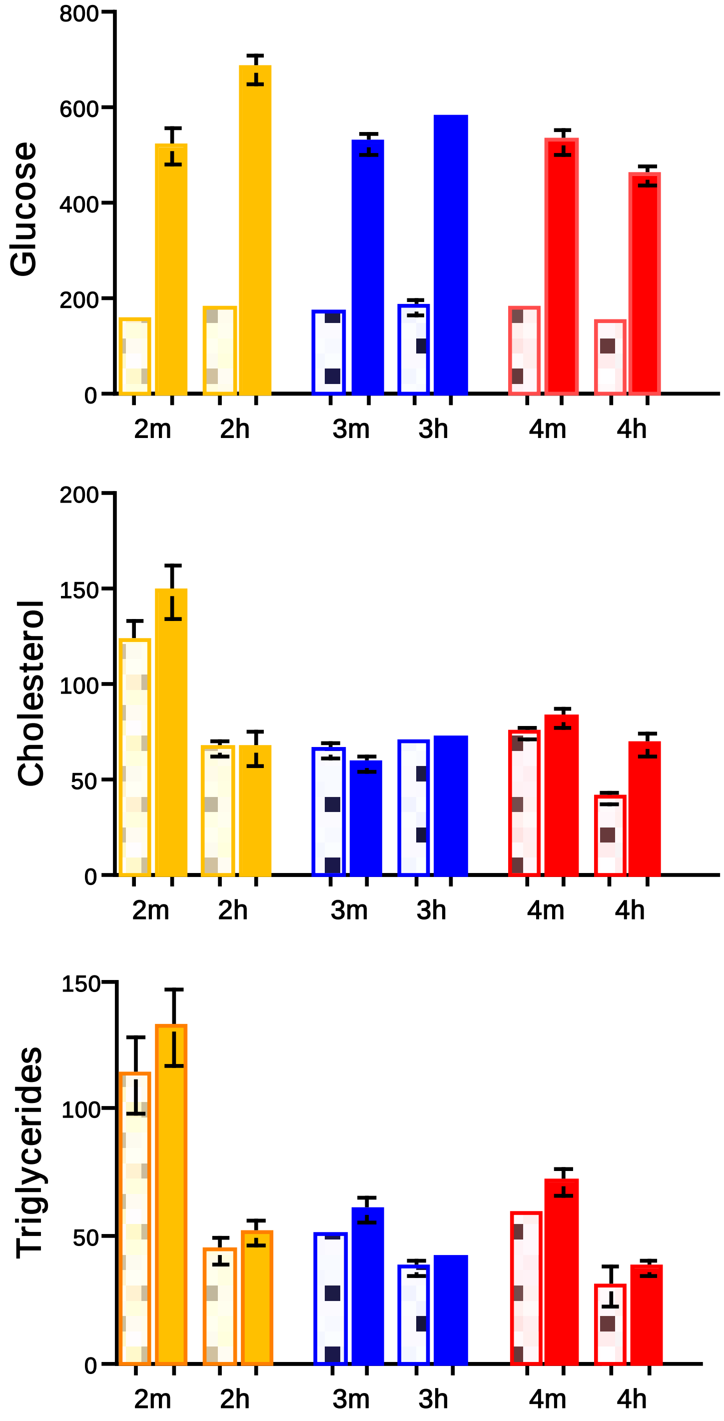
With normal LDLR



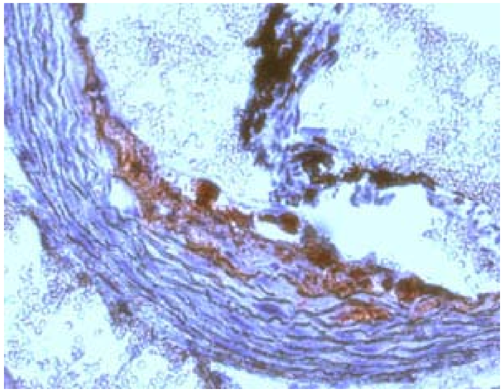
With human LDLR



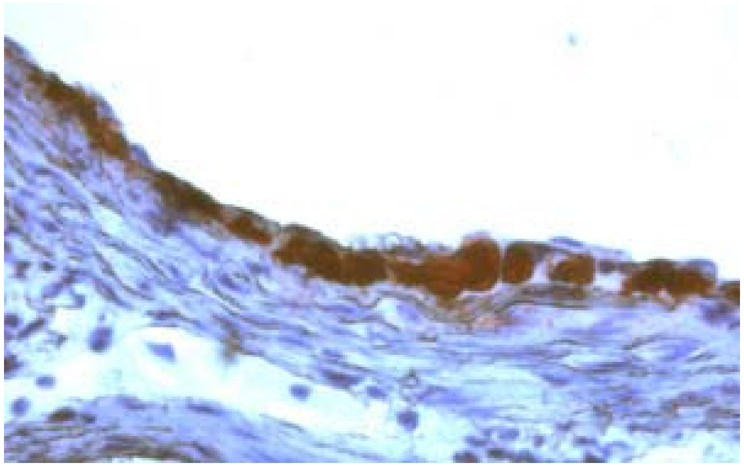
2mAkita

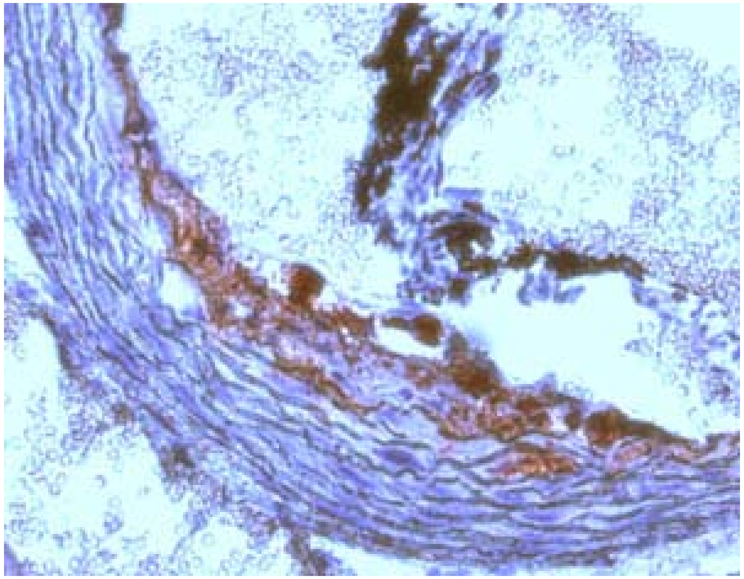


4h-Akita



Two of two -6mo old *4hAkita* mice on a low cholesterol diet had atherosclerosis despite of low plasma cholesterol (<120 mg/dl) levels

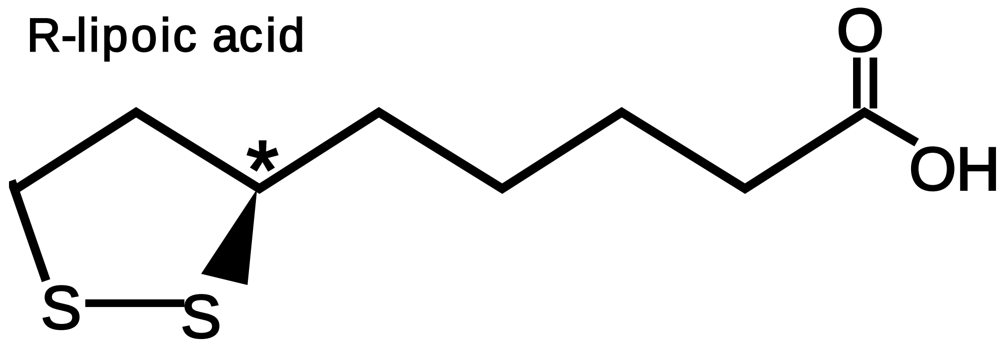




This is the first mouse model of atherosclerosis for which diabetes is required.

Mice with Reduced Lipoic Acid Synthase

Xianwen Yi



- **LA is a water- and fat-soluble antioxidant that can easily cross cell membranes.**

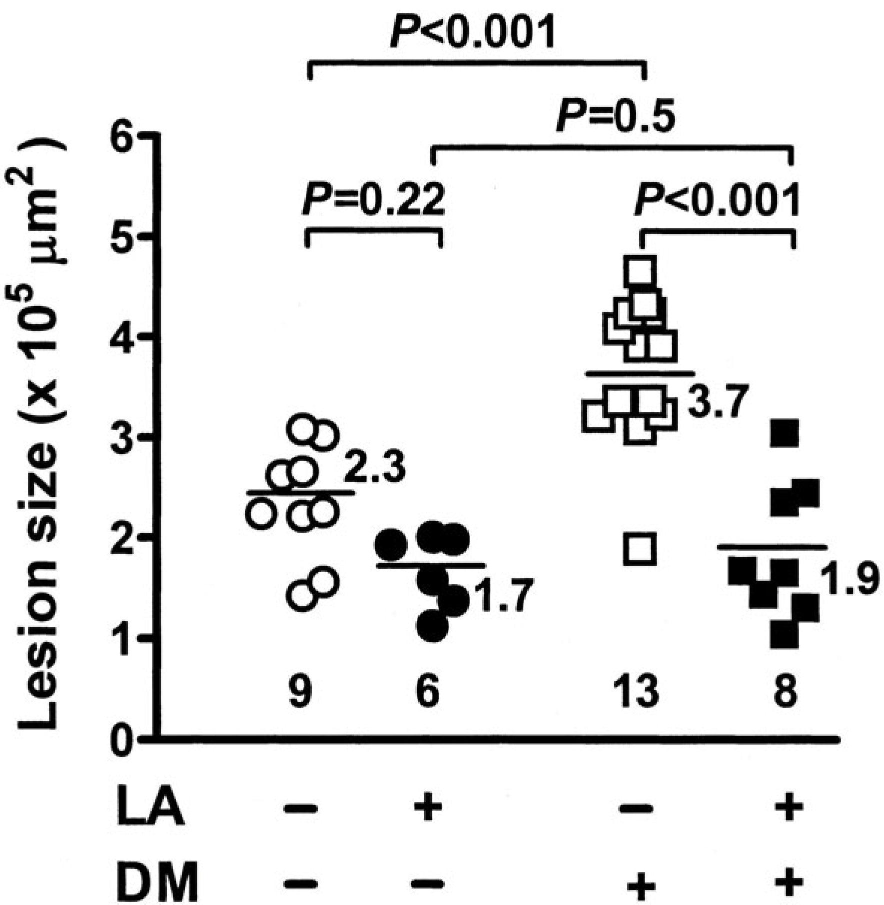
- **Removes catalysts by metal-chelating**

  e.g. Fenton reaction

  $H_2O_2 + Fe^{2+} \longrightarrow OH^* + OH^- + Fe^{3+}$

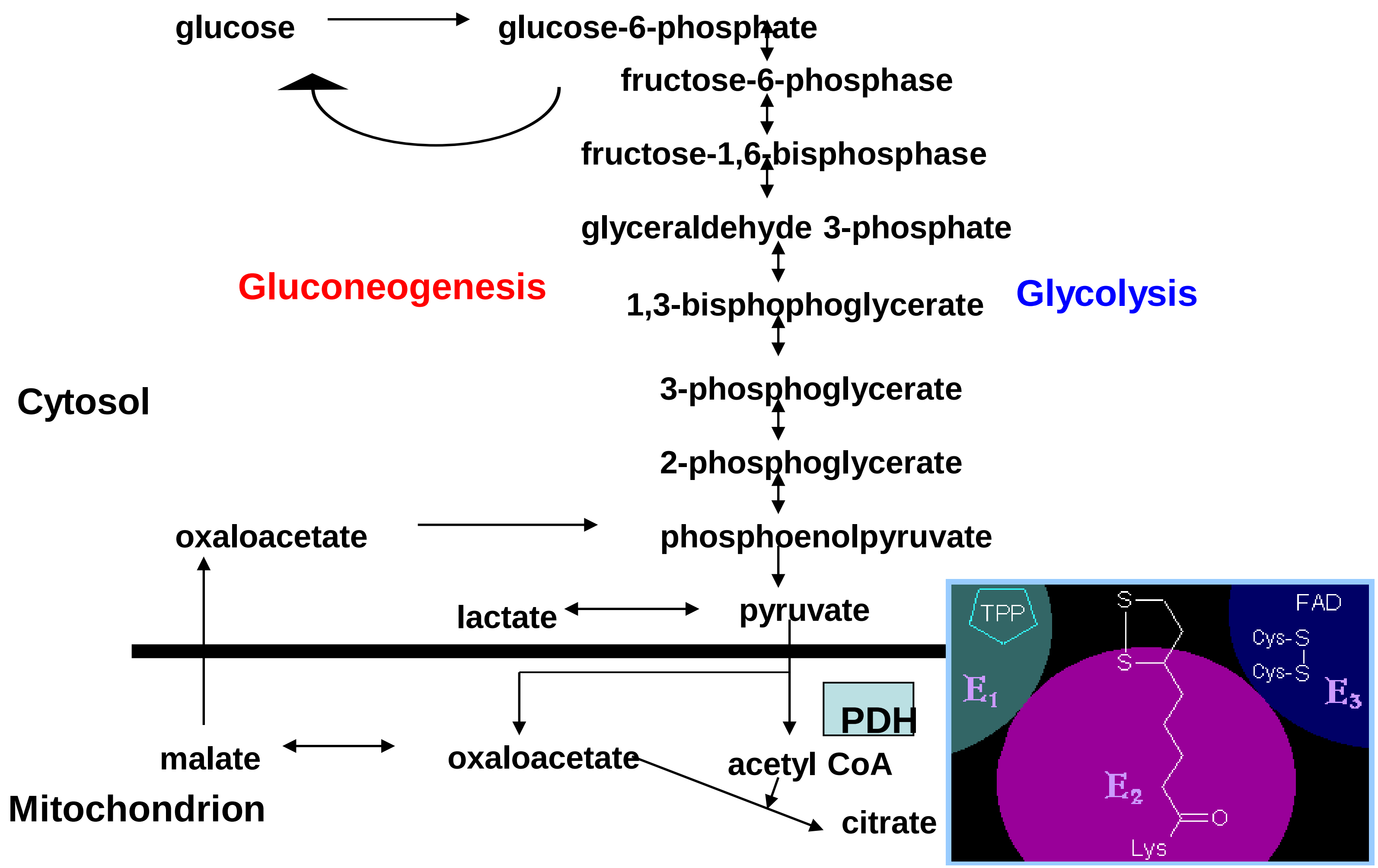
- **A strong reducing agent and recycles other antioxidants like reduced glutathione (GSH)**

LA in diet prevents enhanced atherosclerosis induced by diabetes in apoE-/- mice



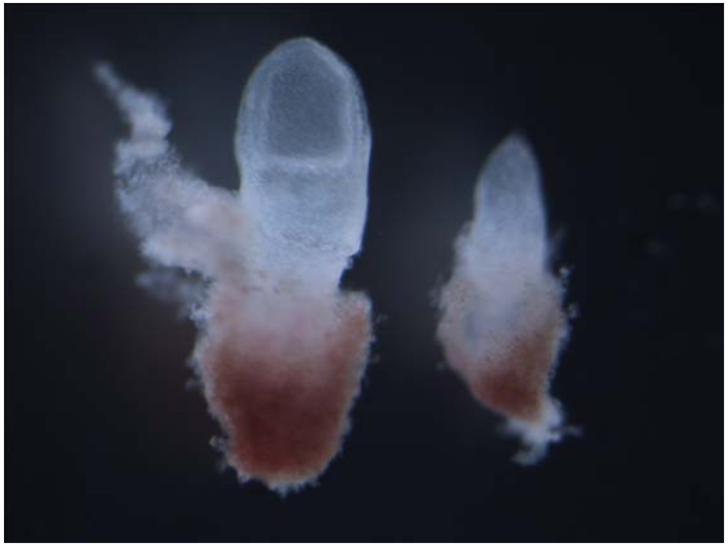
(5mo post STZ)

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

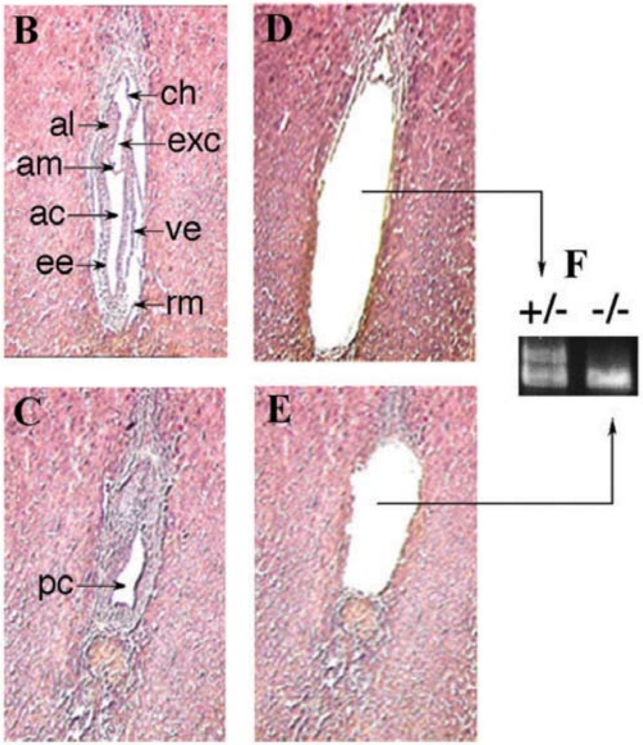


Endogenous production of LA is essential

Lias-/- embryos die immediately after implantation



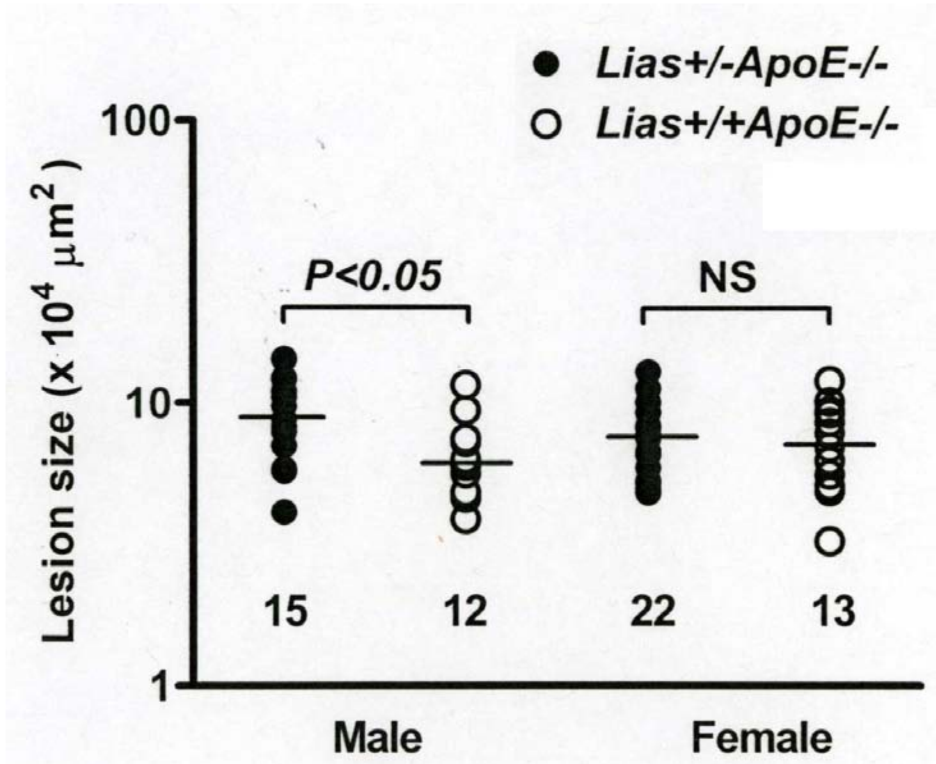
7.5 dpc



***Lias+/-Apoe-/-* mice**

(129SvEvTac)

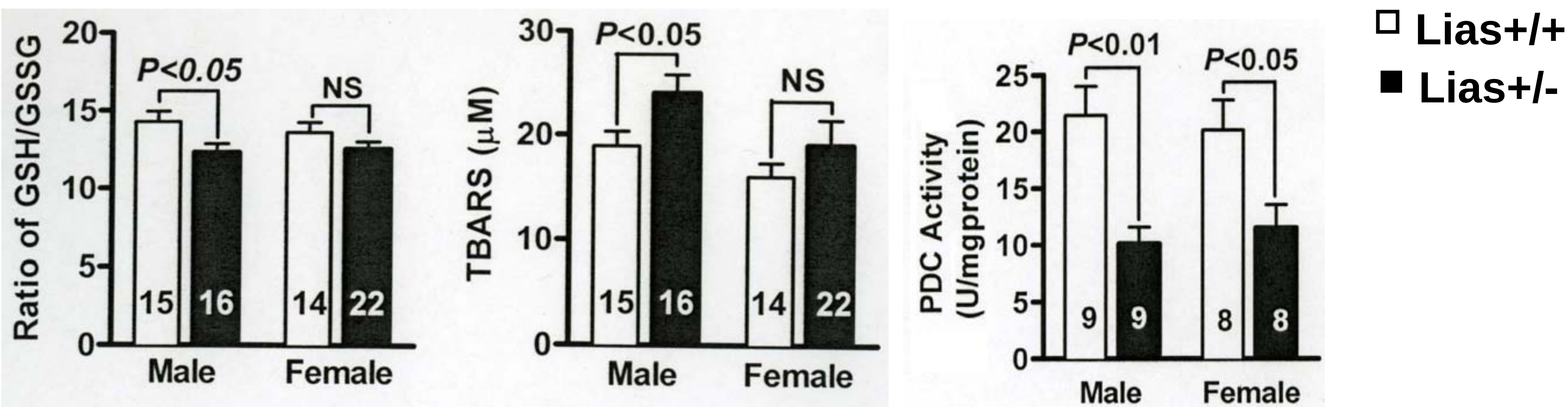
Plaque size at the aortic roots is significantly larger in *Lias+/-Apoe-/-* males but not in females



	Male		Female		P (ANOVA)		
	Lias+/-ApoE-/- n=16	*Lias+/+ApoE-/-* n=15	*Lias+/-ApoE-/-* n=22	*Lias+/+ApoE-/-* n=14	genotype	gender	Interaction
Plasma							
Cholesterol (mg/dl)	746.0±27.7	655.3±16.4	715.3±18.6	664.9±21.9	<0.01	0.63	0.35
Triglyceride (mg/dl)	181.5±20.5	151.8±11.9	153.9±14.5	127.7±11.2	0.08	0.10	0.91
Pyruvate (μM)	30.9±3.0	20.7±2.0	31.2±2.5	21.4±2.5	<0.001	0.95	0.85
Glucose (mg/dl)	196.5±11.1	178.9±6.9	184.2±11.1	176.5±12.0	0.25	0.51	0.65
Body weight (BW, g)	32.3±0.6	30.1±0.6	29.1±0.5	28.2±0.5	<0.01	<0.01	0.21

Blood samples are taken at the end of the study. Data shown are mean values ±SE. The number of animals in each group is parenthesis.

Increased oxidative stress in *Lias+/-Apoe-/-* mice : effects are bigger in males than in females



. Gene expression in aorta

Gene	Male		Female		*P* (ANOVA)		
	Lias+/-ApoE-/- n=9	*Lias+/+ApoE-/-* n=10	*Lias+/-ApoE-/-* n=9	*Lias+/+ApoE-/-* n=9	genotype	gender	Interaction
Sod1	0.57±0.09[a]	1.00±0.15	1.01±0.12	1.24±0.18	<0.01	0.27	0.67
Sod2	0.40±0.13[a]	1.00±0.16	0.97±0.13	1.17±0.18	<0.05	0.81	0.14
Gpx1	0.55±0.06	1.00±0.26	0.71±0.05	0.98±0.30	0.07	0.67	0.65
Lias	0.40±0.07[a]	1.00±0.13	0.46±0.08[b]	1.08±0.15	<0.001	0.53	0.93
IL-6	2.40±0.75	1.00±0.23	1.51±0.35	0.89±0.18	0.11	0.21	0.22

Blood samples are taken at the end of the study. Data shown are mean values ±SE. The number of animals in each group is in parenthesis. [a]:*P*<0.05 vs male *Lias+/+ApoE-/-* mice; [b]:P<0.05 vs female *Lias+/+ApoE-/-* mice.

Lias+/-Apoe-/- mice

A 50% reduction of Lias gene expression.

Significantly increased atherosclerosis in males but not in females.

Higher plasma cholesterol, TG and glucose, and increased body weight --- increased food intake?
LDLR gene expression in the liver is reduced.

Increased oxidative stress markers.

These effects are present in both in males and females, but more pronounced in males.

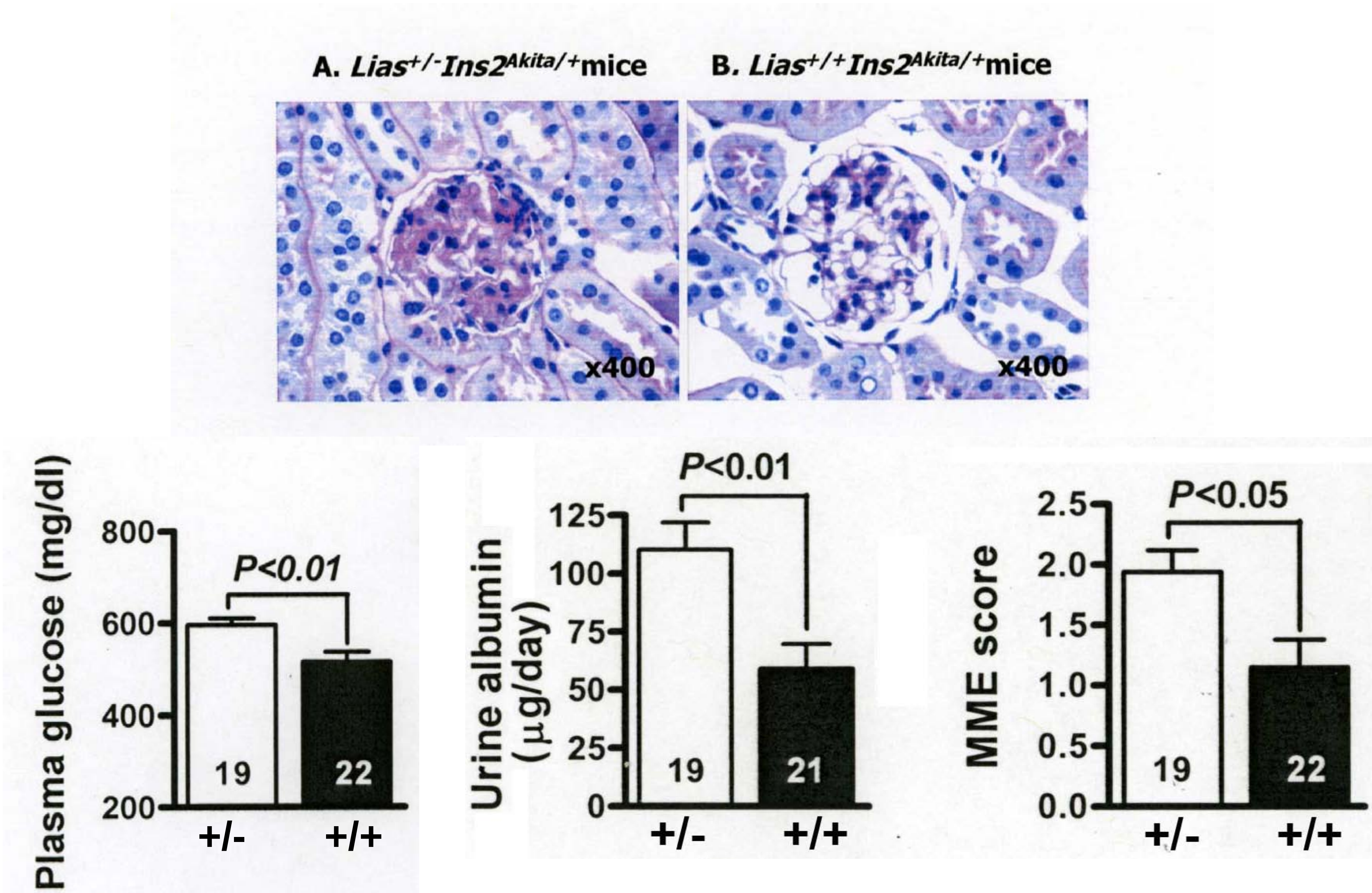
Lias+/-Akita mice

Lias$^{+/-}$
(129)

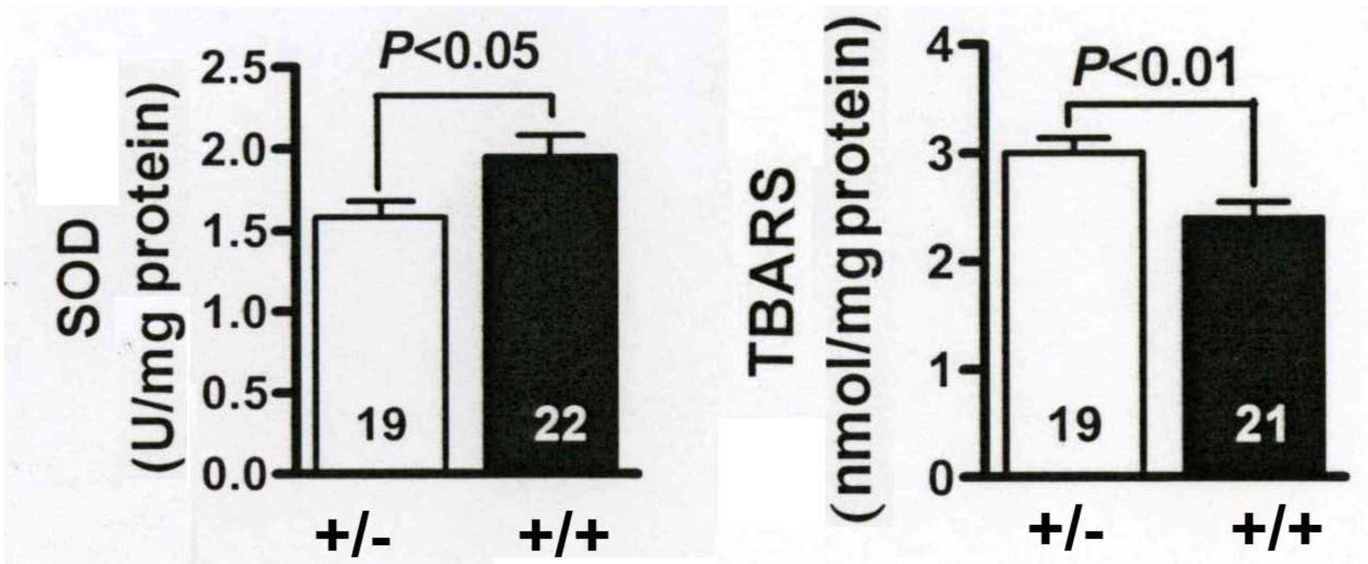
X

Ins2$^{Akita/+}$
(C57BL/6J)

Increased Kidney Damage

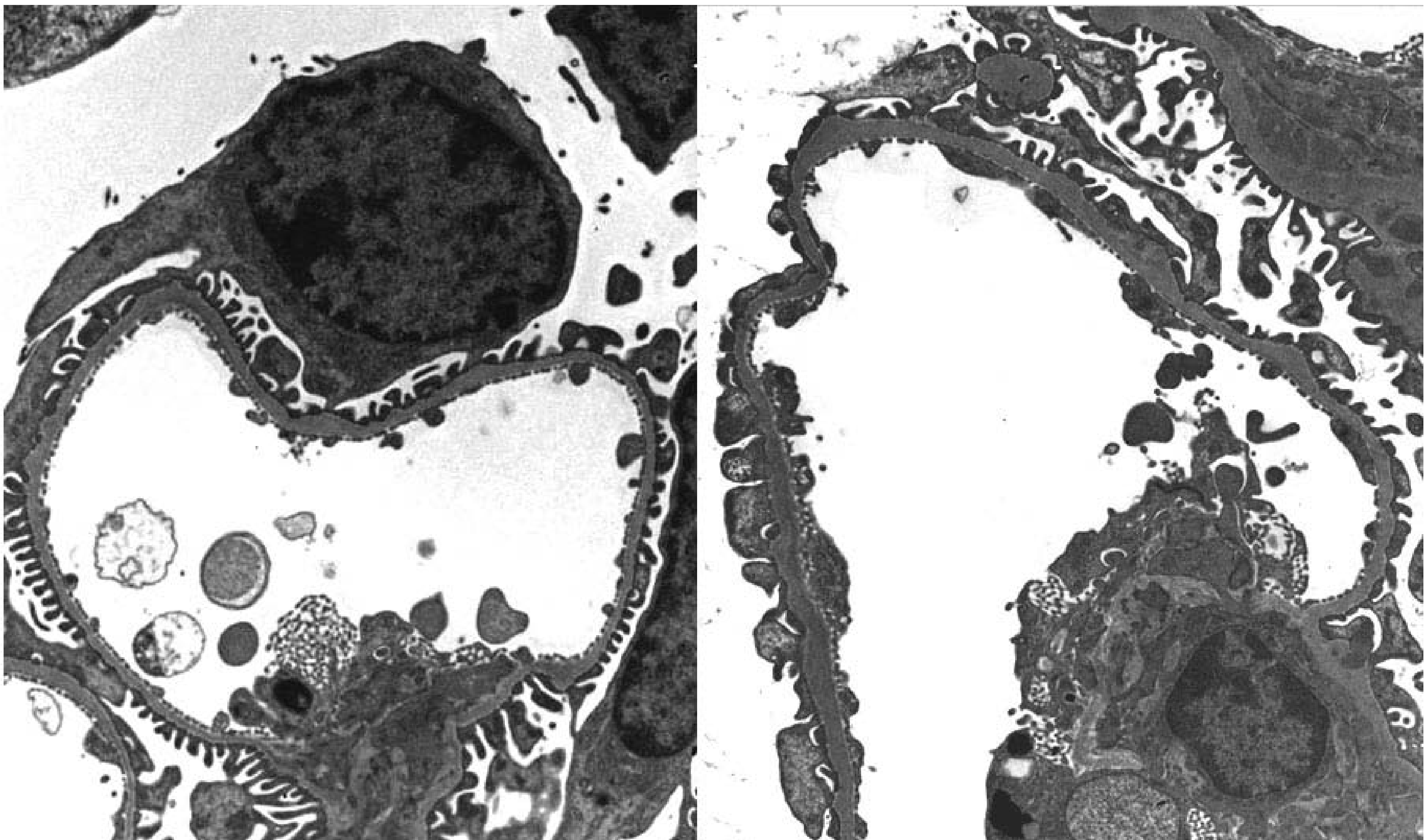


Increased oxidative stress markers in the kidneys



Podocyte Effacement

Lias+/+Akita *Lias+/-Akita*



Mitochondrial damage in the proximal tubular cells

Lias+/+Akita *Lias+/-Akita*

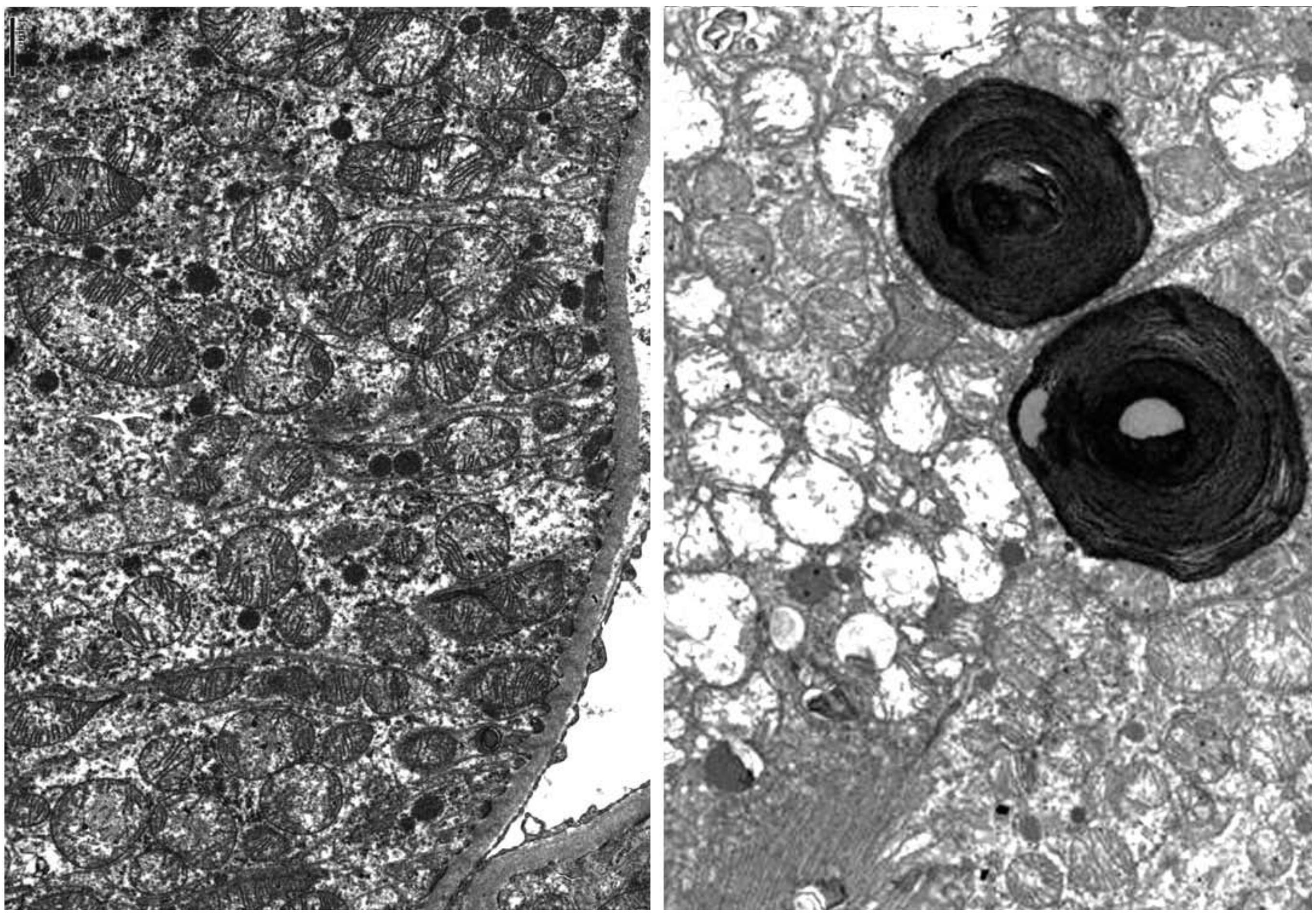


Table 2 Gene expression in kidney

Gene	*Lias*$^{+/-}$*Ins2*$^{Akita/+}$ n=15	*Lias*$^{+/+}$*Ins2*$^{Akita/+}$ n=14	*P* value
Nephrin	0.48±0.07	1.00±0.14	<0.01
Sod1	0.66±0.11	1.00±0.12	<0.05
Sod2	0.65±0.10	1.00±0.11	<0.05
Sod3	0.80±0.10	1.00±0.12	0.09
Col I	1.27±0.18	1.00±0.23	NS
Col IV	1.44±0.19	1.00±0.10	<0.05
Tgfβ1	1.56±0.16	1.00±0.12	<0.05
Lias	0.35±0.03	1.00±0.10	<0.001

mRNA levels in kidney cortex are expressed as mean ± SE relative to the mean levels of the control without LA as 1.00

Conditional Reduction of Lipoic Acid Synthase

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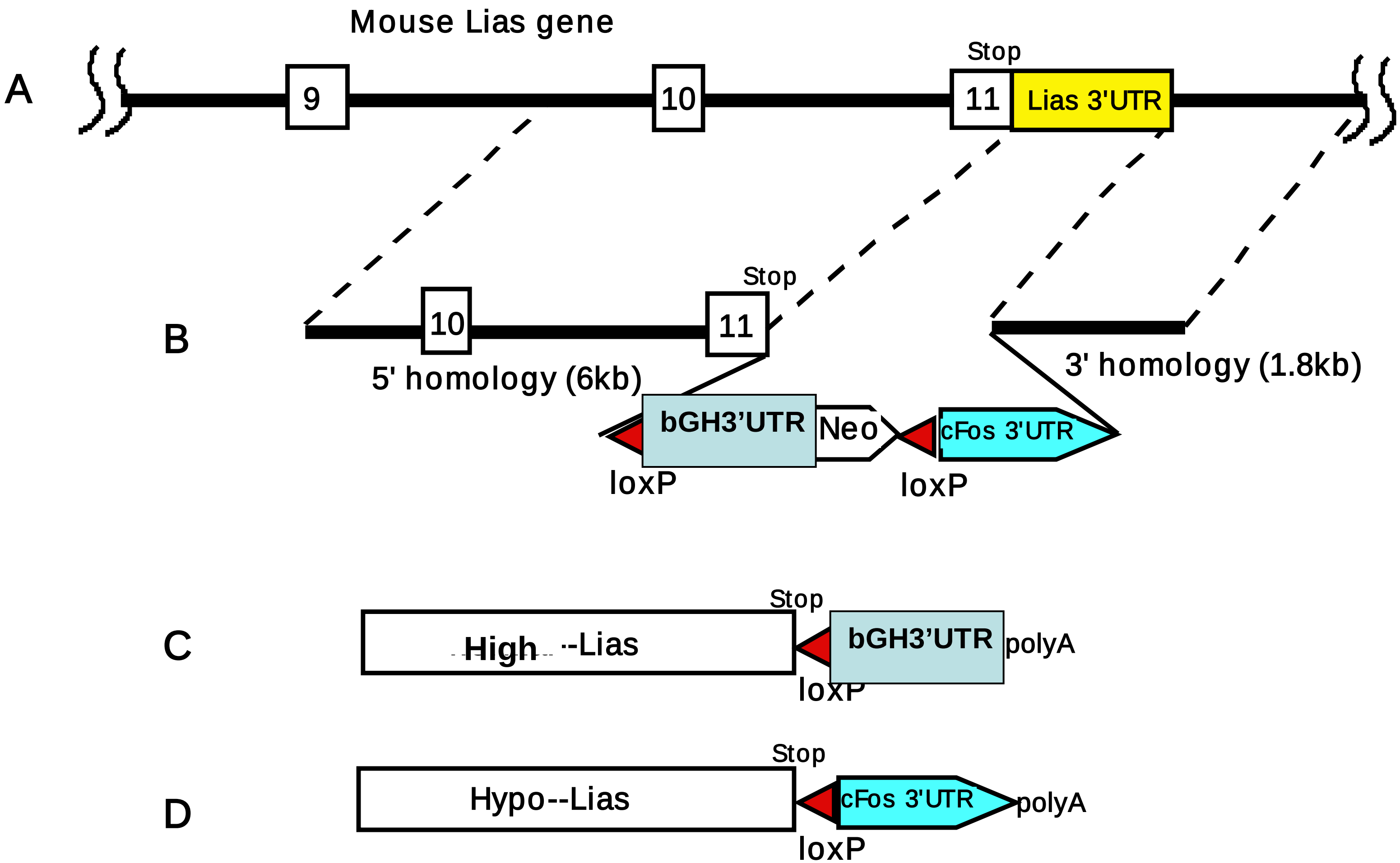
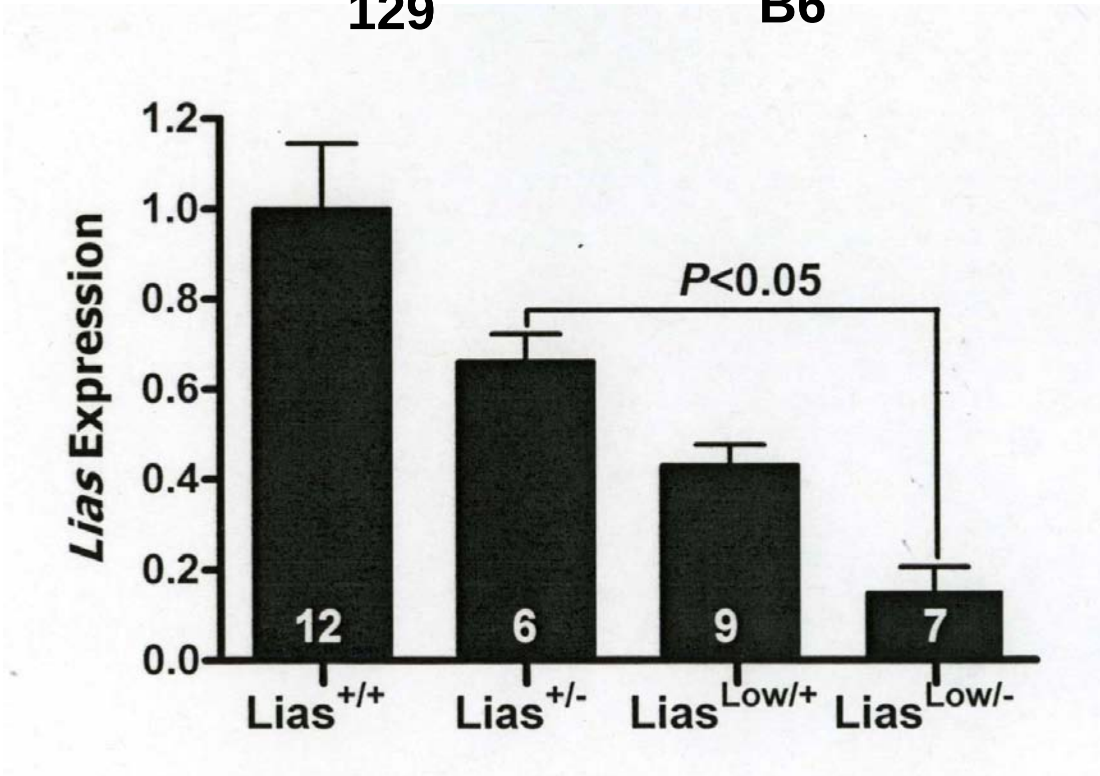
Jackson Lab


Mouse Lias gene
A
9
10
Stop
11
Lias 3'UTR
B
10
Stop
11
5' homology (6kb)
3' homology (1.8kb)
bGH3'UTR
Neo
cFos 3'UTR
loxP
loxP
C
Stop
High --Lias
bGH3'UTR
polyA
loxP
D
Stop
Hypo--Lias
cFos 3'UTR
polyA
loxP

Graded changes of the *Lias* expression in the liver

Lias$^{+/-}$ 129 X *Lias*$^{low/+}$ B6



Conclusions and next directions

Mice expressing human apoE4 and human LDLR develop atherosclerosis when they are diabetic even on a low cholesterol diet.

--- postprandial lipid clearance ?
--- vascular cells ?

A reduced *Lias* gene expression increases diabetic kidney complications.

--- mitochondrial function in proximal tubules?
--- altered metabolism?
--- relationships with atherosclerosis?