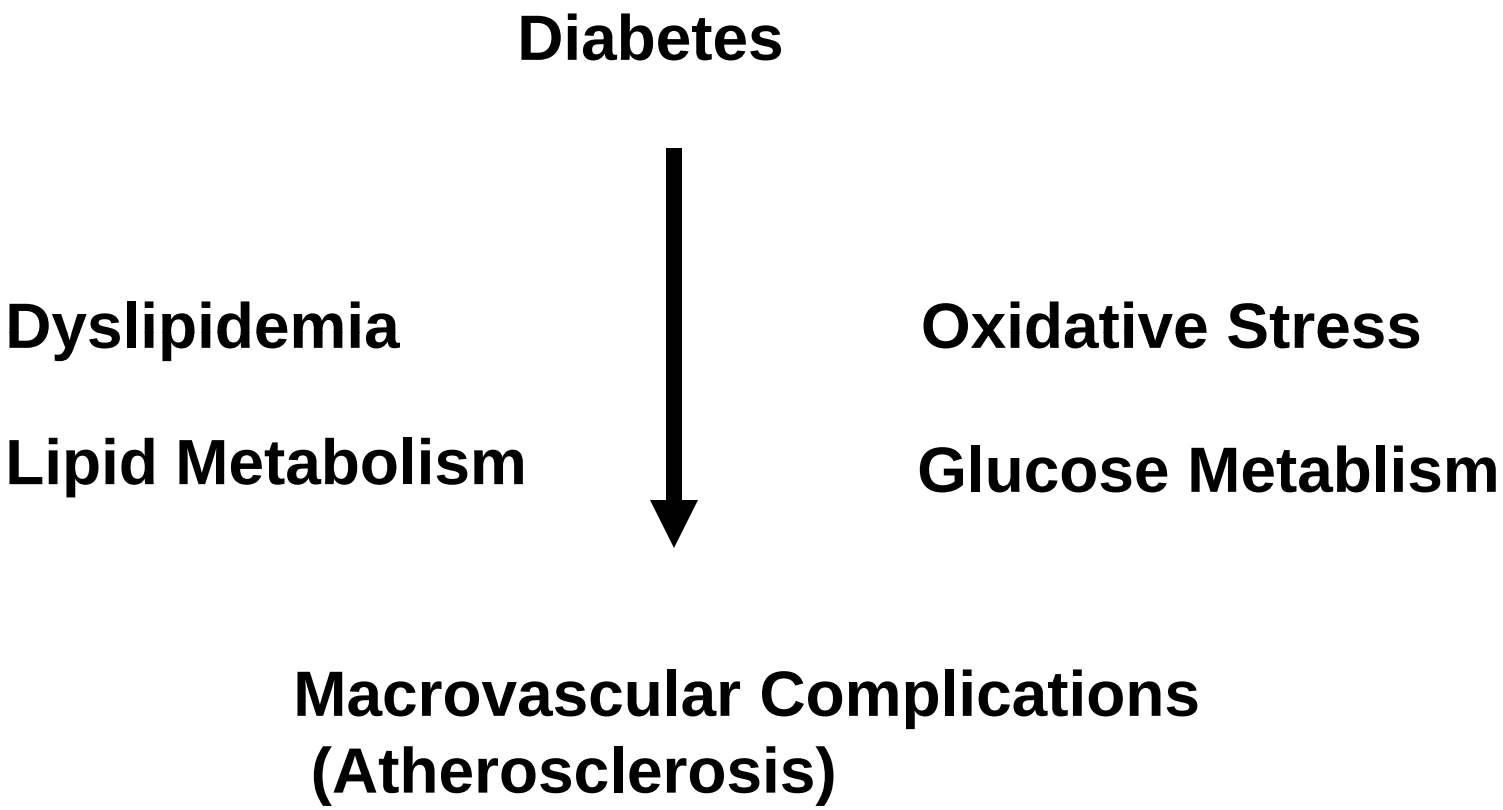
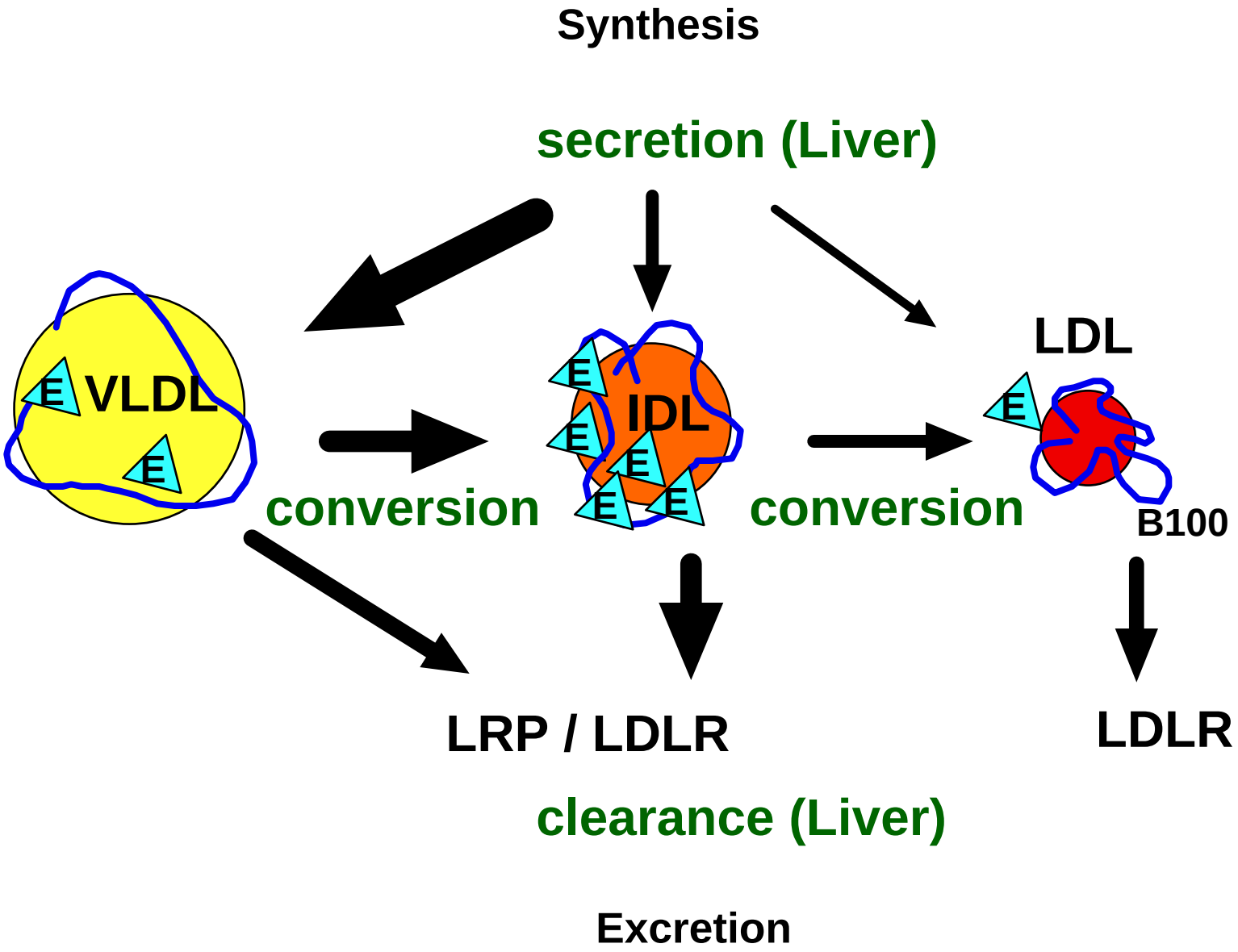

Diabetes
Dyslipidemia
Lipid Metabolism
Oxidative Stress
Glucose Metablism
Macrovascular Complications
(Atherosclerosis)

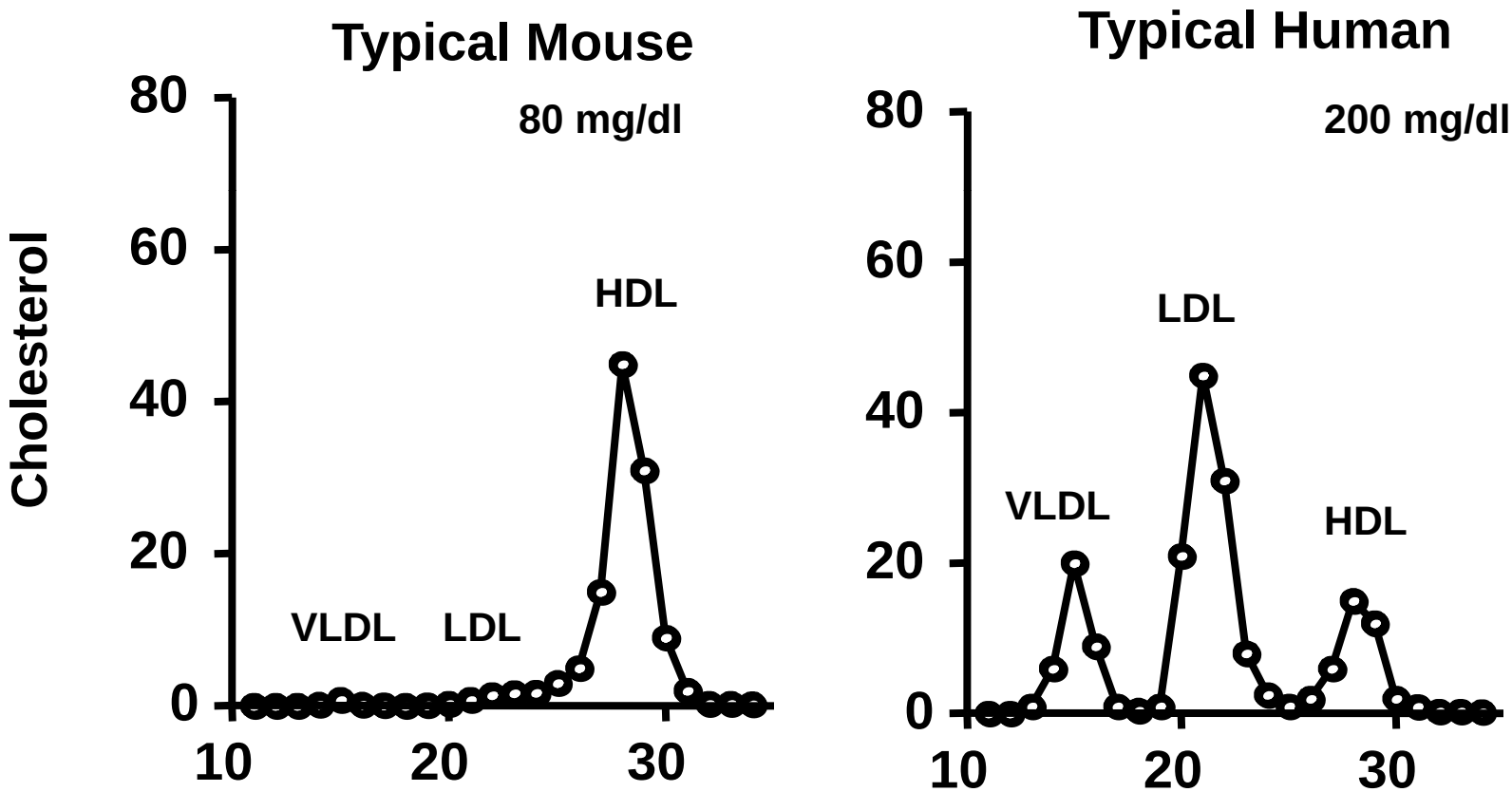
Diabetic dyslipidemia

Increased Triglycerides
Decreased HDL
Small dense LDL

Lipoprotein Metabolism



Plasma Lipoprotein Profiles in Mice and Humans



(Fast Protein Liquid Chromatography)

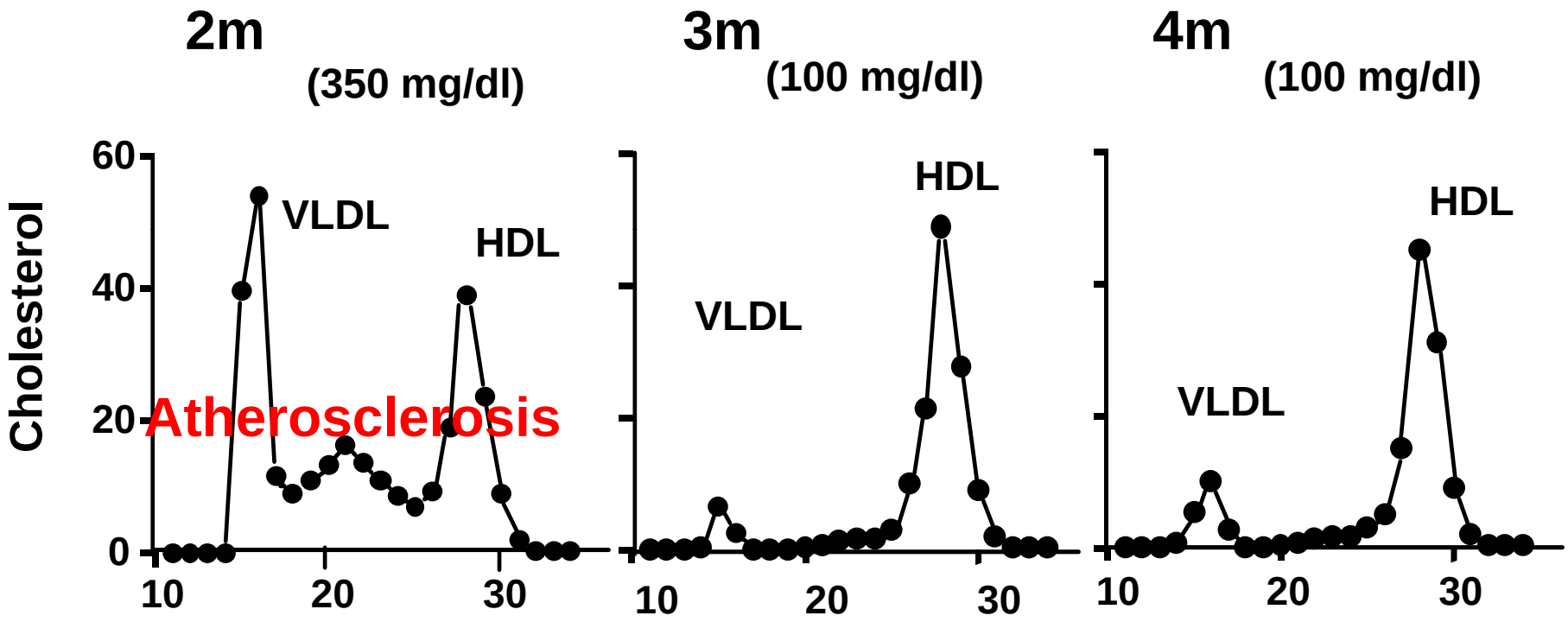
Aim1

Diabetic Dyslipidemia and Atherosclerosis in Humanized Mice

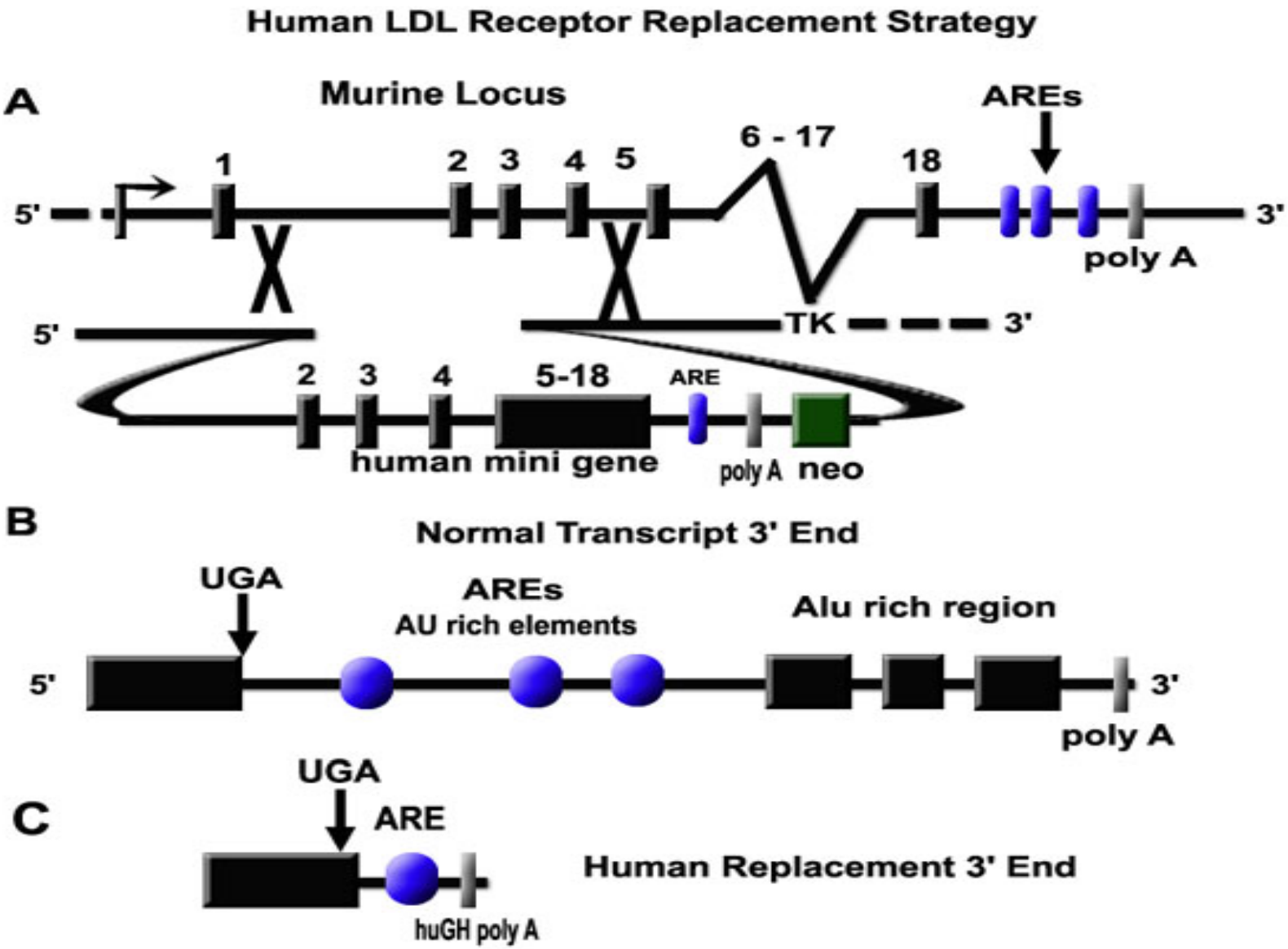
Apolipoprotein E Isoforms

	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	-20mg/dl	ref.	+20mg/dl
MI risk	reduced (type III)	ref.	Increased

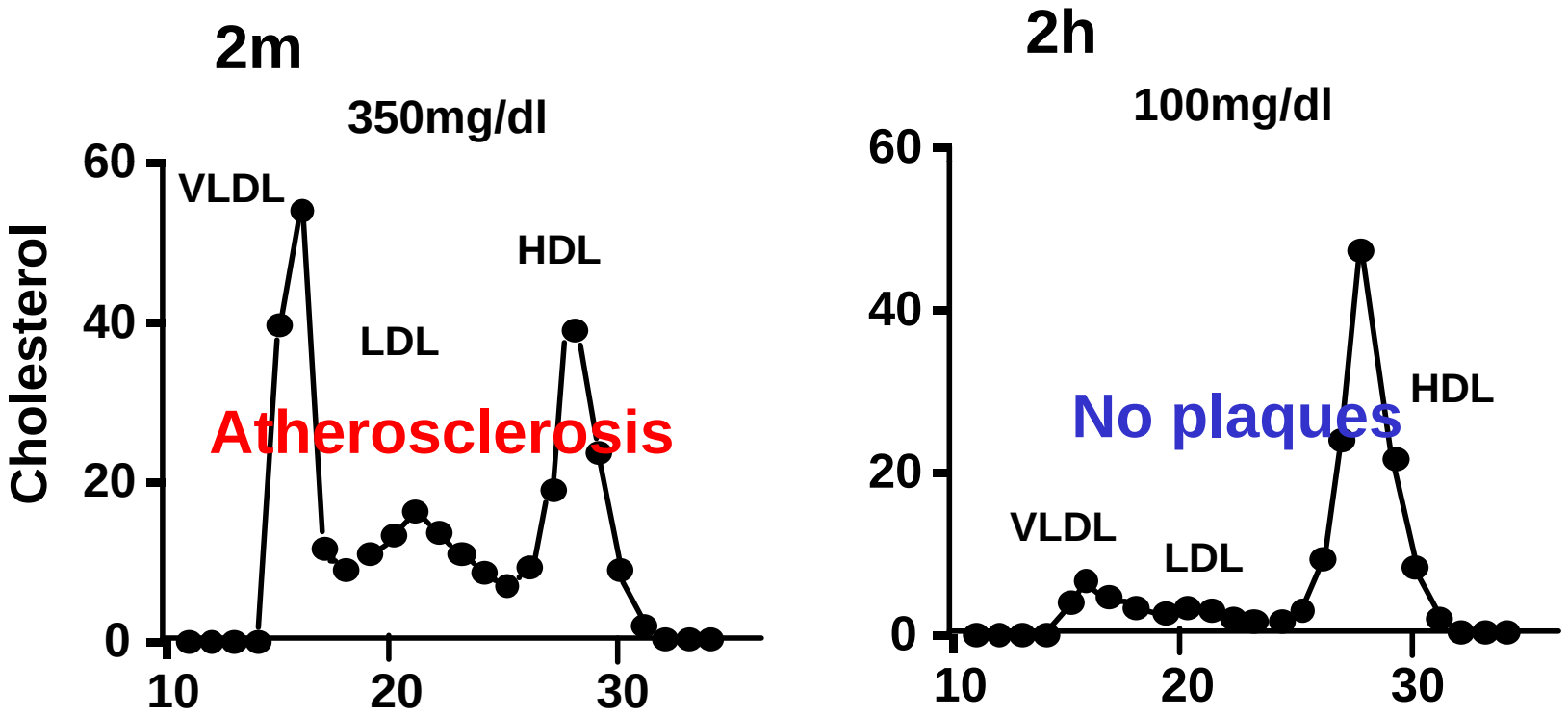
Plasma Lipids in Mice with Human ApoE



Type III hyperlipoproteinemia in E2/2 mice
E3/3 and E4/4 mice are normolipidemic


Human LDL Receptor Replacement Strategy
A
Murine Locus
AREs
1
2
3
4
5
6 - 17
18
5'
3'
poly A
TK
2
3
4
5-18
ARE
human mini gene
poly A
neo
B
Normal Transcript 3' End
UGA
AREs
AU rich elements
Alu rich region
poly A
C
UGA
ARE
Human Replacement 3' End
huGH poly A

Increased LDLR expression ameliorates type III hyperlipoproteinemia in E2/2 mice



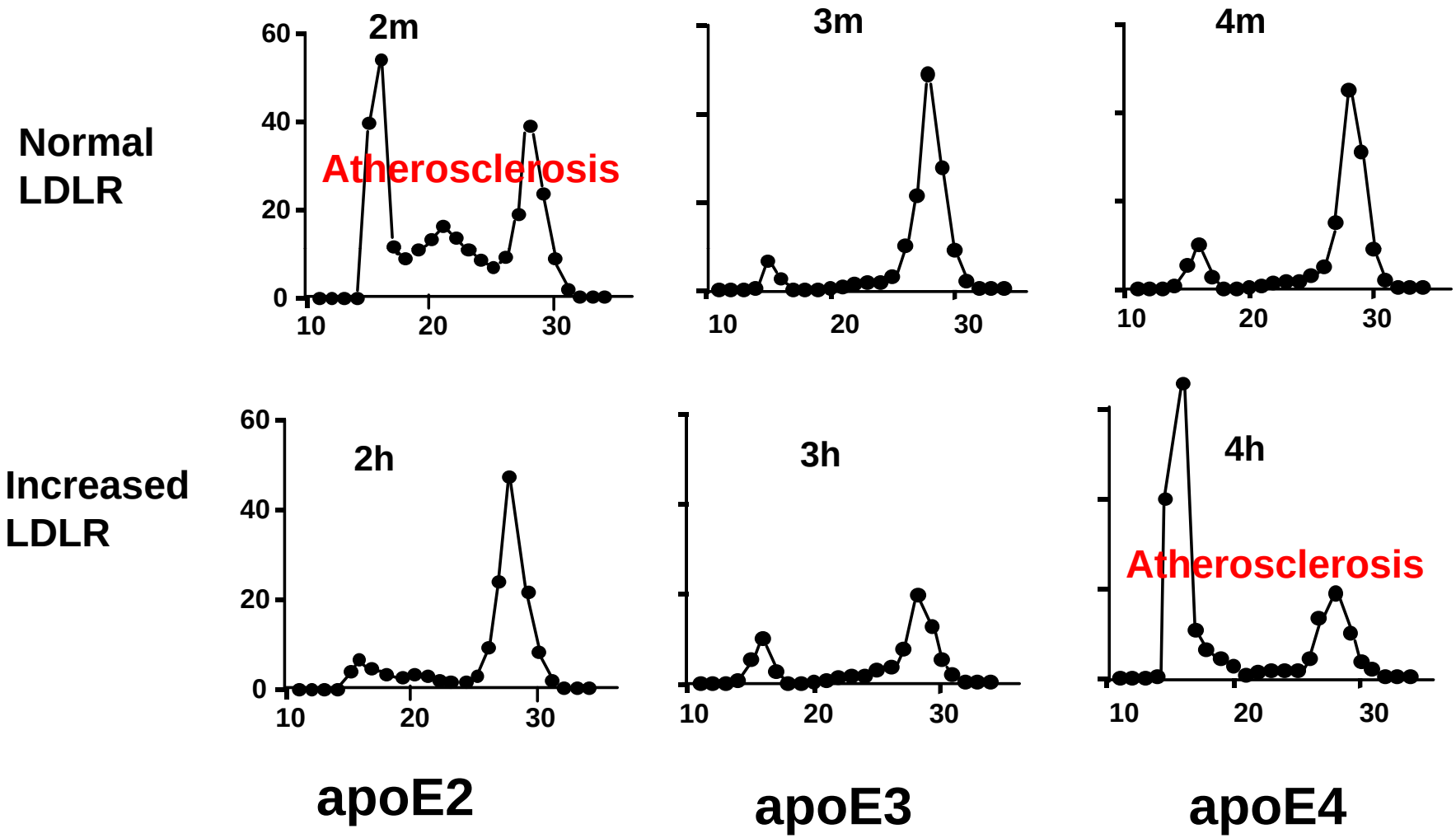
Logic

All E2/2 mice but not all E2/2 humans exhibit type III hyperlipoproteinemia because mice have lower LDLR/apoE ratio relative to average humans.

If so, association of apoE4 with higher plasma cholesterol in humans should also be explained by the same mechanism

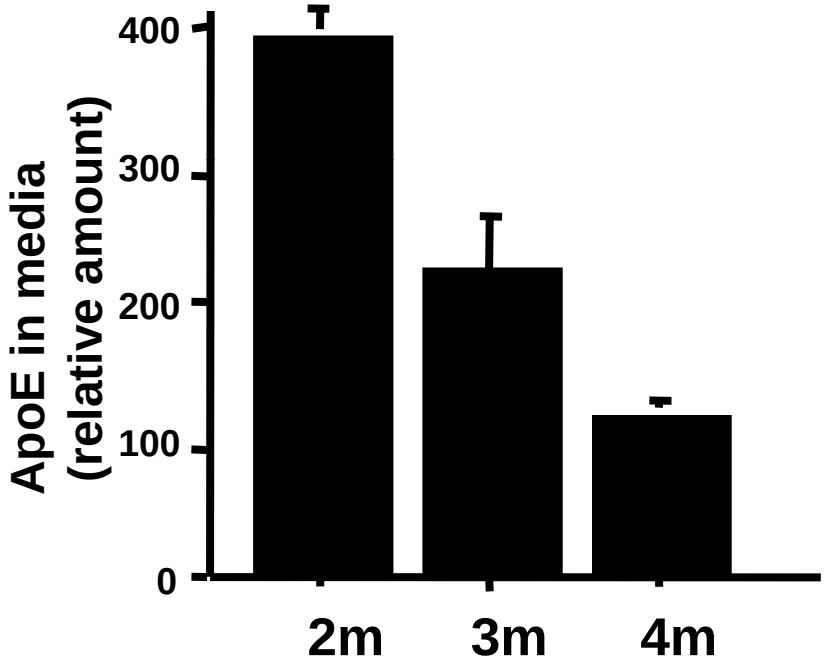
Perhaps too much LDLR expression relative to apoE4 is harmful??

Human-like lipoprotein distributions in mice with increased LDL receptor



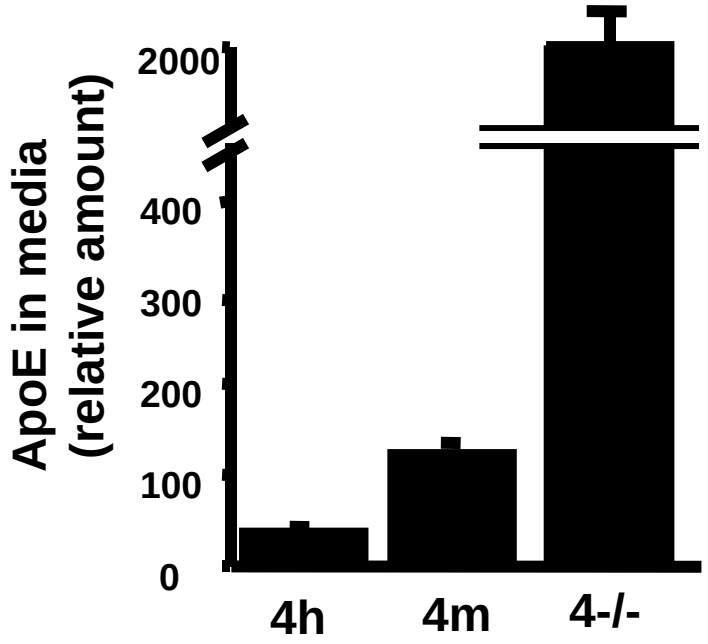
Genotype-Dependent ApoE Secretion

(liver slice culture)

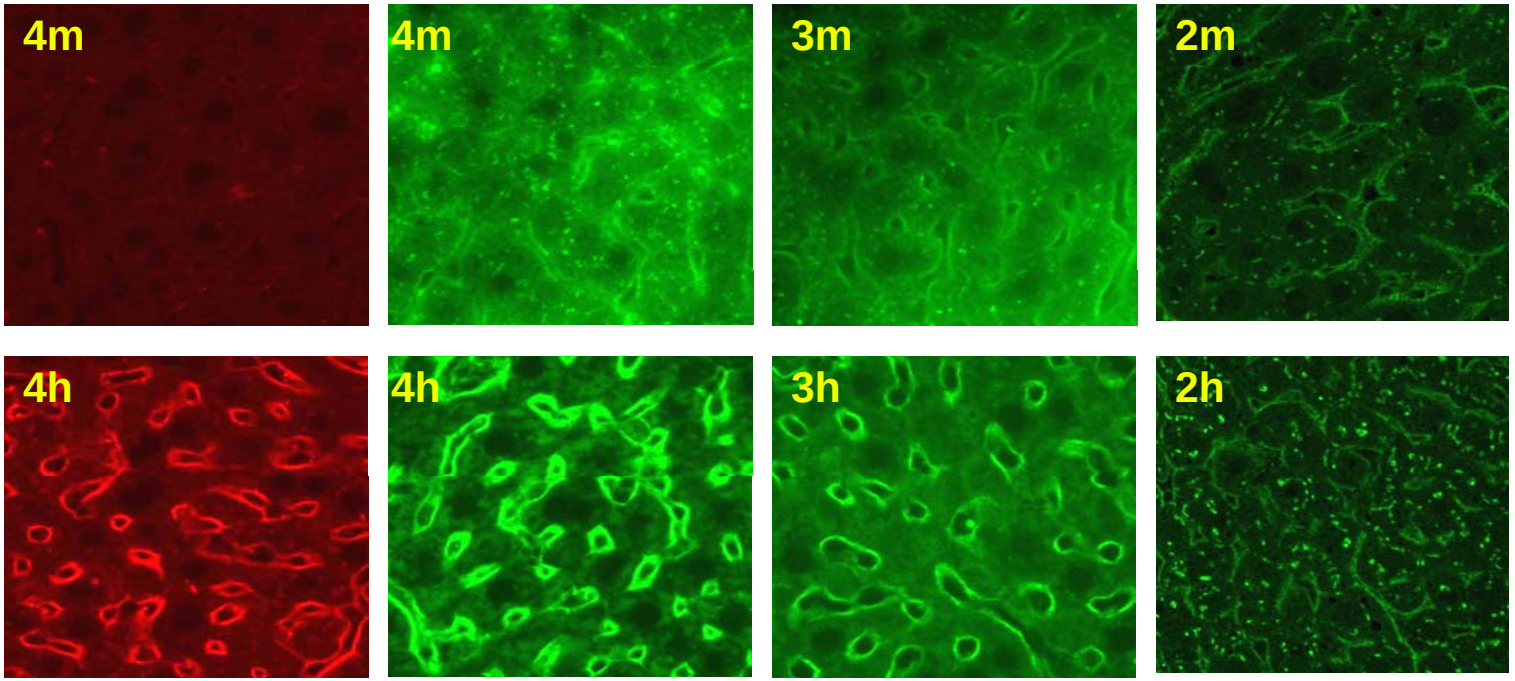


Mike Altenburg

LDLR expression levels influence the secretion of apoE from the liver



Co-localization of ApoE4 with LDLR on the hepatocyte surface

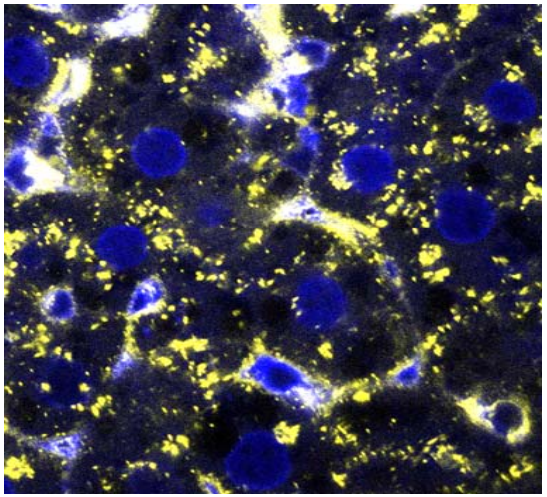


Mike Altenburg

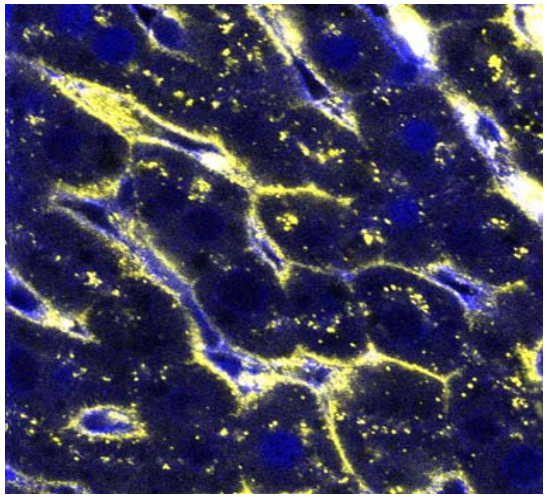
Internalization of DiI-labeled VLDL by the liver

20 min after injection

2h

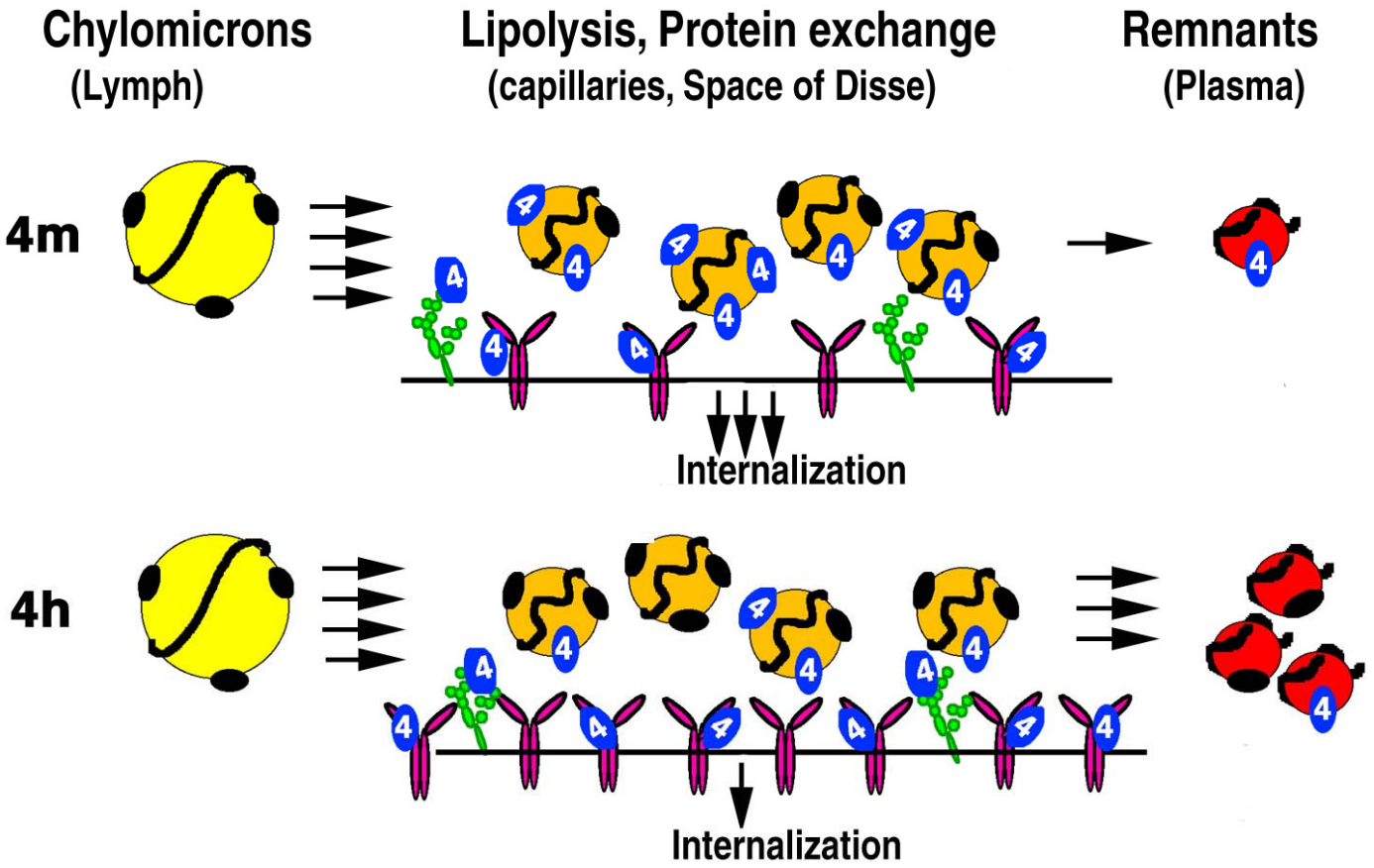


4h



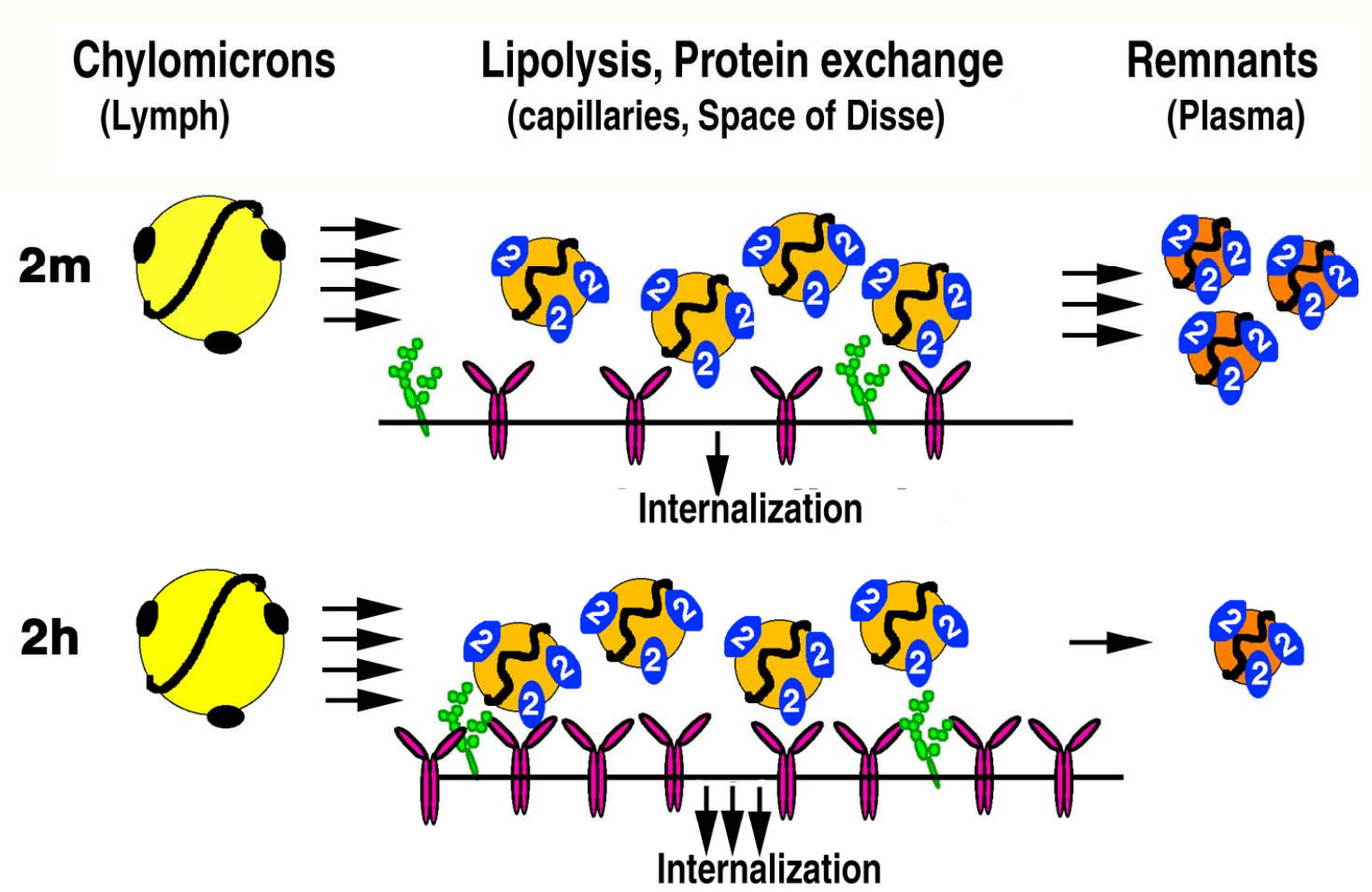
Mike Altenburg

ApoE -Trapping by the LDL receptor



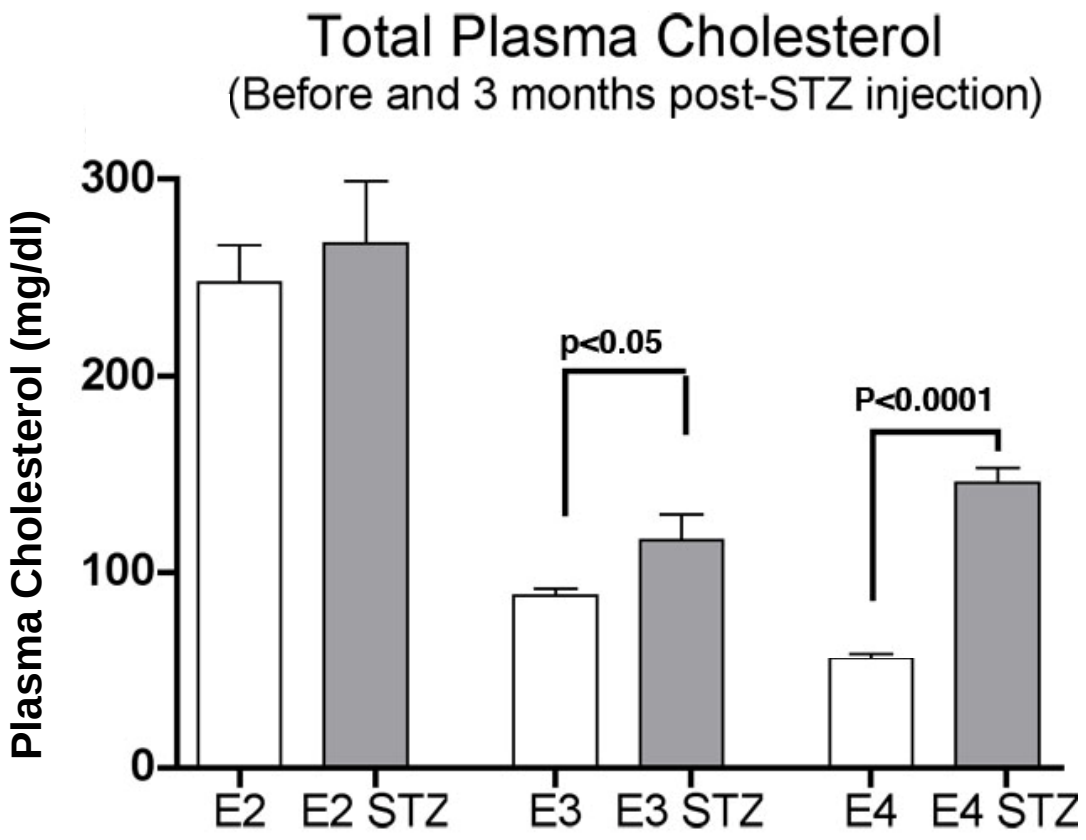
The transfer of apoE4 facilitates internalization of large lipoproteins in 4m mice. In 4h mice, the efficiency of transfer is reduced because apoE4 are trapped by the LDLR on the hepatocyte surface.

E2 is free from trapping

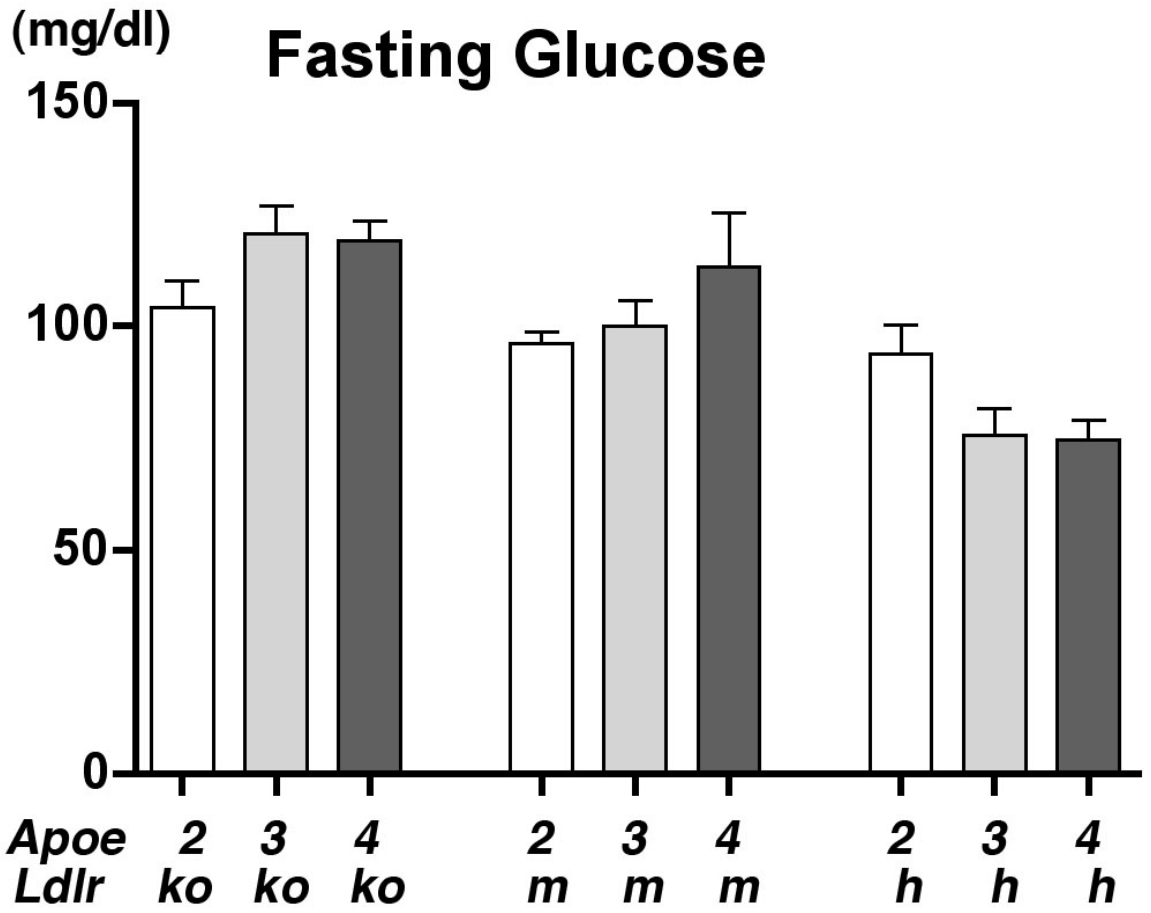


	mice	isotype	lipoprotein profiles (known) without diabetes	atherosclerosis (known) without diabetes
#1	2KO	hum apoE 2/2, LDLR-/-	TG↑, VLDL-C↑, LDL-C↑,	**++++**
#2	3KO	hum apoE 3/3, LDLR-/-	TG↑, VLDL-C↑, LDL-C↑,	**++++**
#3	4KO	hum apoE 4/4, LDLR-/-	TG↑, VLDL-C↑, LDL-C↑,	**++++**
#4	2m	hum apoE 2/2, mouse LDLR	TG↑, VLDL-C↑,	**++**
#5	3m	hum apoE 3/3, mouse LDLR	↔	**--**
#6	4m	hum apoE 4/4, mouse LDLR	↔	**--**
#7	2h	hum apoE 2/2, hum/mouse LDLR	↔	**--**
#8	3h	hum apoE 3/3, hum/mouse LDLR	HDL-C↓	**--**
#9	4h	hum apoE 4/4, hum/mouse LDLR	VLDL-C↑, HDL-C↓	**++**

Mice with humanized apoE with wild type LDLR



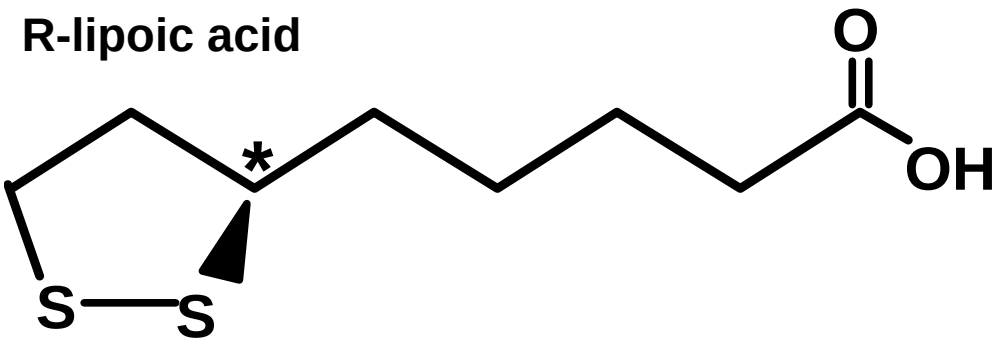
Plasma glucose levels in the humanized mice



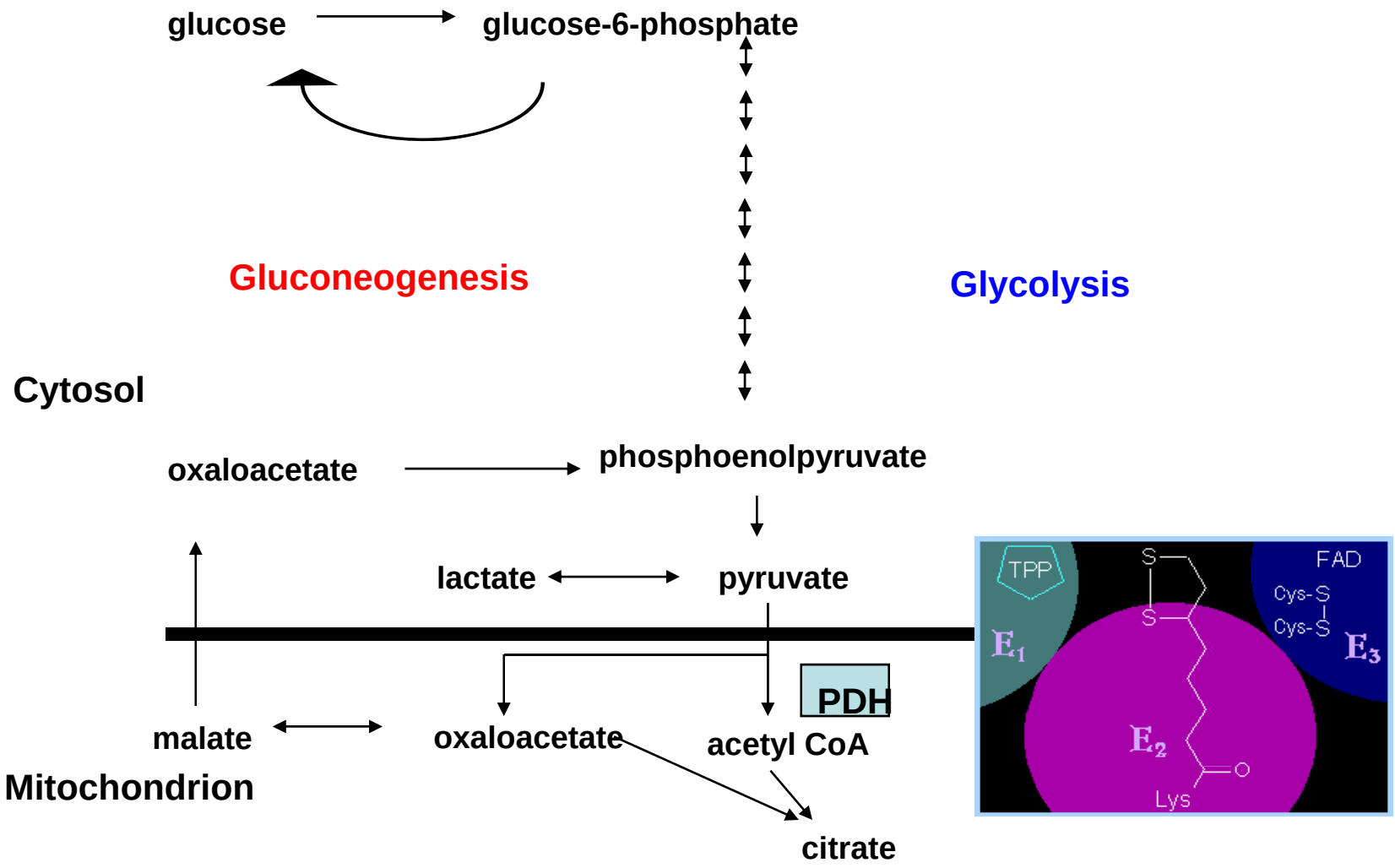
Aim 2

Diabetes, Oxidative Stress and Accelerated Atherosclerosis

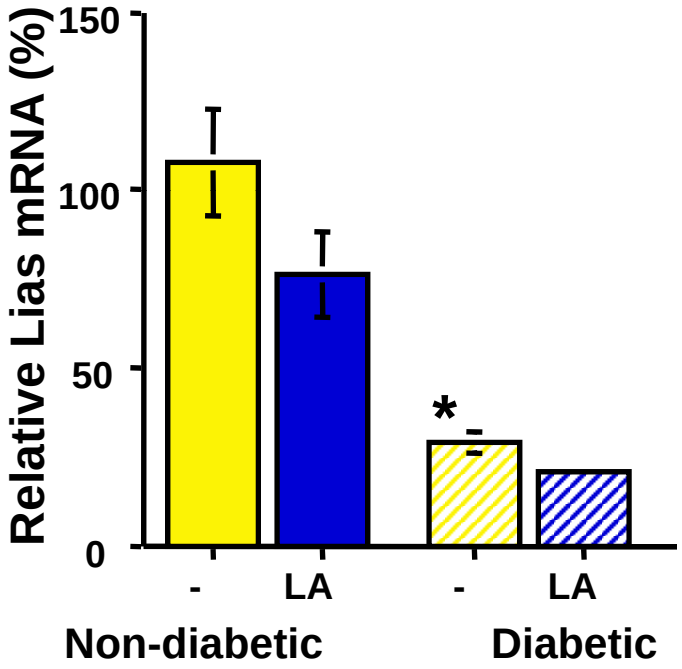
Reduction of Endogenous Lipoic Acid Synthesis



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



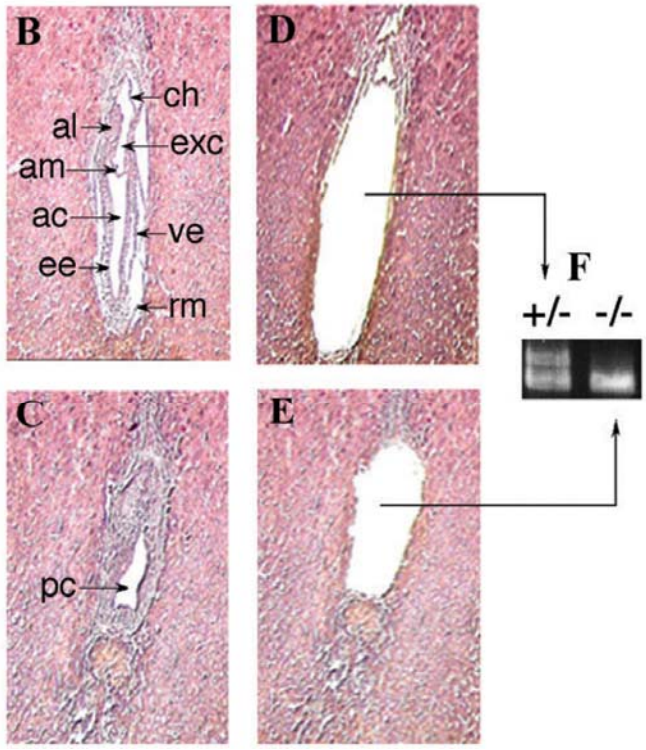
Lias gene expression in the kidney of STZ induced DM mice is 30% normal



Endogenous production of LA is essential

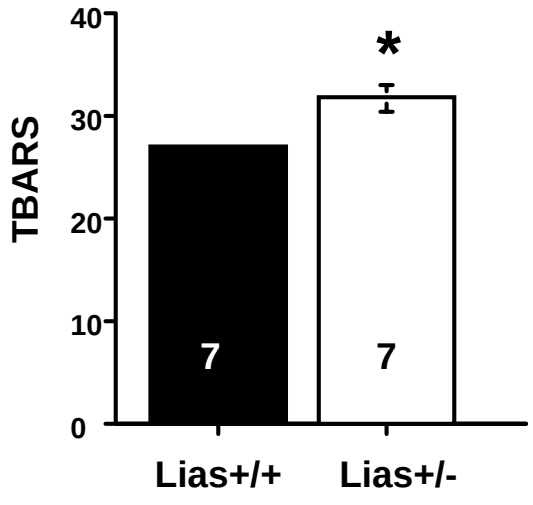
Lias-/- embryos die immediately after implantation

7.5 dpc

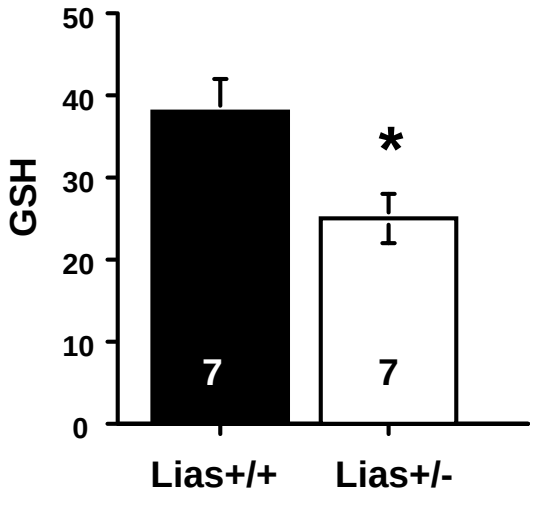


Dietary LA supplement at 1.65 g/kg to *Lias*$^{+/-}$ mothers failed to rescue *Lias*$^{-/-}$ embryo

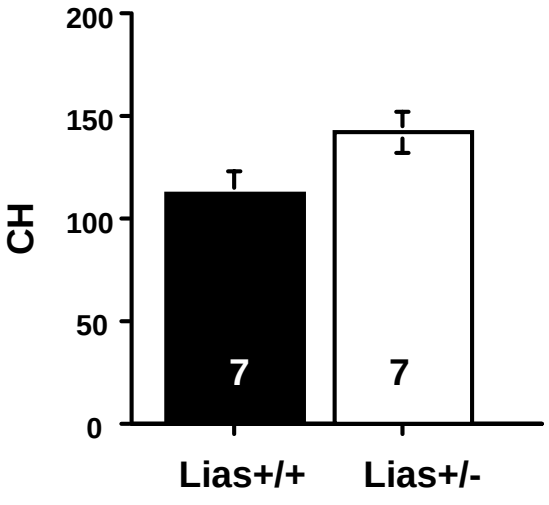
Heterozygous Lias mice (+/-) show a significant increase in oxidative stress after STZ-treated diabetes



5mo



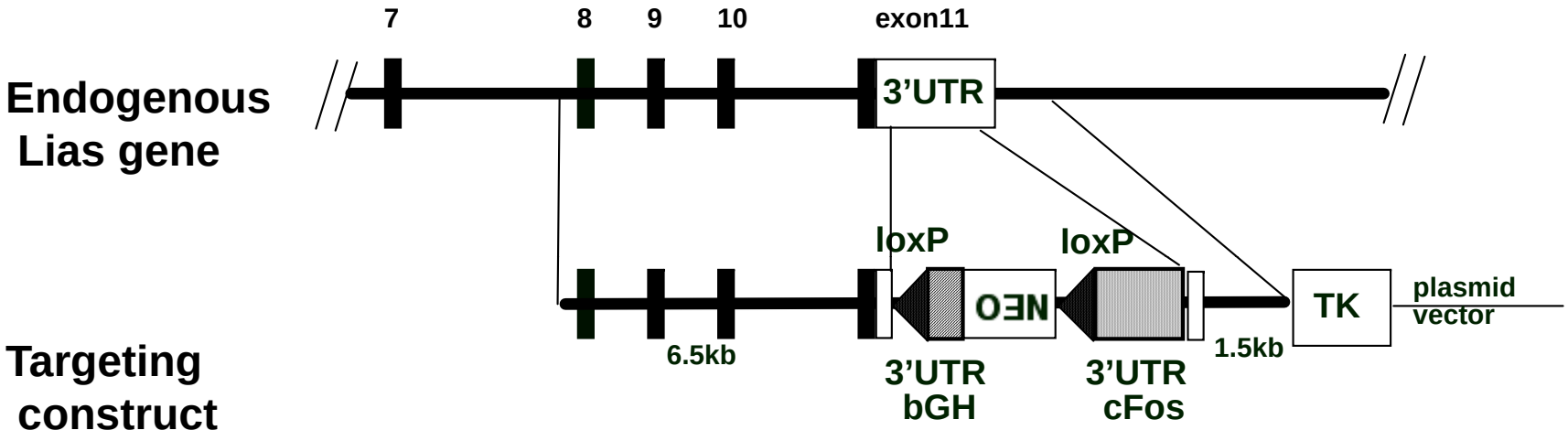
5mo

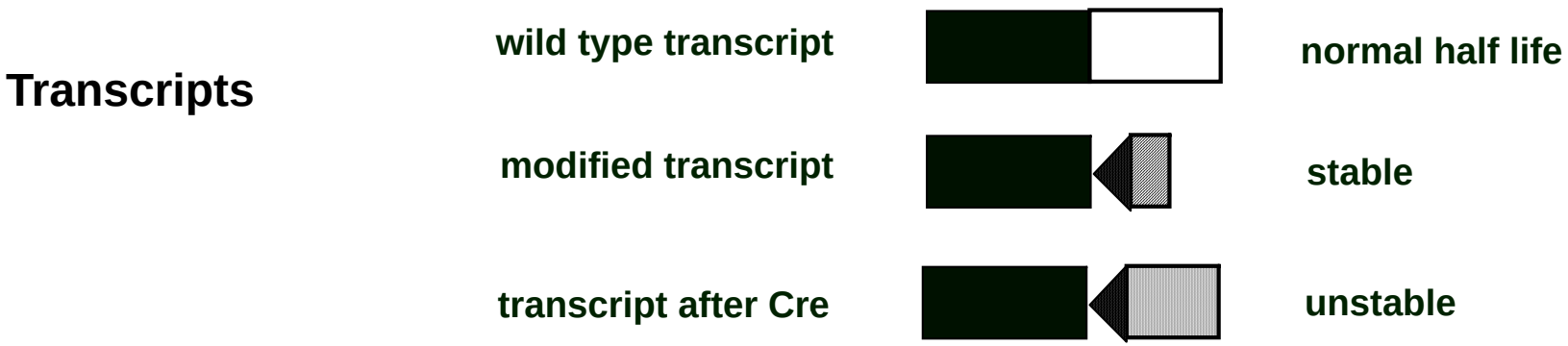


3mo

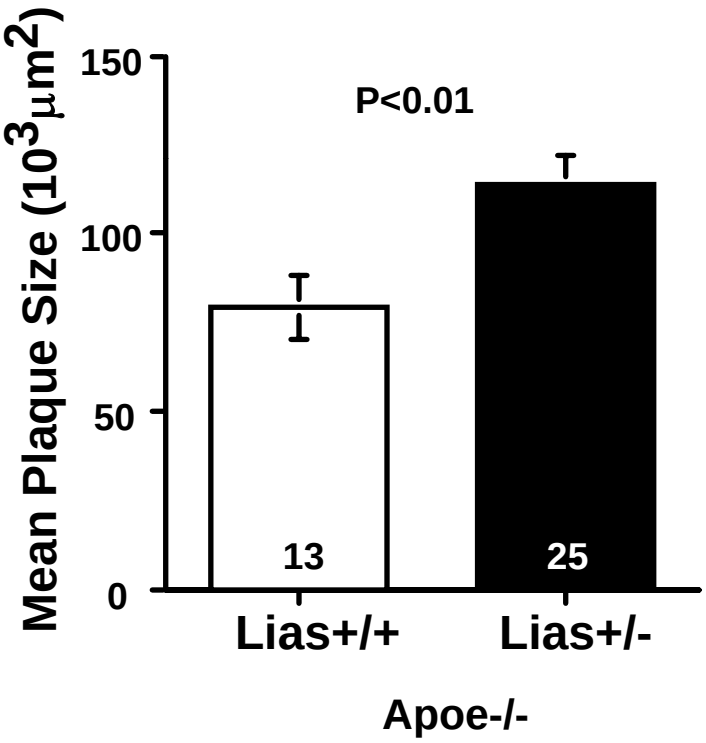
Tissue specific reduction of LA production

High-Low Modification





Increased atherosclerosis in apoE-/- mice with reduced endogenous LA production (non-diabetic)



Current experiments

1. Crossing mice with human apoE2 (or apoE4) and Akita mice

2. 2KO, 3KO and 4KO mice have been treated with STZ for examination of atherosclerosis

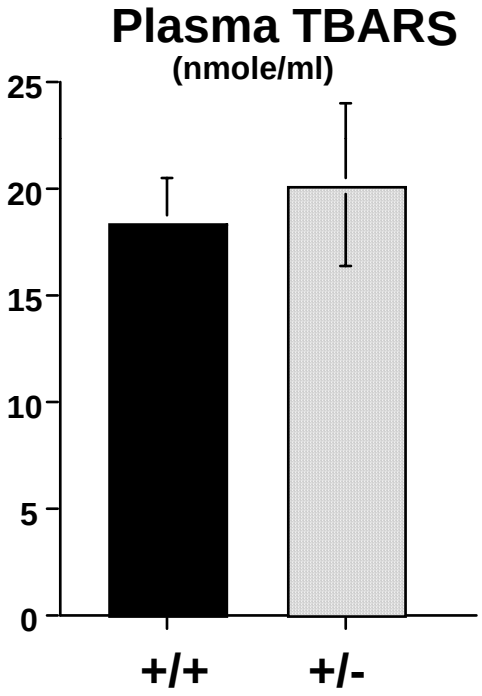
3. Human apoE isoforms and insulin resistance.

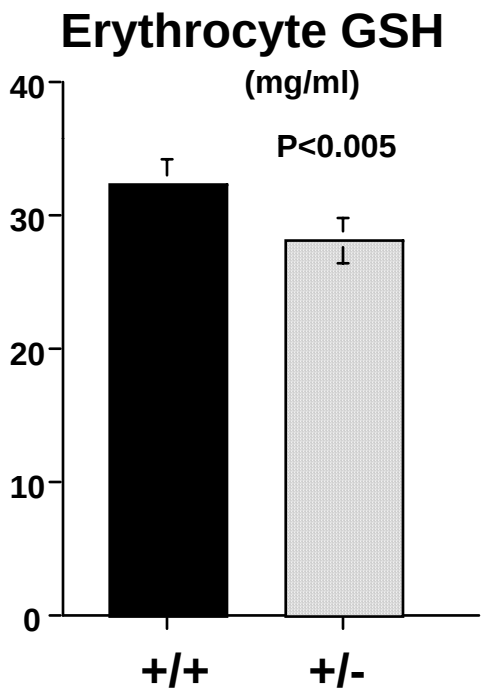
4. Generation of mice with Lias-HL modification (JAX)

5. Evaluation of atherosclerosis in STZ-treated Lias+/-Apoe-/- mice

6. Characterization of Lias+/-Akita mice

Lias+/- heterozygotes have a trend reduced antioxidant capacity

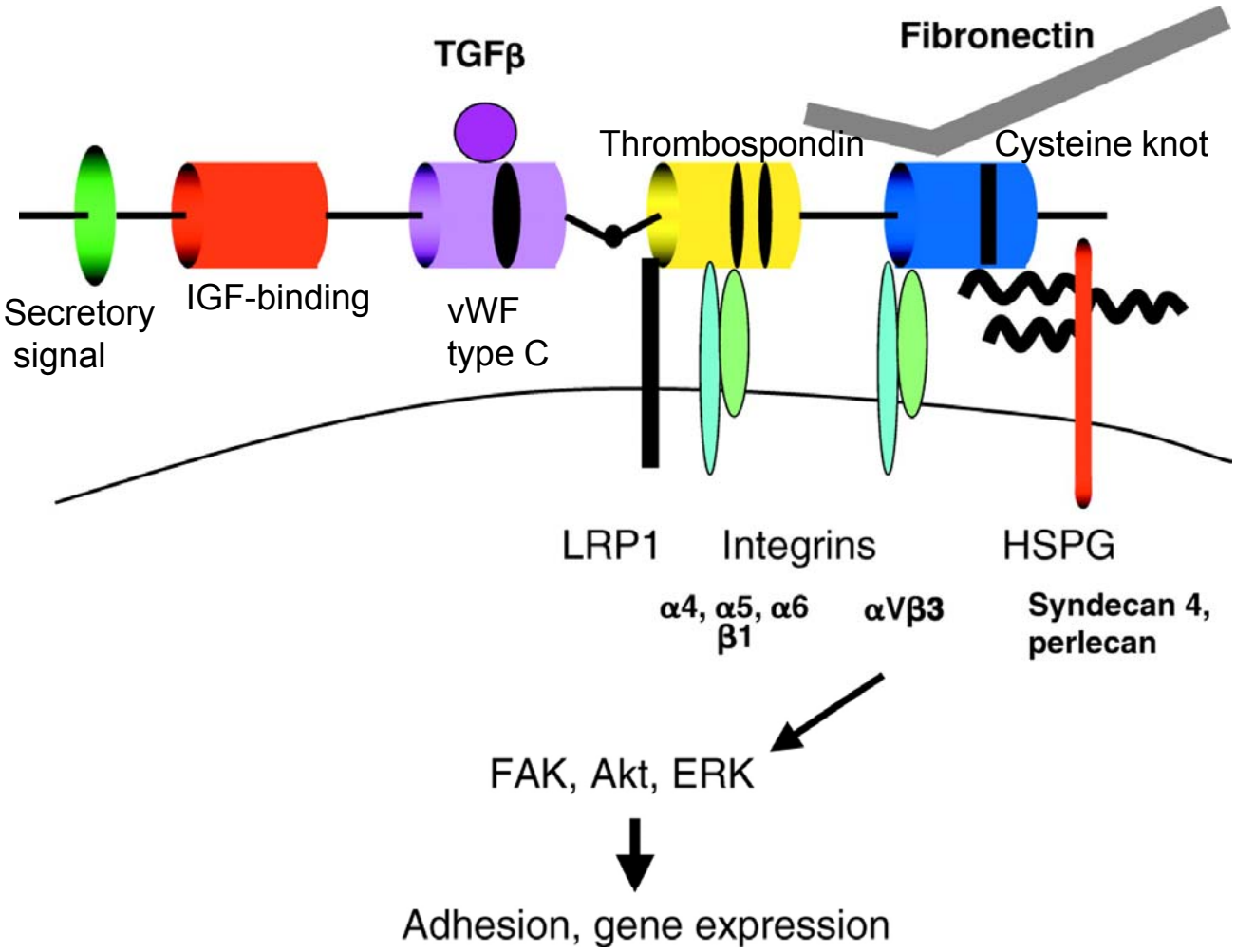




Davignon Hypothesis

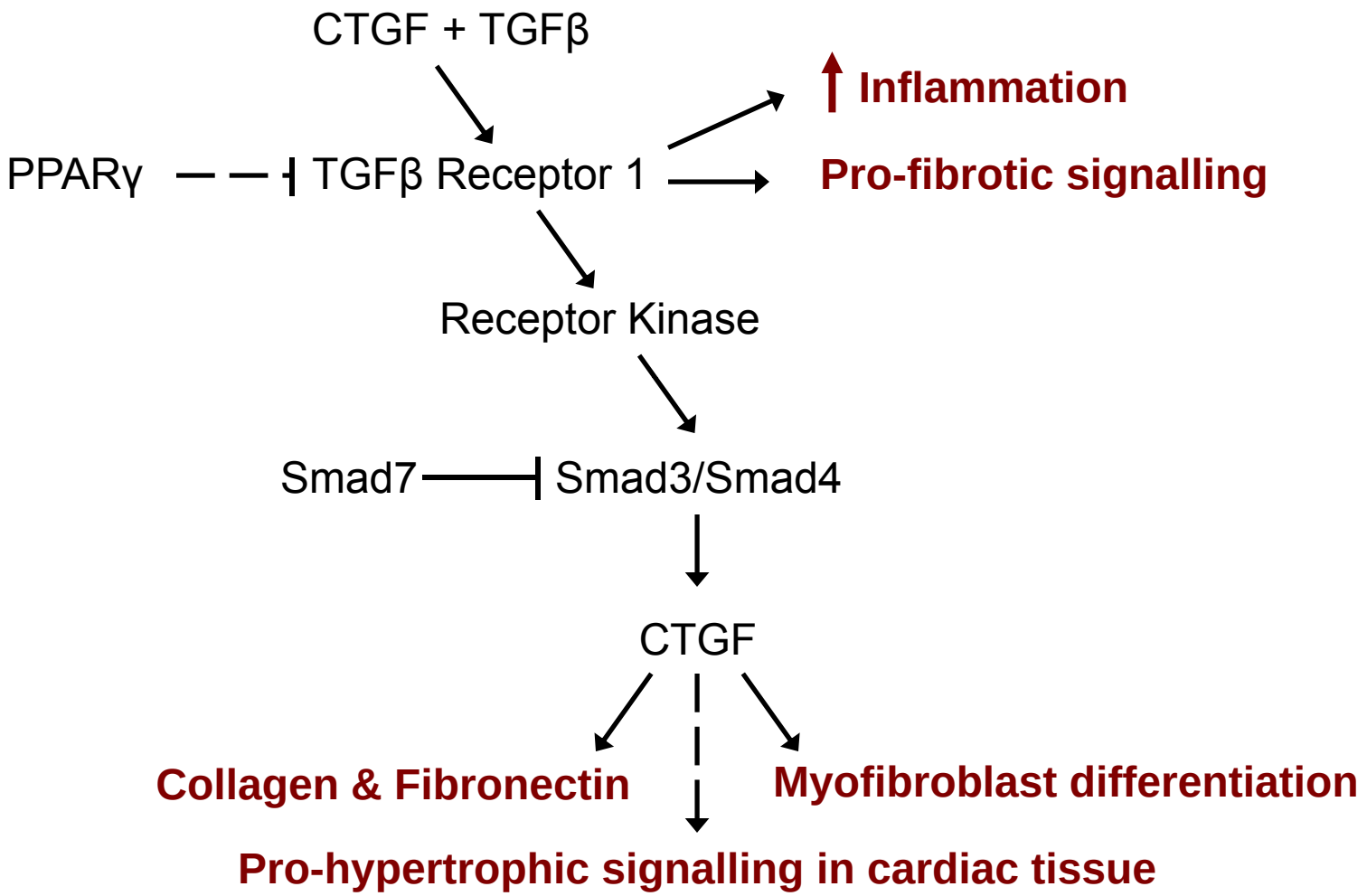
- **Decreased uptake of E2 containing TG-rich remnants lowers intracellular cholesterol pool and up-regulates the LDLR, lowering LDL cholesterol in plasma.**
- **Increased uptake of E4 containing TG-rich remnants raises intracellular cholesterol pool and down-regulates the LDLR, raising LDL cholesterol in plasma.**

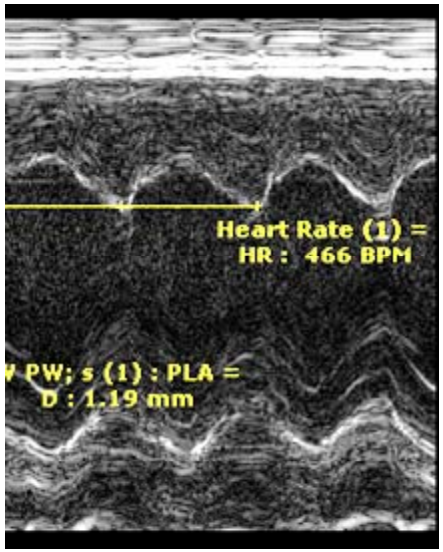
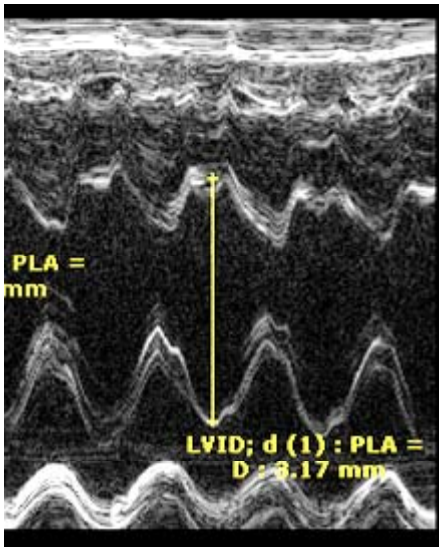
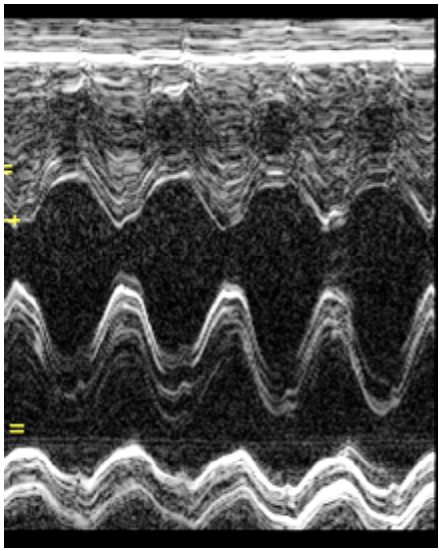
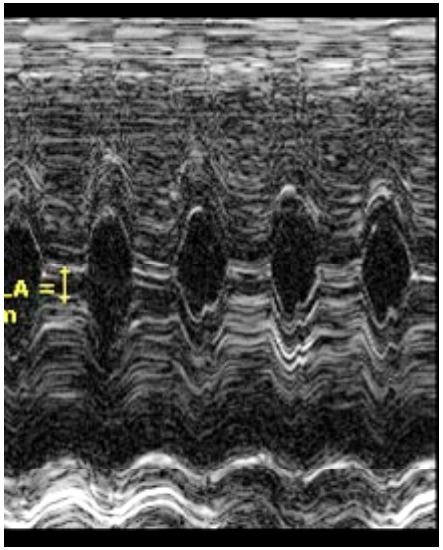
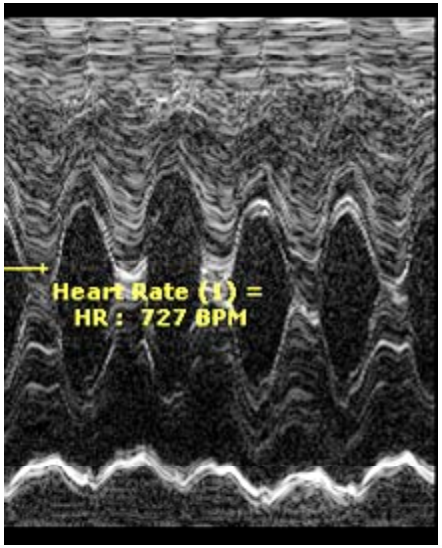
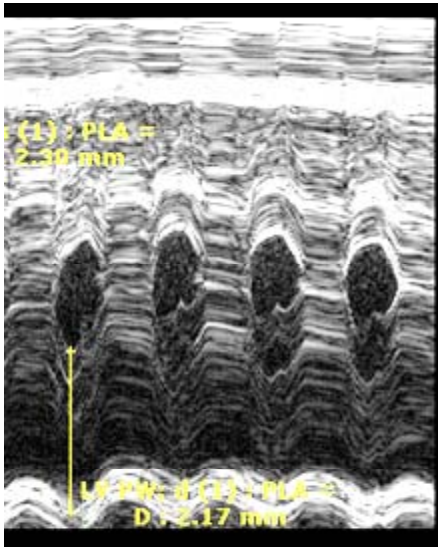
Connective Tissue Growth Factor (CCN2)



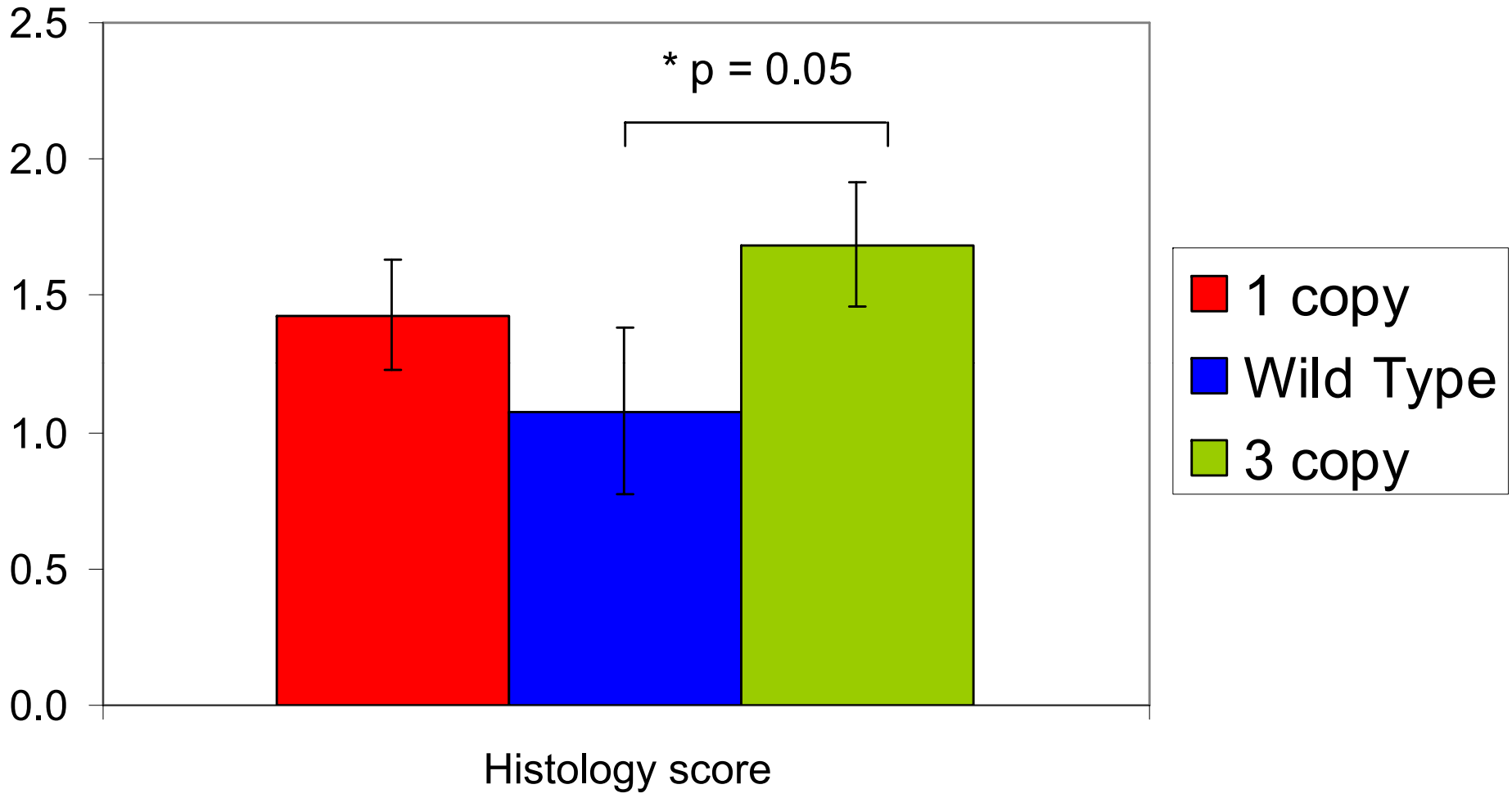
Modified from Leask, A. et al. J Cell Sci 2006;119:4803-4810

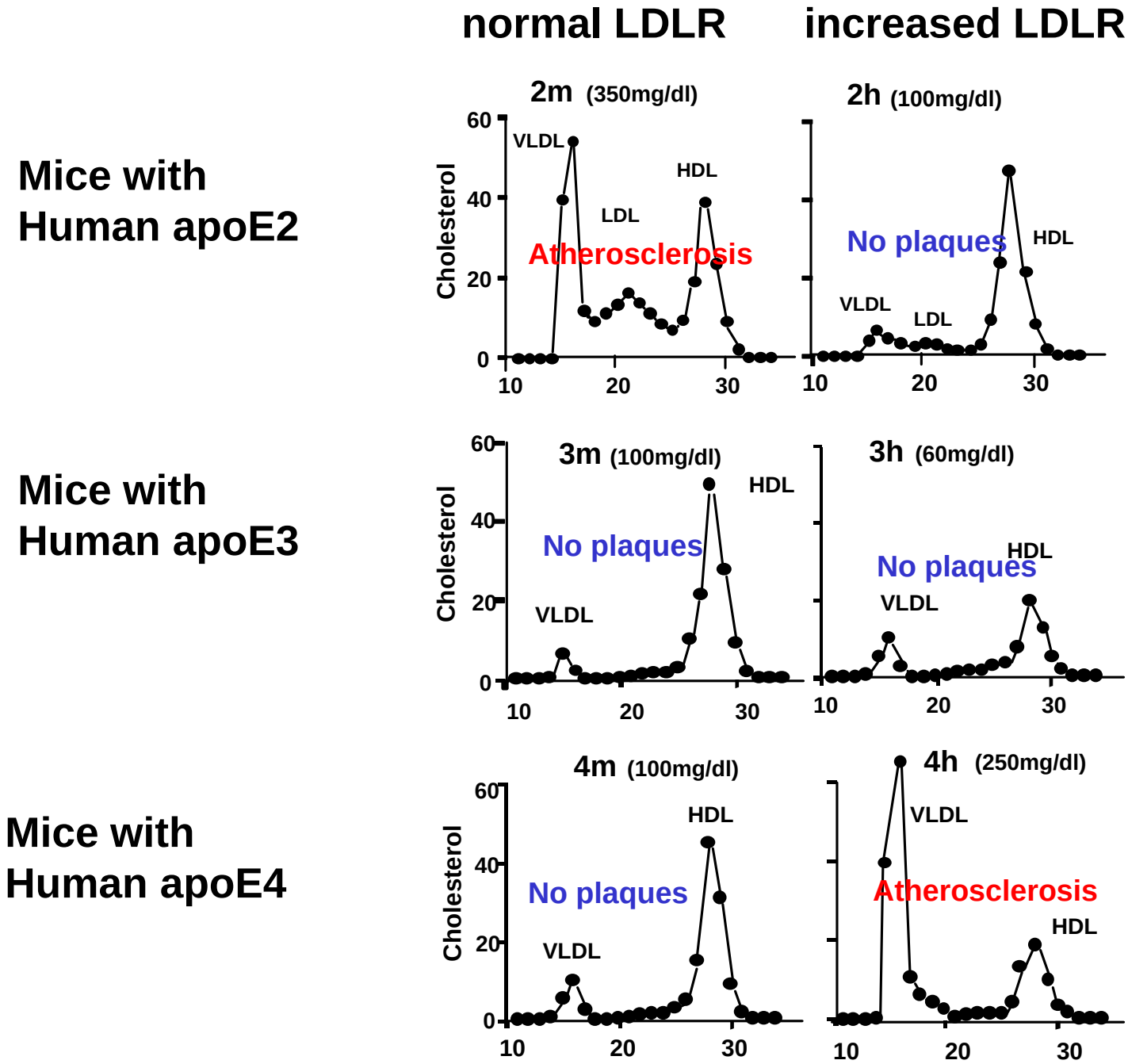
CTGF & Fibrosis

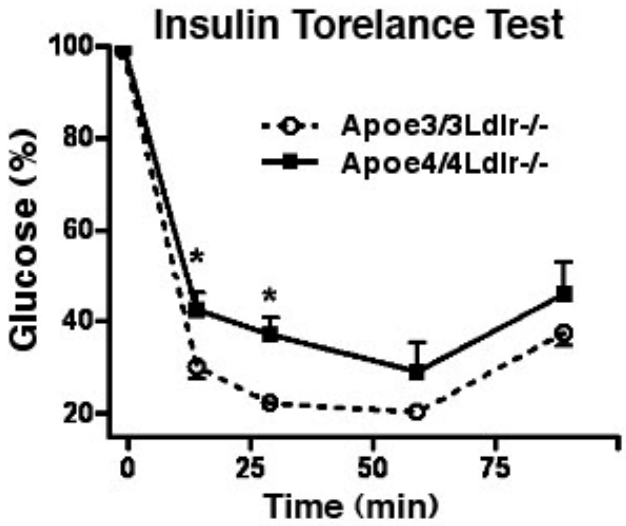


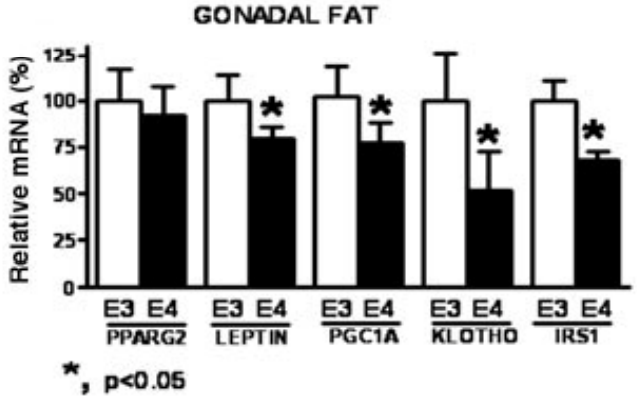
	1 copy	Wild Type	3 copy
Pre-treatment			
2 weeks Ang II			

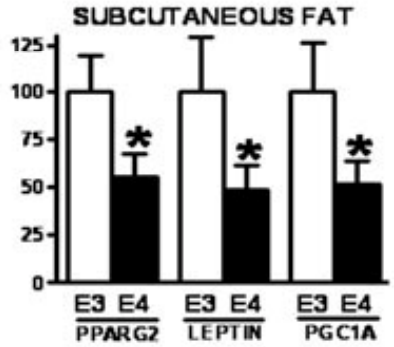
Cardiac Fibrosis Histology Score




normal LDLR
increased LDLR
Mice with Human apoE2
Mice with Human apoE3
Mice with Human apoE4
2m (350mg/dl)
2h (100mg/dl)
3m (100mg/dl)
3h (60mg/dl)
4m (100mg/dl)
4h (250mg/dl)
Cholesterol
VLDL
LDL
HDL
Atherosclerosis
No plaques
0
20
40
60
10
20
30


Insulin Torelance Test
Glucose (%)
100
80
60
40
20
0
25
50
75
Time (min)
Apoe3/3Ldlr-/-
Apoe4/4Ldlr-/-
*
*


GONADAL FAT
Relative mRNA (%)
125
100
75
50
25
0
E3 E4
PPARG2
E3 E4
LEPTIN
E3 E4
PGC1A
E3 E4
KLOTHO
E3 E4
IRS1
*
*
*
*
*
*, p<0.05


SUBCUTANEOUS FAT
125
100
75
50
25
0
E3 E4
PPARG2
E3 E4
LEPTIN
E3 E4
PGC1A
*
*
*