AMDCC: UCLA Cardiovascular Models

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Animal Models

1. Elderly LDLR-/- on Western diet
2. *Hyplip* Mouse
3. Skeletal muscle PPARγ knockout x LDLR-/-
4. Apolipoprotein AII transgenic x apolipoprotein E-/-

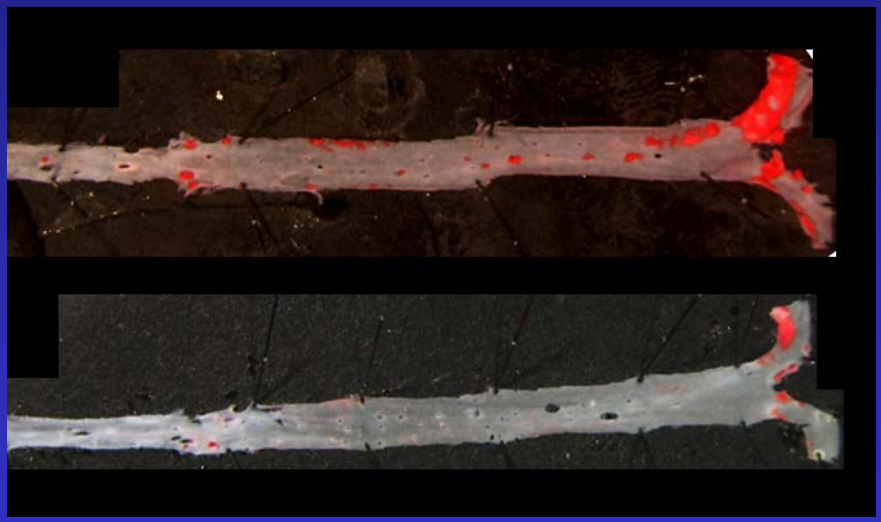
Experimental Protocol

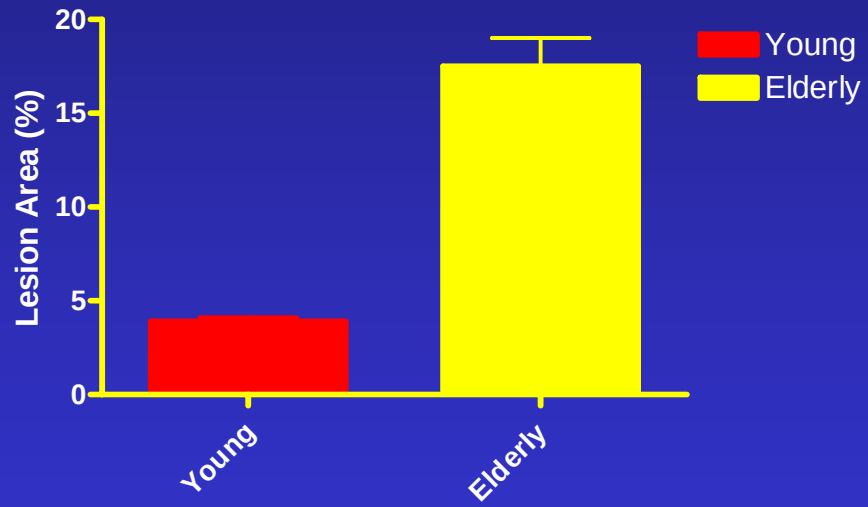
Age at start of Protocol	3 Months	Atherosclerosis

(Young) 3 Months ⟶ Western Diet: Fatty Streaks

(Elderly) 10-12 months Western Diet: Advanced Lesions

Elderly LDLR-/- mice have greater aortic lesion area compared to Young LDLR-/- mice





Lesion Complexity Score in LDLR-/- mice

Young	**6%**
Elderly	**90%**

Defined as the percentage of lesions exhibiting necrotic lipid cores, cholesterol clefts, fibrous caps, etc. of the total lesions

Body Weight, Food Consumption and Blood Pressure

	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Feed Consumption (g/d)	Systolic Blood Pressure (mmHg)
Young LDLR-/-	24±1.1	42±1.5	18	2.5±0.40	101±3
Elderly LDLR-/-	33±1.8 *	51±2.3 *	18	2.8±0.36	105±5

* p<0.05

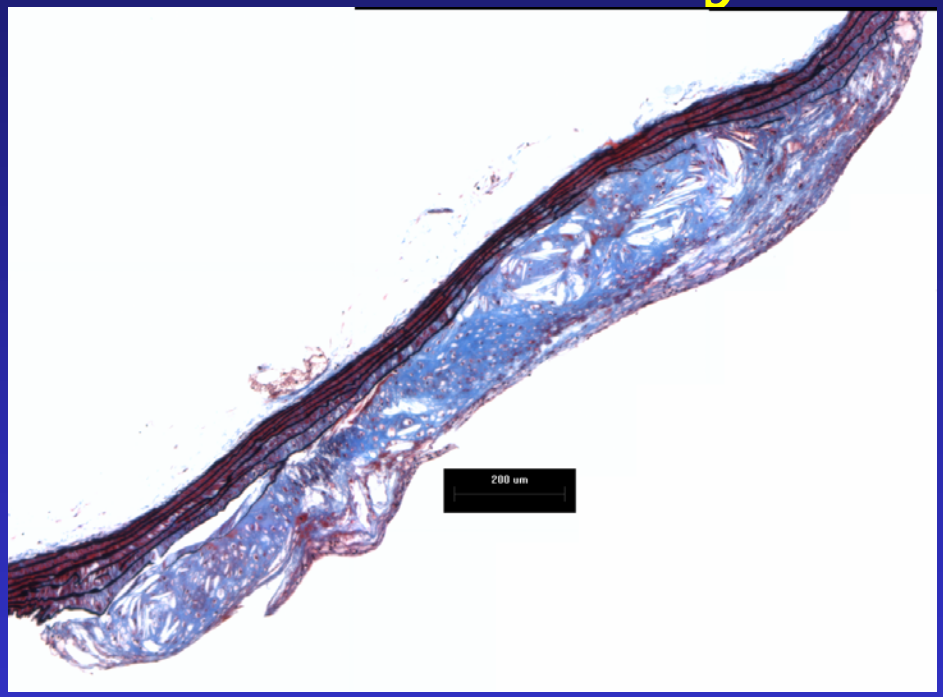
Plasma Metabolic Markers

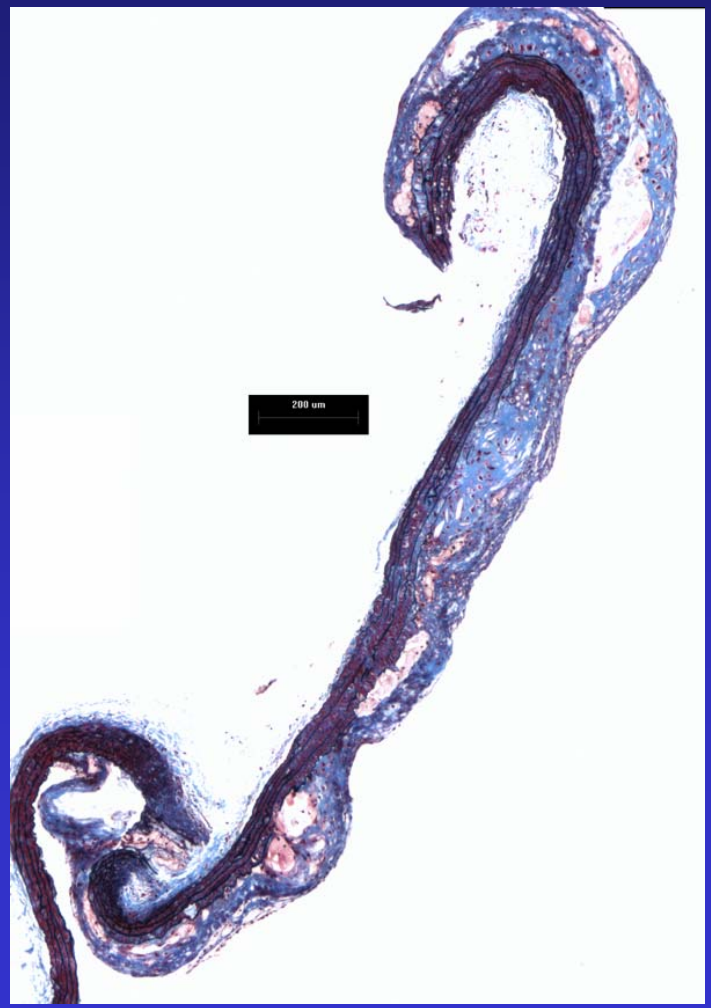
	Total Cholesterol (mg/dl)	HDL-C (mg/dl)	Triglycerides (mg/dl)	Glucose (mg/dl)	Insulin (pg/ml)
Young LDLR -/-	1842±52	105±8	142±8	285±25	1199±150
Elderly LDLR -/-	1928±154	67±5*	371±60*	423±27*	2609±354 *

* p<0.05

Trichrome Stained Lesion from Young LDLR-/- Mouse

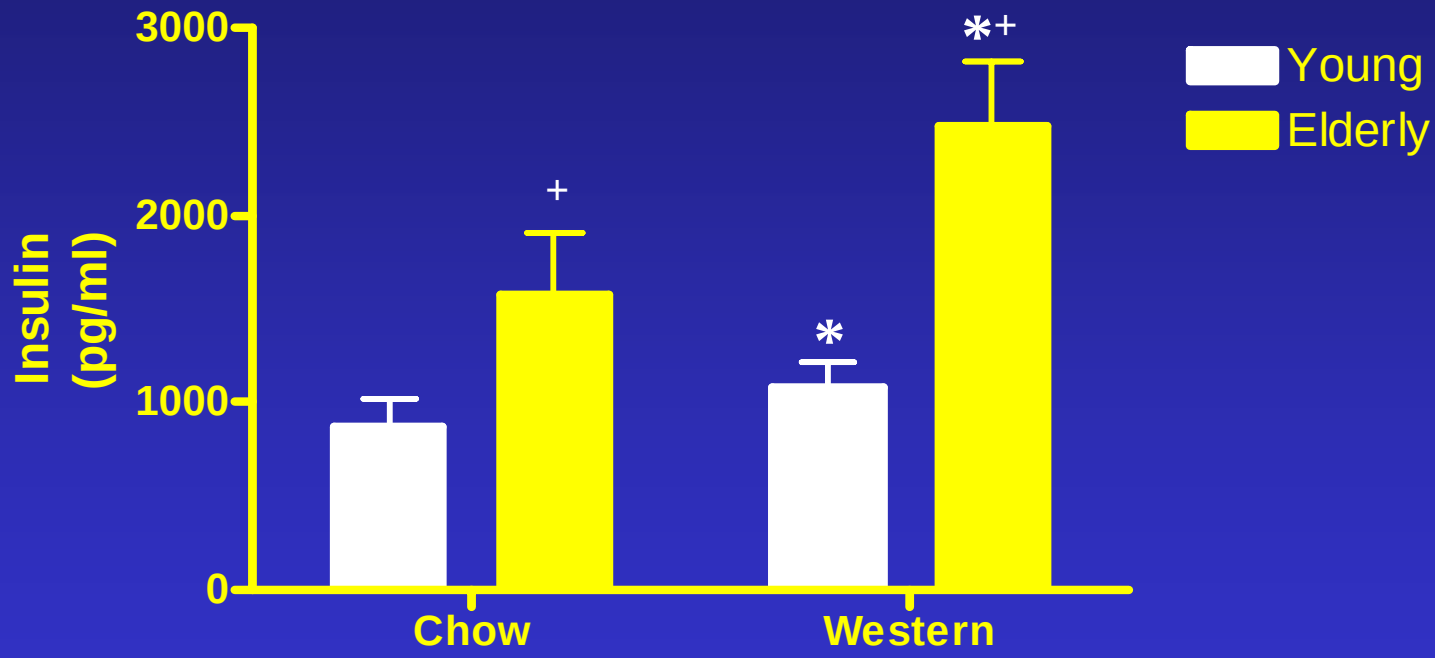
Trichrome Stained Lesions from Elderly LDLR-/- Mice





Black markers are 200 μm in length

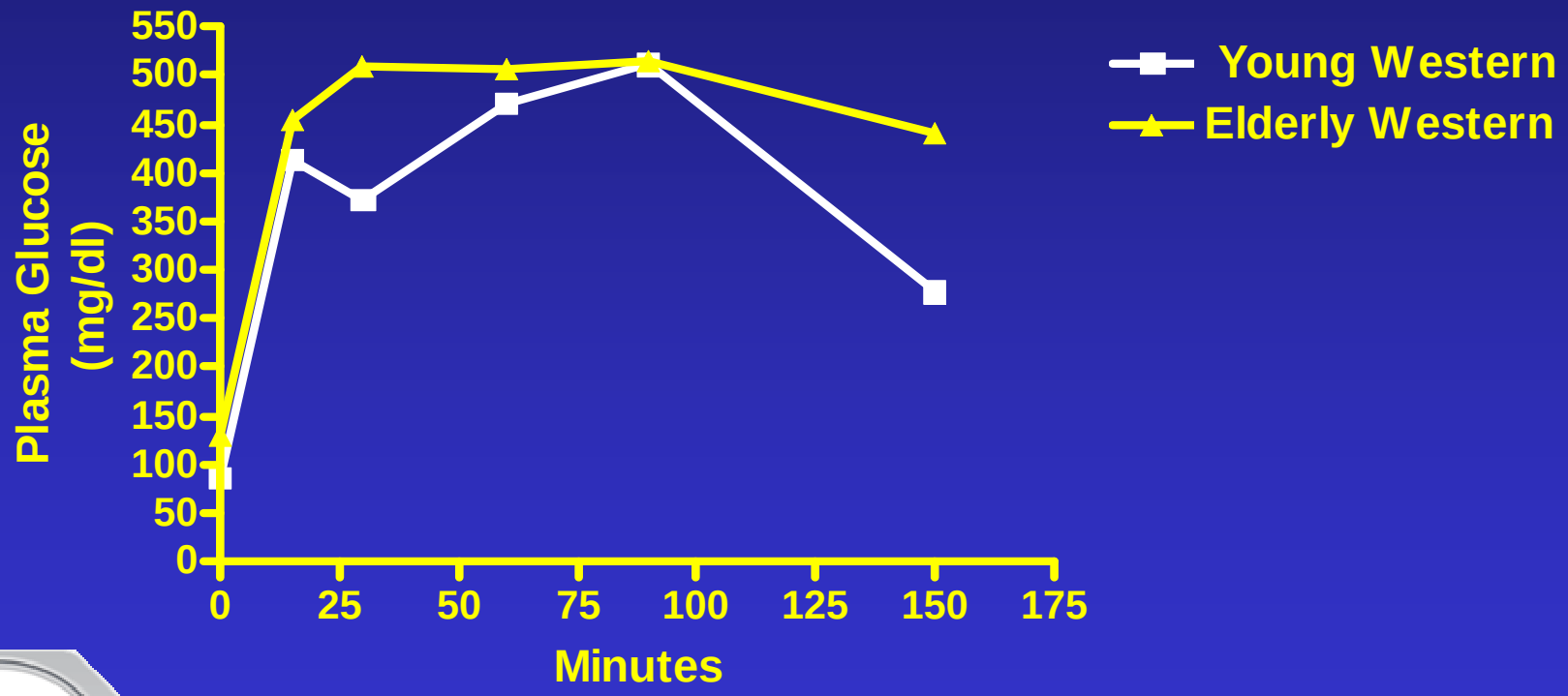
Effects of Age and Diet on Insulin in Male LDLR-/- Mice



* p<0.05 Western vs chow

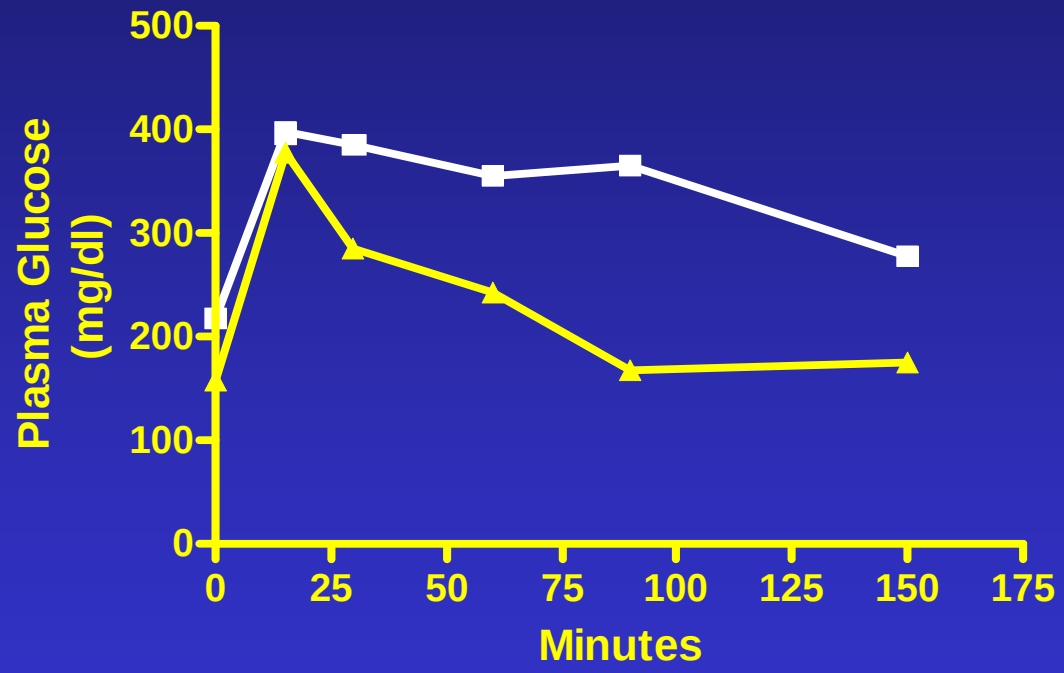
+ p<0.05 Elderly vs young

Oral Glucose Tolerance Test of Elderly and Young LDLR-/- Mice



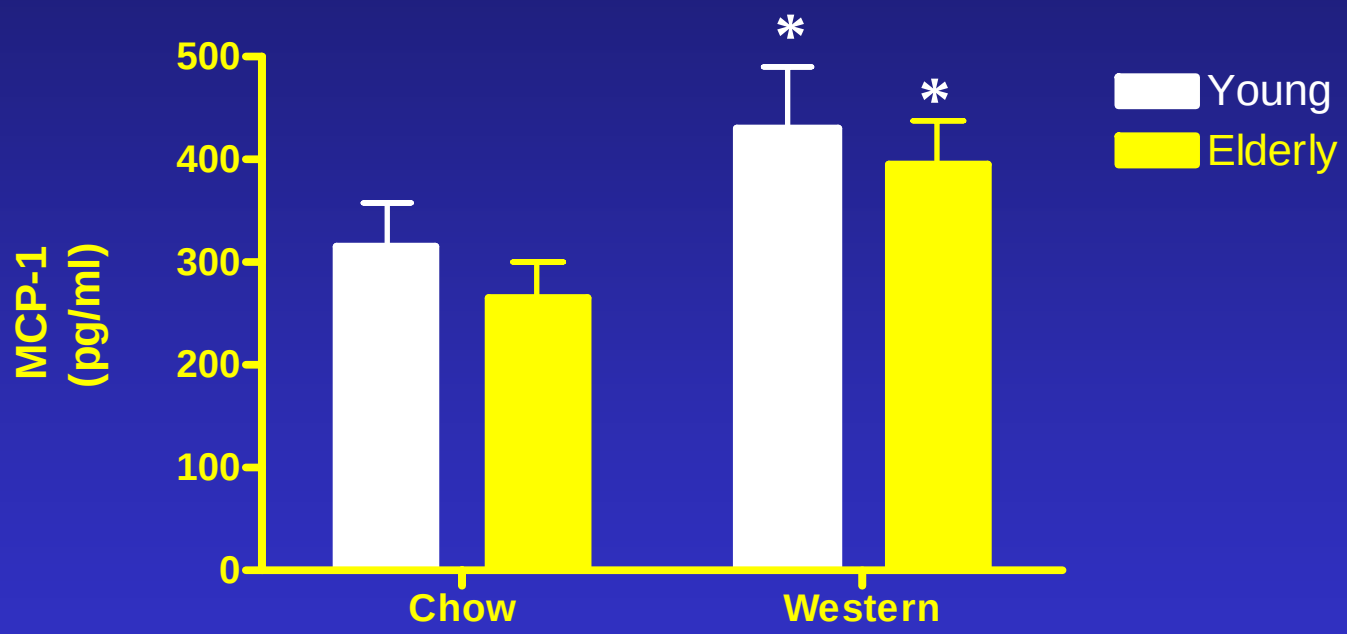
Oral Glucose Tolerance Test (1mg/kg) after 3 months of Western Diet

Oral Glucose Tolerance Test of Elderly LDLR-/- Mice on Western or Clinton-Cybulsky Diets



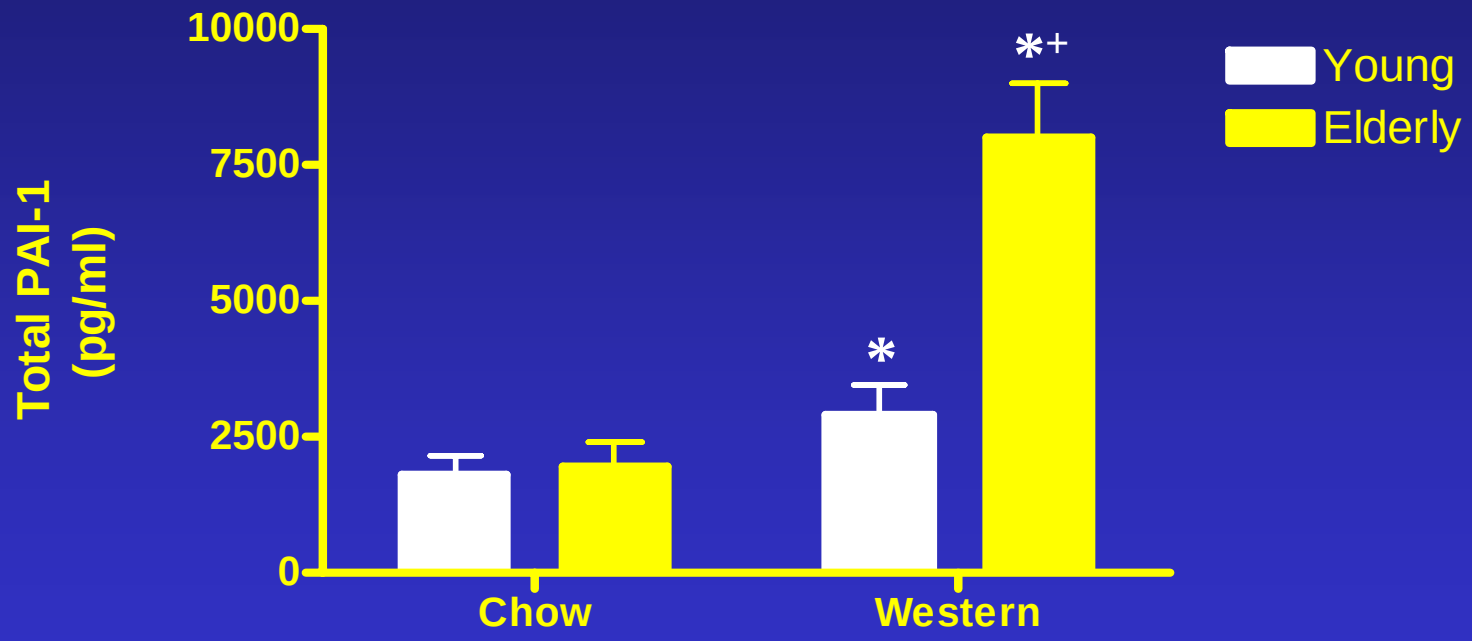
Oral Glucose Tolerance Test (1mg/kg) after 1 month

Effects of Age and Diet in Male LDLR-/- Mice



* p<0.05 Western vs chow

Effects of Age and Diet in Male LDLR-/- Mice



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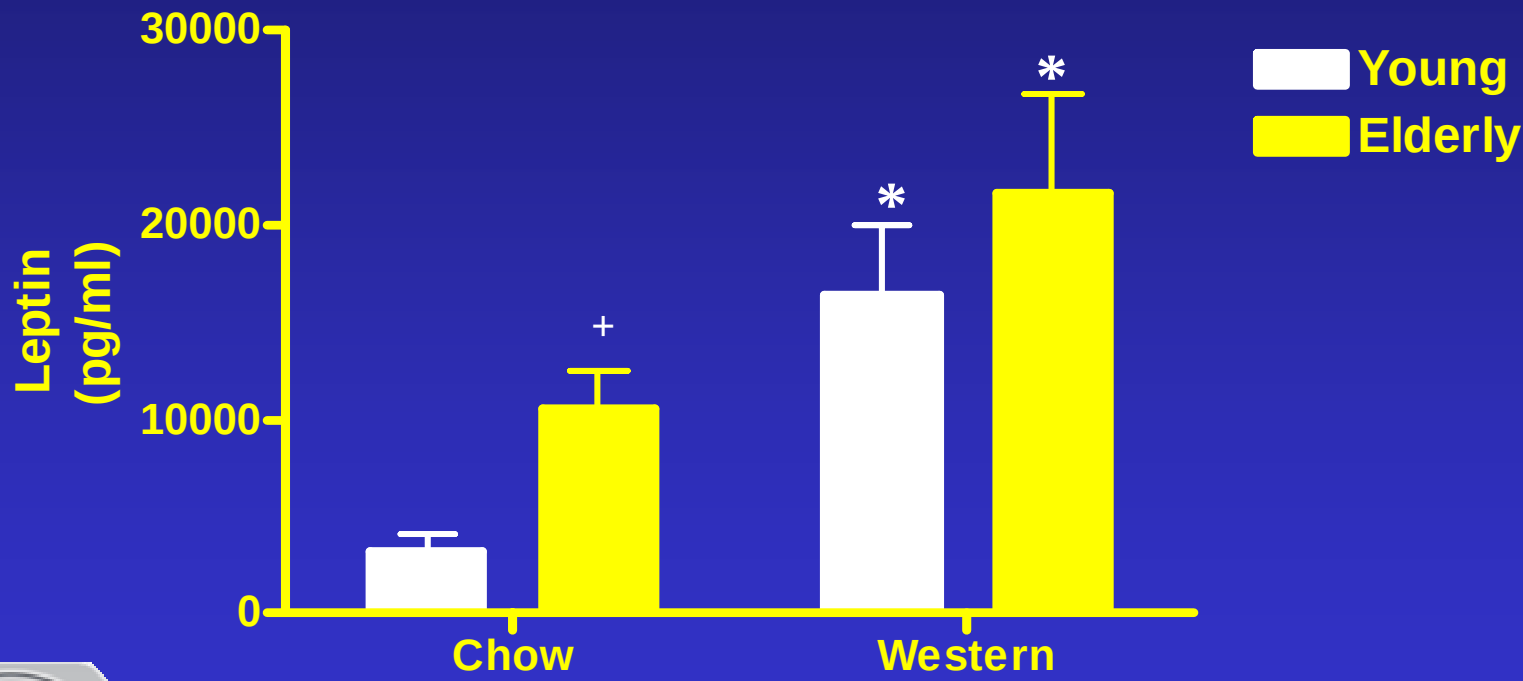
+ p<0.05 Elderly vs young

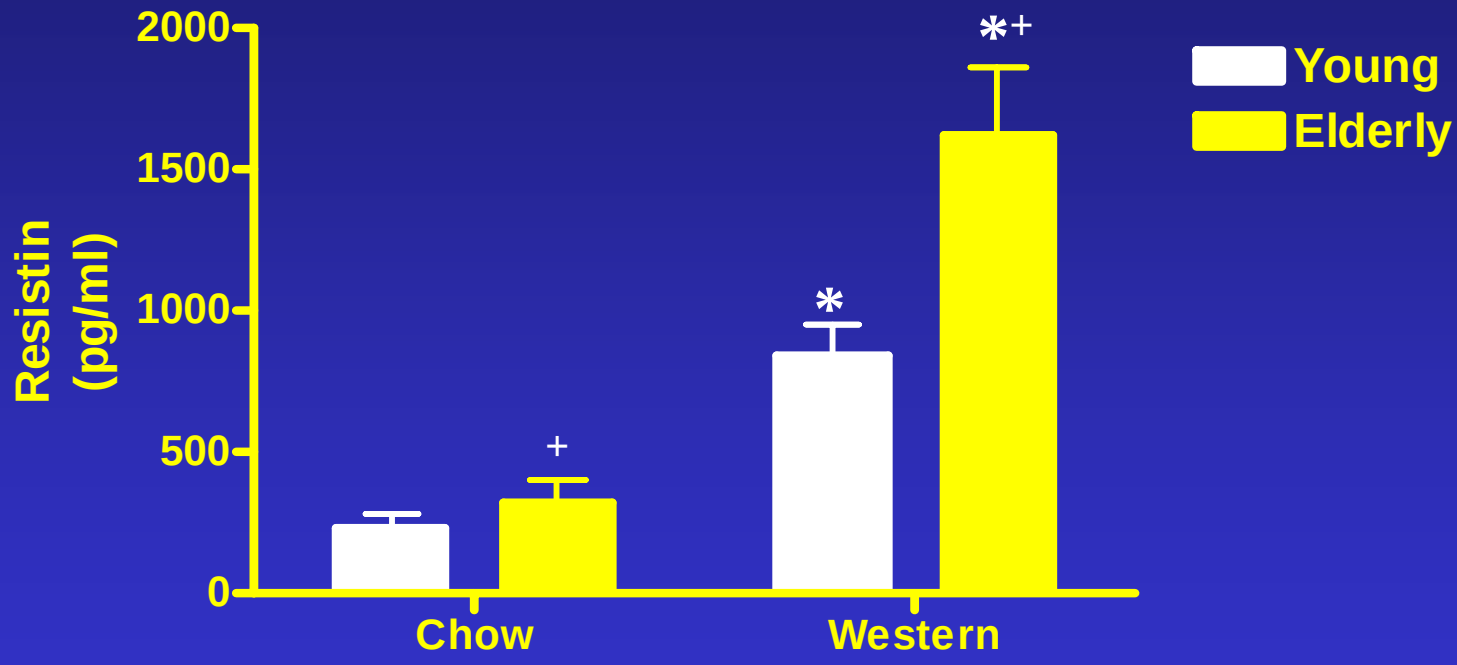
Effects of Age and Diet in Male LDLR-/- Mice



* p<0.05 Western vs chow

+ p<0.05 Elderly vs young

Effects of Age and Diet in Male LDLR-/- Mice



* p<0.05 Western vs chow

+ p<0.05 Elderly vs young

No Differences in Young vs Elderly LDLR-/- Mice

TNF-α

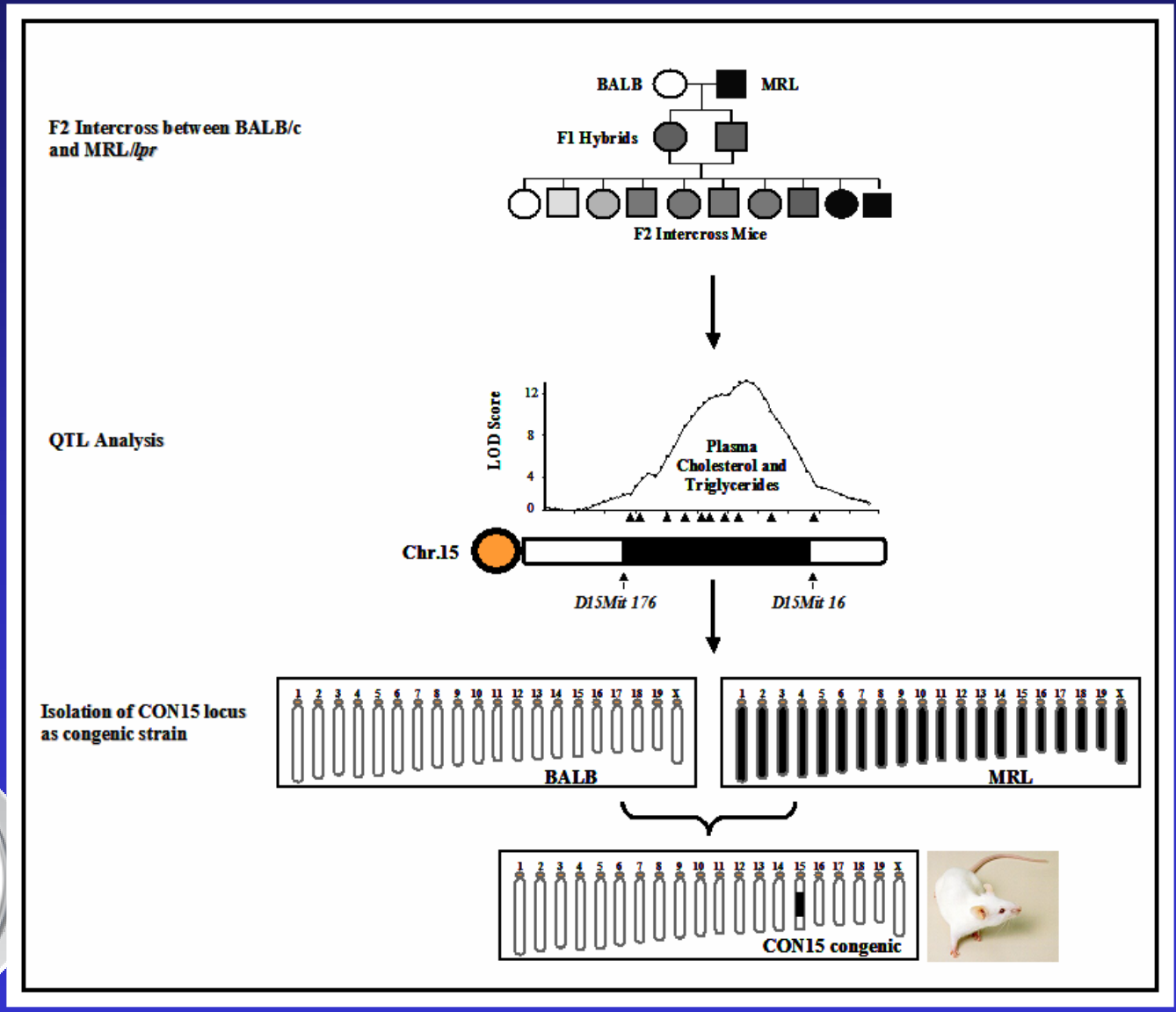
IL-6

Adiponectin

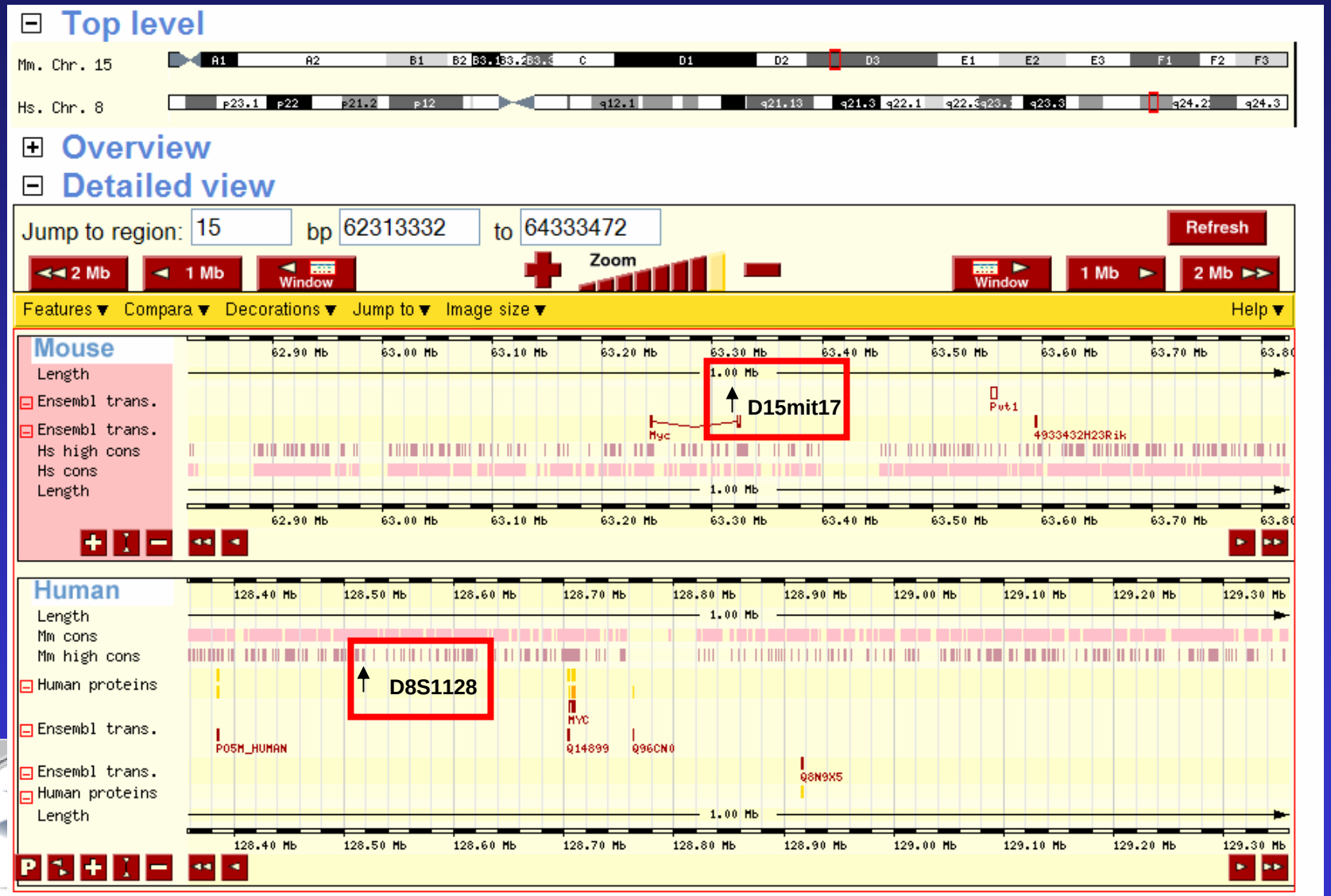
Summary

1. Elderly LDLR-/- have substantial acceleration of the extent and complexity of lesions compared to young LDLR-/- mice
2. Elderly LDLR-/- have Metabolic syndrome
    1. ↑ obesity
    2. ↑ triglycerides
    3. ↓ HDL cholesterol
    4. ↑ hyperglycemia
    5. ↑ hyperinsulinemia
3. Have increased circulating inflammatory biomarkers
    1. ↑ PAI-1
    2. ↑ resistin
    3. ↑ leptin

A congenic strain (CON15) containing the chromosome 15 region from MRL on a BALB/c background

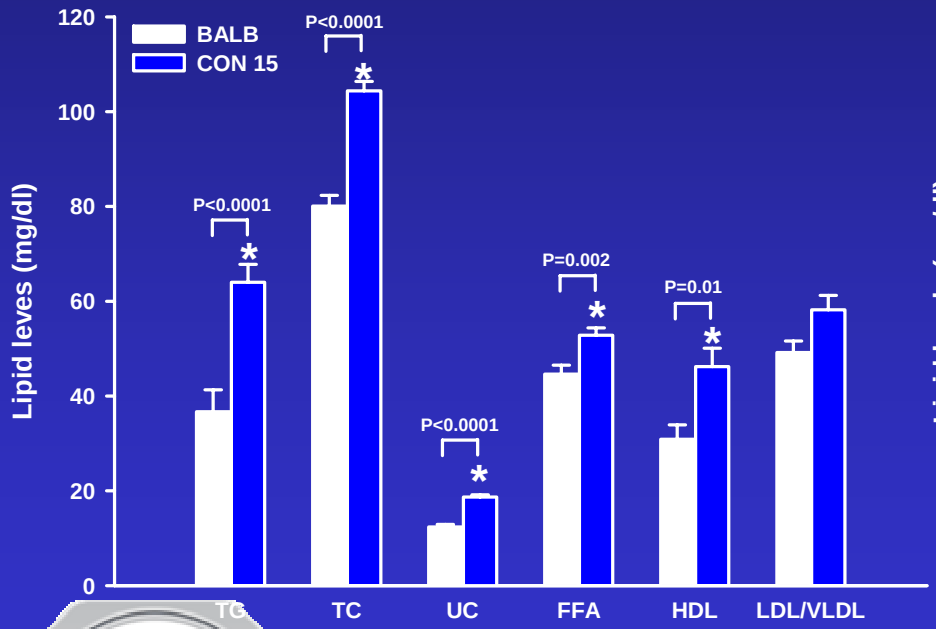


Peak of the *Hyplip2* locus maps within 1Mbp of human syntenic Chr. 8 FCHL locus

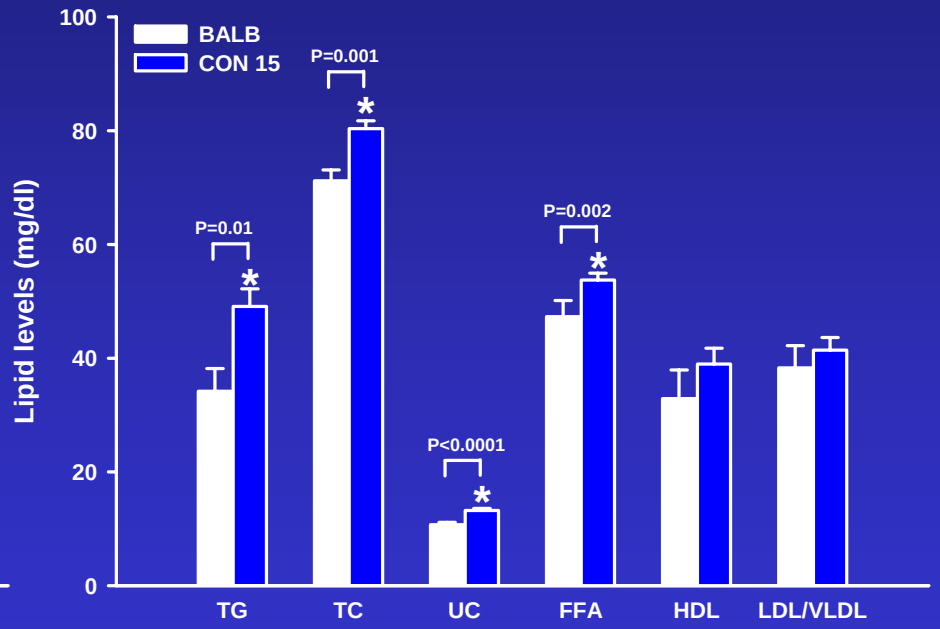


CON15 vs BALB plasma lipid levels on chow diet

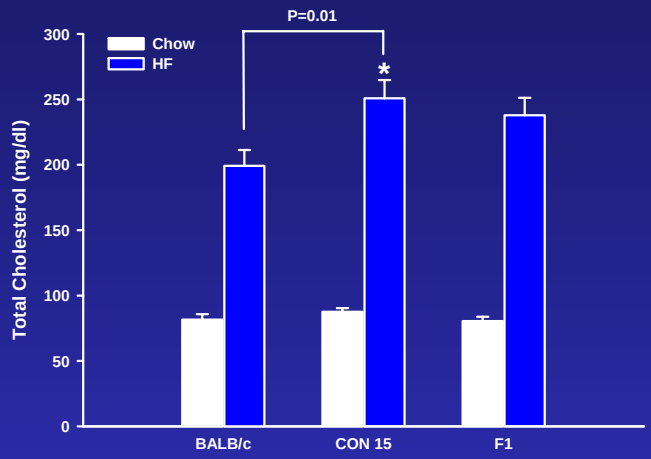
Males

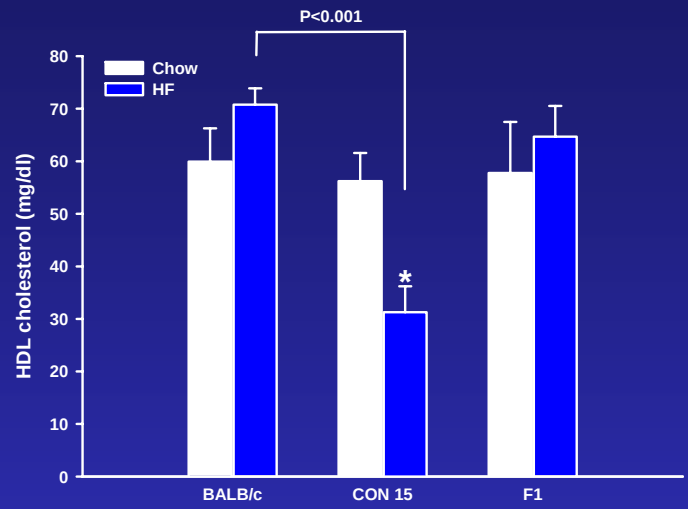


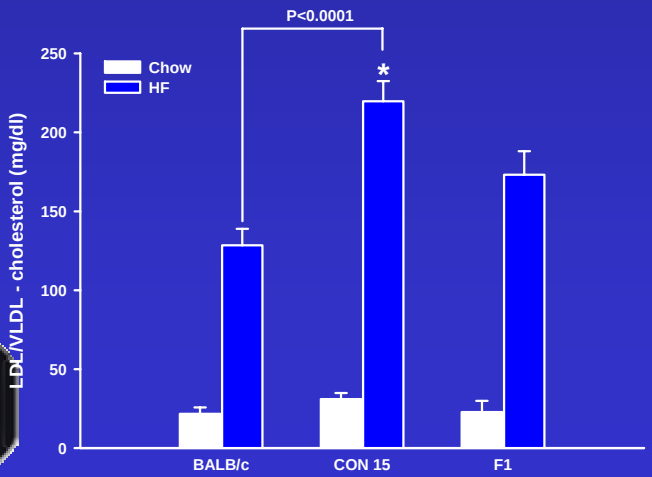
Females

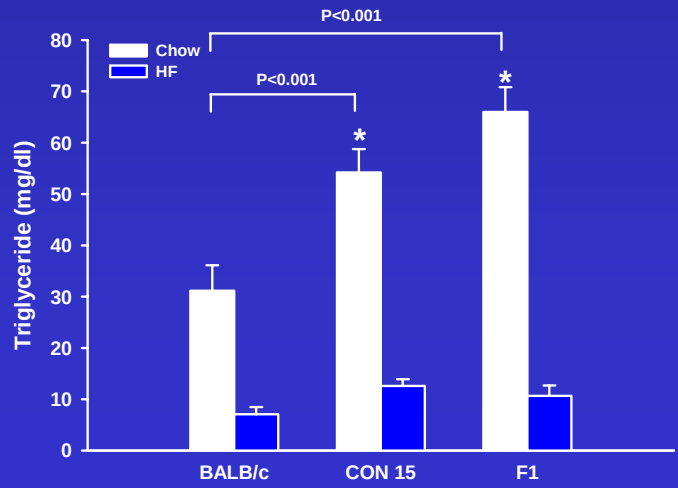


Plasma lipid levels on HF diet

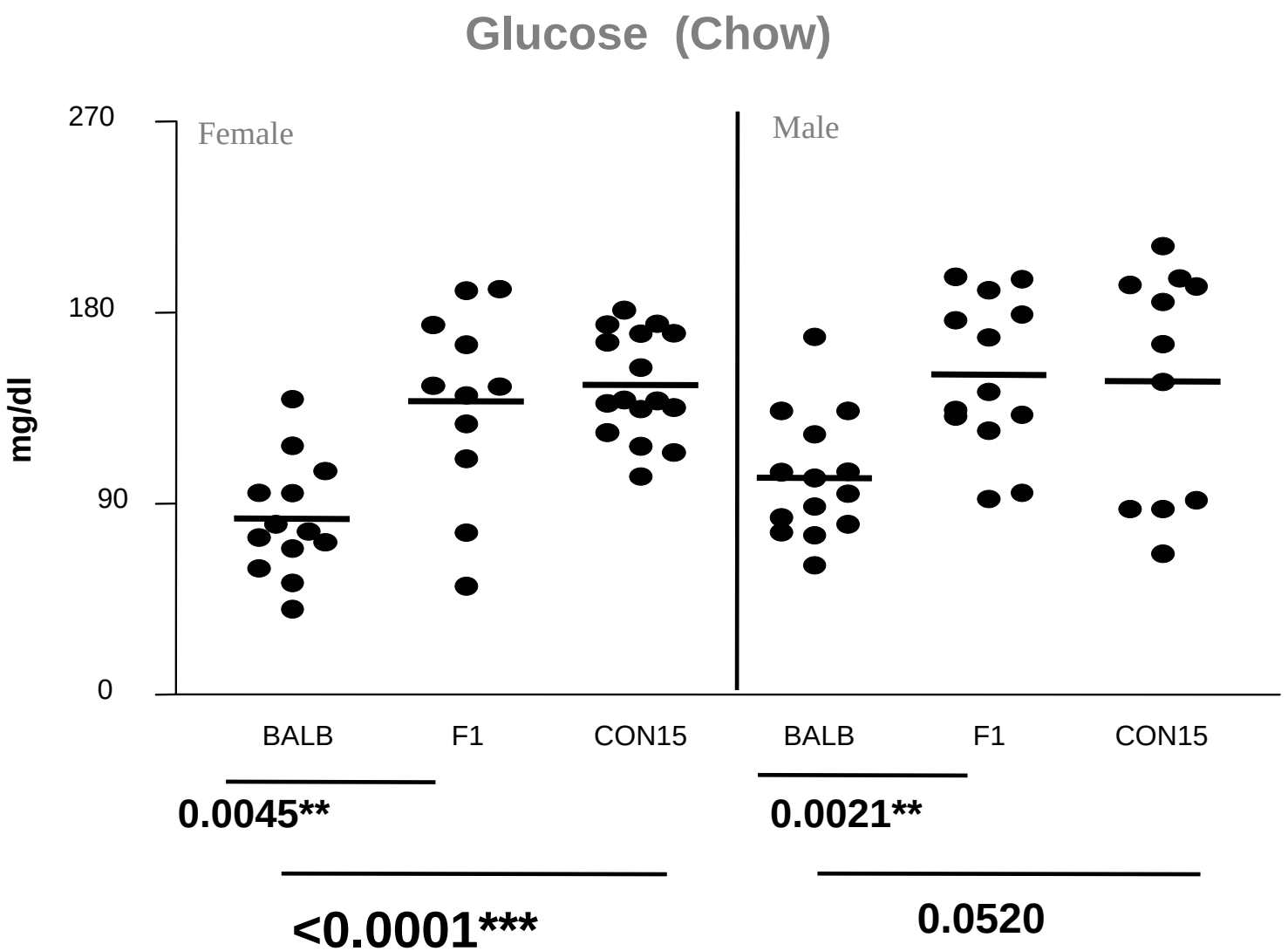




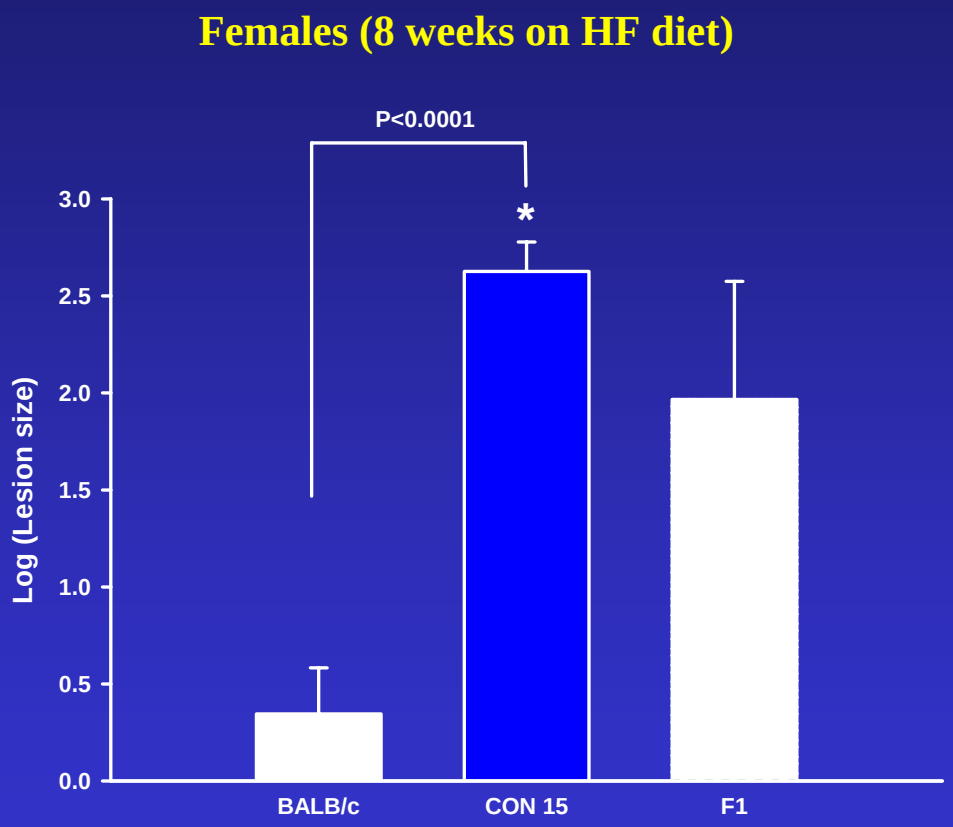




CON15 mice have elevated glucose levels



CON15 mice exhibit a 30-fold increase in atherosclerotic lesions



Wang, Gargalovic et al. ATVB 2004 24:1928-1934

Renal Injury in the *Hyplip 2 (LDLR-/- BALB)* Mouse

- **STZ induced Diabetes**
  - **51 mg/dl BUN vs 36 for BALB STZ mice $p<0.01$**
  - **No differences in blood glucose**
  - **No differences in glomerular cellularity or ECM or inflammation**
- **STZ induced Diabetes and Western Diet**
  - **Increased cholesterol (724 vs 139 mg/dl, BUN (69 vs 32 mg/dl) compared to BALB STZ/Western Diet mice**
  - **Increased cellularity and ECM in the glomerulus**
  - **Increased macrophages infiltration of the glomerulus (1.9 vs 0.5 macrophages/gcs)**

Spencer et al. *AJN* 24:20, 2004

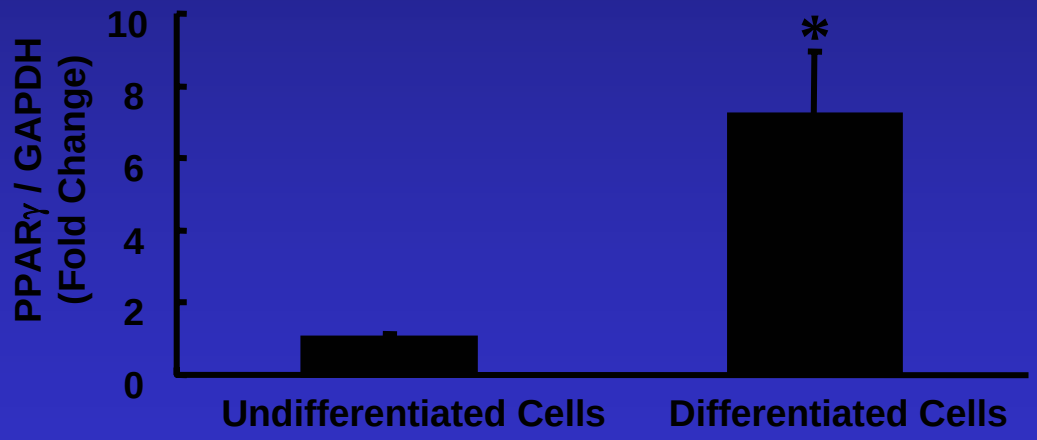
Hyplip2 Present studies

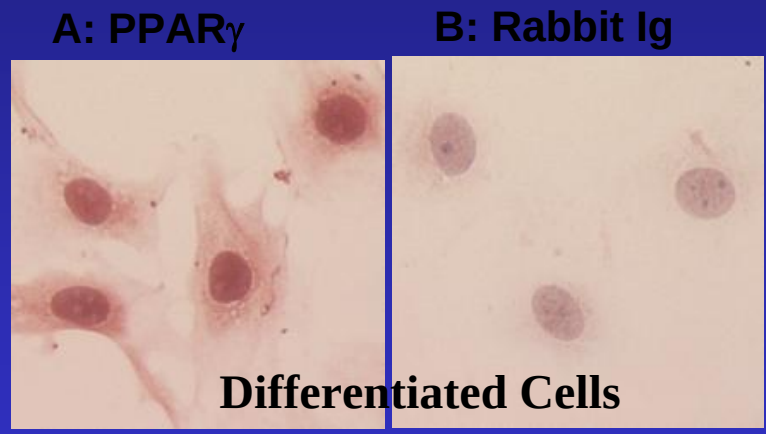
-We are attempting to identify the gene underlying the Chromosome 15 region by fine mapping with subcongenic mice and by expression array analysis

-The CON15 mouse is being bred to BALB/c.LDLR$^{-/-}$ mice to test for effects on advanced atherosclerotic lesions and other pathologies associated with diabetes

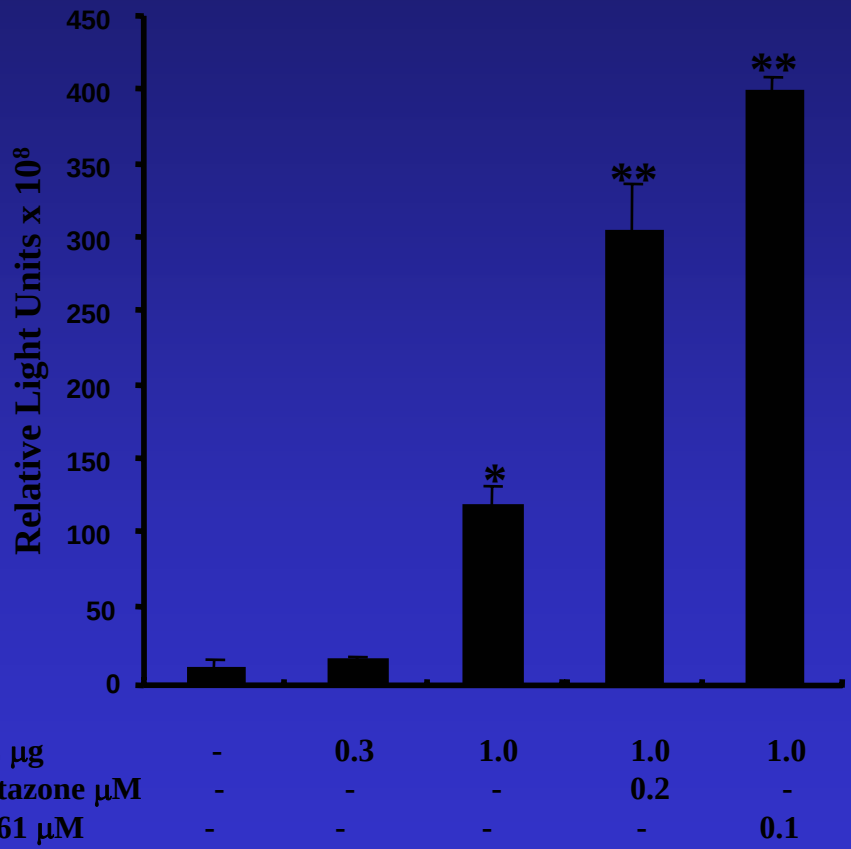
-The CON15/LDLR$^{-/-}$ mice on a BALB/c background may be a useful model of atherosclerosis and nephropathy in a hyperlipidemic, hyperglycemic mouse

PPARγ receptors are expressed in podocytes

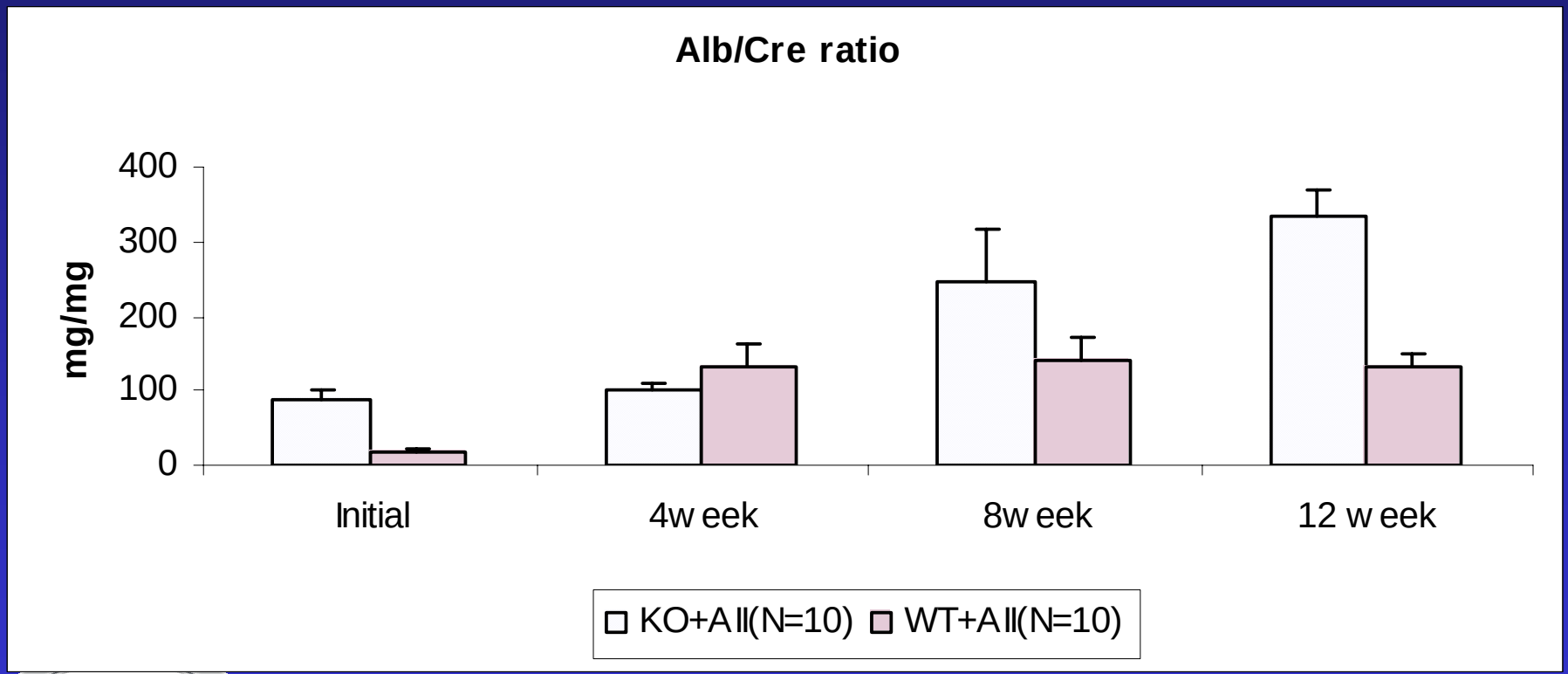




PPARγ in Podocytes is Functional



Neph2Cre × PPARγflox develop albuminuria with AngII infusion



Skeletal Muscle Specific PPARγ Knockout Mice

- Obtained from Jerry Olefsky at University of California San Diego
- Model of Insulin Resistance and Type 2 DM

Skeletal Muscle Specific PPARγ Knockout Mice Breeding Scheme

skCRE$^{+/o}$ PPARγfl$^{+/+}$ LDLR$^{+/+}$ X skCRE$^{o/o}$ PPARγfl$^{-/-}$ LDLR$^{-/-}$ → skCRE$^{+/o}$ PPARγfl$^{+/-}$ LDLR$^{+/-}$ X skCRE$^{o/o}$ PPARγfl$^{-/-}$ LDLR$^{-/-}$

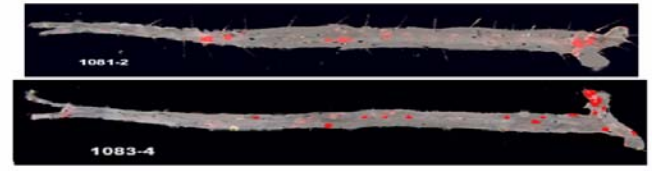
skCRE$^{+/o}$ PPARγfl$^{+/-}$ LDLR$^{-/-}$ X skCRE$^{+/o}$ PPARγfl$^{+/-}$ LDLR$^{-/-}$ → skCRE$^{+/o}$ PPARγfl$^{+/+}$ LDLR$^{-/-}$ & skCRE$^{o/o}$ PPARγfl$^{+/+}$ LDLR$^{-/-}$

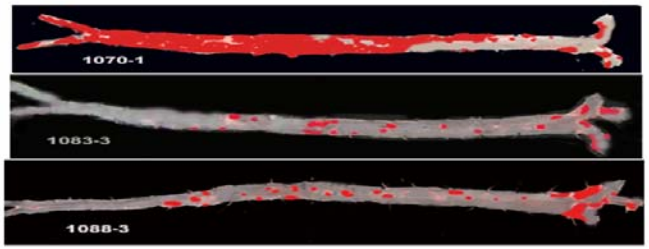
Knockouts **Littermate Controls**

We are currently expanding the numbers of knockouts and littermate controls

Apolipoprotein AII Trangenic Mice

- Develop insulin resistance and exhibit increased atherosclerotic lesion formation
- May have more pronounced atherosclerosis on an apoE-/- background compared to the apoE-/- alone
- May be a model of dyslipidemia and FFA accelerated atherosclerosis

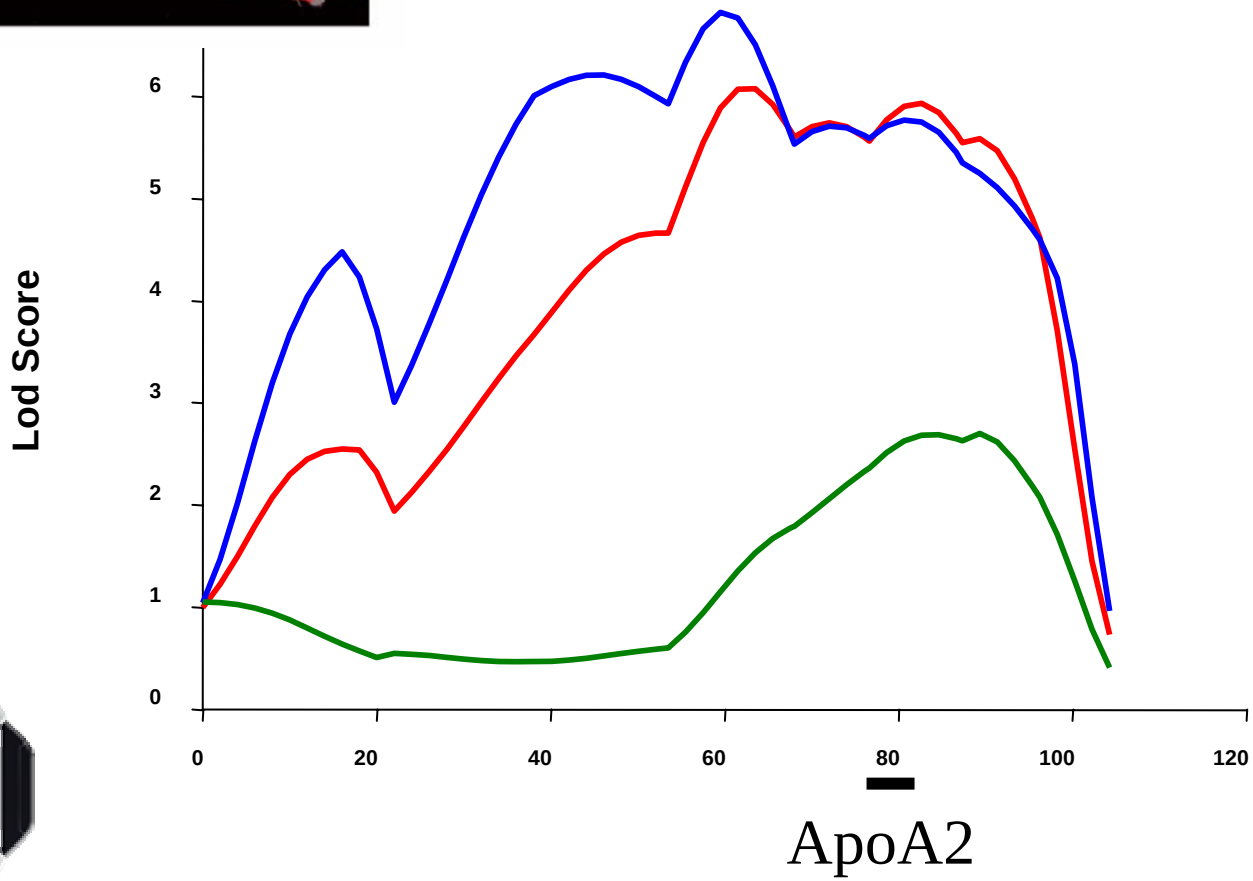




Lesion QTLs

females & males
females
males

Chromosome 1

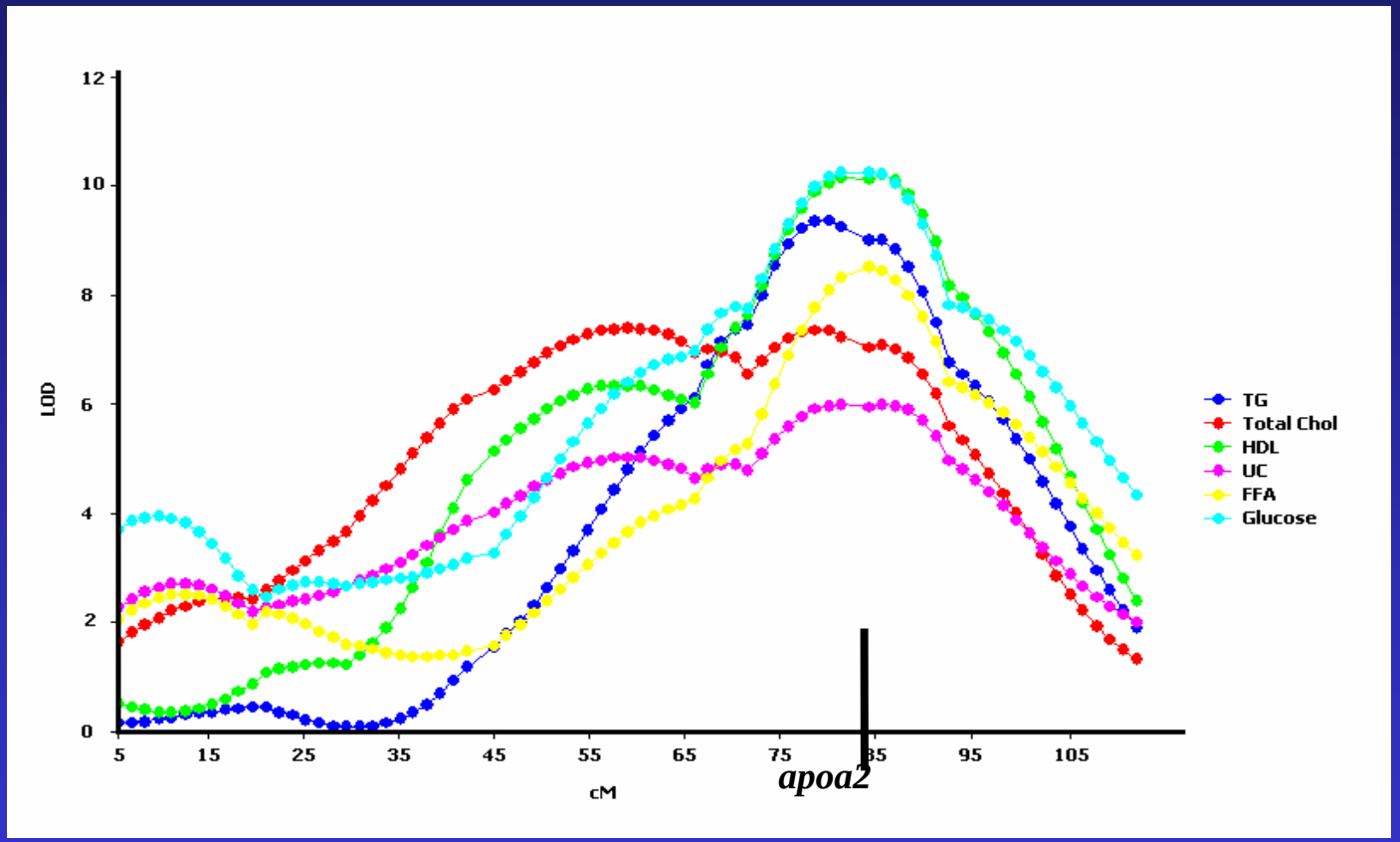


C57BL/6 X C3H apoE-/- Cross

- **F2s fed Western diet – 16 weeks**
- **Sac at 24 weeks**
- **328 F2s; 164 males, 164 females**
- **Genotyping by Marshfield (140 markers, 10cM map) & ParAllele (2,000 markers, 2cM map)**

- **Note: C3H mice have an allelic variant of *apoa2* that results in plasma apoAII concentrations that are approximately double those of the other parental strain, C57BL/6.**

LOD Scores for Metabolic and Lipid Traits on Murine Chromosome 1



LOD scores for plasma lipids and glucose concentrations over the *apoa2* gene locus on mouse Chromosome 1. Genotyping by Marshfield (140 markers, 10cM map) and ParAllele (2,000 markers, 2 cM map) on 382 F2 mice (164 males, 164 females) from a cross between strains C57BL6 (B) and C3H (H), in which both inbred strains were on an apoE knockout (apoE ko) background. The mice were fed Western diet for 16 weeks and sacrificed at 24 weeks of age.

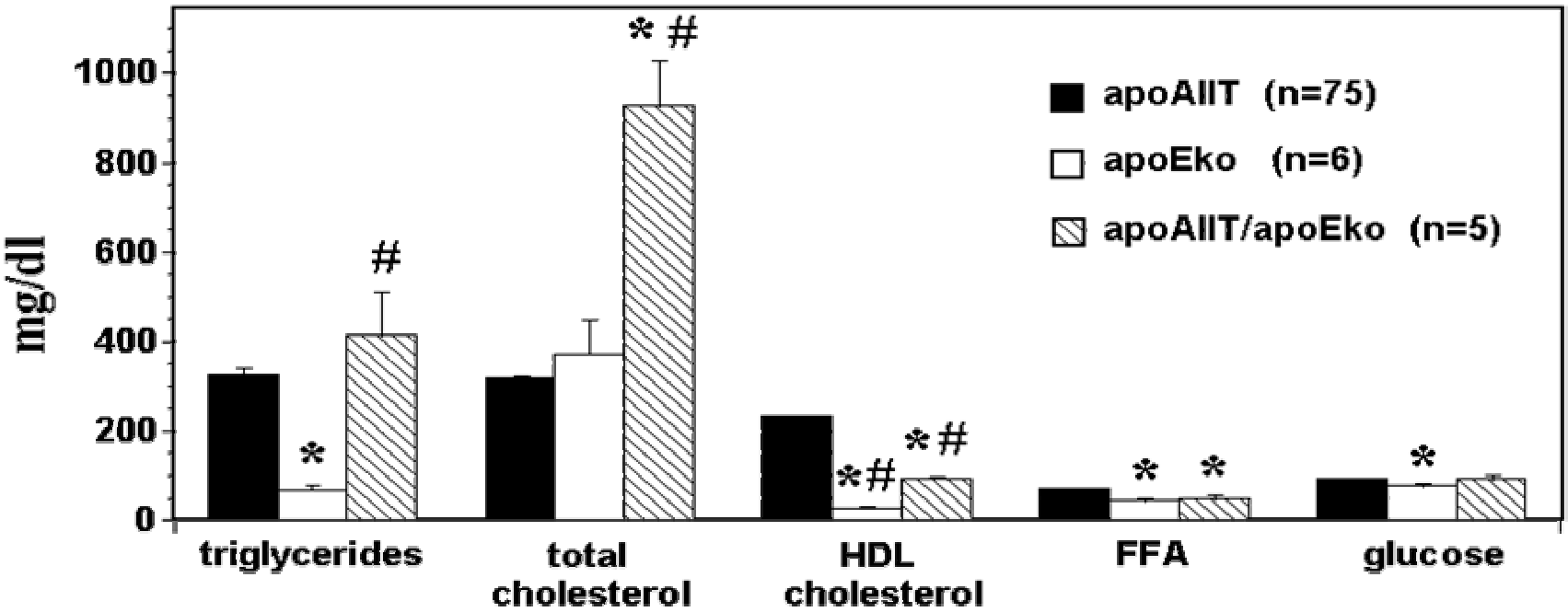
We previously reported that C3H mice exhibit an increase in plasma apoAII concentrations compared to strain C57BL6. In the present cross, the C3H apoa2 allele was associated with significant increases in all of the traits described above. Apparently, only a small increase in plasma apoAII concentrations on the apoE knockout background had very dramatic effects on the traits in question.

We now have the apoAII transgene on the apoE knockout background. Consistent with the data in the F2 cross described above, the apoAII transgene on the apoE knockout background has produced a markedly more atherogenic lipoprotein phenotype than that observed in either the apoAII transgenic or apoE knockout mice.

Triglycerides were markedly increased in the combined apoAIIT/apoE-/- mice compared to the apoEko mice, and there was a trend towards an increase in triglycerides compared to the apoAIIT mice. Total cholesterol was markedly increased in the combined apoAIIT/apoE-/- mice compared to either the apoAIIT or apoEko mice. This increase was primarlily in apoB containing lipoproteins since, the apoE null mutation significantly reduced the HDL cholesterol in the combined apoAIIT/apoEko mice compared to the apoAIIT mice. The lipid values presented are from very young mice (~6 weeks old) that had been maintained on a standard low fat mouse chow diet. The lipoprotein profile in the combined apoAIIT/apoEko mice appears to be dramatically more atherogenic than in either the apoAIIT or apoEko mice.

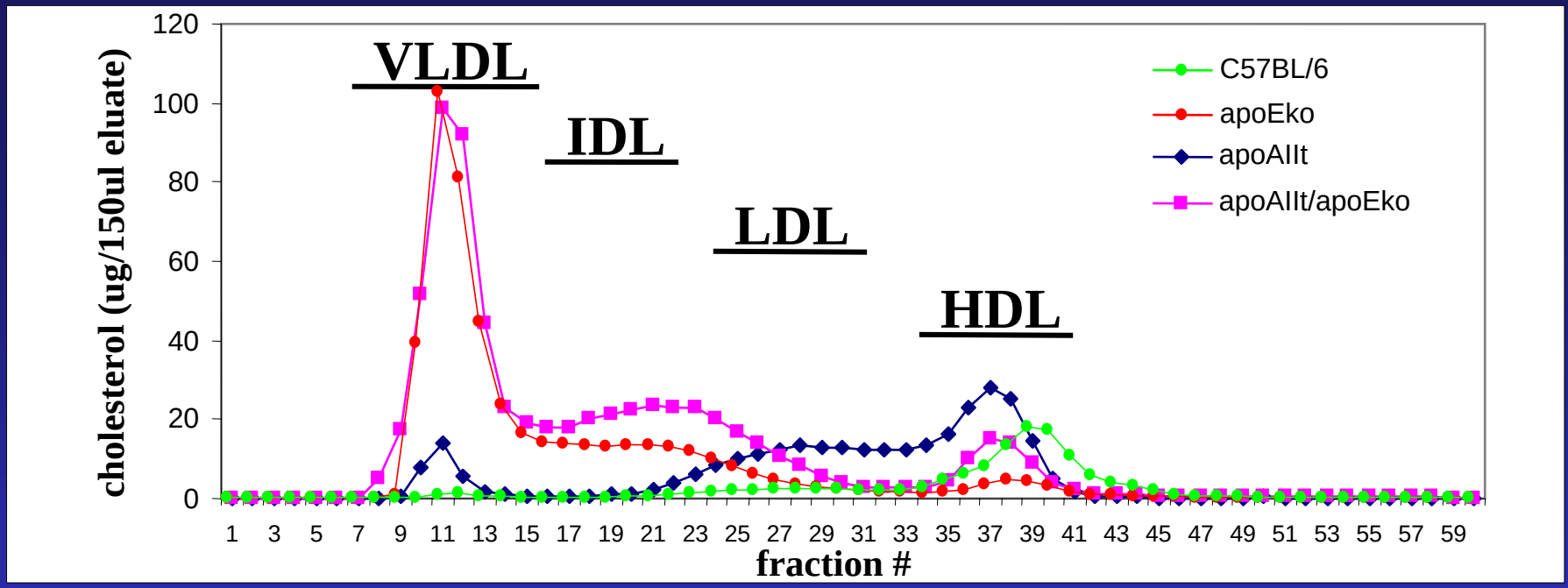
The lipoprotein profile of the apoAIIt and combined apoAIIt/apoEko mice was determined by FPLC analysis using gel filtration chromatography to separate the lipoprotein classes by size. The FPLC lipoprotein profiles are consistent with the lipid assay data and confirm a markedly more atherogenic lipoprotein profile in the apoAIIt/apoEko mice than in the apoAIIt mice.

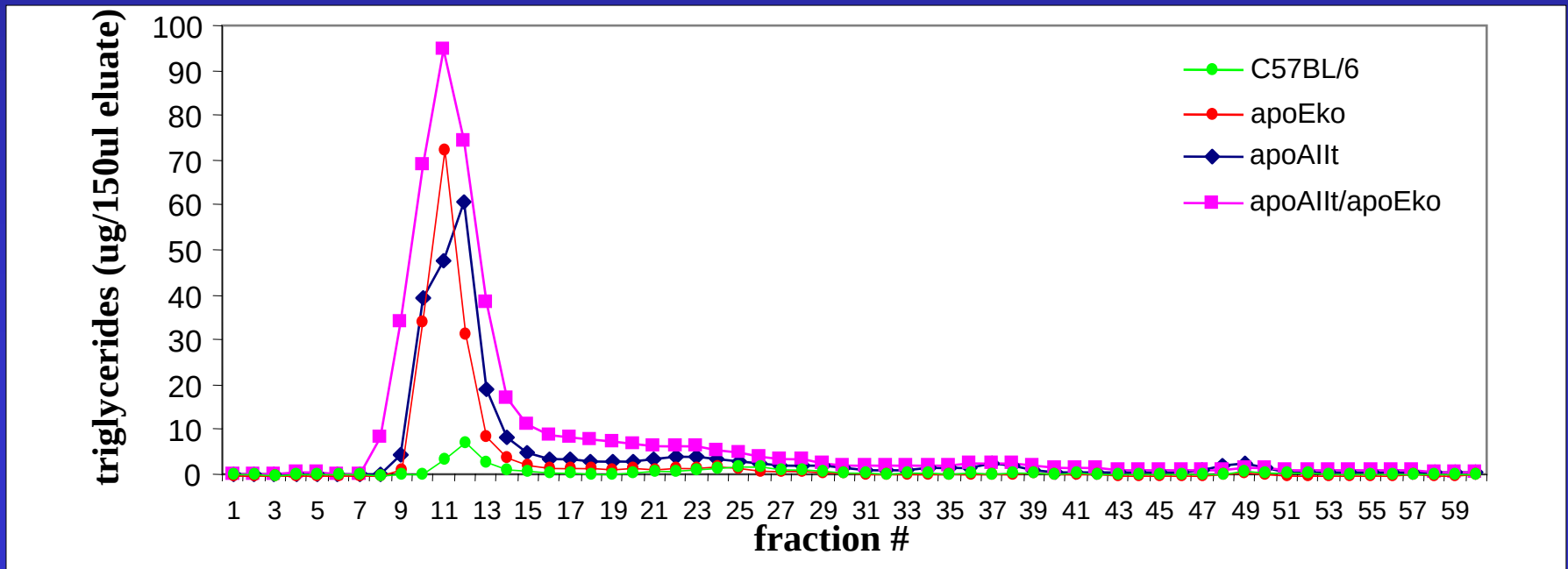
Fasting plasma lipid and glucose concentrations in apoAII transgenic (apoAIIT), apoE knockout (apoEko), and combined apoAII transgenic/apoE knockout (apoAIIT/apoEko) mice.



All values are means ± SEM expressed in mg/dl. All animals were 6 weeks (± 1 week) of age and had been maintained on a low fat (4.5%) standard mouse chow diet. * denotes values that are significantly different (p< 0.05) than the apoAIIT mice. # denotes values that are significantly different (p<0.05) than apoEko mice.

Gel Filtration Lipoprotein Profiles of ApoAIIt and ApoAIIt/apoEko mice





Assessing renal function/disease in the apoAII transgenic mice:

The basis for our interest in renal function in the apoAII transgenic mice stems from correlations between plasma apoAII concentrations and plasma creatinine observed in one of our human studies, as well as a study by another group.

Our Human Study Population

- 390 members from 77 families of Hispanic descent.
- Probands had primary hypertension without evidence of a secondary cause.

Plasma apoAII concentrations are positively correlated with:

Insulin resistance	p=0.034
Fasting insulin	p=0.013
Plasma TG	p=0.027
HDL chol	p=0.019
LDL chol	p=0.0003
Total chol	p<0.0001
Plasma apoB	p=0.02
Plasma creatinine	p<0.0001

Note: Although plasma concentrations of apoAI and apoAII were highly correlated, of the traits listed above apoAI was correlated significantly only with HDL cholesterol

A search of the literature revealed another human study in which plasma apoAII concentrations were highly correlated with plasma creatinine concentrations (Cerne *et al*., *Ren. Fail*., 22:799-808, 2000).

In this study plasma apoAII concentrations also served as the best indicator of the degree of atherosclerotic lesion progression. The effect of apoAII plasma concentrations in the human studies described above, suggest that the apoAII transgenic mice could exhibit renal complications, in addition to the atherosclerosis and insulin resistance.

Future Plans:

Assess insulin resistance and atherosclerosis in the apoAIIt/apoEko mice using the methodologies established by the AMDCC.

We should have enough animals to get preliminary lesion data in the next 2-3 months.

Future Plans

- **Assess insulin resistance and atherosclerosis in the apoAIIt/apoEko mice using the guidelines established by the AMDCC. (We should have enough animals to sacrifice a few for preliminary data on lesions in approximately two months)**
- **Assessing renal function/disease in the apoAIIt and apoAIIt/apoEko mice. (We are currently collecting urine samples from the apoAII transgenic mice for analysis of creatinine and albumin. We should have results within 1 month. We should have enough of the apoAIIt/apoEko mice to perform similar studies in about two months)**