AMDCC: UCLA Cardiovascular Models

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Importance of the AMDCC

- Foster greater cooperation among scientists and institutions throughout the country resulting in high quality collaborations and improved consistency among models.
- Develop cross disciplinary relationships, i.e. renal, cardiovascular and other complications.
- Allow creation of a larger number of novel and diverse animal models of diabetes complications.
- Provide for more efficient and greater distribution of well characterized animal models of diabetes complications.

Specific Examples of Benefits for AMDCC

1. Collaboration on models of diabetic cardiomyopathy: Dale Abel, University of Utah.

2. Creatinine Technology: Matt Breyer, Vanderbilt University.

3. Provision of mouse models for nephropathy: Frank Brosher and Larry Holdsman, University of Michigan.

4. Mouse vascular injury technique: Hayes Dansky, Mount Sinai, New York.

5. db/db phenotyping.

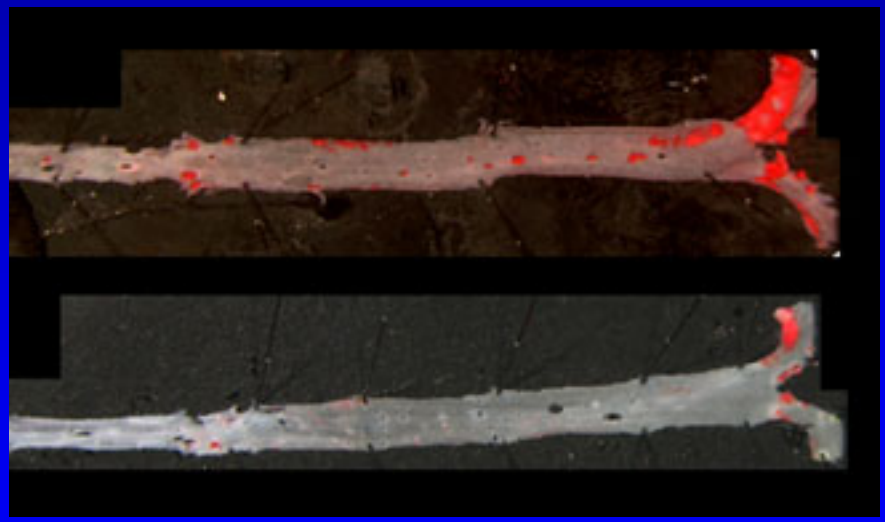
Animal Models

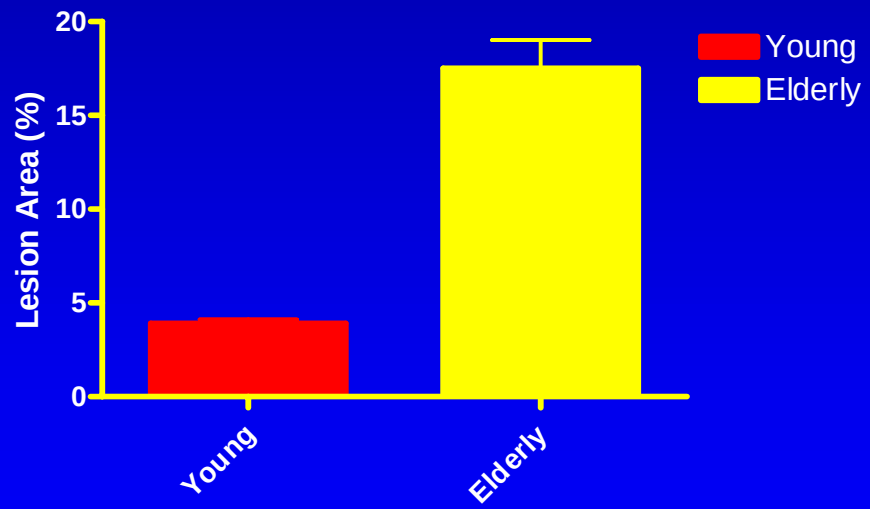
1. Elderly LDLR-/- on Western diet
2. Apolipoprotein A2 transgenic x LDLR-/-
3. Skeletal muscle PPARγ knockout x LDLR-/-
4. Con 15 from MRL on BALB/c

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

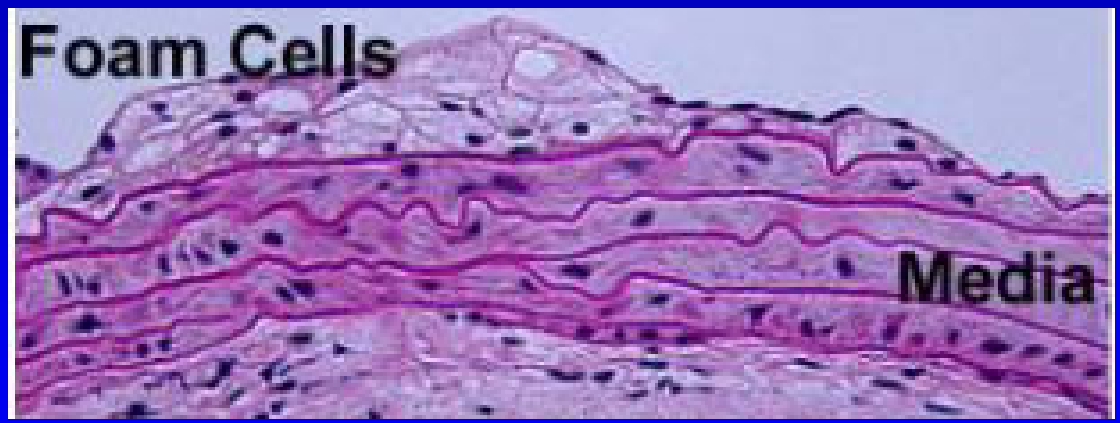
Elderly LDLR-/- mice have greater aortic lesion area compared to Young LDLR-/- mice





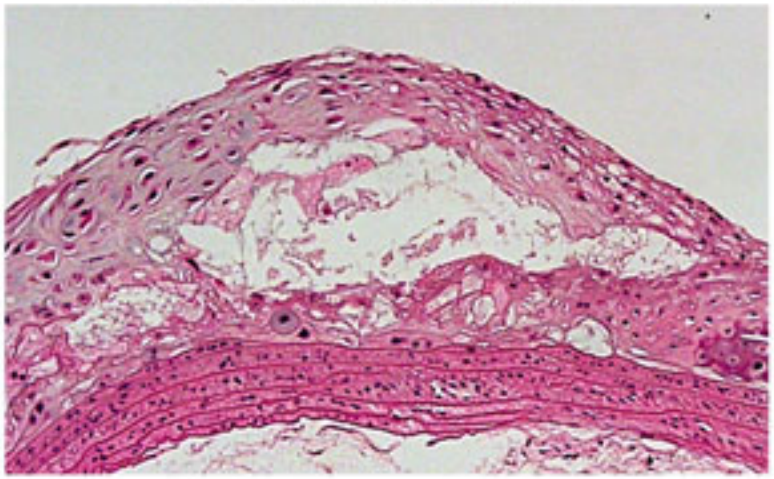
Presented at 2004 ADA meeting

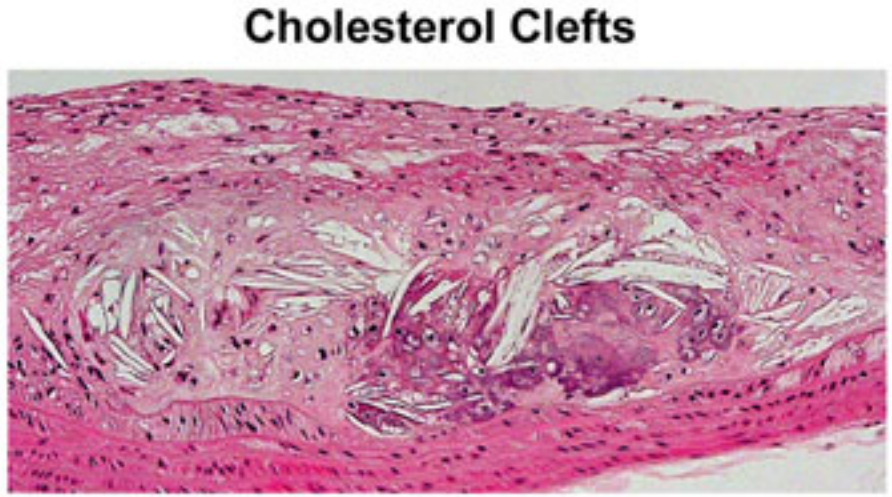
Lesion Histology of Young Male LDLR-/- Mice



Presented at 2004 ADA meeting

Lesion Histology of Elderly Male LDLR-/- Mice





Presented at 2004 ADA meeting

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*

* $p<0.05$

Conclusion

- Age worsens the diabetes induced by the western diet
- Extent and complexity of atherosclerosis are accelerated in elderly LDLR-/- mice exposed to the Western diet.
- These results suggest that the elderly male LDLR-/- mouse may be an important model of diabetes-accelerated vascular injury

Studies Planned for the Future

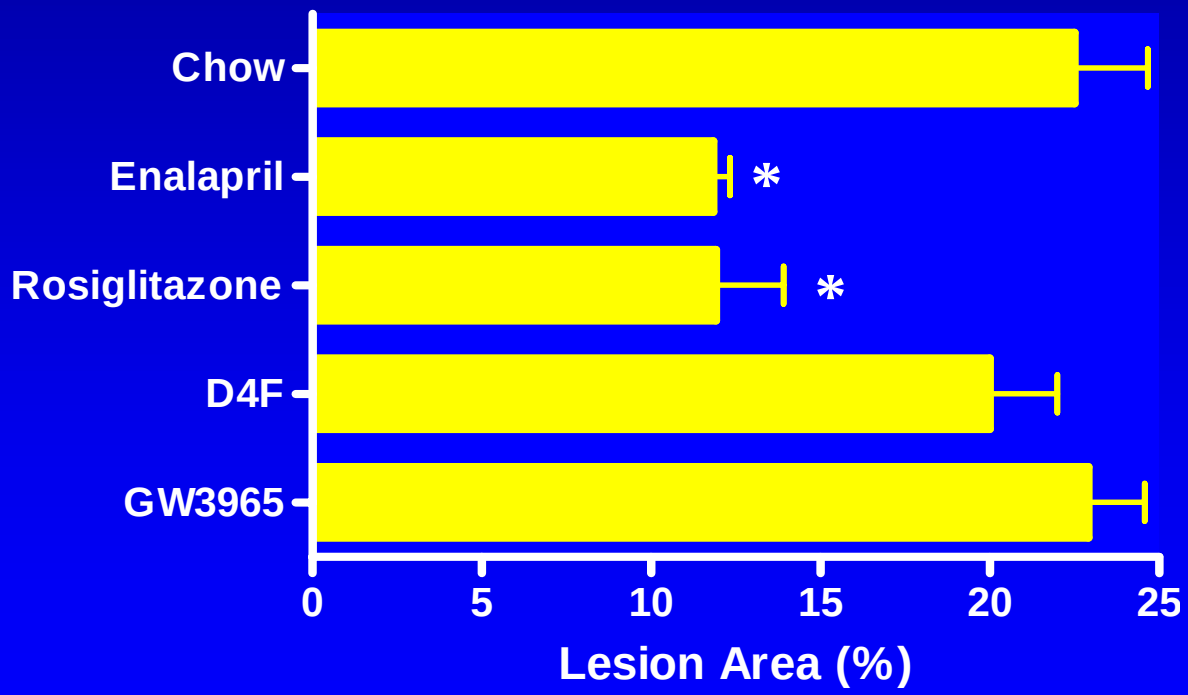
- Response to AIN high and low fat diets with matched cholesterols
- Plasma adipokine measurements, body composition and detailed lipid analyses
- Identification of genes responsible for the age-induced susceptibility to atherosclerosis and diabetes (microarray analysis of artery wall and adipose)

Regression of Pre-existing Atherosclerosis in Elderly LDLR-/- Mice

Protocol

- 12 month old male LDLR-/- mice placed on Western diet for 3 months
- Mice are placed on normal chow for 3 additional months
- Following compounds were added to the chow or drinking water during the chow fed period
  - Enalapril
  - Rosiglitazone
  - D4F
  - GW3965 (LXR ligand)

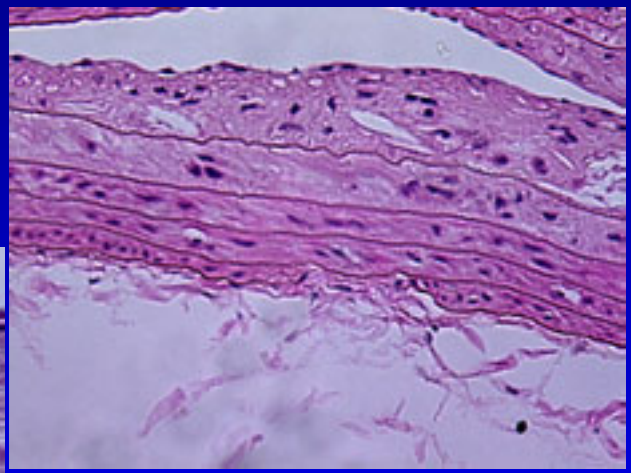
Lesion Regression in Elderly LDLR-/- Mice



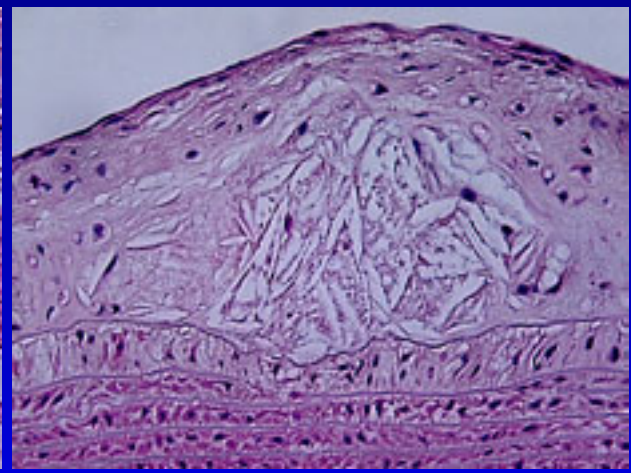
* p<0.05 vs chow

Lesion Histology

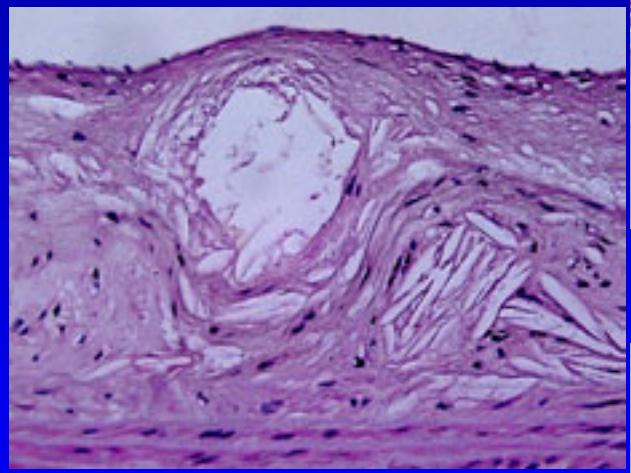
Enalapril



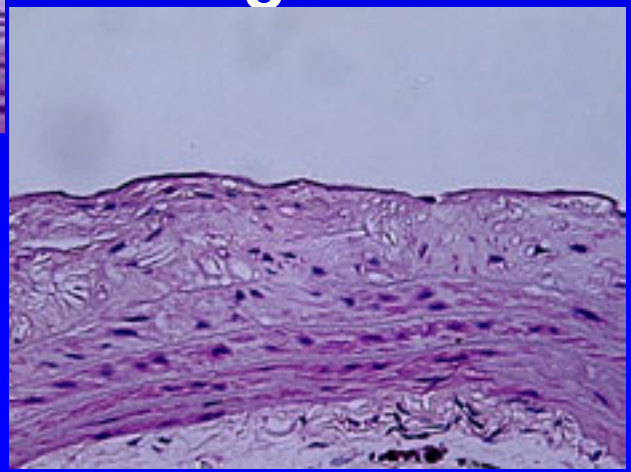
D4F



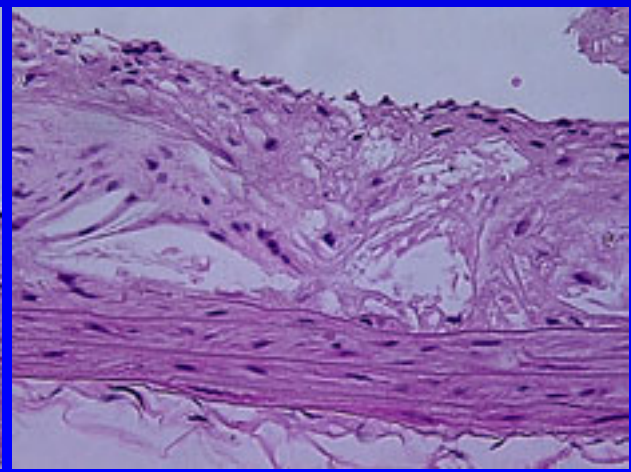
Chow



Rosiglitazone



GW3965



All Photographs are produced at the same magnification

Lesion Complexity Score

Chow Fed Controls	94%
Enalapril treated	12%
Rosiglitazone Treated	45%
D4F Treated	89%
GW3965 Treated	92%

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

Body Weight and Blood Pressure

	Chow Controls	Enalapril	Rosiglita-zone	D4F	GW3965
Final Body Weight (g)	35±1.0	31±1.8	34±2.3	30±2.2	40±1.2
Systolic Blood Pressure (mmHg)	106±4	85±4*	100±3	102±5	103±3

* $p<0.05$ vs chow

Plasma Metabolic Markers

	Total Cholesterol (mg/dl)	HDL-C (mg/dl)	Triglycerides (mg/dl)	Glucose (mg/dl)	Insulin (pg/ml)
Chow	304±11	96±3	240±24	225±25	608±96
Enala-pril	308±15	86±6	122±7*	266±20	616±89
Rosiglit-azone	360±13*	98±8	100±11*	247±27	1112±260
D4F	428±21*	79±4*	79±15*	180±41	763±178
GW3965	1163±36*	64±2*	260±12	252±8	1711±101*

* $p<0.05$ vs chow

Conclusion

In a model of accelerated atherosclerosis in elderly LDLR-/- mice

- **Chow (low cholesterol and fat) diet is not associated with lesion regression**
- **Inhibition of the RAS or rosiglitazone is associated with substantial lesion regression**
- **Enalapril appears to deplete lesion lipid content**
- **Rosiglitazone appears to shrink lesion size**

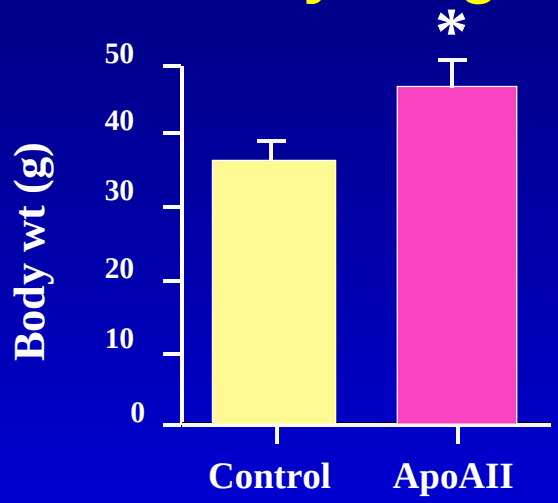
Plasma Metabolic Parameters in 5 month old apoA2 Tg Mice

	Female C57	Female apoA2tg	Male C57	Male apoA2tg
Total Cholesterol (mg/dl)	90±3	216±4*	101±4	357±19*
HDL-cholesterol (mg/dl)	71±3	167±4*	74±3	240±5*
Triglycerides (mg/dl)	57±10	156±16*	59±5	345±24*
FFA (mg/dl)	54±5	82±4*	56±2	107±5*
Glucose (mg/dl)	95±6	106±8	101±7	139±11*
Insulin (pg/ml)	453±105	1018±397*	537±98	1752±476*
apoA2 (mg/dl)	15±5	66±5*	17±2	92±7*

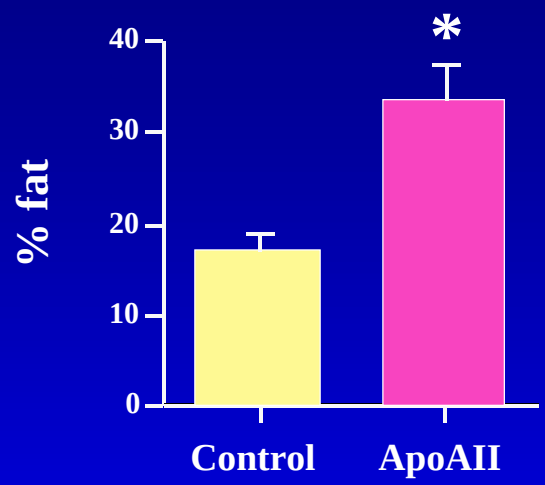
Boisfer et al. *J Lipid Res.* 43:732-41, 2002.

The apoAII transgenic mice become obese

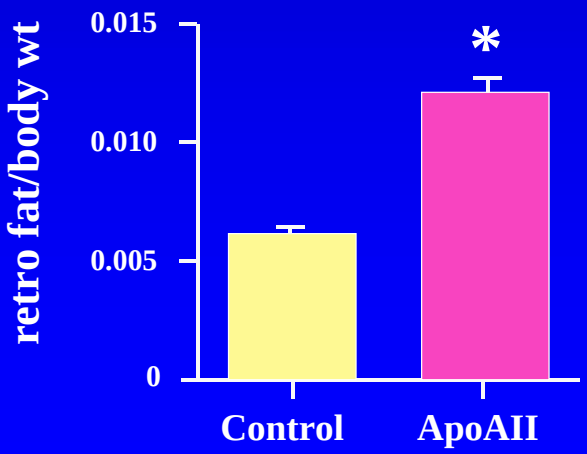
Body weight



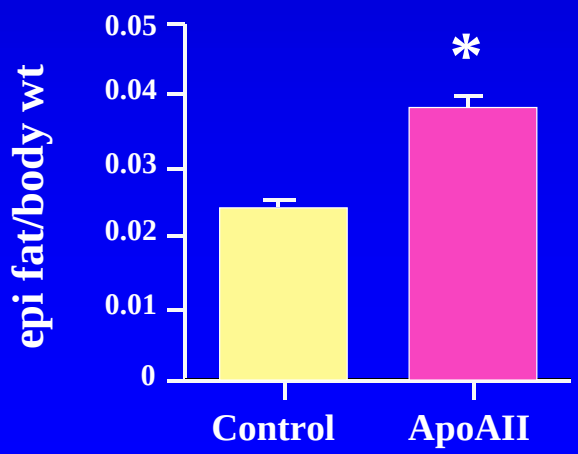
% total fat



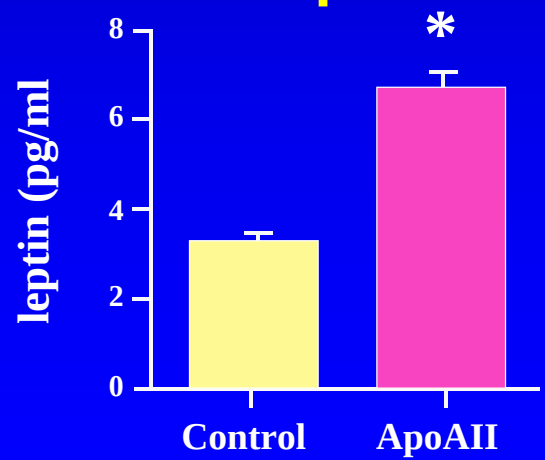
Retroperitoneal fat



Epididymal fat

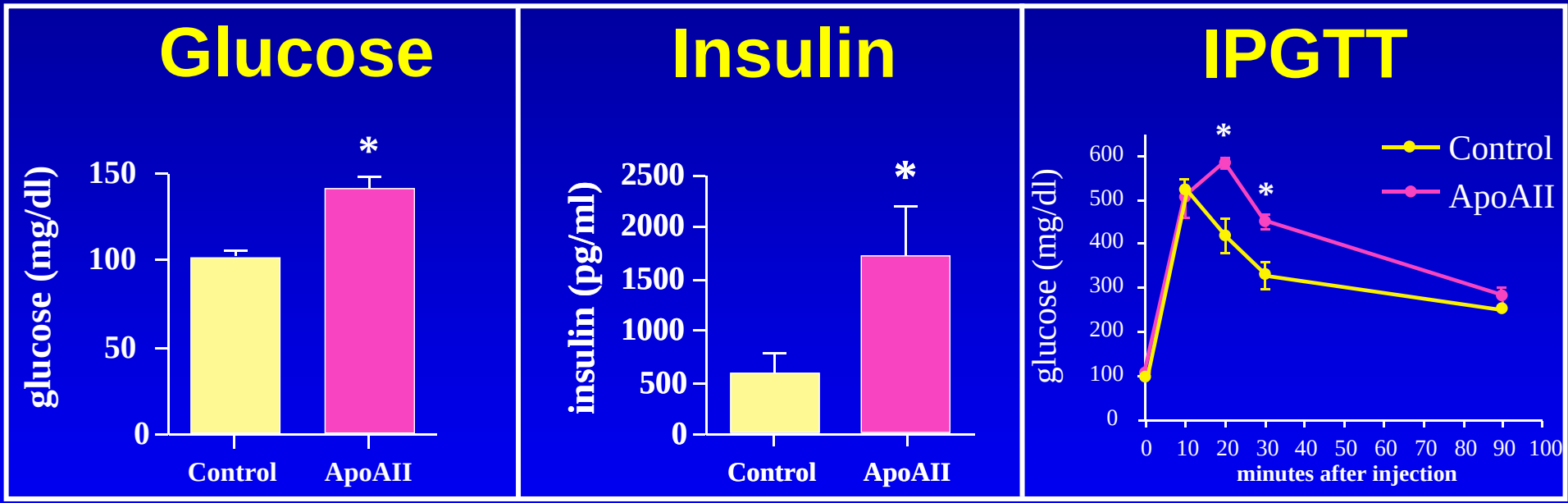


Leptin



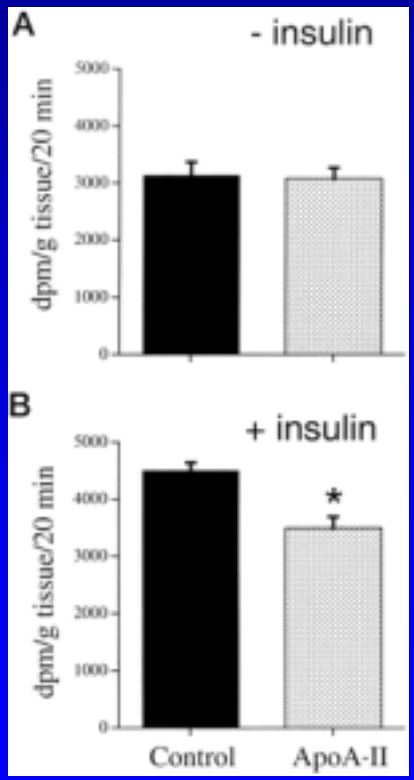
Castellani et al. *Diabetes* 50:643, 2001

The apoAII transgenic mice are insulin resistant



Castellani et al. *Diabetes* 50:643, 2001

ApoA2Tg Mice Exhibit Skeletal Muscle Insulin Resistance and Increased Triglyceride Content



Glucose uptake by isolated soleus muscle

Skeletal Muscle Triglycerides

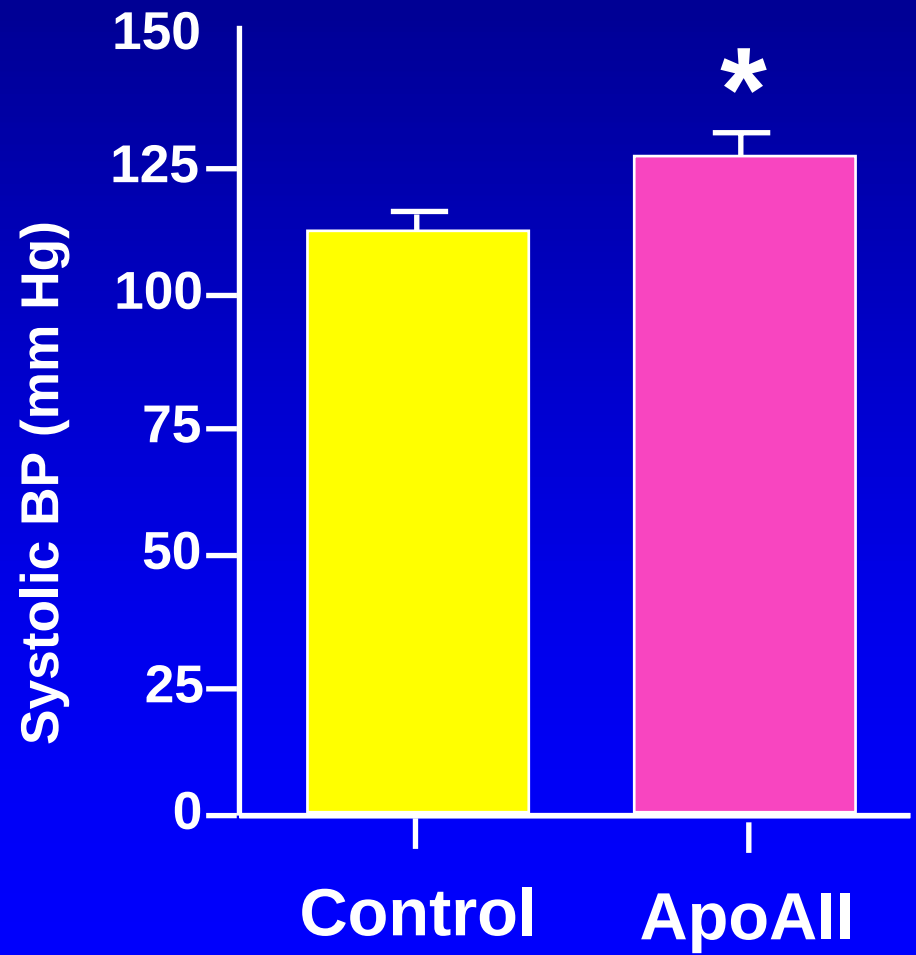
(mg/g of muscle)

C57	**12.98±0.45**
apoA2Tg	**18.58±1.79***

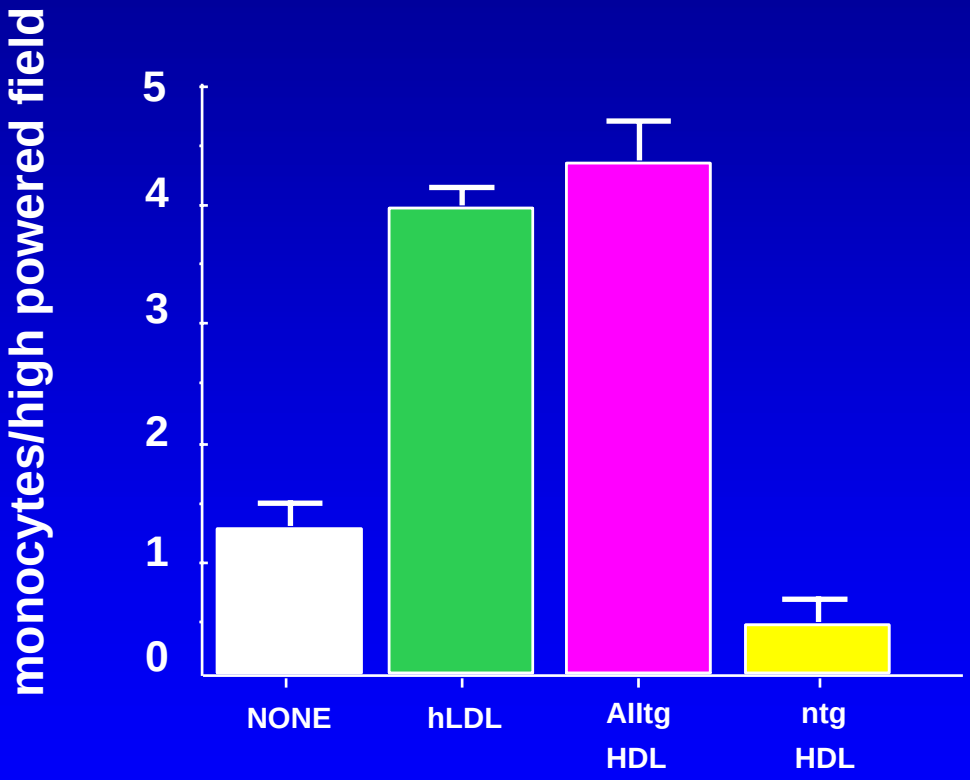
***p<0.05**

Castellani et al. *Diabetes* 50:643, 2001

Blood pressure is increased in the ApoAII transgenic mice



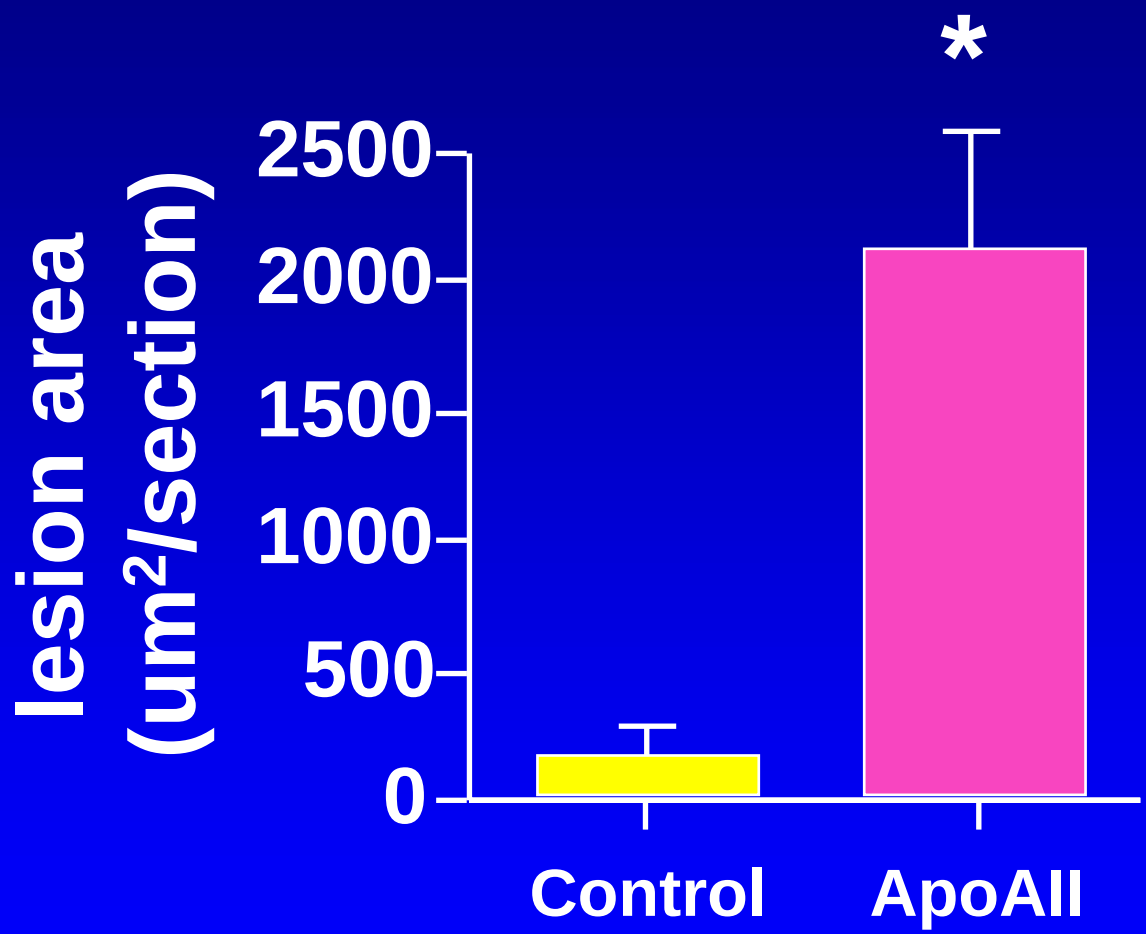
ApoA-II HDL promotes MCP-1 production by endothelial cells and stimulates monocyte transmigration



Castellani et al., *J. Clin. Invest.* 100: 464-474, 1997

Boisfer et al. *J Lipid Res.* 43:732-41, 2002.

ApoAII transgenic mice have increased atherosclerosis



Warden et al., *Science*. 261: 469-472, 1993

Shultz et al. *Nature* 365: 762-764, 1993

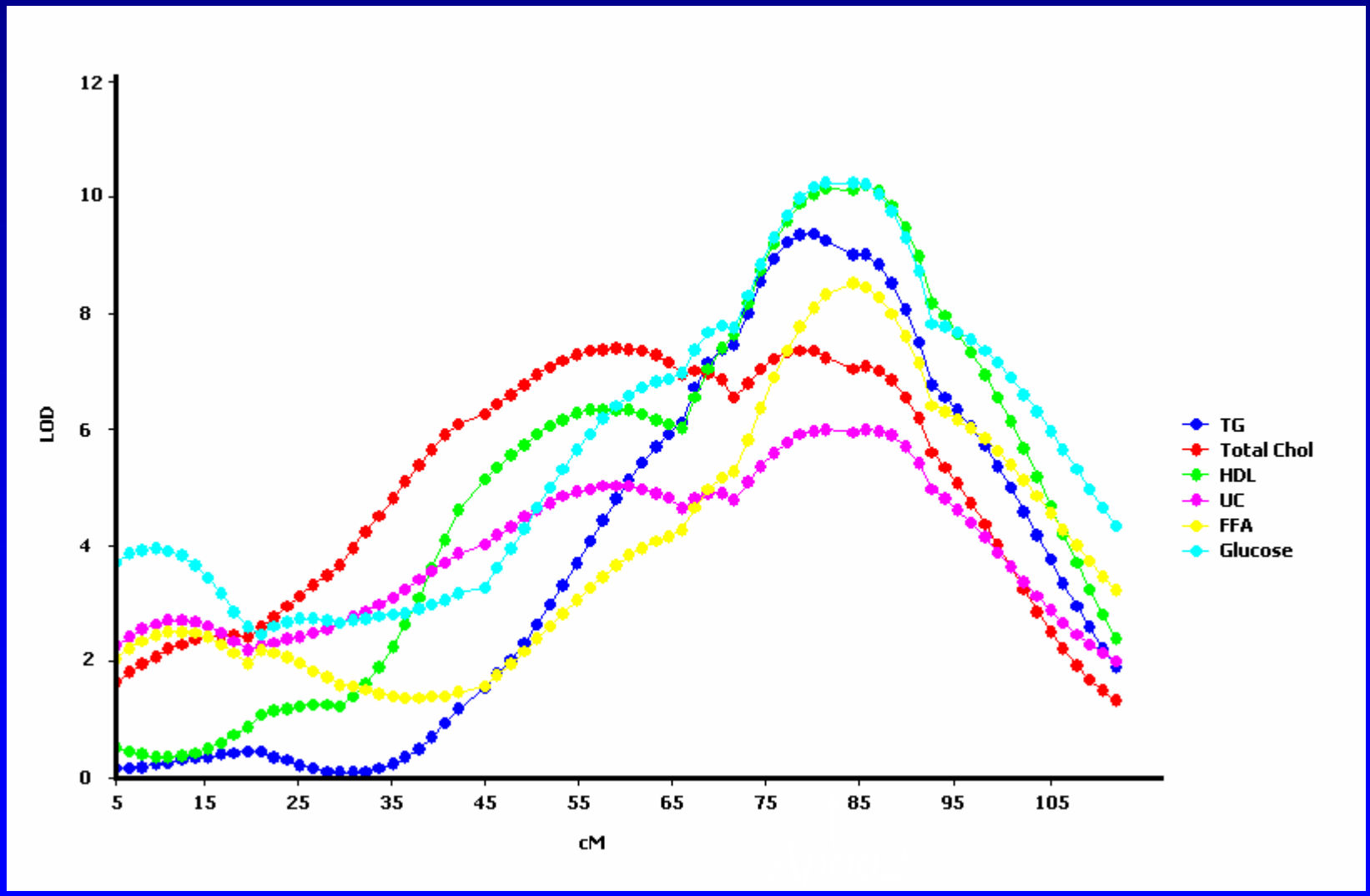
Treatment of apoA2Tg Mice with Rosiglitazone for One Week

	Pre-treatment	Post-treatment
Total Cholesterol (mg/dl)	252±20	216±17
HDL-cholesterol (mg/dl)	176±12	137±9*
Triglycerides (mg/dl)	212±28	98±14*
FFA (mg/dl)	41±2	32±2*
Glucose (mg/dl)	142±9	109±8*
Insulin (pg/ml)	1880±318	718±110*
apoA2 (mg/dl)	93±8	85±7
apoA1 (mg/dl)	140±13	127±8

*p<0.05

Boisfer et al. *J Lipid Res.* 43:732-41, 2002.

QTLs over ApoAII gene in a cross between C3H.Apoe$^{-/-}$ and C57BL/6.Apoe $^{-/-}$



Apo A2 Tg Present studies

- **ApoAII transgenic mice are being bred to apoE$^{-/-}$ to generate combined apoAII transgenic/apoE $^{-/-}$ mice**

- **Based on the results of our apoAII Tg/apoE $^{-/-}$ cross we expect all aspects of the apoAII transgenic phenotype to be dramatically exacerbated, including glucose levels and atherosclerosis, providing a new model of diabetic complications.**

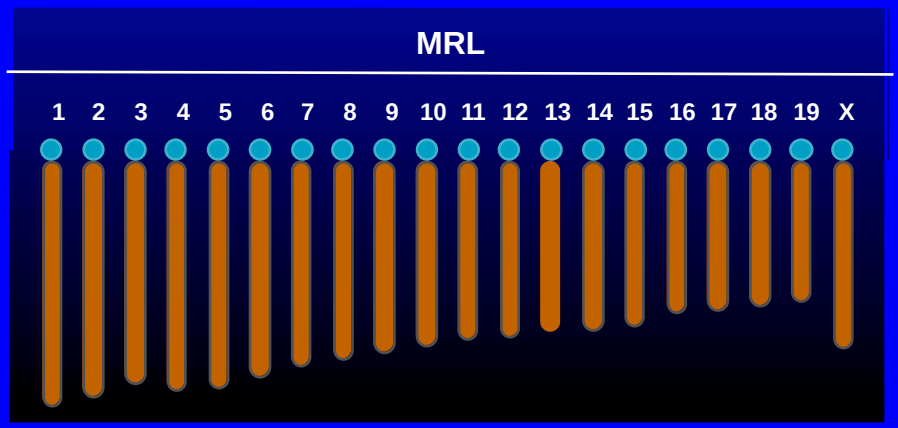
Skeletal Muscle Specific PPARγ Knockout Mice

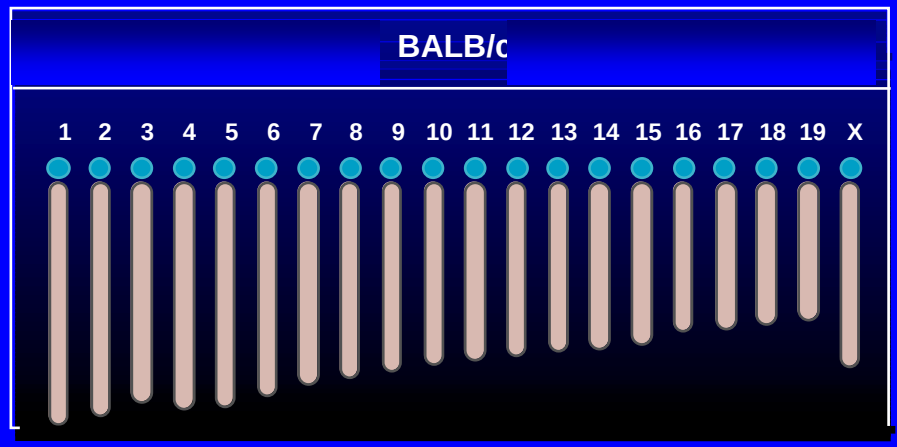
- Obtained from Jerrold Olefsky at University of California San Diego
- Model of Insulin Resistance and Type 2 DM
- Being Bred into LDLR-/- mice for cardiovascular complications (F2 currently)

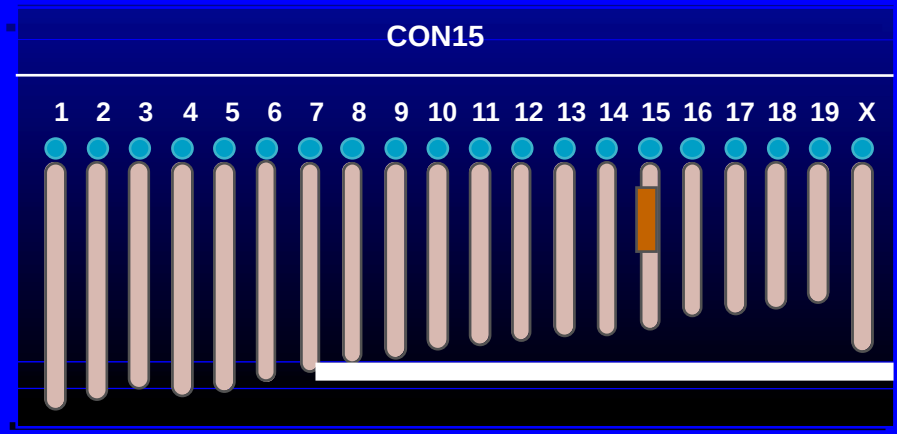
Skeletal muscle PPARγ knockout compared to adipose 11βHSD1 transgenic

- PPARγ KO
  - Glucose 139 mg/dl
  - Insulin 1.5 ng/ml
  - Triglycerides 209 mg/dl
  - Glucose intolerance at 4 months of age
  - Insulin resistant at 14 months of age
- 11βHSD1 Tg
  - Glucose 135 mg/dl
  - Insulin not reported
  - Triglycerides reduced compared to controls
  - Total Cholesterol 80 mg/dl
  - Insulin resistant at 18 weeks of age

A congenic strain (CON15) containing the chromosome 15 region from MRL on a BALB/c background Hyplip2



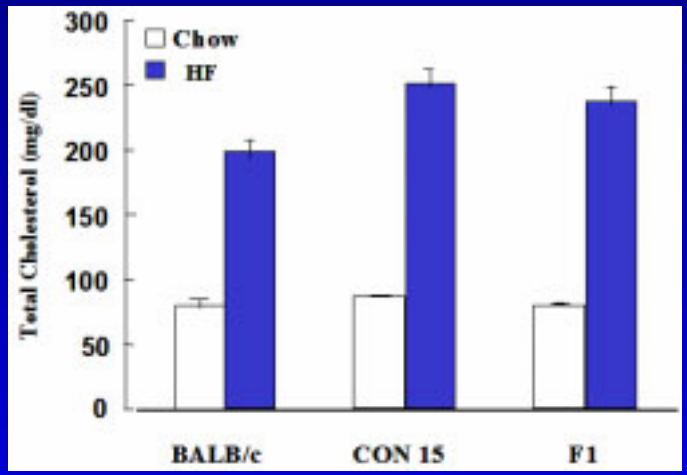




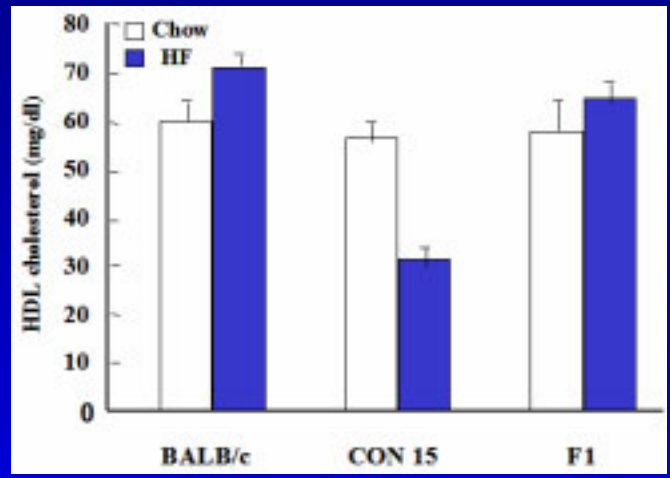
Lingjie Gu et al. Arterioscler Thromb Vasc Biol. 1999;19(3):443-52

Effect of the CON15 locus on lipid parameters

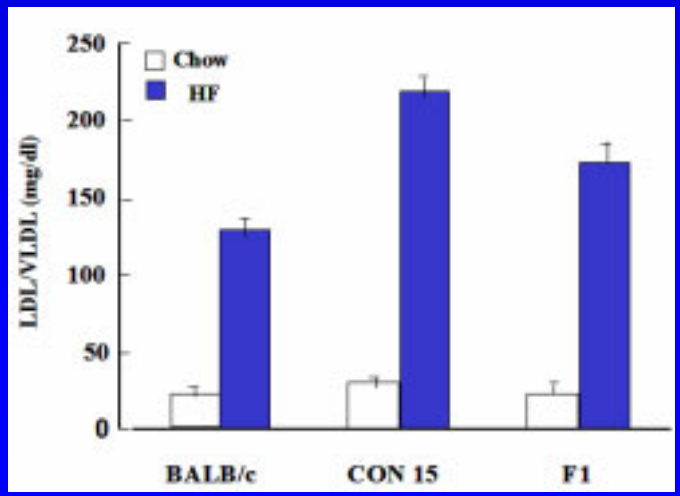
TC



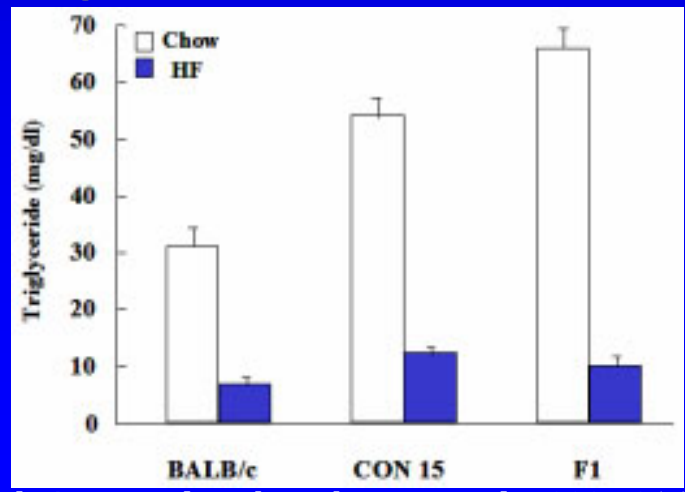
HDL



LDL/VLDL

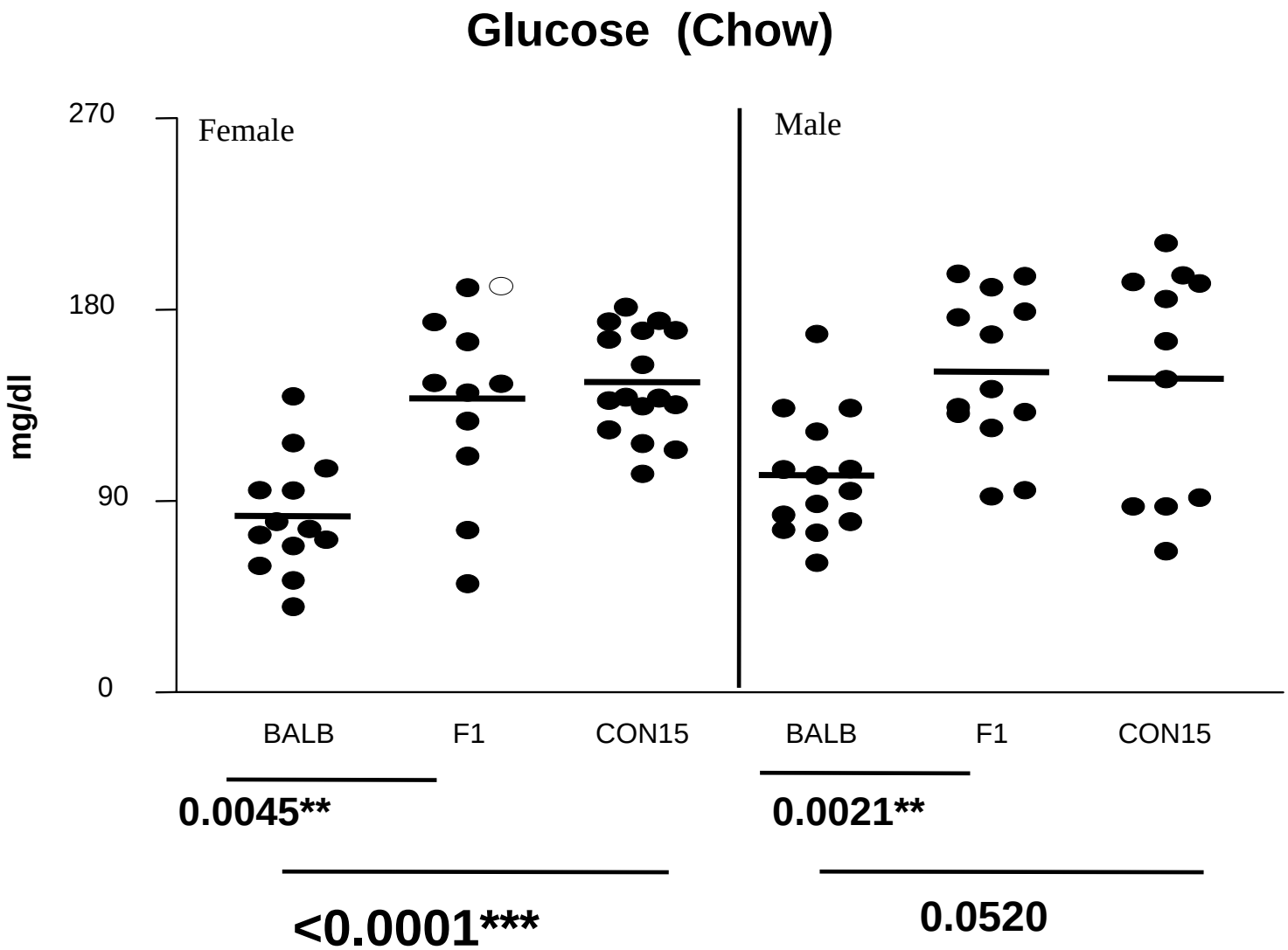


Triglycerides



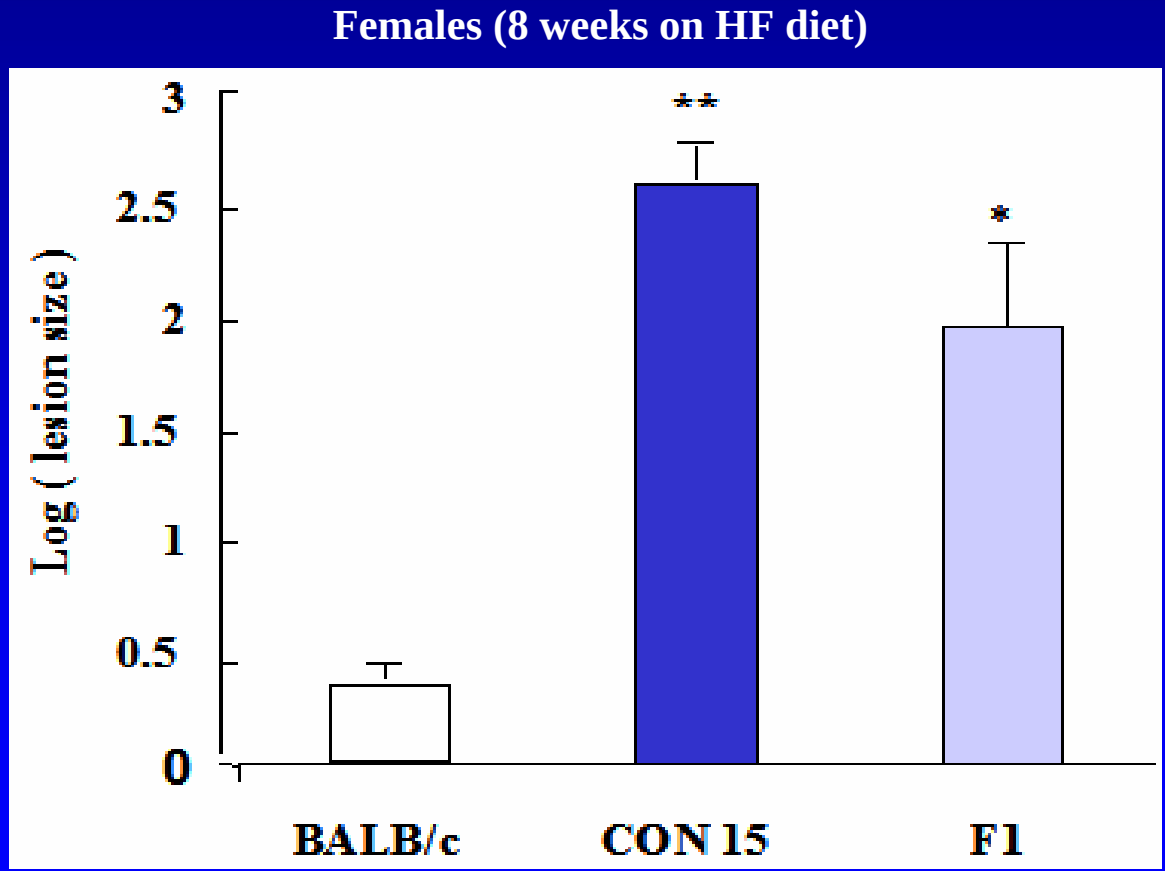
Lingjie Gu et al. Arterioscler Thromb Vasc Biol. 1999 19(2):442-53

CON15 mice have elevated glucose levels



Lingjie Gu et al. Arterioscler Thromb Vasc Biol. 1999 19(2):442-53

CON15 mice exhibit a 30-fold increase in atherosclerotic lesions



Renal Injury in the *Hyplip (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 diabetes-related atherosclerosis and nephropathy