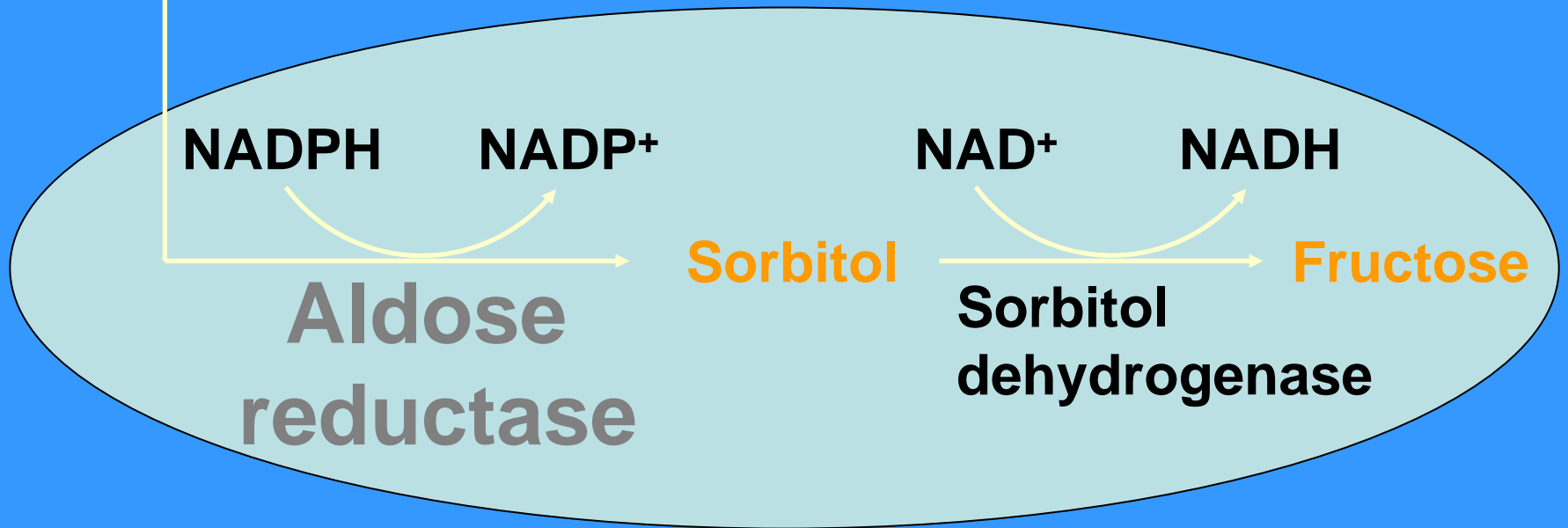
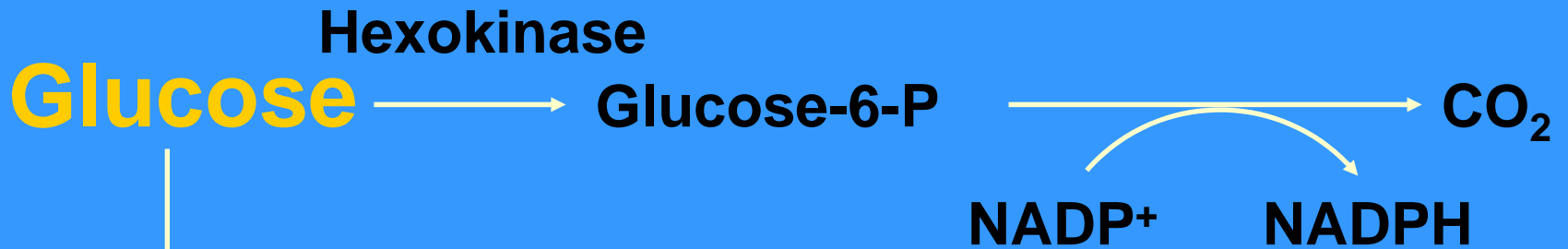
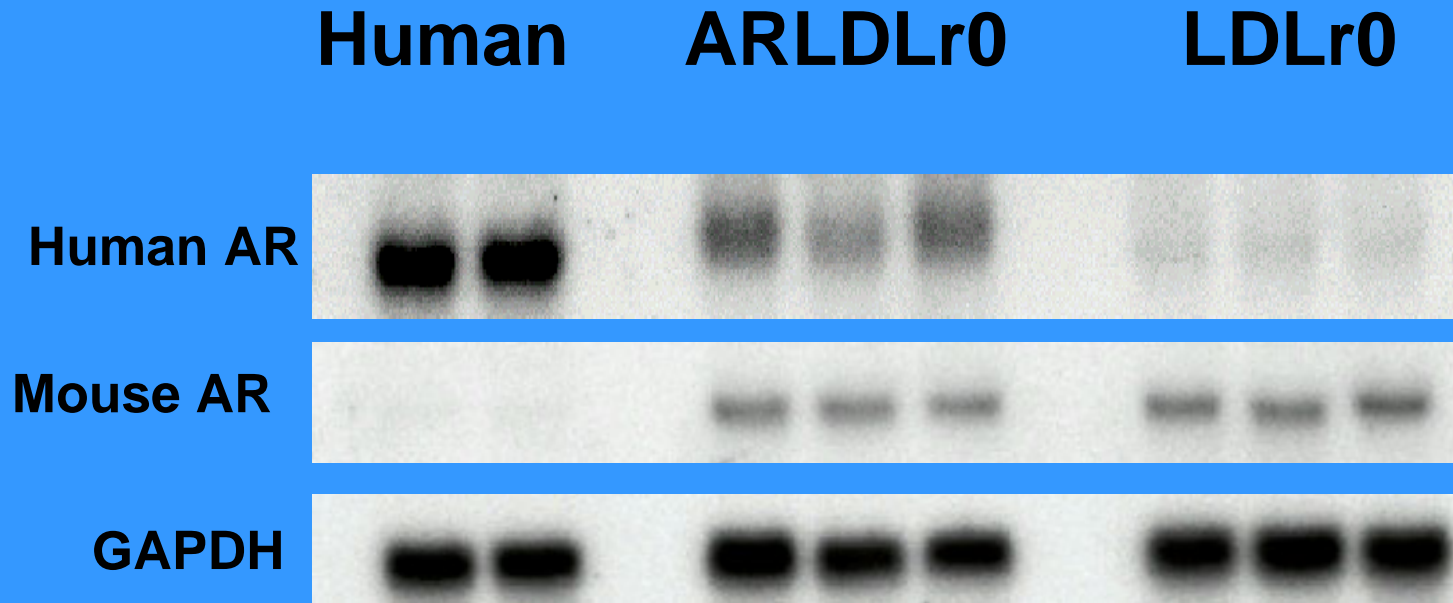


Hypothesis: Diabetic mice are deficient in a gene required for toxic effects of glucose



Transgenic expression of Aldose Reductase is restorative, not pharmacologic

Northern blot of heart RNA



Study Design

hARtg mice X Ldlr^{-/-} mice

hAR/Ldlr^{-/-} : Con & STZ
Ldlr^{-/-} : Con & STZ

hAR/Ldlr^{+/-} : Con & STZ
Ldlr^{+/-} : Con & STZ

High cholesterol diet

0.02% cholesterol

Chow diet

Paigen diet

6 wks

8 wks

12 wks

12 wks

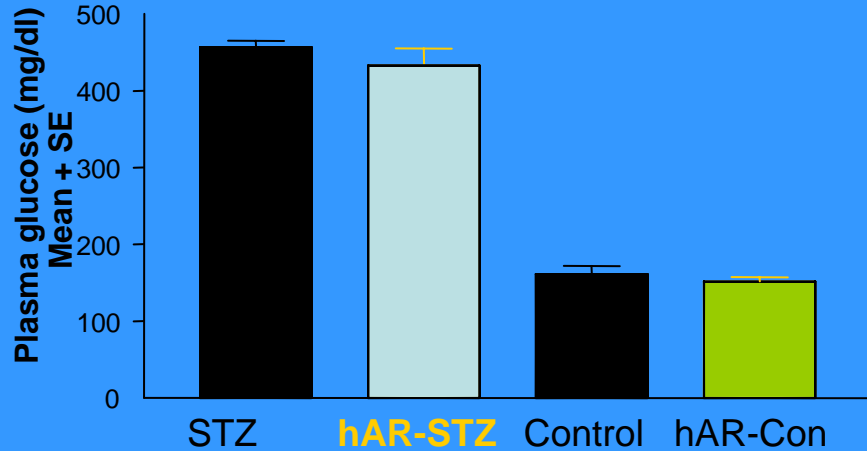
12 wks

12 wks

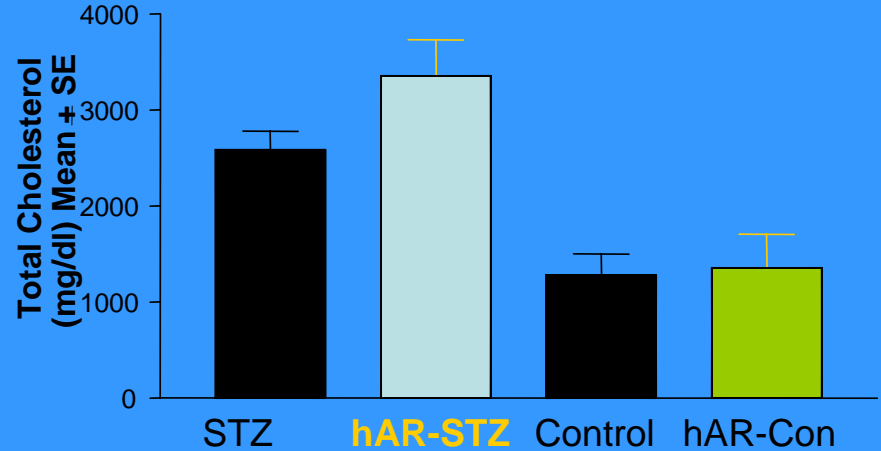


Plasma glucose and lipids after 6 weeks on 0.15% cholesterol diet – all Ldlr-/-

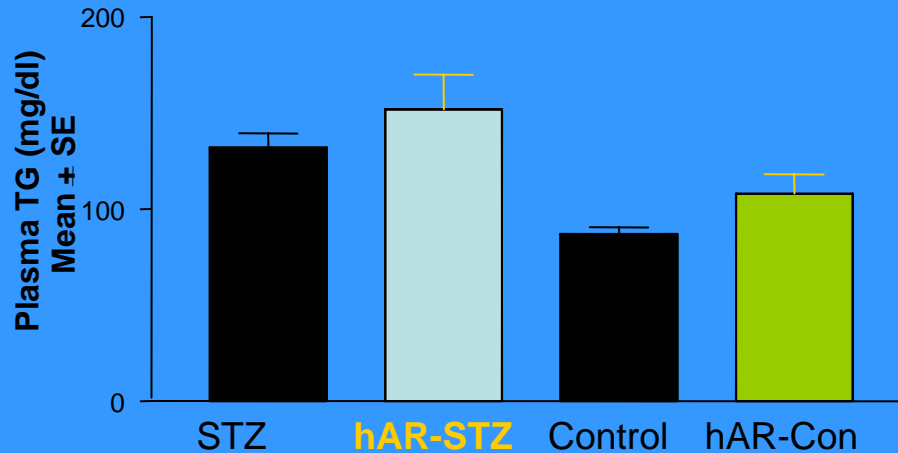
Plasma glucose



Total cholesterol



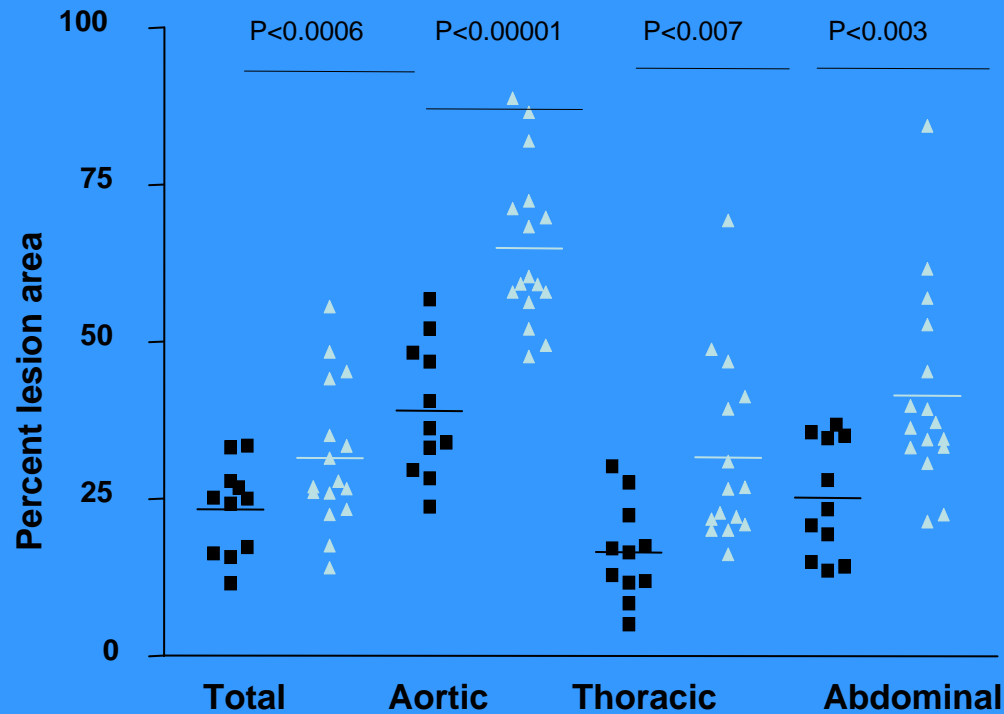
Triglyceride



En face lesion area in diabetic mice fed 0.15% cholesterol diet

■ Ldlr^{-/-} ▲ hAR/Ldlr^{-/-}

6 weeks

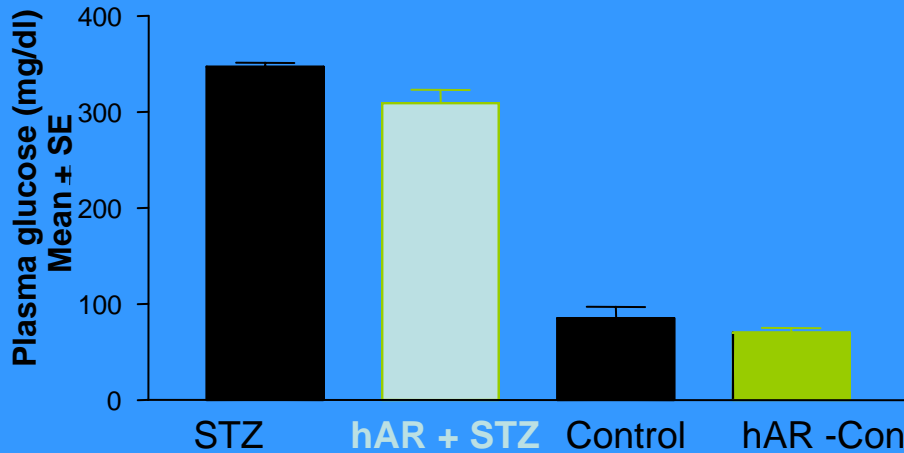


Ldlr+/- and hAR/Ldlr+/- mice
on 12 weeks of Paigen diet

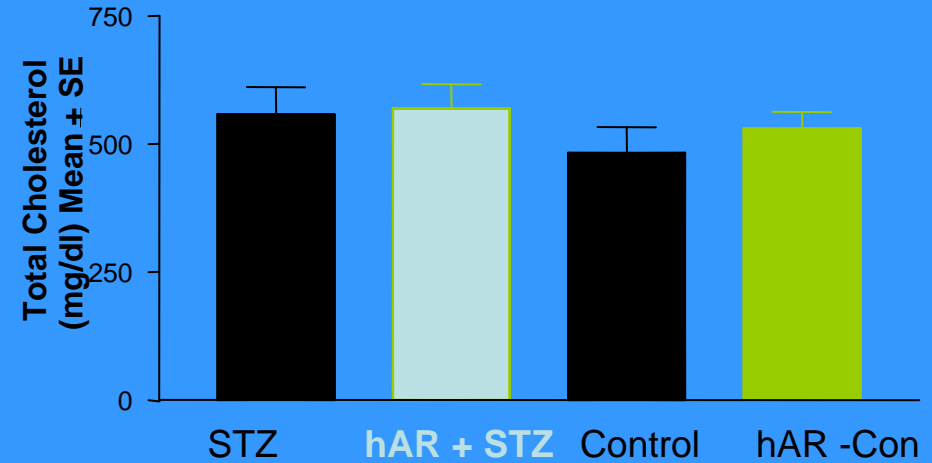
Plasma glucose and lipids after 12 weeks on Paigen diet

All mice are Ldlr+/-

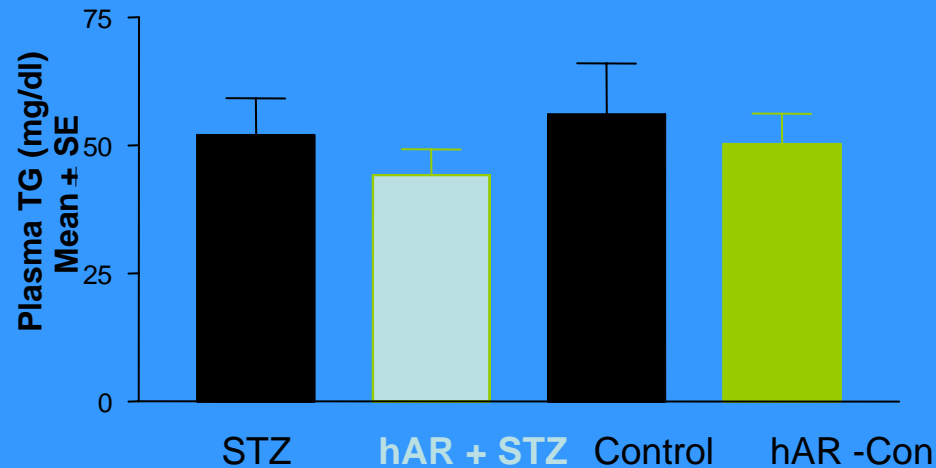
Plasma glucose



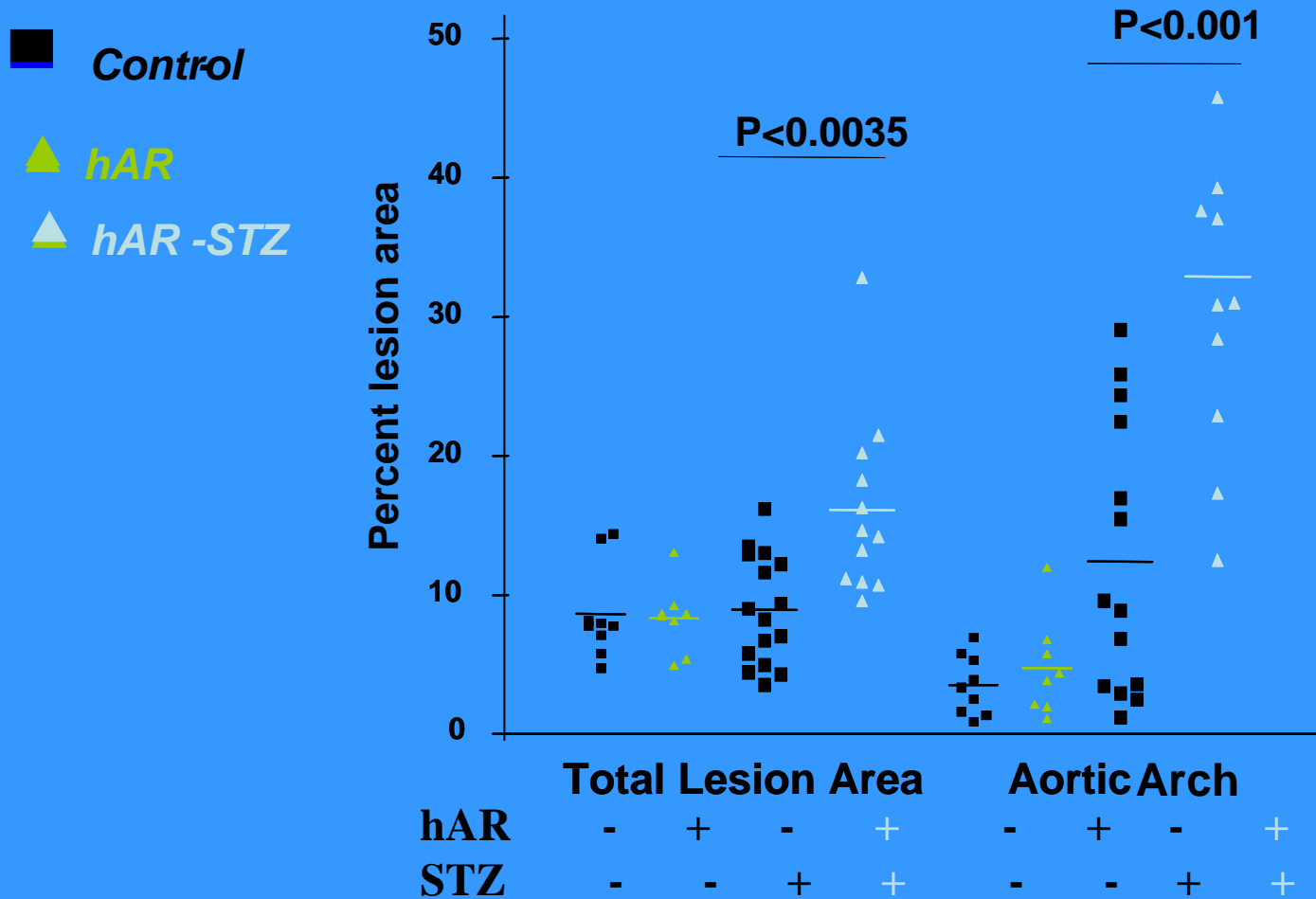
Total cholesterol



Triglyceride



En face lesion area in Non-diabetic and Diabetic Ldlr+/- and hAR/Ldlr+/- Mice fed Paigen diet for 12 weeks



Plans

- To determine if hAR increases atherosclerosis in other murine models of diabetes.
- To determine which cells - macrophages, endothelial cells, or smooth muscle cells - are responsible for increased atherosclerosis.
- To determine the mechanisms by which hAR increases atherosclerosis

Foxa-2

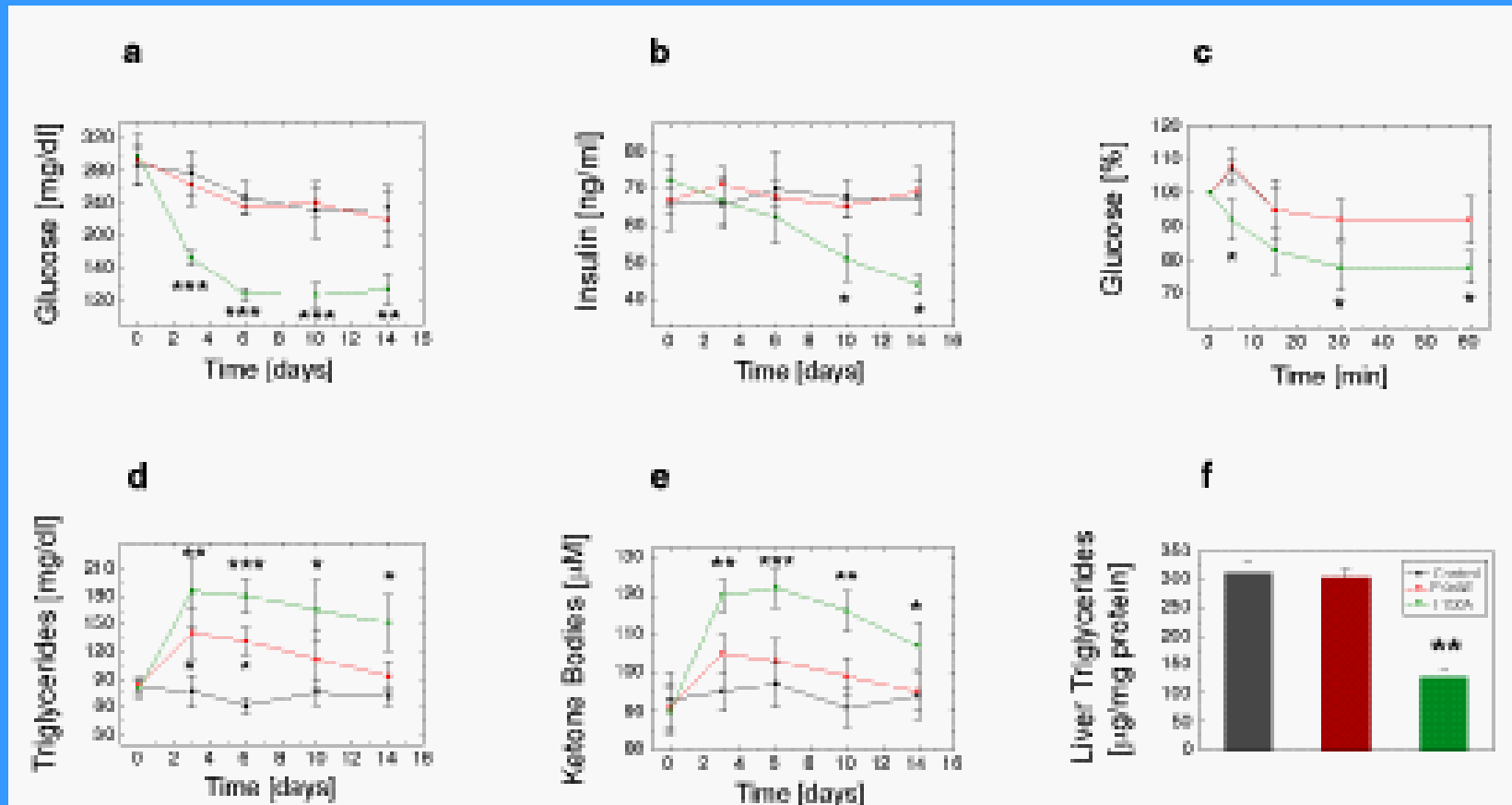
Transcription factor of the winged-helix forkhead family

Activation of insulin – PI3-kinase – Akt pathway induces Foxa-2 phosphorylation at a single, highly conserved tyrosine residue (T156)

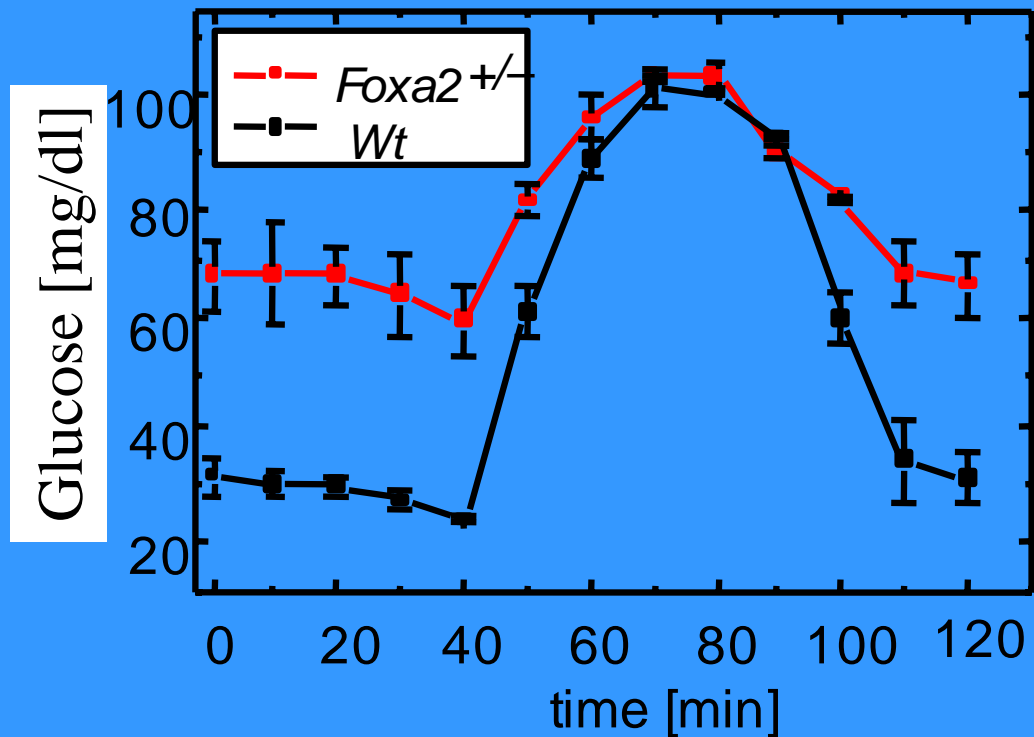
Insulin – PI3-kinase – Akt signaling leads to nuclear exclusion and inhibition of Foxa-2 target gene expression in liver

Constitutive-active form of Foxa2 (T156A) in the liver leads to activation of β -oxidation, ketogenesis and glycolytic genes

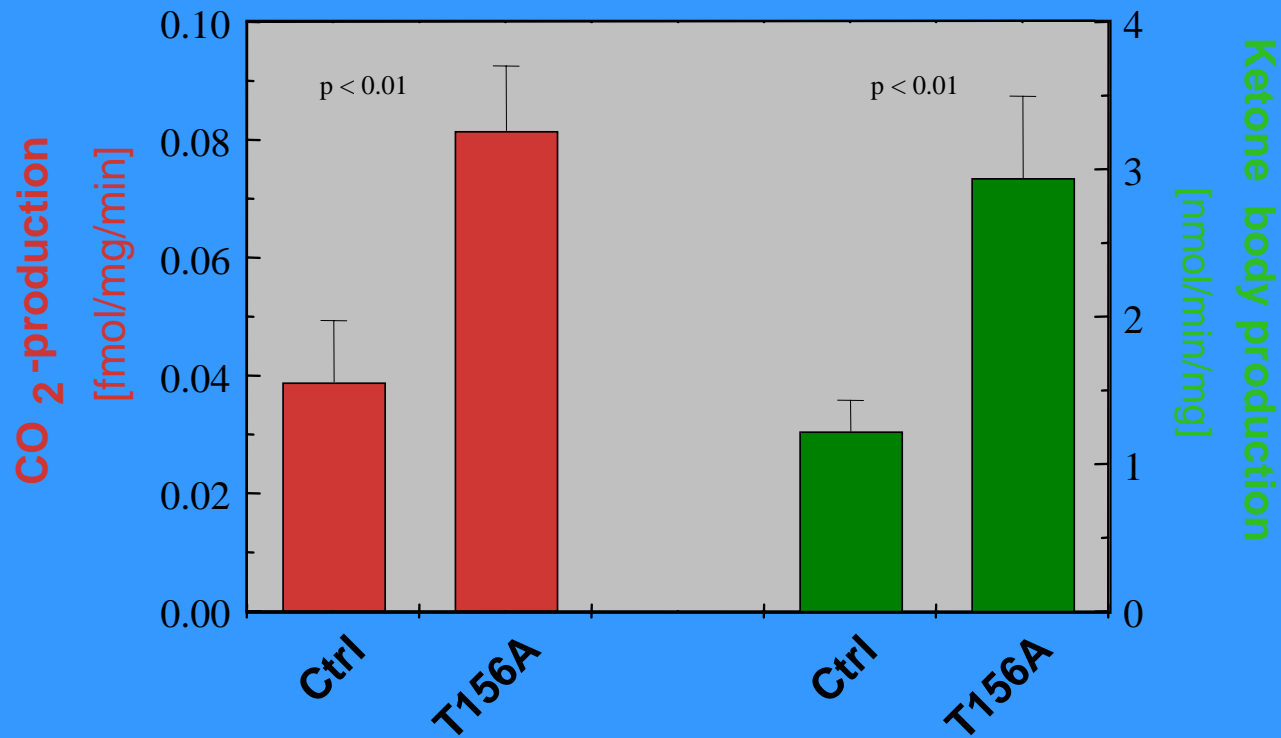
Hepatic expression of Foxa2T156A in *ob/ob* mice increases insulin sensitivity and normalizes blood glucose concentrations



Increased hepatic glucose production in *Foxa2*^{+/-} mice



Increased β -oxidation and ketone production in livers expressing Foxa2T156A



Summary

Foxa2 is inactive (cytoplasmic localization) in hepatocytes of insulin resistant/diabetic animal models

Constitutive active Foxa2 (adenoviral delivery) decreases hepatic triglyceride content, increases hepatic insulin sensitivity, reduces glucose production, normalizes plasma glucose and reduces plasma insulin.

These changes are associated with an induction of genes of fatty acid oxidation, ketogenesis and glycolysis.

Ongoing/future studies:

Study the metabolic changes of conditional expression of constitutive active expression of Foxa2 and investigate the effect on atherosclerotic lesion formation and regression (with Breslow, Dansky, Fisher)

Study the metabolic changes of dominant negative Foxa2 expression in the liver and investigate the effect on atherosclerotic lesion formation and regression (with Breslow, Dansky, Fisher)

Will diet induce obesity/diabetes increase neointimal formation in response to arterial injury ?

- C57BL/6 mice fed a chow or high fat diet for 9 weeks
- Metabolic parameters measured
- endothelial function assessed in the femoral artery using ex vivo myography
- bilateral femoral artery injury performed to assess neointimal formation

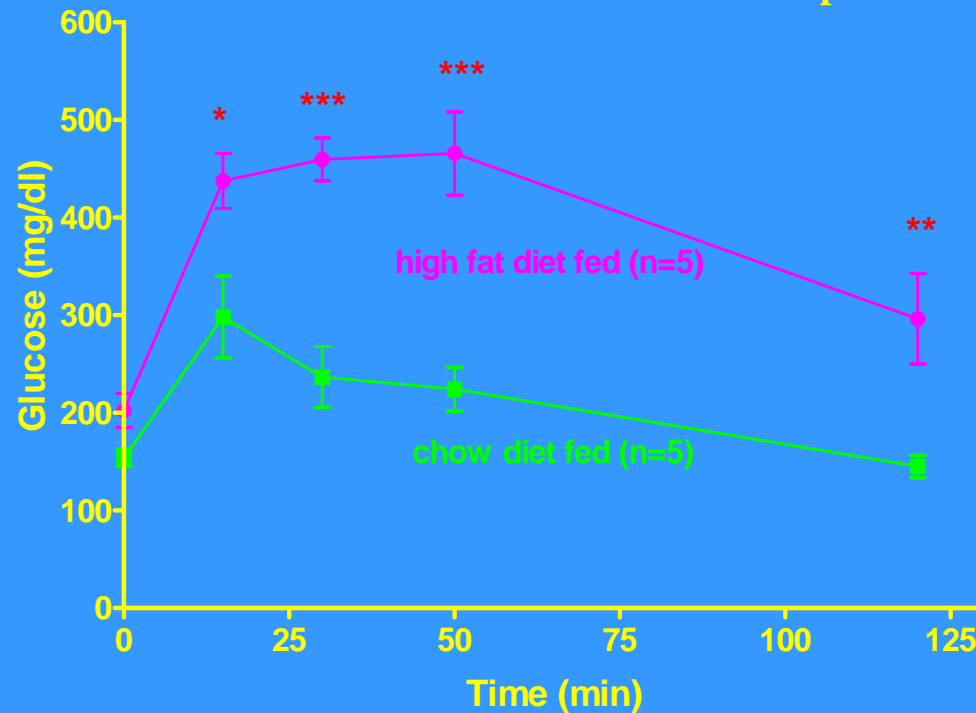
Diet induced obesity in C57BL/6 mice resulted in elevations in body weight, leptin, glucose, insulin and total cholesterol

<u>Parameter</u>	<u>Chow</u>	<u>High fat</u>	
weight	32±1 (17)	39±1(30)*	↑
Leptin	4±1(10)	56±5(20)*	↑
Glucose	135±7(10)	226±9(20)*	↑
Insulin	0.28±0.04 (10)	2.5±0.4(25)*	↑
total chol	79±4(7)	139±6(10)*	↑
Triglyceride	46±2(7)	14±1(11)*	↓
FFA	0.59±0.04(10)	0.47±0.06(10)	

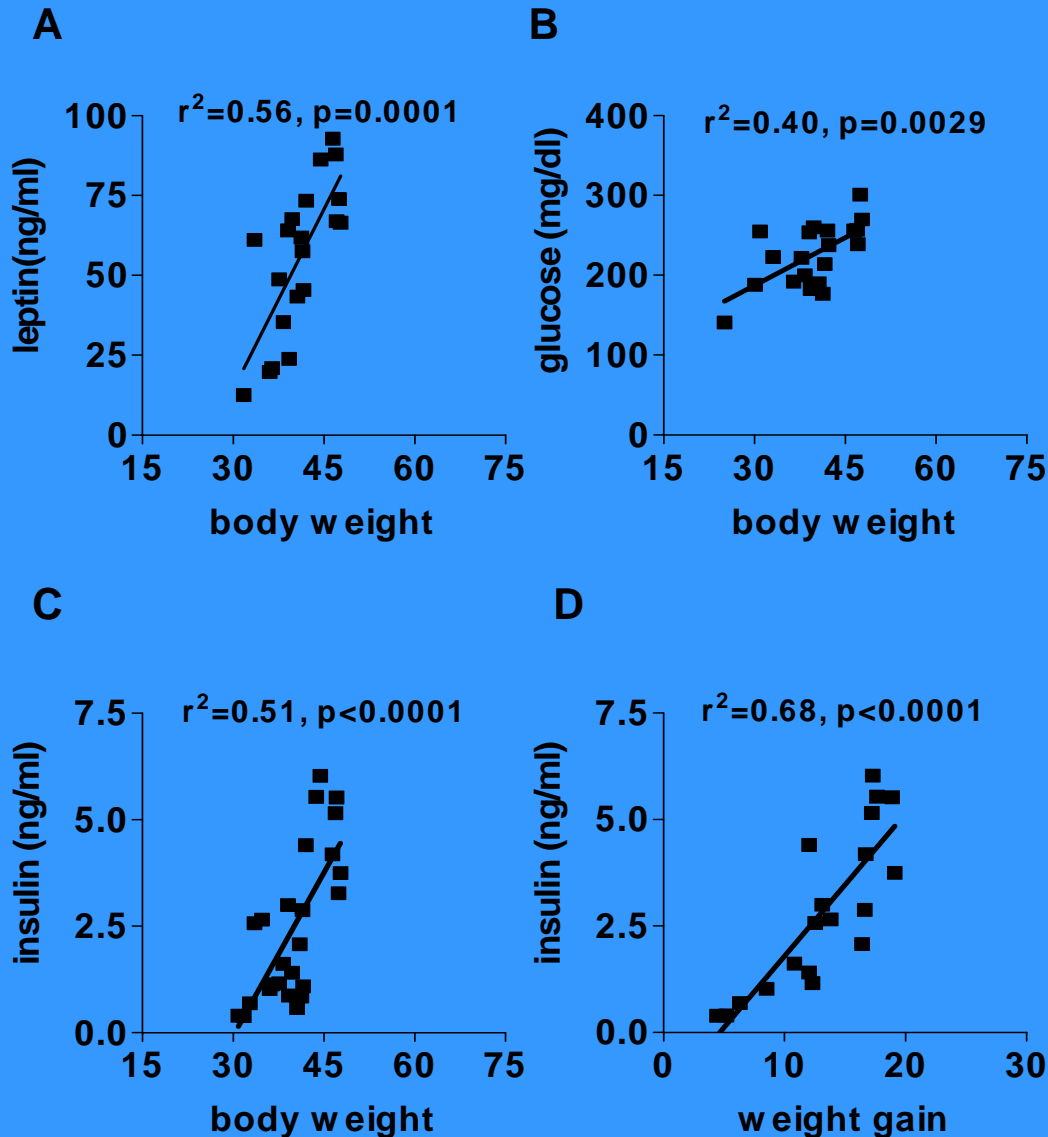
* p<0.0001

High Fat Feeding Results in Impaired Glucose Tolerance in C57bl/6 Wild Type Mice

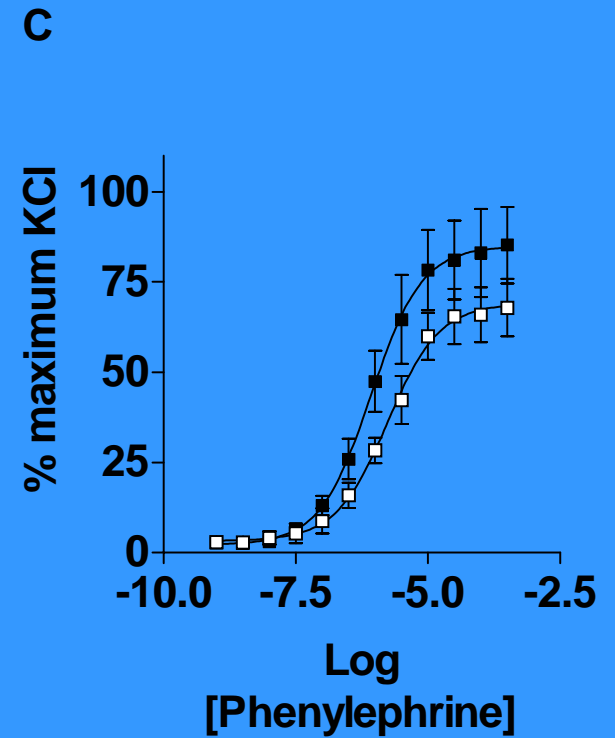
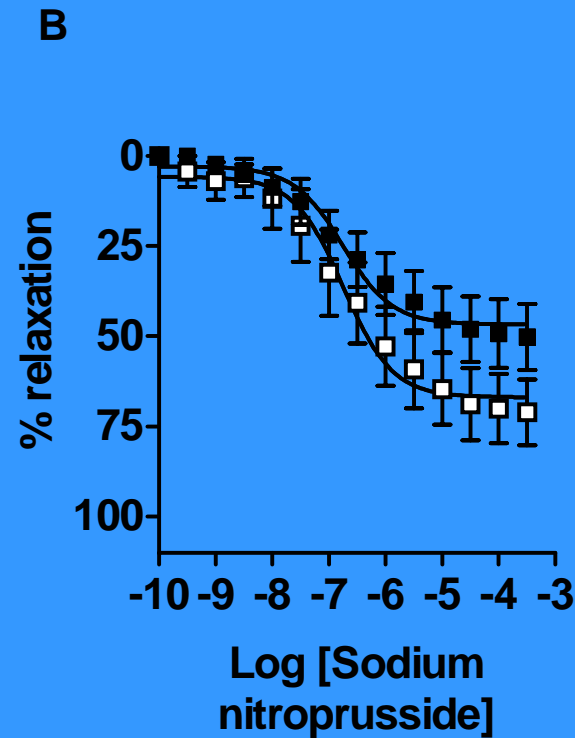
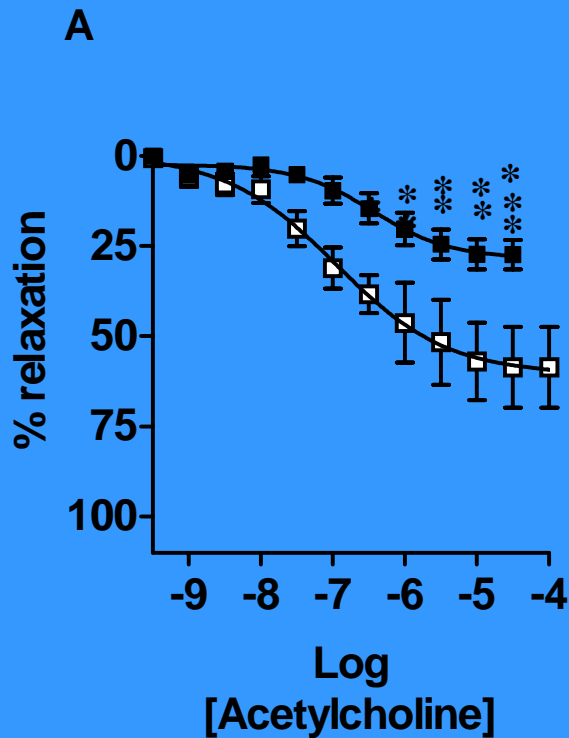
1mg/g body weight glucose
i.p. after 4h fast



Strong correlations between body weight and metabolic parameters in high fat diet fed C57Bl/6 mice.

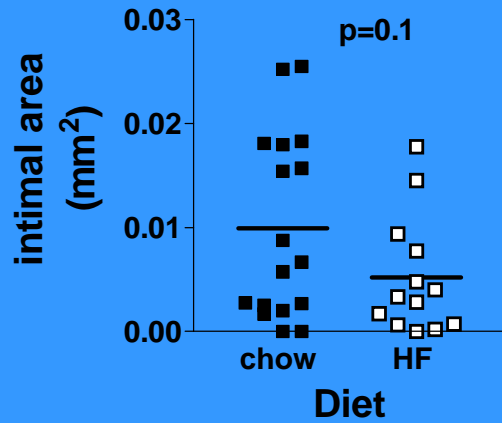


Impaired endothelium dependent relaxation and increased sensitivity to alpha adrenergic stimuli in the femoral arteries of high fat diet fed diabetic mice

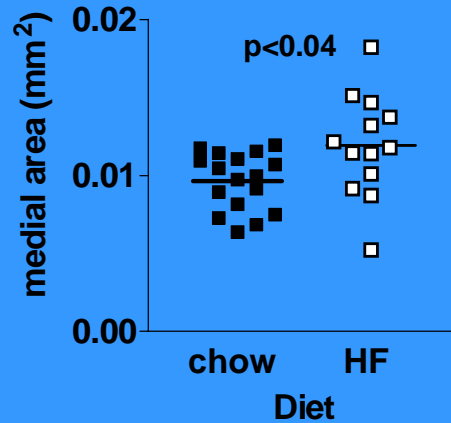


Diabetes/insulin resistance did not increase neointimal formation in C57Bl/6 wild type mice

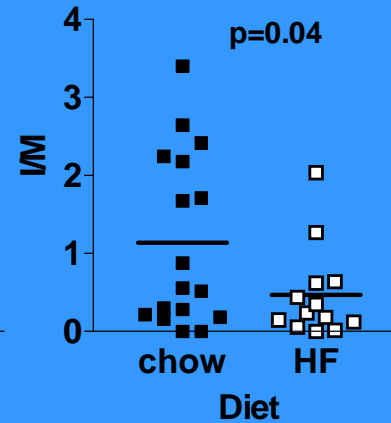
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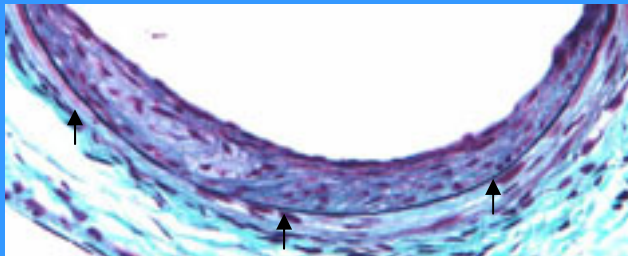
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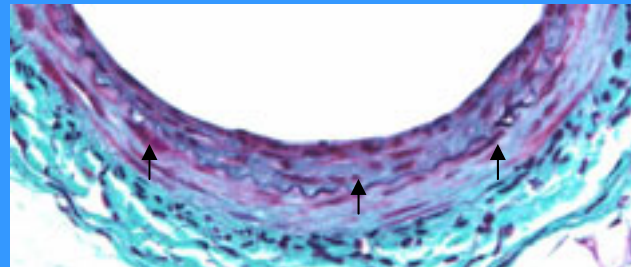
C



D



E



Conclusions

- High fat diet feeding of C57Bl/6 mice induced obesity and a type 2 diabetes phenotype.
- diabetes impaired endothelium dependent vasodilation and to a small extent endothelium independent relaxation. Diabetes increased vasoconstriction to alpha adrenergic agents.
- Neointimal formation decreased in diabetic mice. This decrease in neointimal formation in diet induced obese/diabetic mice was consistent with our previous study in which neointimal formation also decreased in diabetic db/db mice.