Mouse Models of Diabetic Macrovascular Disease RU/Columbia/NYU Group

1. **“Create new mouse models of diabetes” Markus Stoffel-RU**
2. **“Create mouse models of diabetic dyslipidemia” Ira Goldberg-Columbia**
3. **“Effect of diabetes on atherosclerosis progression” Jan Breslow-RU**
4. **“Effects of diabetes on atherosclerosis regression/remodeling” Ed Fisher-NYU**
5. **“Effect of diabetes on arterial injury/restenosis” Hayes Dansky-Columbia**

Publications 05-06

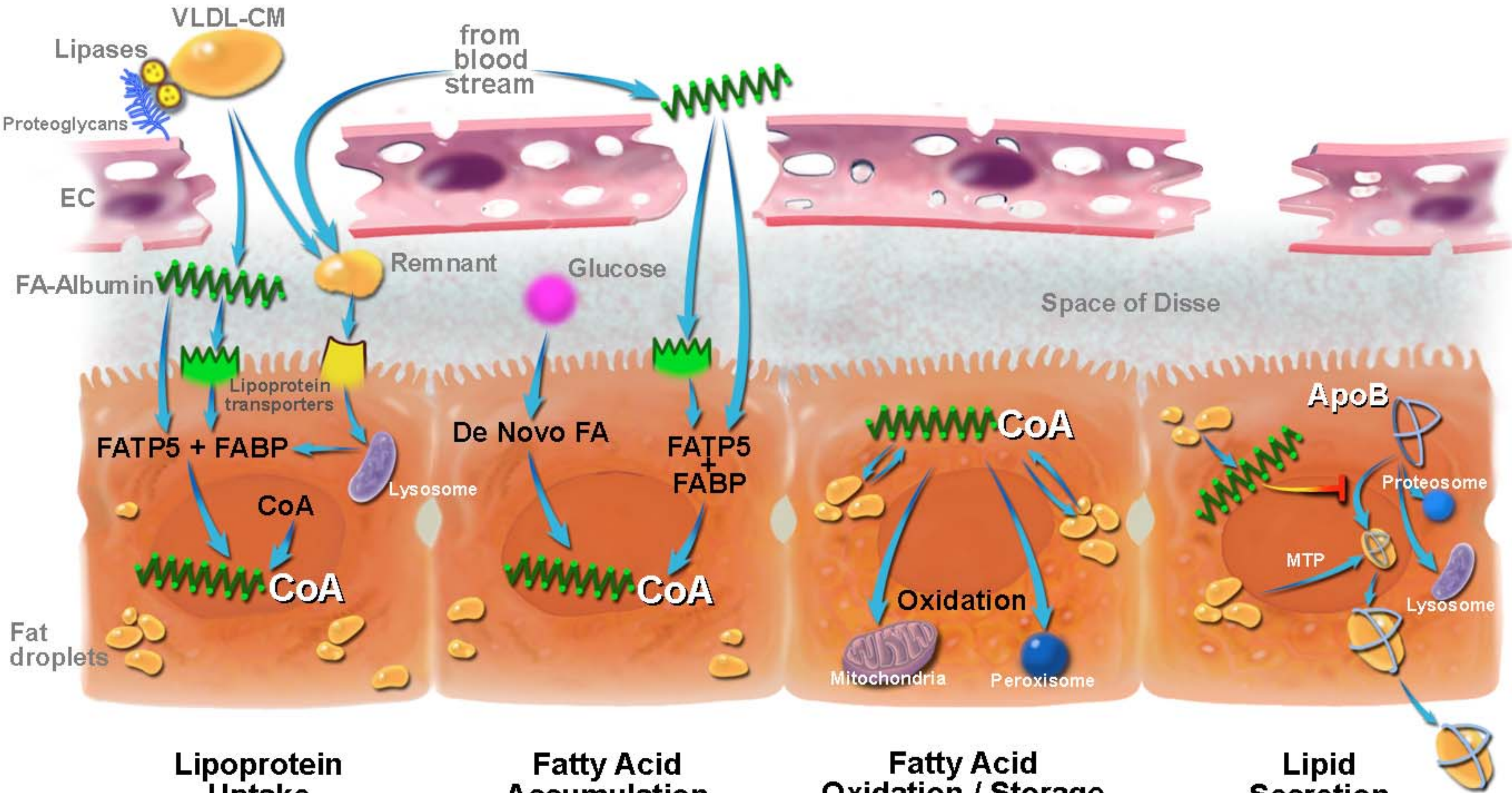
- Wolfrum, C and Stoffel, M. Coactivation of Foxa2 through Pgc-1β promotes liver fatty acid oxidation and triglyceride/VLDL secretion. *Cell Metabolism* **3**:99-110, 2006.
- Vikramadithyan RK, Hu Y, Noh H-L, Liang C-P, Hallam K, Tall AR, Ramasamy A, Goldberg IJ. Human Aldose Reductase Expression Accelerates Diabetic Atherosclerosis in Transgenic Mice. J Clin Invest, 115 2434-2443, 2005
- Teupser D, Tan M, Persky AD, Breslow JL. Atherosclerosis quantitative trait loci are sex- and lineage-dependent in an intercross of C57BL/6J and FVB/N low-density lipoprotein receptor -/- mice. *Proc Natl Acad Sci USA*. 2006;**103**:123-128.
- Eugene Trogan, Jonathan E. Feig, Snjezana Dogan, George H. Rothblat, Véronique Angeli, Frank Tacke, Gwendalyn Randolph, and Edward A. Fisher. Gene Expression Changes in Foam Cells and the Role of the Dendritic Cell Migration Factor CCR7 During Atherosclerosis Regression in ApoE-deficient Mice, PNAS, early edition, 2006.
- Eugene Trogan and Edward A. Fisher. Laser capture microdissection for analysis of macrophage gene expression from atherosclerotic lesions. Methods Mol Biol. 2005;293:221-31.
- Molnar J, Yu S, Mzhavia N, Pau C, Chereshnev I, Dansky HM. Diabetes induces endothelial dysfunction but does not increase neointimal formation in high-fat diet fed C57BL/6J mice. Circ Res. 2005 Jun 10;96(11):1178-84.
- Salzberg S, Filsoufi F, Anyanwu A, von Harbou K, Karlof E, Carpentier A, Dansky HM, Adams DH. Neointimal formation is increased in the setting of type 2 diabetes mellitus in a murine model of vein grafting. Circulation supplement (in press)

Markus Stoffel

Create new mouse models of diabetes

- Studies of transcription factors mediating insulin actions
- Focus on Fox2a and its actions in the liver
- Insights in a diabetic complication, NAFLD

Pathogenesis of NAFLD



Foxa2

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Fasting

Low Insulin

↓

Foxa2
(Nuclear)

Pgc1β

Mcad/CptI ***Mtp/Dgat2***

β-oxidation ketogenesis ***VLDL synthesis***

Fed

High Insulin

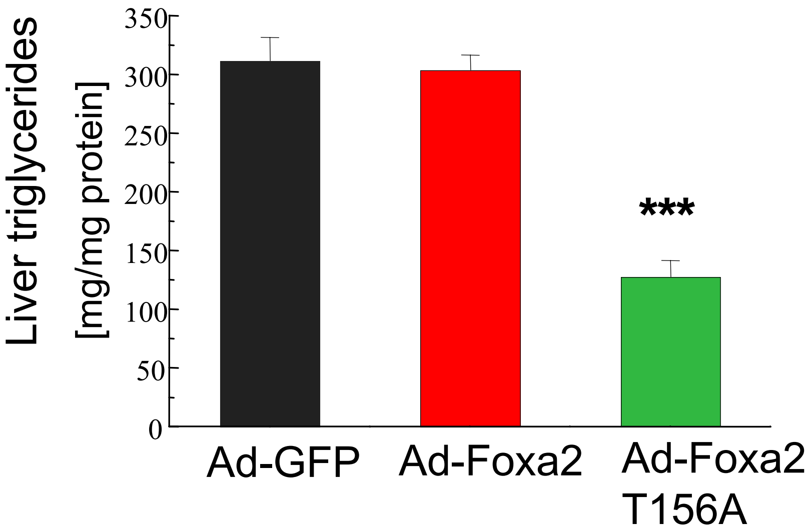
↓

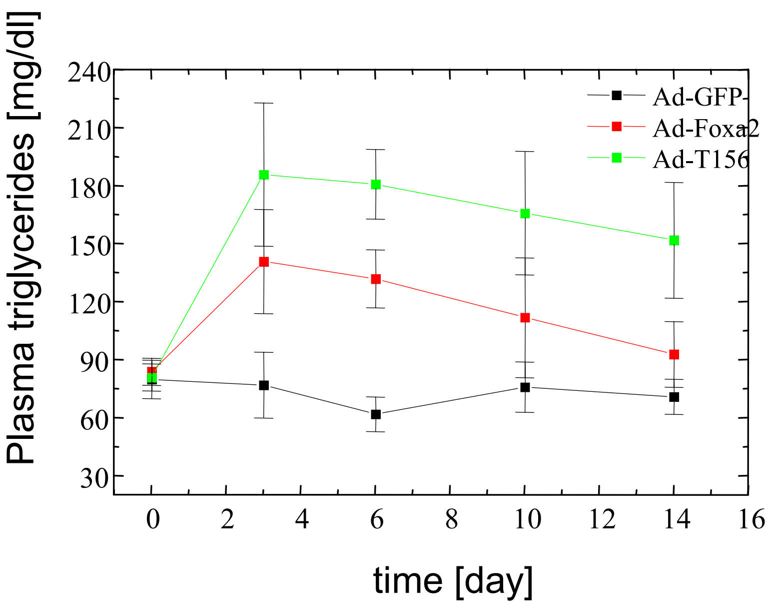
Phosphorylated Foxa2
(Nuclear Exclusion)

↓

↓VLDL synthesis

Increased plasma triglycerides in *ob/ob* mice injected with Ad-Foxa2T156A (constitutively active – not phosphorylated)





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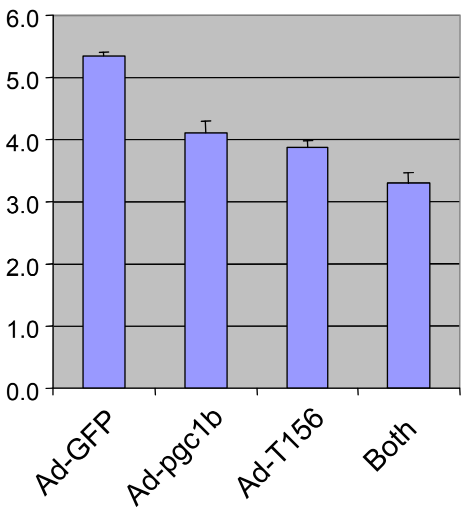
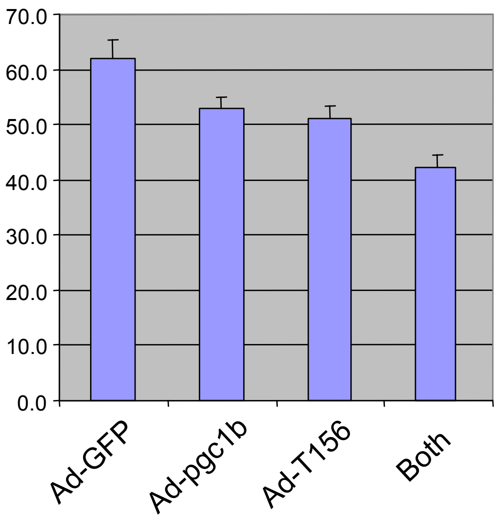
Metabolic parameters of Ad-Pgc1β and Ad-Foxa2 injected ob/ob mice

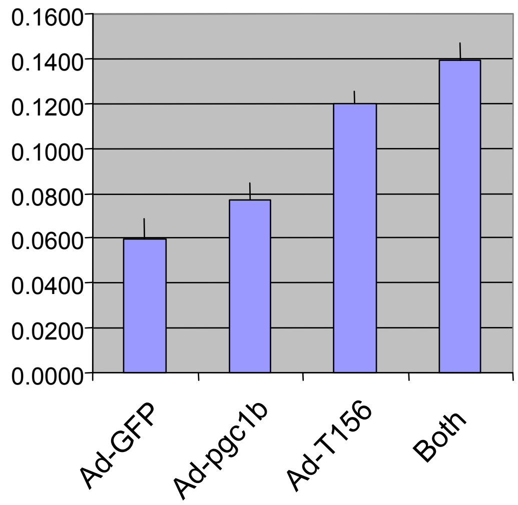
Liver weight



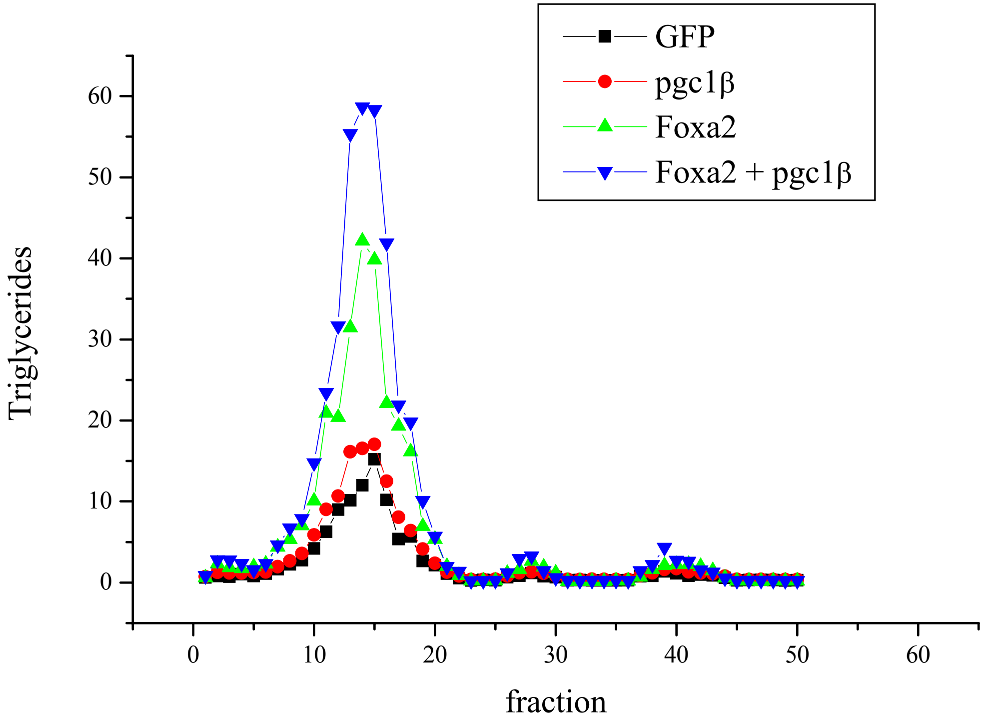
Liver TG



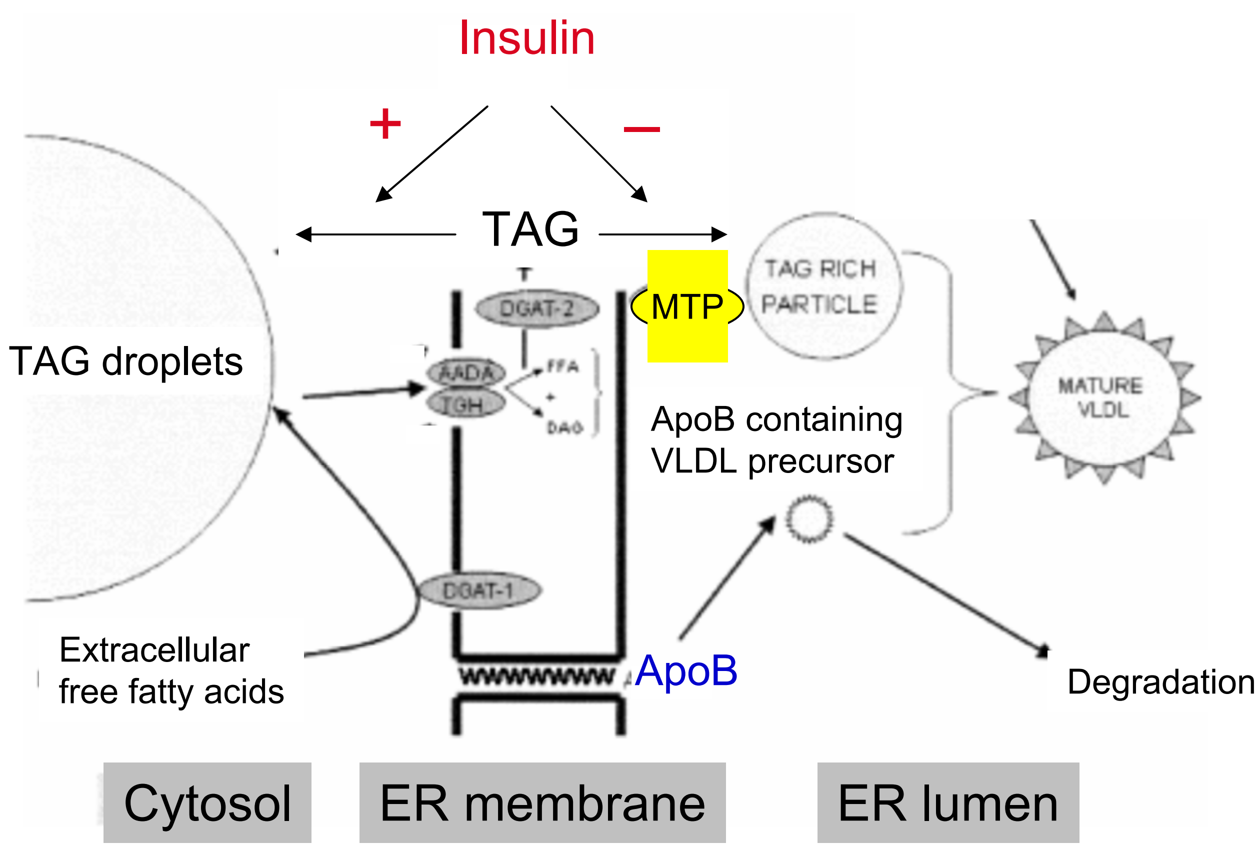
β-oxidation



Plasma lipoproteins of Ad-Pgc1β and Ad-Foxa2 injected *ob/ob* mice

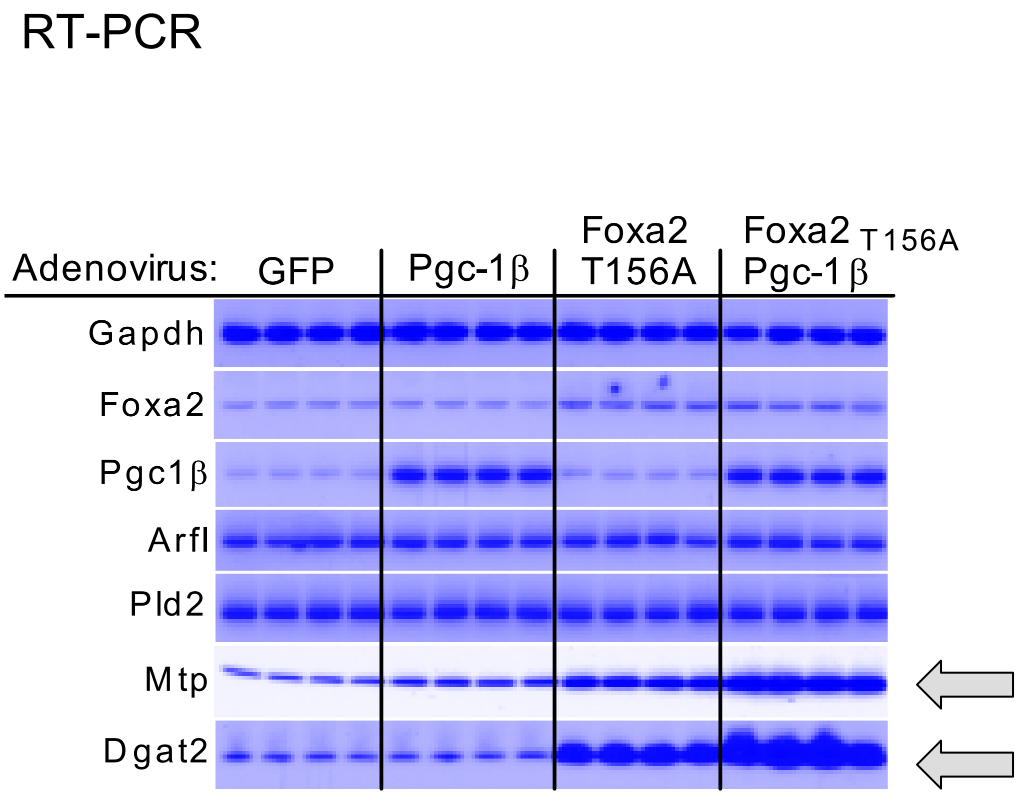


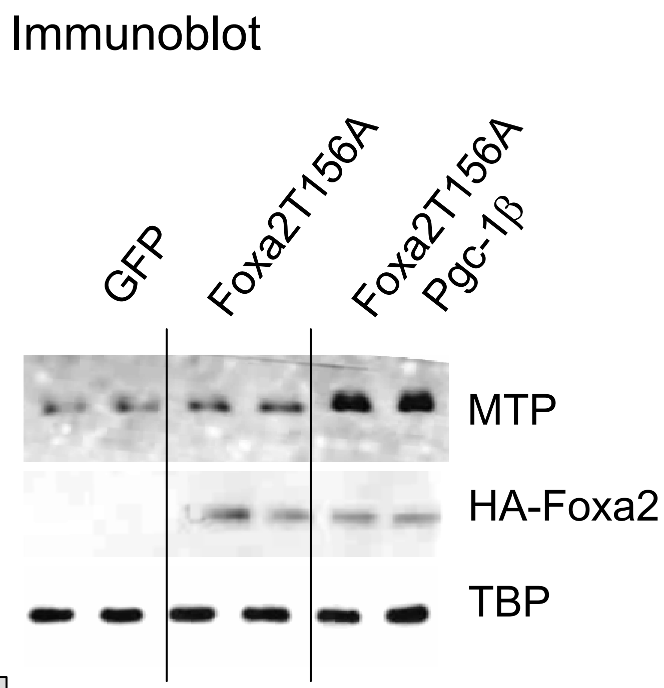
Regulation of VLDL secretion by insulin



Modified from G.F.Gibbons et al, 2004

Increased MTP expression in *ob/ob* mice infected with Ad-Foxa2T156A





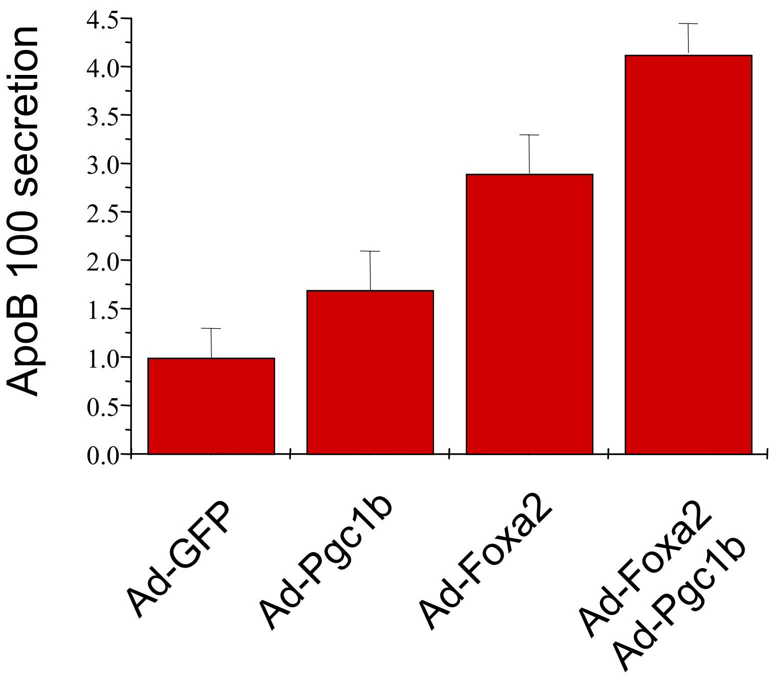
Induction of MTP expression by Foxa2/Pgc1β leads to increased ApoB secretion in HepG2 cells

HepG2 cells are infected with Pgc1β and/or Foxa2

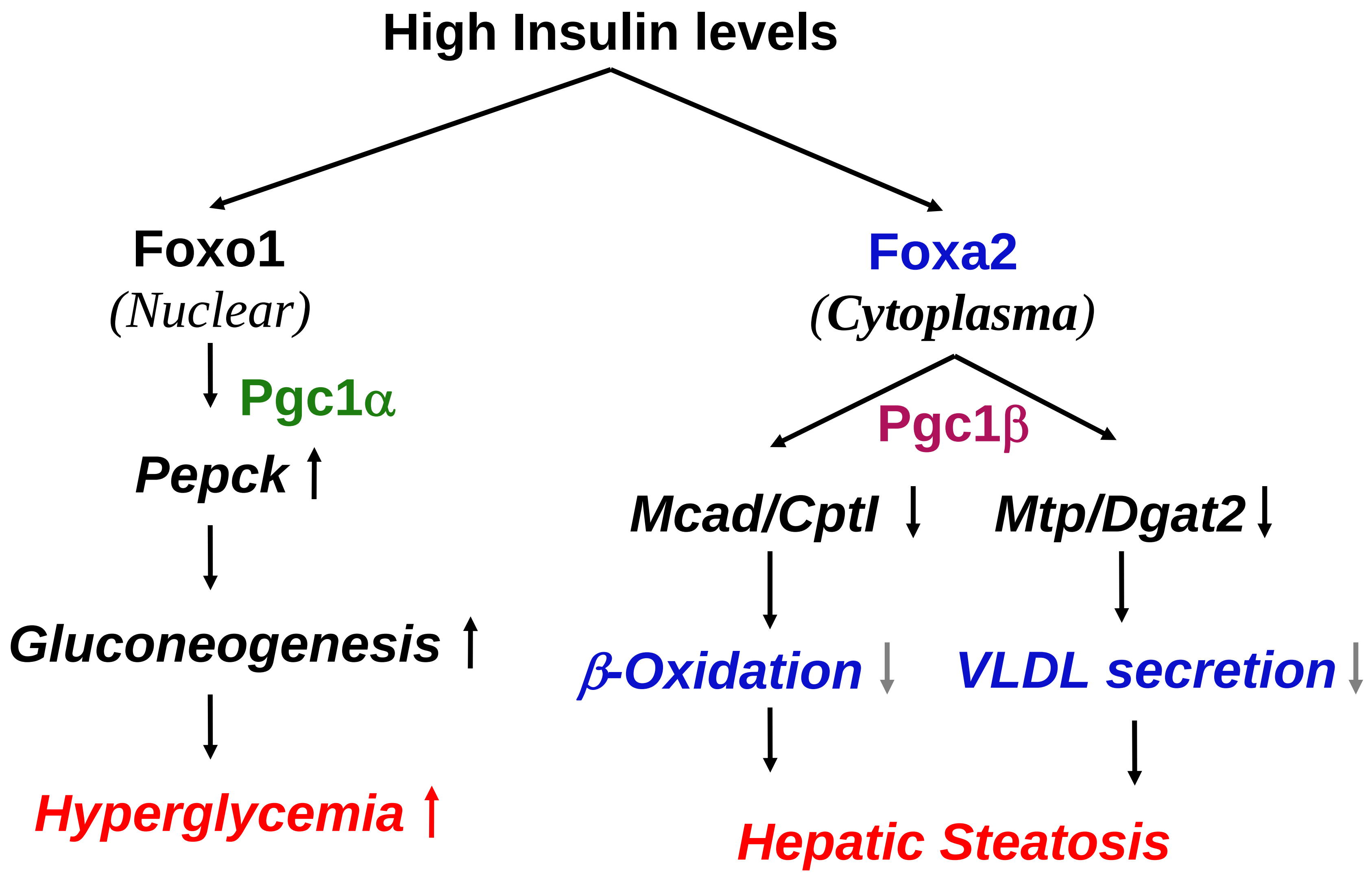
24h postinfection cells are labeled with 35S-Methionin (2h pulse)

Lipoprotein media is added to the cells and samples are taken after 2h

ApoB100 is precipitated and detected on a SDS-PAGE by autoradiography



Role of Foxa2 in Insulin Resistance



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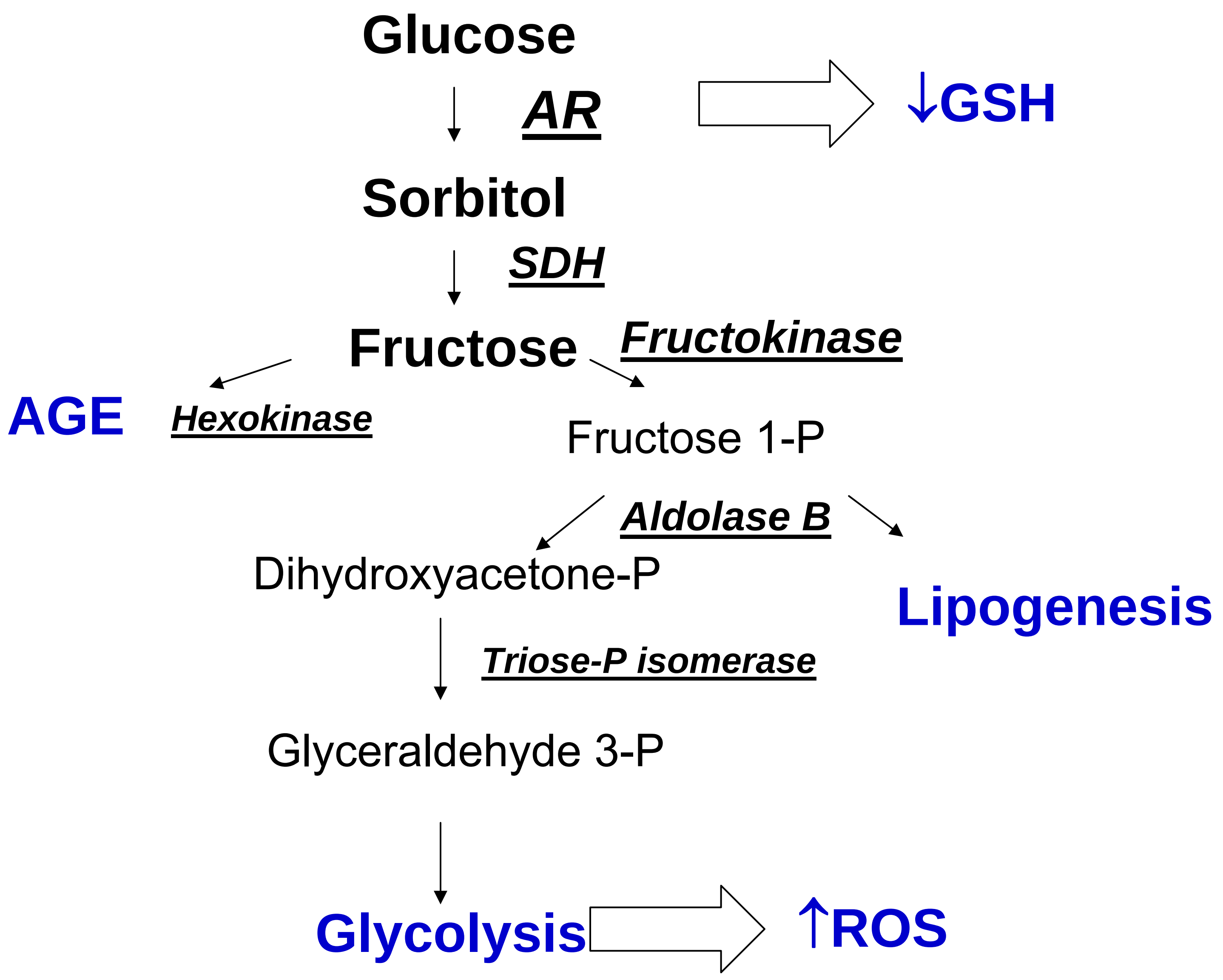
Reeba
Vikramadithyan
Yunying
Winnie Hu
Hye-Lim Noh

Aortas of hAR-Ldlr-/- and Ldlr-/- STZ mice
(6 weeks on 0.15% cholesterol diet)

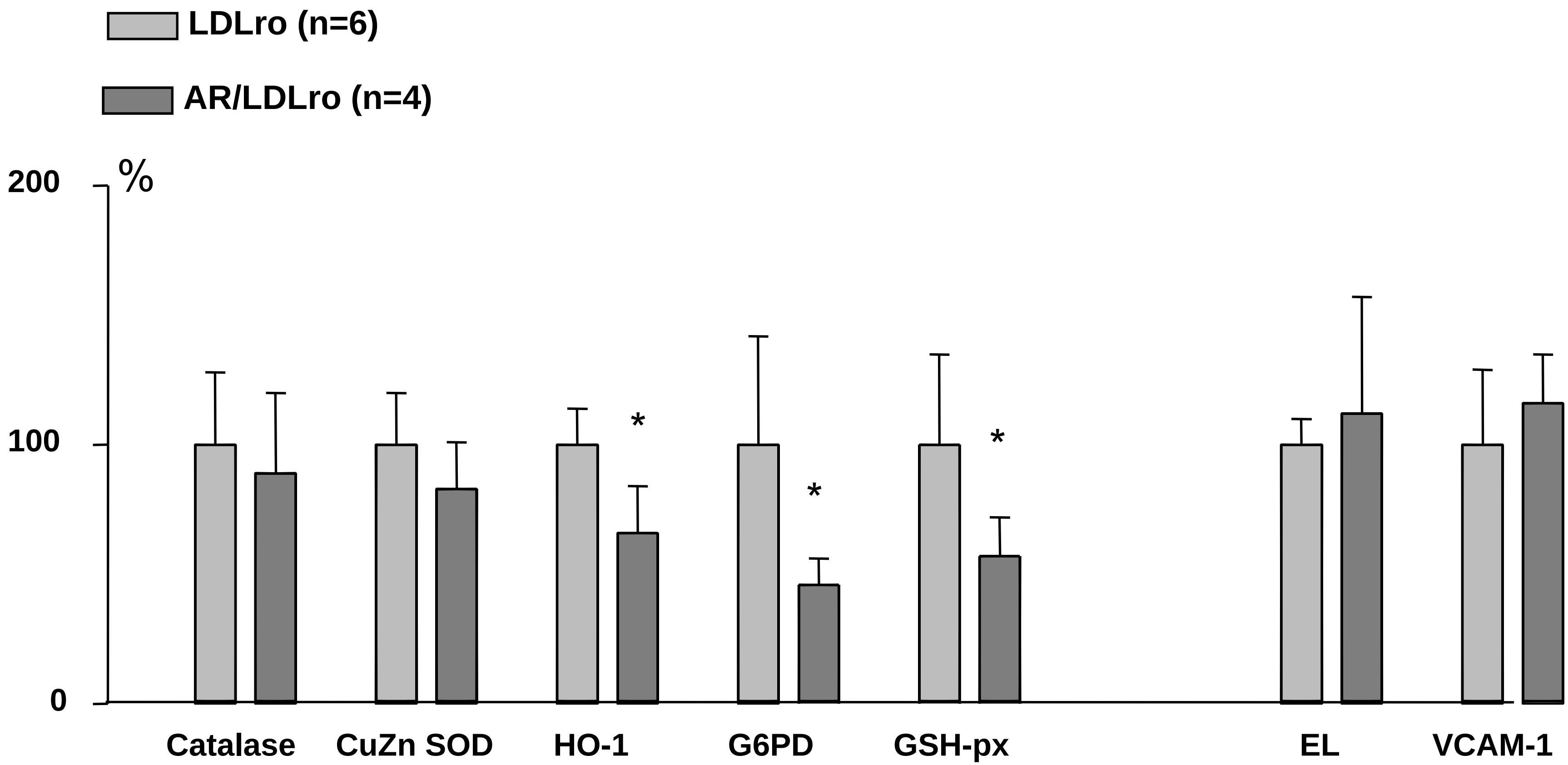
Ldlr-/- + STZ

hAR-*Ldlr-/- + STZ*

Polyol Pathway



Gene expression in Aorta
(n=4-6/groups in the diabetic mice, a month after STZ tx)



*** p<0.05 compared to LDLr0 mice**

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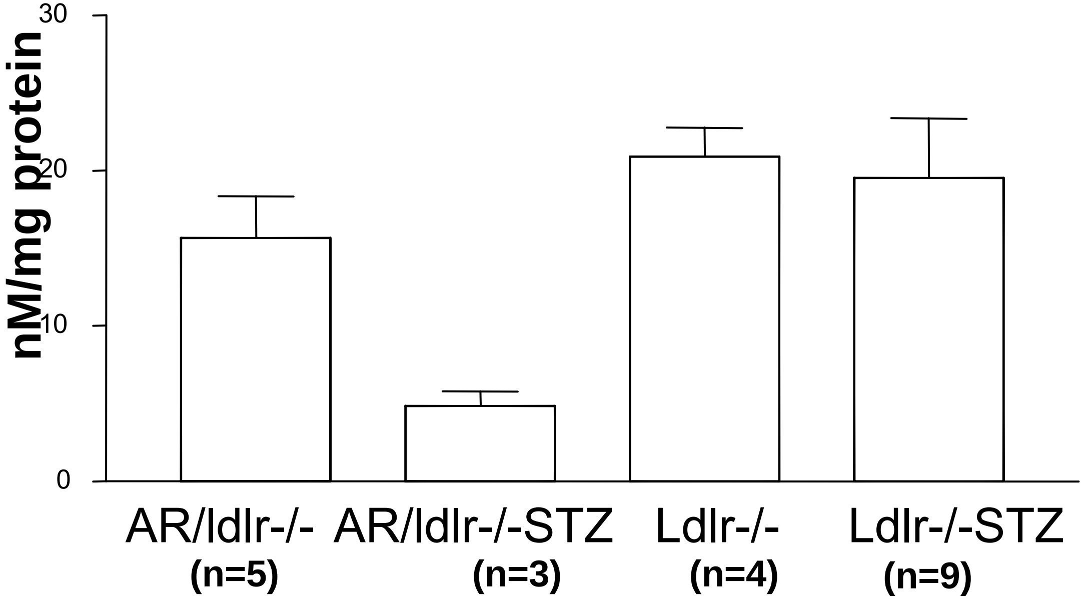
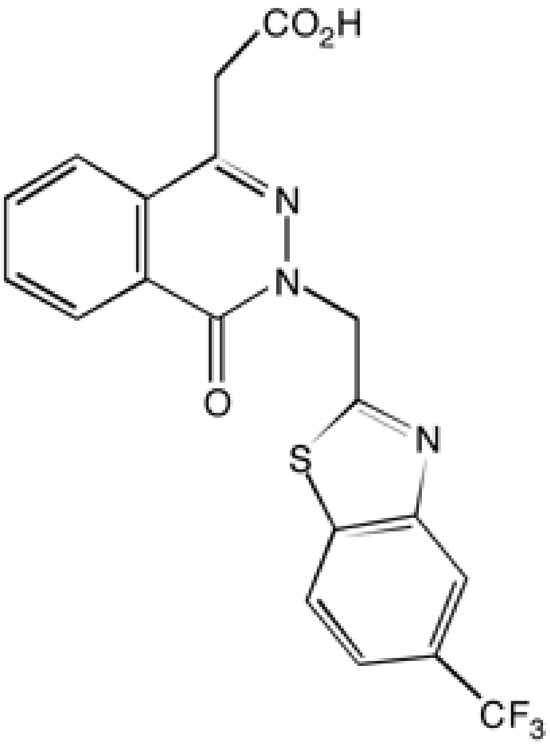
Aortic GSH levels are reduced in STZ-Rx hAR mice

The mice with 0.15% cholesterol diet for 6weeks



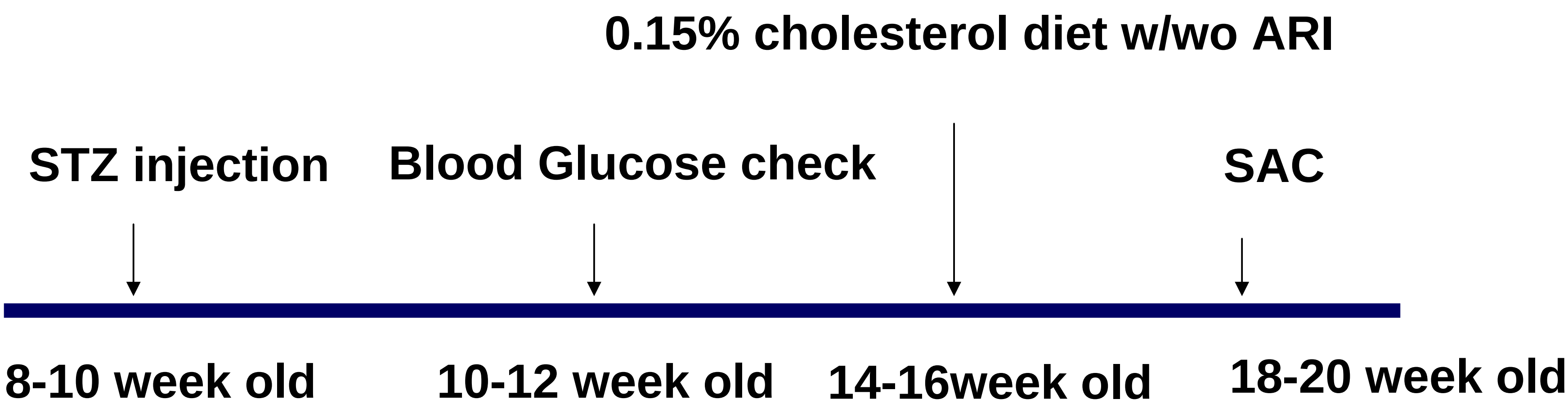
Aldose reductase inhibitor -- Zopolrestat



ZOPOLRESTAT

ARI treatments Methods

Experiment groups : all AR/Ldlr-/- mice with 0.15% cholesterol for 4weeks
Control, ARI, STZ, STZ-ARI
ARI treatments : 4weeks, zopolrestat - 80mg/kg/day (mixed with food)



The atherosclerosis lesion size in the aortic root area

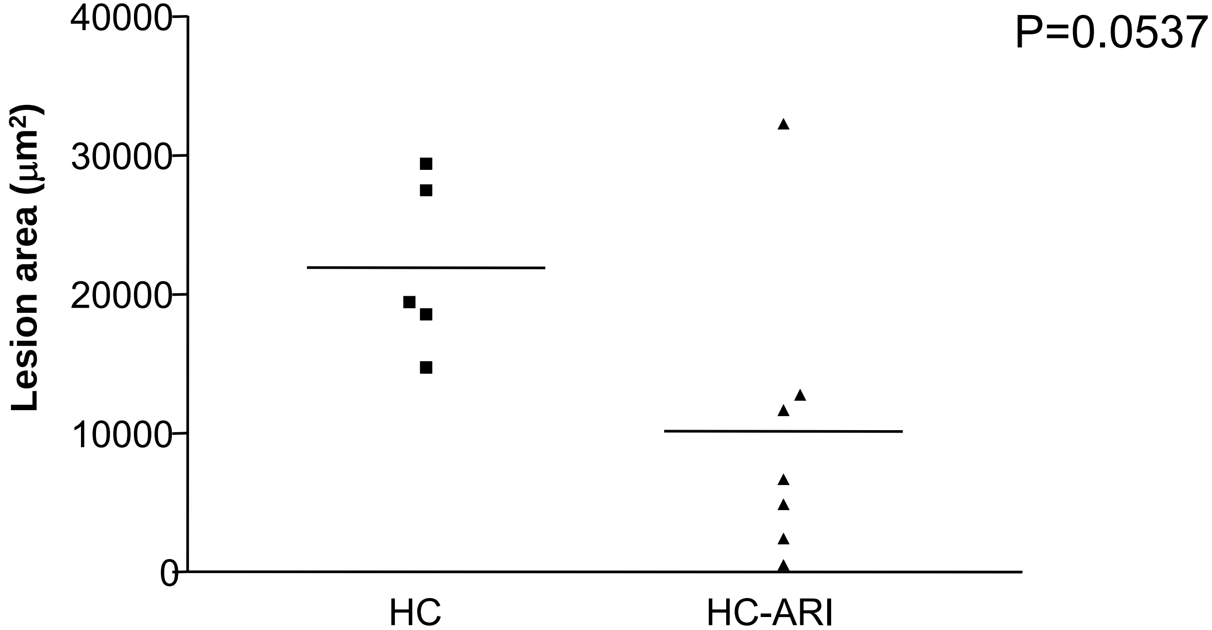
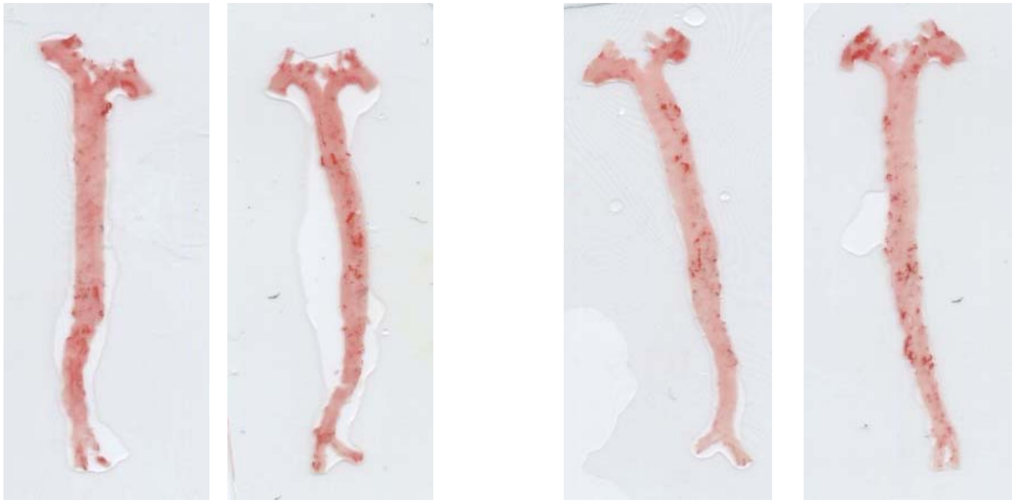


Fig. The atherosclerosis lesion size in the aortic root area in male hAR-*Ldlr*–/– diabetic mice fed the HC or HC-ARI for 4 weeks.

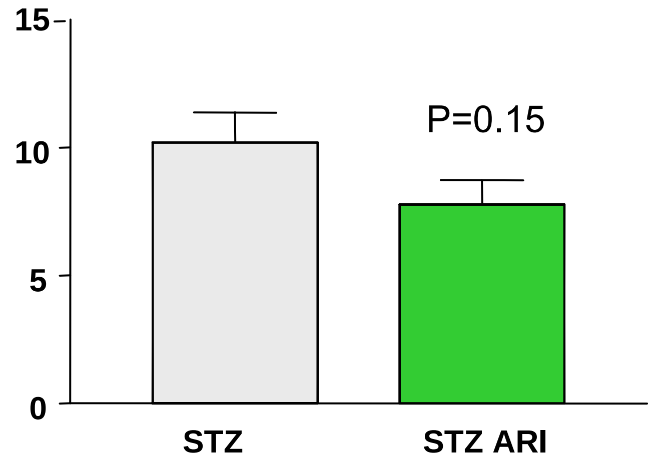
(A) Oil red O staining of en face lesion area



STZ

STZ- ARI

(B) Total lesion area (%)



Will ARI treatment improve heart function in hAR/Ldlr-/- diabetic mice?

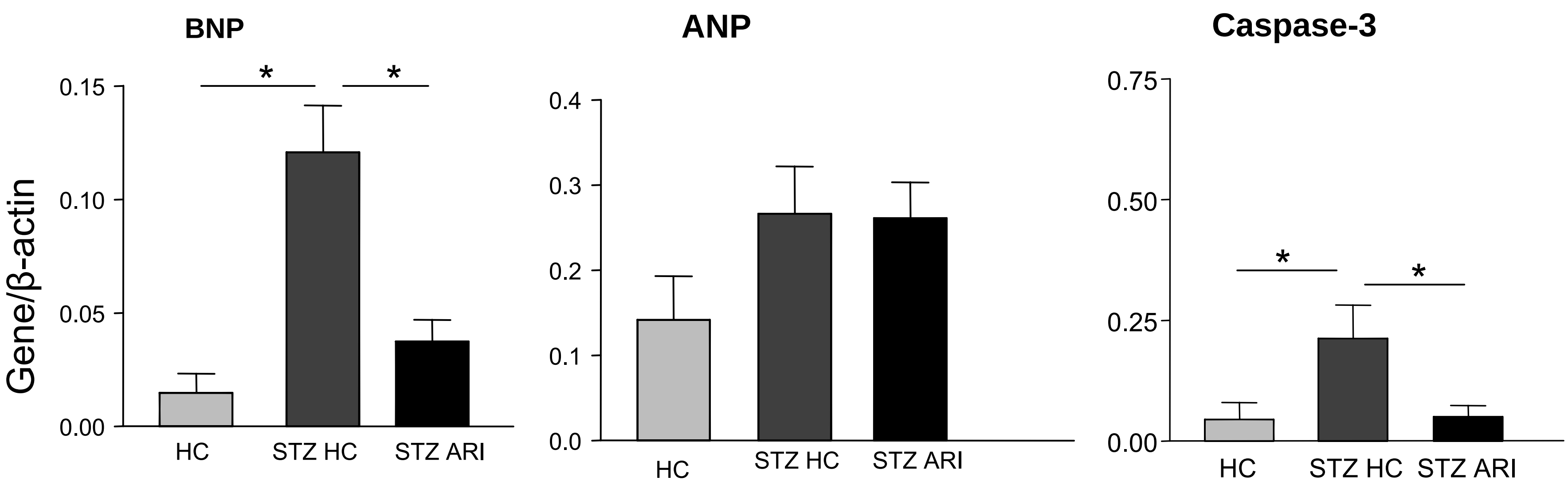
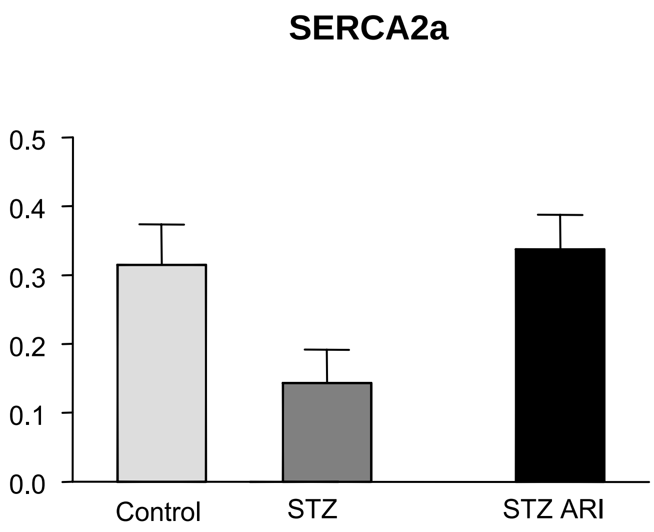
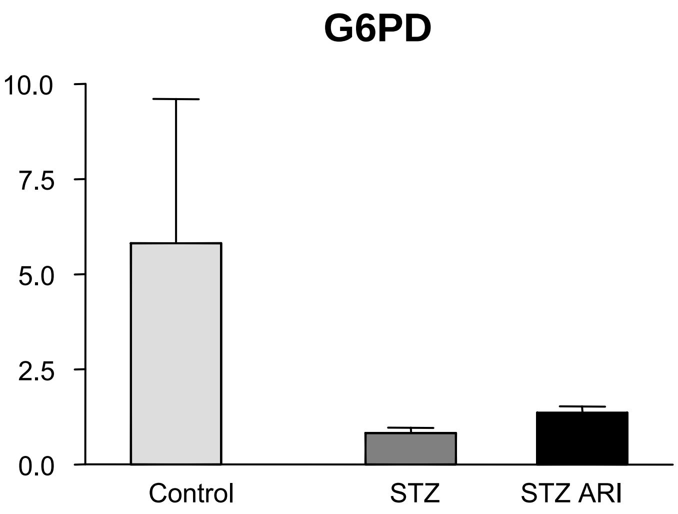


Fig. Heart gene expression in the hAR-*Ldlr–/–* diabetic and non-diabetic mice fed HC or HC-ARI diet for 4 weeks *p<0.05

Heart gene expression in hAR/Ldlr-/- Mice





Future Plan

ARI effects : Fructose, Sorbitol level in tissues and plasma

Atherosclerosis study : Add more samples

- Biochemical data
- TBARS in plasma
- GSH level in Blood and Aorta tissues
- Histology : atherosclerosis lesion area in aorta and heart roots

Heart Function study

Histology :

Trichrome for fibrosis, Caspase-3 and TUNEL for apoptosis

Gene expression :

- ROS defense enzyme (GSH-px, HO-1, catalase, SOD)
- Stress signaling related genes (c-fos, c-jun, HSP-70, nf-kappaB)
- AGE related gene (RAGEs)
- Glucose metabolism (Glut1, Glut4)

Dr. J.

Diabetes & Atherosclerosis in ob/ob; LDLR-/- C57BL/6J Mice

Data, Figures, & Staining

AMDCC, Baltimore, March 21-22, 2006

C57BL/6J LDLR-/- Ob/Ob Males 20 week

	Male LDLR-/- n=14	Male ob/ob; LDLR-/- n=14	Significance t-test
Lesion Area μm^2(x10^4)	13.2±5.8	55.4±16.3	p=0.000***
Body Weight g	26.6±4.0	50.8±3.2	p=0.000***
GTT 0' mg/dl	191±36	185±30	p=0.668
GTT 15' mg/dl	382±67	417±82	p=0.249
GTT 30' mg/dl	371±92	464±106	p=0.025*
GTT 60' mg/dl	288±97	363±121	p=0.094
GTT 120' mg/dl	165±50	211±99	p=0.154
Insulin ng/ml	0.85±0.48	7.09±2.76	p=0.000***
Cholesterol mg/dl	442±120	2724±349	p=0.000***
Non-HDL Chol mg/dl	385±125	2654±345	p=0.001***
HDL Chol mg/dl	56±11	71±22	p=0.038*
Triglycerides mg/dl	87±46	662±210	p=0.000***

* P<0.05; **P<0.01; ***P<0.001

C57BL/6J LDLR-/- Ob/Ob Females 20 week

	Female LDLR-/- n=10	Female ob/ob; LDLR-/- n=11	Significance t-test
Lesion Area $\mu m^2 (x10^4)$	28.0±9.7	50.3±5.9	p=0.000***
Body Weight g	19.1±1.5	49.0±3.3	p=0.000***
GTT 0' mg/dl	140±50	208±42	p=0.003**
GTT 15' mg/dl	284±57	472±81	p=0.000***
GTT 30' mg/dl	239±61	505±77	p=0.000***
GTT 60' mg/dl	167±40	383±117	p=0.000***
GTT 120' mg/dl	103±23	285±137	p=0.001**
Insulin ng/ml	0.80±0.61	15.74±10.17	p=0.000**
Cholesterol mg/dl	690±158	2420±424	p=0.000***
Non-HDL Chol mg/dl	655±163	2334±400	p=0.000***
HDL Chol mg/dl	35±8	86±40	p=0.002**
Triglycerides mg/dl	186±94	612±307	p=0.001**

* P<0.05; **P<0.01; ***P<0.001

C57BL/6J LDLR-/- Ob/Ob Males 32 week

	Male LDLR-/- n=6	Male ob/ob; LDLR-/- n=11	Significance t-test
Lesion Area $\mu m^2 (x10^4)$	39.6±7.7	130.6±4.7	p=0.000***
Body Weight g	30.0±3.1	56.3±10.8	p=0.000***
GTT 0' mg/dl	180±25	210±139	p=0.636
GTT 15' mg/dl	404±59	460±105	p=0.274
GTT 30' mg/dl	505±73	544±65	p=0.306
GTT 60' mg/dl	443±97	468±120	p=0.684
GTT 120' mg/dl	272±153	320±176	p=0.603
Insulin ng/ml	1.0±0.5	6.8±4.4	p=0.039*
Cholesterol mg/dl	536±127	2195±639	p=0.000***
Non-HDL Chol mg/dl	483±131	2115±639	p=0.000***
HDL Chol mg/dl	53±16	80±12	p=0.006**
Triglycerides mg/dl	102±41	315±170	p=0.100*

* P<0.05; **P<0.01; ***P<0.001

C57BL/6J LDLR-/- Ob/Ob Females 32 week

	Female LDLR-/- n=10	Female ob/ob; LDLR-/- n=11	Significance t-test
Lesion Area $\mu m^2 (x10^4)$	75.8±14.1	114.1±14.3	p=0.000***
Body Weight g	23.5±2.0	57.5±6.1	p=0.000***
GTT 0' mg/dl	129±40	148±43	p=0.329
GTT 15' mg/dl	369±101	422±108	p=0.279
GTT 30' mg/dl	438±98	520±130	p=0.133
GTT 60' mg/dl	355±119	465±188	p=0.140
GTT 120' mg/dl	149±30	540±214	p=0.001**
Insulin ng/ml	0.2±0.1	9.7±4.6	p=0.021*
Cholesterol mg/dl	760±201	2206±658	p=0.000***
Non-HDL Chol mg/dl	737±212	2129±659	p=0.001***
HDL Chol mg/dl	28±7	77±10	p=0.000***
Triglycerides mg/dl	185±100	574±435	p=0.111

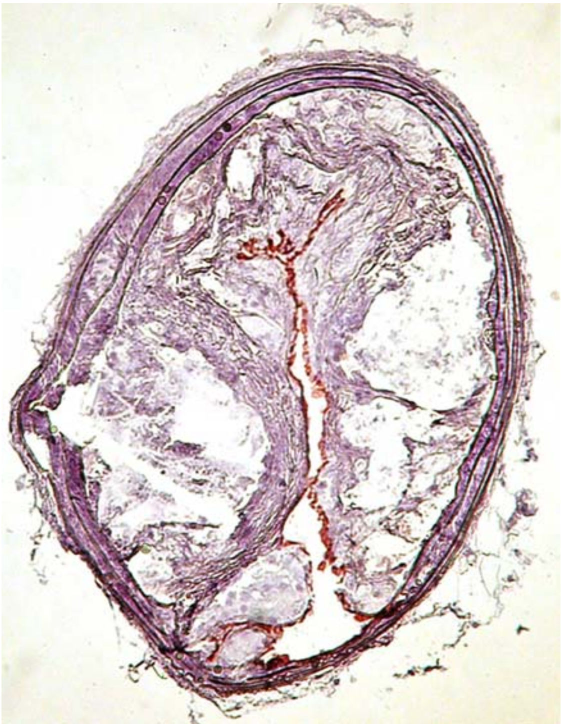
* P<0.05; **P<0.01; ***P<0.001

Movat’s Pentachrome Stain

- **Nuclei and elastic fibers (Black)**
- **Cholesterol clefts/ Proteoglycan (Blue)**
- **Muscle (Red)**
- **Collagen/Fibrous cap (Yellow)**
- **Fibrin (Intense Red)**

Immunohistochemistry

Brachiocephalic artery sections from 32 week-old diet-fed males



CD31
(endothelium cells)

CD68
(macrophages)

α-actin
(smooth muscle cells)

Plaque Instability?

- Cross of hAR x Ldlr-/-^{AKITA}
- Cross of MnSOD+/- onto Ldlr-/-^{AKITA}

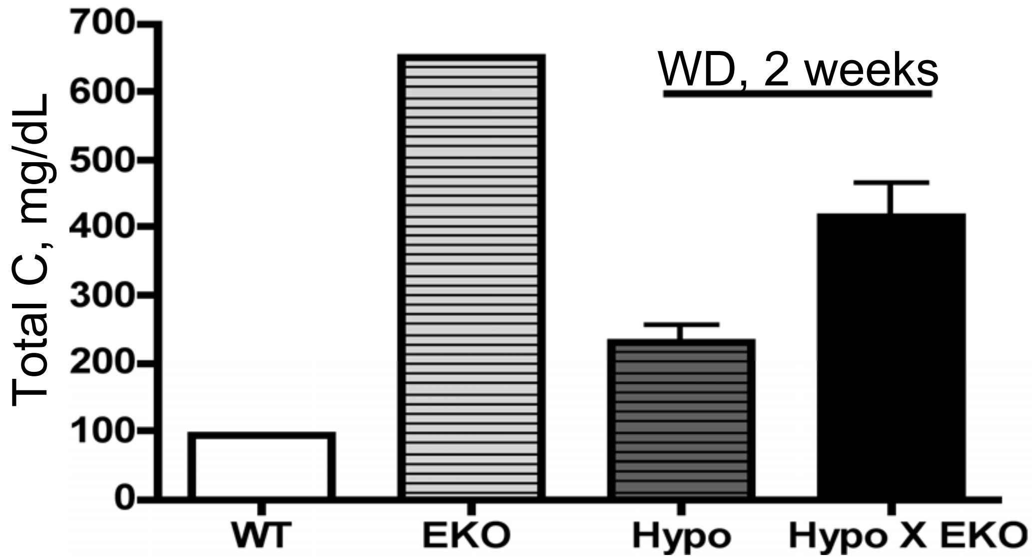
Ed Fisher

Non-surgical Model of Regression Project

- We had proposed to use “hypomorphic” apoE mice
- These mice have low expression of apoE from an engineered apoE gene knocked into both WT loci
- Previous studies used a Paigen (toxic) diet to induce atherosclerosis; apoE expression can be conditionally increased in these mice and total cholesterol falls, resulting in regression
- Our goal: use a non-toxic western diet (WD) and then begin a STZ-study to examine the effects of hyperglycemia on the regression process

- Problem: on WD, TC in hypomorphs are only in the 200 mg/dL range, too low to develop atherosclerosis (next slide)
- Solution: cross hypomorphs to apoE-/- mice- the heterozygotes will have a greater deficiency of apoE and should respond better to WD, but when the expression of apoE is conditionally increased, normalization of TC should still occur

Crossing the Hypomorph Mouse to the apoEKO Mouse Increases the Response to Western Diet

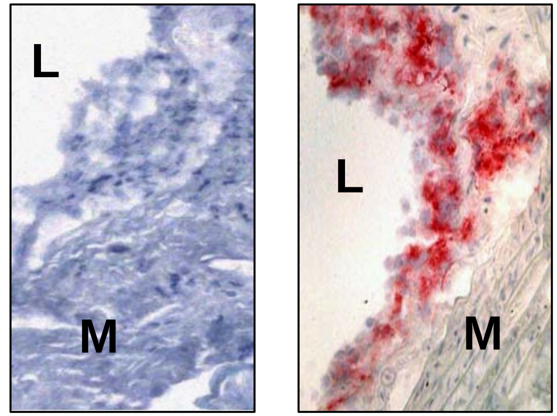


- A new (non-surgical) model, the hypomorph/apoE-/- mouse, is sufficiently responsive to WD to develop atherosclerosis in a short time period.
- This colony will be expanded and used for the hyperglycemia-regression studies, even though such a study may fall outside the funding period.

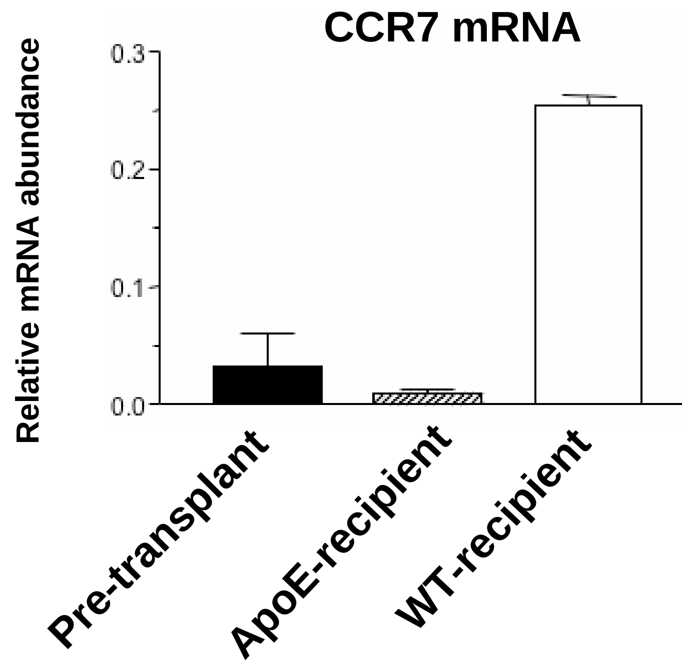
Molecular Regulation of Regression

- In the original grant proposal, we described some approaches to analyze regressing plaques on the molecular level
- We have refined our methods to discover in the transplant regression model that foam cells can leave plaques in large numbers
- Molecular analysis of these plaques using laser capture microdissection and Real Time -PCR has recently shown (Trogan et al., PNAS 2006) that associated with migration was induction of the dendritic cell factor CCR7 in foam cells in regressing plaques (next slide)

CCR7 protein



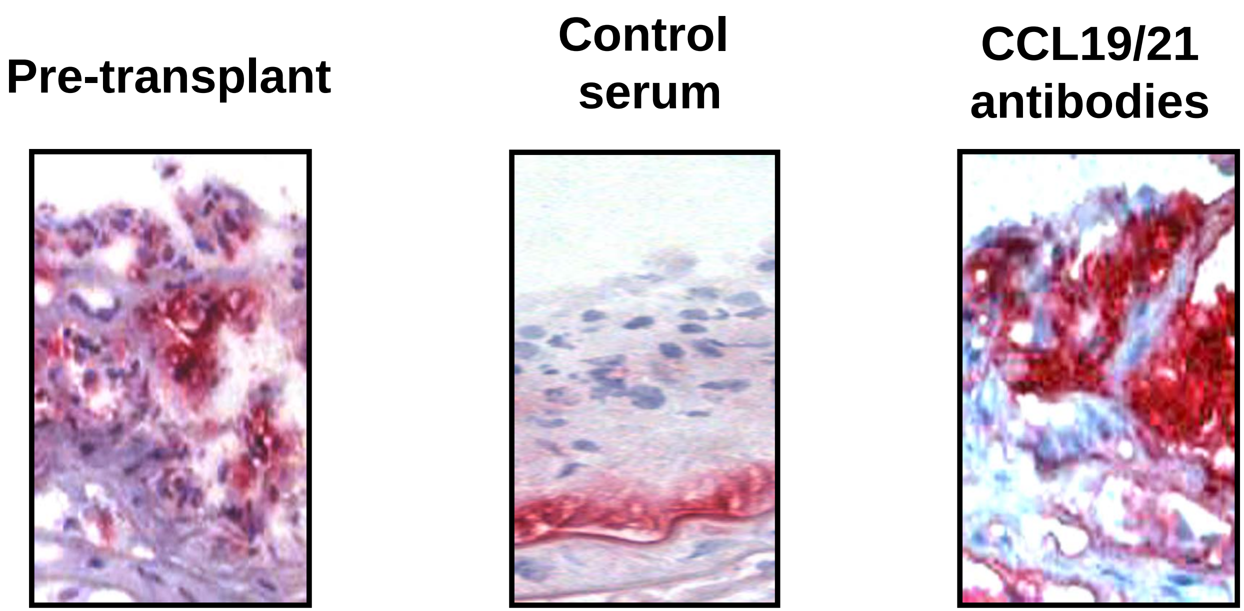
Reminder: transfer of a plaque into an apoE-/- mouse continues progression; into a WT mouse induces regression

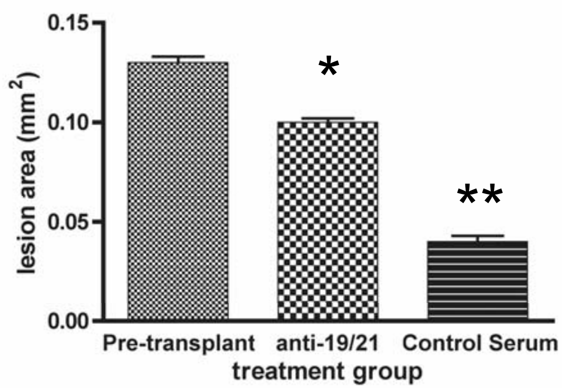


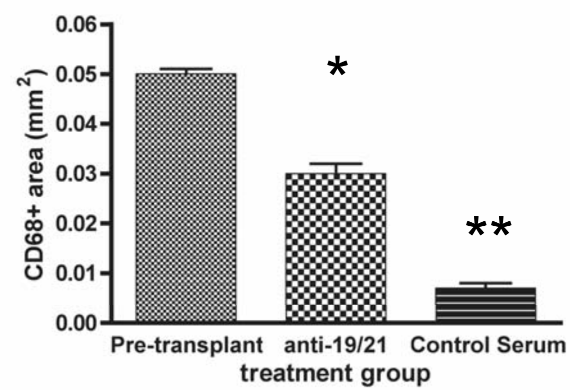
Is CCR7 Functionally Required for Regression?

- Plan: block the CCR7 pathway by injecting transplant recipients with antibodies to the 2 ligands required for DC emigration (CCL19, CCL21). Will foam cells be retained?
- Control is injection of pre-immune serum. Foam cells should leave.

Red = CD68+ foam cells in intima







Conclusion: blocking the CCR7 pathway results in substantial retention of foam cells

Conclusions- Fisher Project

- By modifying the hypomorph mouse, we will have a relatively convenient model of regression and will test the effect of hyperglycemia
- Foam cell emigration from mouse plaques occurs during regression and this process requires CCR7

Hayes Dansky

<u>Major Goal:</u> Create Mouse Models In which Diabetes Worsens Atherosclerosis

<u>Hypotheses</u>

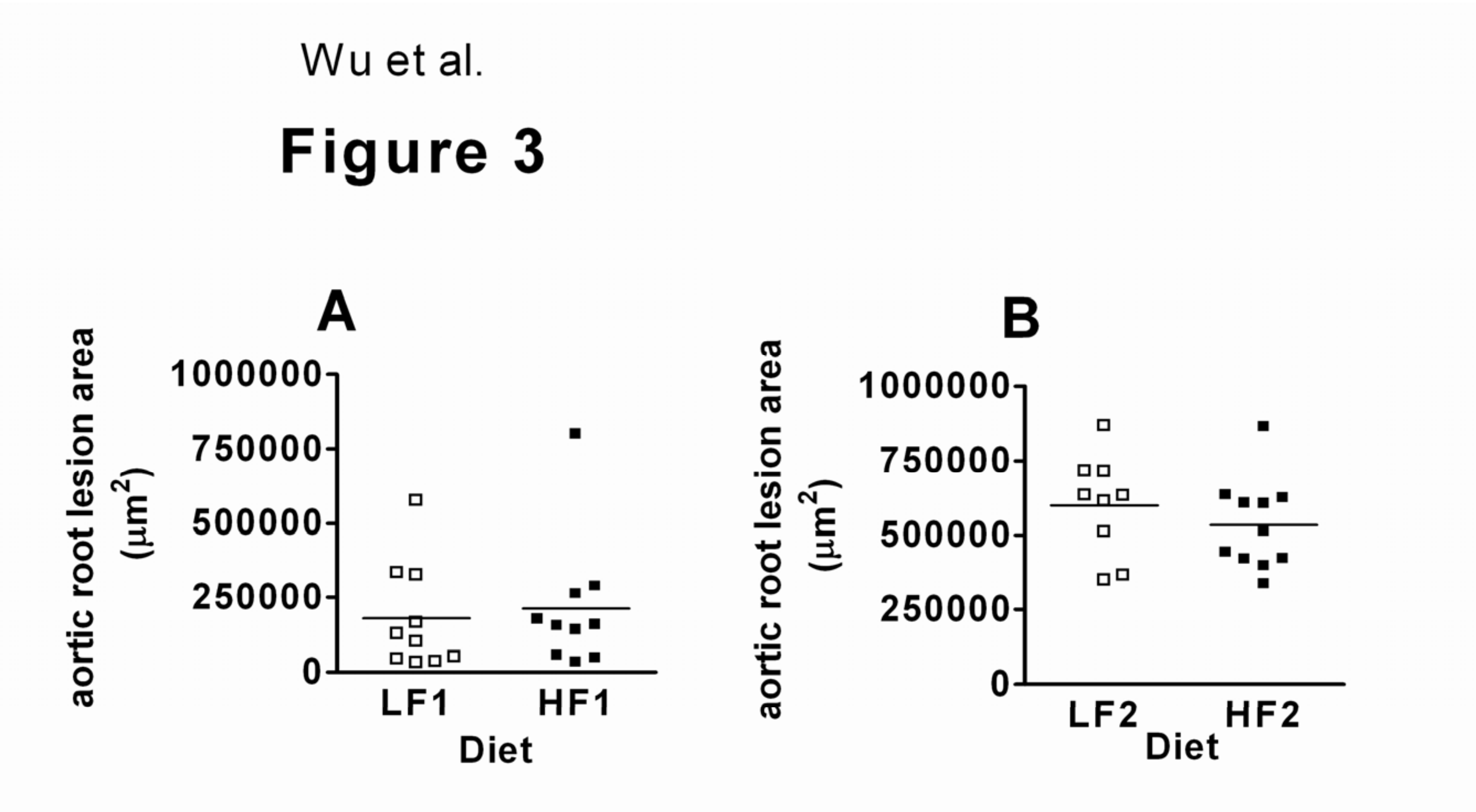
1. Mice do not develop diabetes-induced acceleration of macrovascular disease because they are genetically deficient in a glucose sensitive gene.
2. Increased atherosclerosis in diabetic mice is due to a defect in lesion regression.
3. **Diabetic macrovascular disease is due to hyperinsulinemia, insulin resistance.**

Effects of HF diets on glucose and atherosclerosis

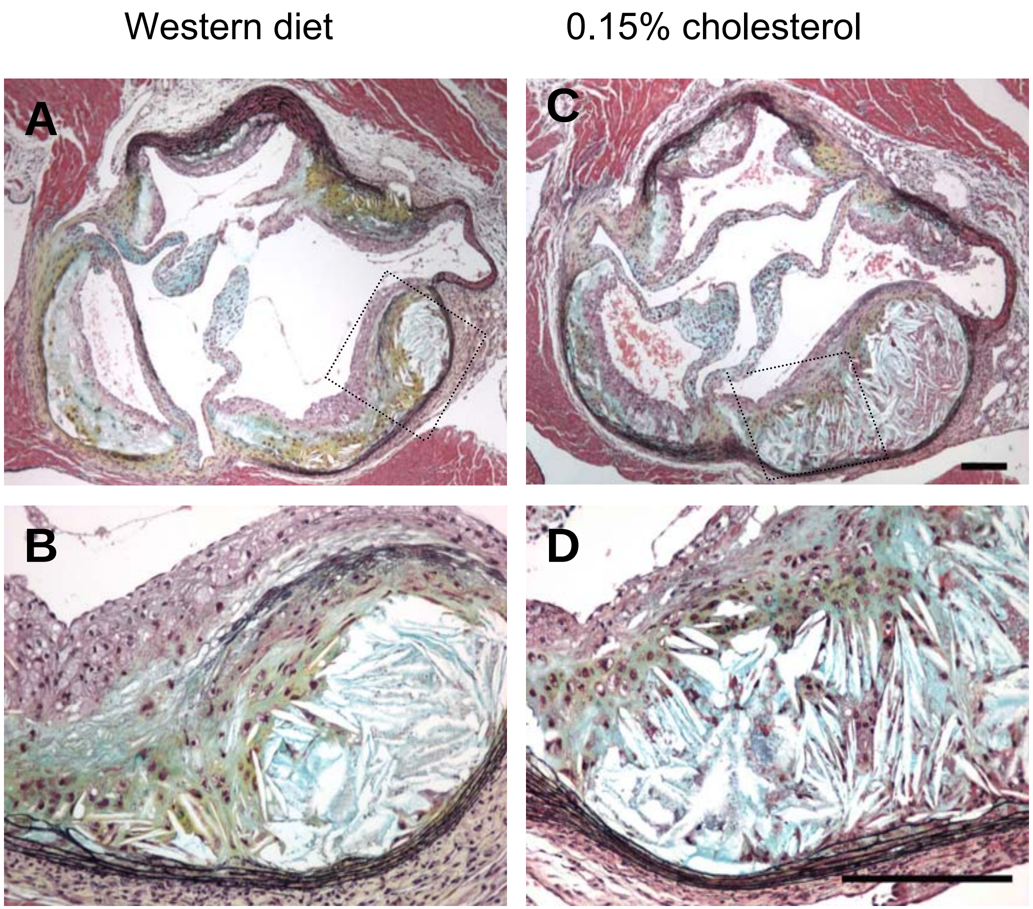
	LF1	HF1 35% lard	LF2	HF2 21% milk fat
Chol	0.02%	0	0.15%	0.15%

Glucose 129 ± 7	169 ± 5	122 ± 9	182 ± 9
Cholesterol 505 ± 48	489 ± 34	633 ± 2	701 ± 10
Triglyceride 155 ± 24	226 ± 36	109 ± 8	250 ± 26

There was no effect of high fat (and insulin resistance) on atherosclerosis



Movat's staining for LDLR-/- mice on the western diet and 0.15%HF diet (aortic root)



Ongoing

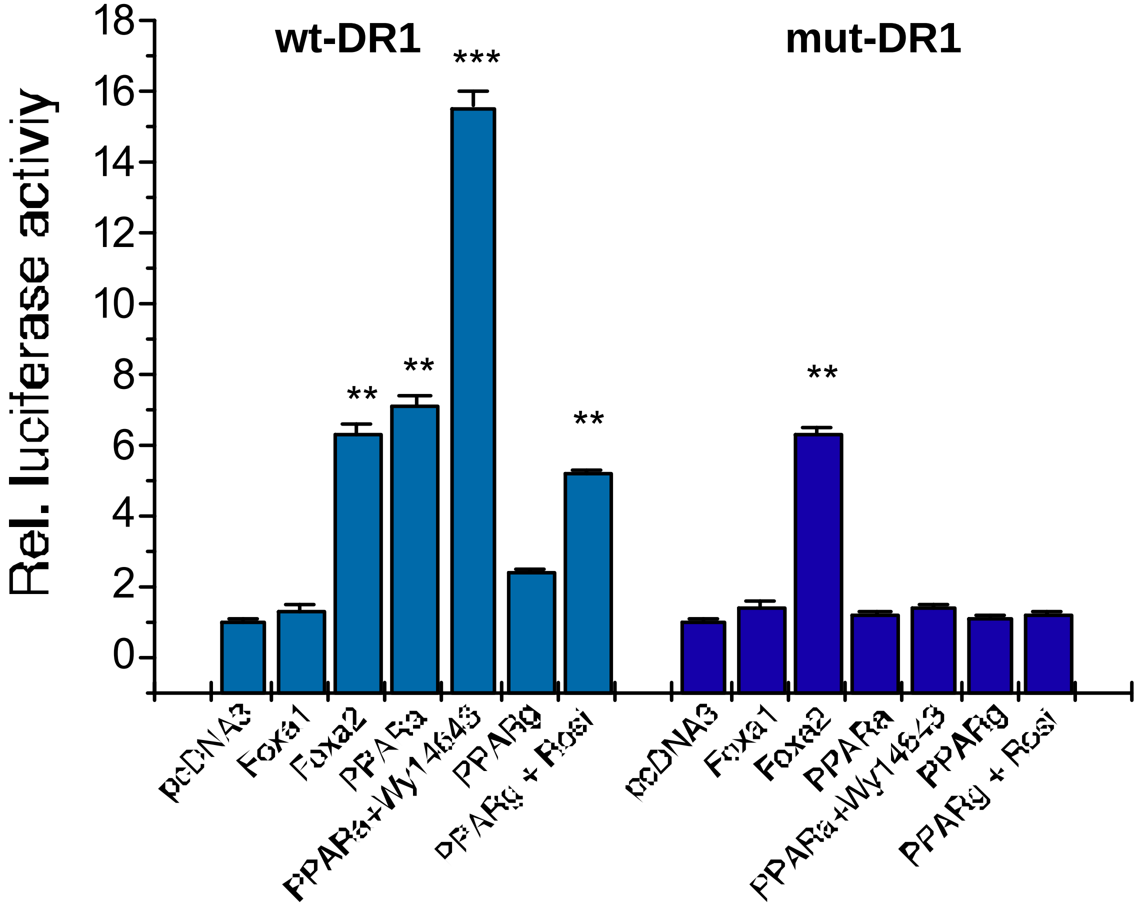
- Study effects of ARI (Columbia).
- Develop data on effects of hAR/Ldlr-/- AKITA (Rockefeller). MnSOD-/- / Ldlr-/- has difficulty breeding.
- Effects of diabetes on regression using hypomorphic apoE ± hAR.

Goldberg
Dansky

Breslow
Stoffel

Fisher

Foxa2/Pgc1b activates Mtp expression independent of Ppar-α



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Constitutive Foxa2 activity leads to increased apoB secretion

Pulse-chase experiment: