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

Major Goal: Create Mouse Model Diabetes Worsens Atherosclerosis

Strategy:

1. Create mice with diabetic dyslipidemia
2. Cross with models of hyperglycemia, insulin resistance or both
3. Assess effects on atherosclerosis progression, regression and response to arterial injury

Platform Models: C57BL/6J LDLR-/- Mouse

- **Hyperglycemia: Pdx1+/-; $Ins2^{Akita/+}$**
- **Insulin Resistance: IRS1-/-; Akt2-/-***
- **Combination: Ob/Ob**

Founders: * 10% out bred and currently backcrossing

Evaluation of Platform Models

1. Determine weight, FBS, fasting insulin, IPGTT, triglycerides and total, VLDL, LDL, HDL cholesterol
2. Evaluate lesion progression at aortic root and BCA and lesion morphology after 16 weeks on AIN76a diet plus 0.02% cholesterol (20 wks of age)
3. Evaluate regression of advanced aortic lesions
4. Evaluate response to arterial injury
5. At sacrifice, tissues distributed to AMDCC cores to assess nephropathy, retinopathy, neuropathy, and uropathy
6. Extra mice bred and shipped to AMDCC core laboratories for functional studies (neuropathy, uropathy, cardiomyopathy)

C57BL/6J LDLR-/- Pdx1+/- Females

	Female Pdx+/+ n=16	Female Pdx+/- n=13	Signif t-test
Lesion Area μm^2(x10-4)	35.3±7.9	24.5±8.4	p=0.010
Body Weight g	20.1±1.4	20.2±1.7	p=0.885
GTT 0’ mg/dl	132±17	182±27	p=0.000***
GTT 15’ mg/dl	341±45	484±77	p=0.000***
GTT 30’ mg/dl	332±67	528±70	p=0.000***
GTT 60’ mg/dl	224±61	408±119	p=0.000***
GTT 120’ mg/dl	135±18	161±59	p=0.125
Insulin ng/ml	0.60±0.32	0.31±0.15	p=0.010**
Cholesterol mg/dl	508±150	399±113	p=0.218
Non-HDL Chol mg/dl	478±146	366±112	p=0.180
HDL Chol mg/dl	30±10	33±10	p=0.220
Triglycerides mg/dl	121±68	86±23	p=0.499

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

C57BL/6J LDLR-/- Pdx1+/- Males

	Male Pdx+/+ n=21	Male Pdx+/- n=14	Signif t-test
Lesion Area µm2(x10-4)	12.2±6.8	16.6±13.1	p=0.272
Body Weight g	27.7±2.4	27.4±1.6	p=0.683
GTT 0’ mg/dl	139±16	214±41	p=0.000***
GTT 15’ mg/dl	343±35	500±80	p=0.000***
GTT 30’ mg/dl	341±68	523±50	p=0.000***
GTT 60’ mg/dl	267±80	472±86	p=0.000***
GTT 120’ mg/dl	164±28	271±69	p=0.000***
Insulin ng/ml	0.92±0.46	0.45±0.21	p=0.001***
Cholesterol mg/dl	447±151	352±89	p=0.048*
Non-HDL Chol mg/dl	404±151	308±85	p=0.043*
HDL Chol mg/dl	43±8	44±10	p=0.786
Triglycerides mg/dl	109±36	73±17	p=0.002**

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

C57BL/6J LDLR-/- Pdx1+/+ vs. Pdx+/-

1. In female mice the Pdx1+/- trait decreases aortic root lesion area (p=0.01), but has no significant effect on plasma lipids or lipoproteins
2. In male mice the Pdx1+/- trait has no significant effect on aortic root lesion area (weak trend up), but causes decreased cholesterol (p=0.048), non-HDL cholesterol (p=0.043), and triglycerides (p=0.002)
3. The results in female Pdx1+/- mice contrast with those in males. In females hyperglycemia decreases aortic root lesion area and in males, despite lower levels of atherogenic lipoproteins, there is a weak trend for hyperglycemia to increase aortic root lesion area.

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

	Female +/+ n=10	Female ob/ob n=11	Signif t-test
Lesion Area µm2(x10-4)	In progress	In progress	
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±105	472±81	p=0.000***
GTT 30’ mg/dl	239±95	505±77	p=0.000***
GTT 60’ mg/dl	167±65	383±117	p=0.000***
GTT 120’ mg/dl	103±39	285±137	p=0.003**
Insulin ng/ml	0.80±0.61	15.74±10.17	p=0.001**
Cholesterol mg/dl	687±263	2420±424	p=0.000***
Non-HDL Chol mg/dl	652±257	2334±400	p=0.000***
HDL Chol mg/dl	35±14	86±40	p=0.003**
Triglycerides mg/dl	186±107	612±307	p=0.002**

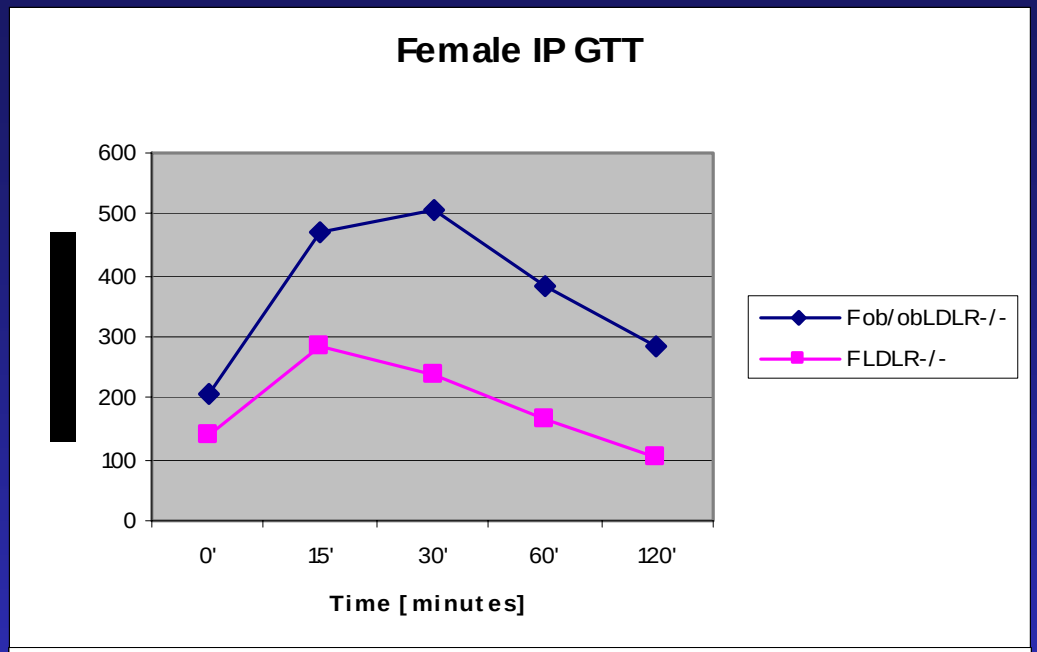
* P<0.05; **P<0.01; ***P<0.001

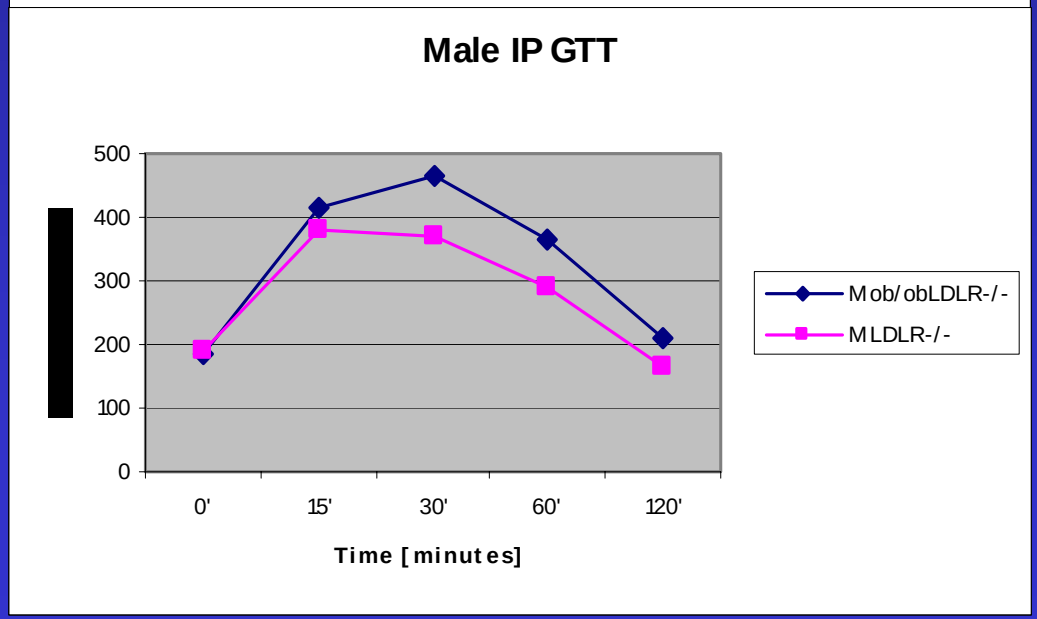
C57BL/6J LDLR-/- Ob/Ob Males

	Male +/+ n=14	Male ob/ob n=14	Signif t-test
Lesion Area µm2(x10-4)	In progress	In progress	
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=0.095
GTT 120’ mg/dl	165±50	211±99	p=0.158
Insulin ng/ml	0.85±0.48	7.09±2.76	p=0.000***
Cholesterol mg/dl	434±118	2724±349	p=0.000***
Non-HDL Chol mg/dl	379±122	2654±345	p=0.000***
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

LDLR-/-Ob/Ob IPGTT





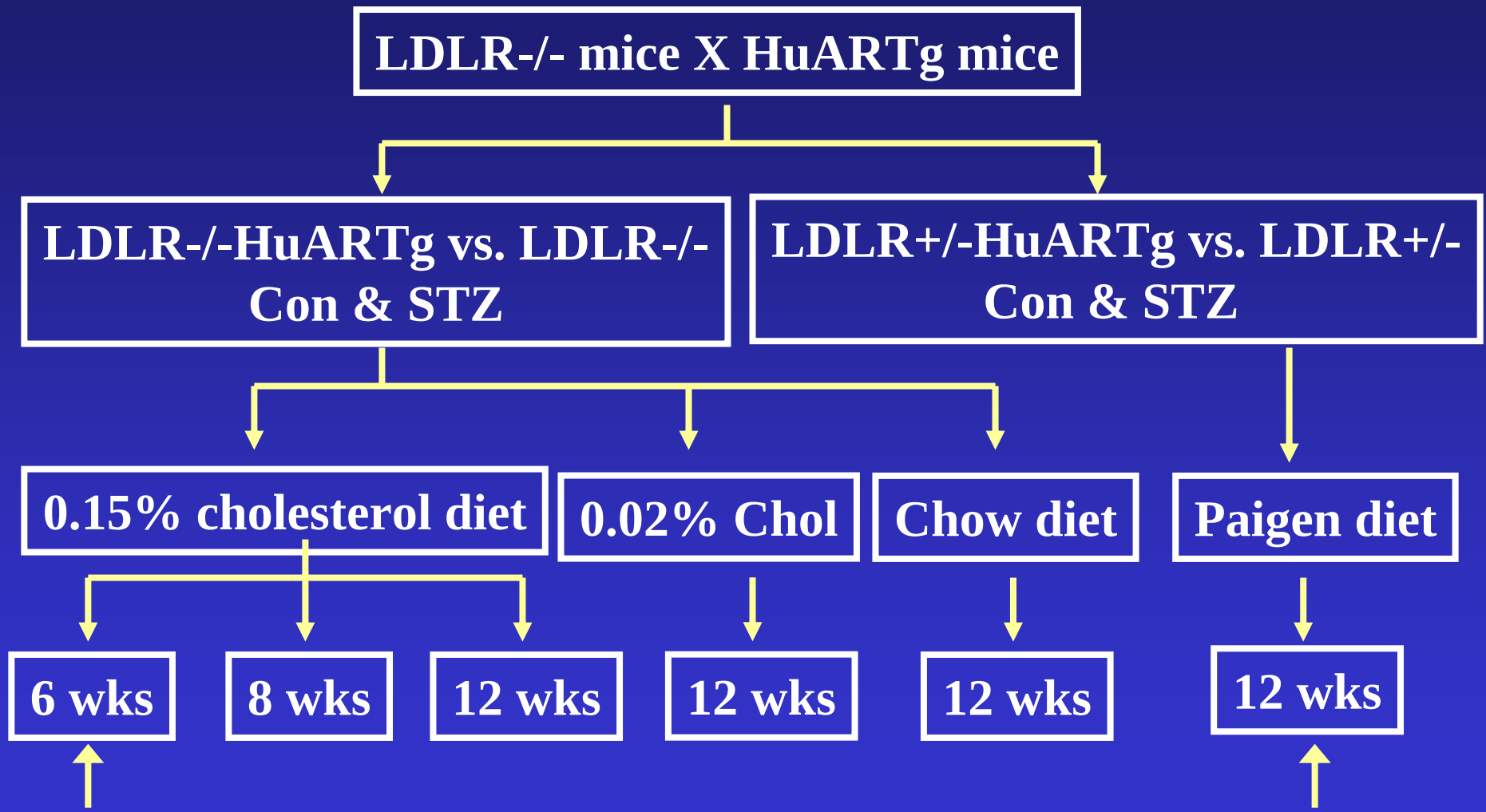
C57BL/6J LDLR-/-
+/+ vs. Ob/Ob

1. The Ob/Ob trait resulted in ~2 times higher fasting insulins in females compared to males. The Ob/Ob trait in females caused elevated glucose levels at each time point of the IPGTT (p~0.001), whereas in males glucose was only increased at the 30' time point (p=0.025). This indicates that female Ob/Ob mice are more insulin resistant than males.
2. The Ob/Ob trait elevated total and non-HDL cholesterol and triglycerides in both genders (p~0.001). The Ob/Ob trait raised HDL cholesterol much more in females (p=0.003) than males (p=0.038).
3. The effects of the Ob/Ob trait on atherosclerosis, neuropathy, retinopathy, nephropathy, and uropathy are under evaluation.

Diabetic Dyslipidemia

- **Diabetic dyslipidemia mimicked by: LDLR-/-, LDLR-/-apoAI-/-, HuBTg, HuCETPTg, LPL+/- and high fat-high cholesterol diets**
- **Mice made hyperglycemic with low dose STZ protocol**
- **No evidence of diabetes effect on atherosclerosis progression, exclusive of lipid abnormalities**
- **Hypothesis: A gene present in humans but not mice mediates toxic effect of diabetes/hyperglycemia**

Study Design (in progress)



LDLR-/-HuARTg vs. LDLR-/- Control vs. STZ

0.15% cholesterol diet 6 wks

	***Ldlr*-/-** (n=14)	**hAR*Ldlr*-/-** (n=13)	**L*dlr*-/- STZ** (n=11)	**hAR*Ldlr*-/- STZ** (n=16)
Glucose (mg/dl)	147 ± 6	148 ± 8	530 ± 32*	473 ± 22*
TC (mg/dl)	1569 ± 223	1358 ± 158	3033 ± 275*	3302 ± 269*
TG (mg/dl)	90 ± 4	93 ± 9	130 ± 10	149 ± 16
HDL (mg/dl)	58 ± 4	51 ± 5	58 ± 9	67 ± 5
Total lesion (%)	8.0 ± 0.6	10.7 ± 1.2	23.4 ± 2.2	37.5 ± 2.7
Aortic arch (%)	14.3 ± 2.0	23.9 ± 2.4	39.1 ± 3.2	65.0 ± 3.0#

Mean ± SEM. *P<0.05 vs. non-diabetics, #P<0.05 vs. non-transgenic diabetics

LDLR+/-HuARTg vs. LDLR+/- Control vs. STZ

Paigen diet 12 wks

	Ldlr+/- (n=8)	hAR*Ldlr*+/- (n=8)	*Ldlr*+/- STZ (n=16)	hAR*Ldlr*+/- STZ (n=12)
Glucose (mg/dl)	127 ± 9.34	108 ± 6	436 ± 38*	420 ± 34*
TC (mg/dl)	483 ± 51	530 ± 33	543 ± 43	525 ± 54
TG (mg/dl)	47 ± 6	50 ± 5	49 ± 4	49 ± 6
VLDL-C (mg/dl)	269 ± 40	319 ± 27	318 ± 34	310 ± 38
LDL-C (mg/dl)	106 ± 10	138 ± 3	125 ± 13	147 ± 10
HDL-C (mg/dl)	77 ± 4	63 ± 7	70 ± 9	58 ± 11
Total lesion(%)	8.2 ± 1.0	7.9 ± 0.9	8.9 ± 1.0	16.1 ± 1.9#
Aortic arch (%)	3.3 ± 0.7	4.5 ± 1.2	15.0 ± 2.8	30.7 ± 4.2#

Mean ± SEM. *P<0.05 vs. non-diabetics, #P<0.05 vs. non-transgenic diabetics

C57BL/6J LDLR-/- & LDLR+/- HuARTg mice show STZ induced enhancement of en face lesion area

Plan:

1. STZ-induced hyperglycemia studies: optimize diet and duration and extend analysis to aortic root and BCA
2. Extend model to Pdx1+/- induced hyperglycemia:
   LDLR-/-Pdx1+/- X LDLR-/-HuARTg

LDLR-/-; LDLR-/-Pdx1+/-; LDLR-/-HuARTg; LDLR-/-Pdx1+/-HuARTg

3. Atherosclerosis: Quantitation: Aortic Root, BCA; Assess lesion phenotypes
4. Tissue distribution to AMDCC core laboratories: Assess nephropathy, retinopathy, neuropathy, uropathy
5. Ship additional mice to AMDCC core laboratories for functional studies (neuropathy, uropathy, cardiomyopathy)

Platform Models: C57BL/6J LDLR-/- Mouse

- **Hyperglycemia: Pdx1+/-; $Ins2^{Akita/+}$**
- **Insulin Resistance: IRS1-/-; Akt2-/-***
- **Combination: Ob/Ob**
- **Sensitizer: HuARTg**; SOD2+/-****

Founders: * 10% outbred; ** 20% outbred and currently backcrossing

C57BL/6J LDLR-/- Hyperglycemic Mice PDX1+/-; Ins2$^{Akita/+}$ Sensitizers: HuARTg, SOD2

Plan:

1. Cross C57BL/6J LDLR-/- **Pdx1+/-** with C57BL/6J LDLR-/- **SOD2+/-** and compare metabolic profile and lesion area and characteristics on offspring of the 4 genotypes
2. Cross C57BL/6J LDLR-/- **Ins2Akita/+** with C57BL/6J LDLR-/- **HuARTg** and compare metabolic profile and lesion area and characteristics on offspring of the 4 genotypes.
3. Atherosclerosis: Quantitation Aortic Root, BCA; Assessment of plaque vulnerability phenotypes
4. Tissues distributed to AMDCC core laboratories for assessment of nephropathy, retinopathy, neuropathy, uropathy
5. Extra mice bred for shipment to AMDCC core laboratories for functional studies (neuropathy, uropathy, cardiomyopathy)

C57BL/6J LDLR-/- SOD2 PDX1 Males

	S+/+ P+/+ (n=3)	S+/+ P+/- (n=4)	S+/- P+/+ (n=5)	S+/- P+/- (n=3)	Signif ANOVA
Aortic Root Area	pending	pending	pending	pending	p=?
Body Weight g	29.3±2.0	27.5±1.8	28.1±1.7	30.2±0.6	p=0.317
GTT 0’ mg/dl	148±25	211±13	140±12	219±26	p=0.000***
GTT 15’ mg/dl	279±36	479±70	264±62	473±99	p=0.004**
GTT 30’ mg/dl	282±76	521±73	247±65	528±57	p=0.000***
GTT 60’ mg/dl	207±82	449±87	193±65	487±35	p=0.000***
GTT 120’ mg/dl	122±36	229±38	132±22	319±37	p=0.000***
Insulin ng/ml	1.1±0.5	0.7±0.3	1.4±0.4	1.6±0.7	p=0.379
Cholesterol	355±56	319±17	345±37	442±3	p=0.016*
Non-HDL Chol	297±50	258±14	286±34	376±5	p=0.017*
HDL Chol	58±6	61±5	58±4	67±2	p=0.225
Triglycerides	111±36	129±44	120±29	157±50	p=0.621

* p<0.05; **p<0.01; ***p<0.001

Movat's Pentachrome Stain

- Nuclei and elastic fibers (Black)
- Ground substance (Blue)
- Muscle (Red)
- Collagen (Yellow)
- Fibrin (Intense Red)