Plans for the first 9 months

- Model: C57BLKS ApoE -/- db/db mice
- Pancreatic function in C57BLKS mice
- Impact of 5-LO on pancreatic function
- Construction of 5-LO transgenic and knockout on C57BL/6 ApoE db/db background
- Gene Expression in C57BL6 db/db vs C57BLKS db/db mice

C57BLKS ApoE -/- db/db mice

- Strategy: Backcross ApoE-/- to C57BLKS
- Goal: Use high density SNP mapping to assess any residual C57BL/6 or 129 contamination deriving from the C57BL/6 ApoE-/- parent.

C57BLKS ApoE -/- db/db mice

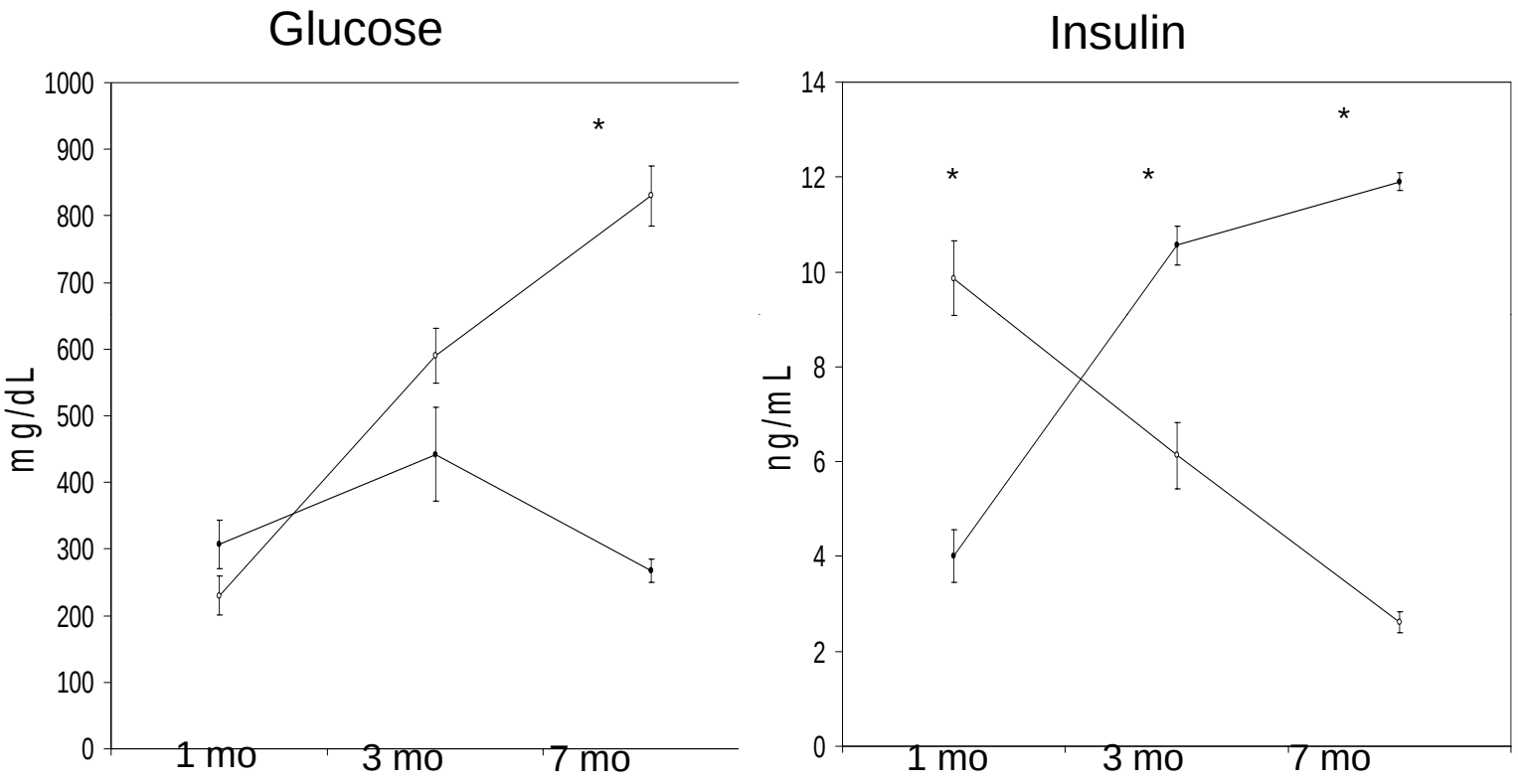
- N10 Mice from Vanderbilt now at UCLA and bred to homozygosity for Apo E and carrying the db+/- mutation
- UCLA mice at N7
- 5K Mouse SNP chip analysis
- Breeding stocks of
  C57BLKS db/db ApoE-/-
  C57B6 db/db ApoE-/-

  Comparison: Relative susceptibility to diabetes and atherosclerosis

Strain-specific effects of the db/db mutation

- Inactivation of the leptin receptor
- Hyperphasia and extreme obesity
- Strain specific differences
  - C57BL/6 vs C56BLKS
    - Insulin resistance
    - Beta cell failure and diabetes

B6-*db* vs BKS-*db*: Difference in Diabetes Susceptibility



Time-course of blood glucose and plasma insulin levels in B6-*db* (filled symbols) and BKS-*db* (open symbols) mice. 4-h fasted male mice are shown (n = 6-10, mean ± SEM, *, $p<0.05$)

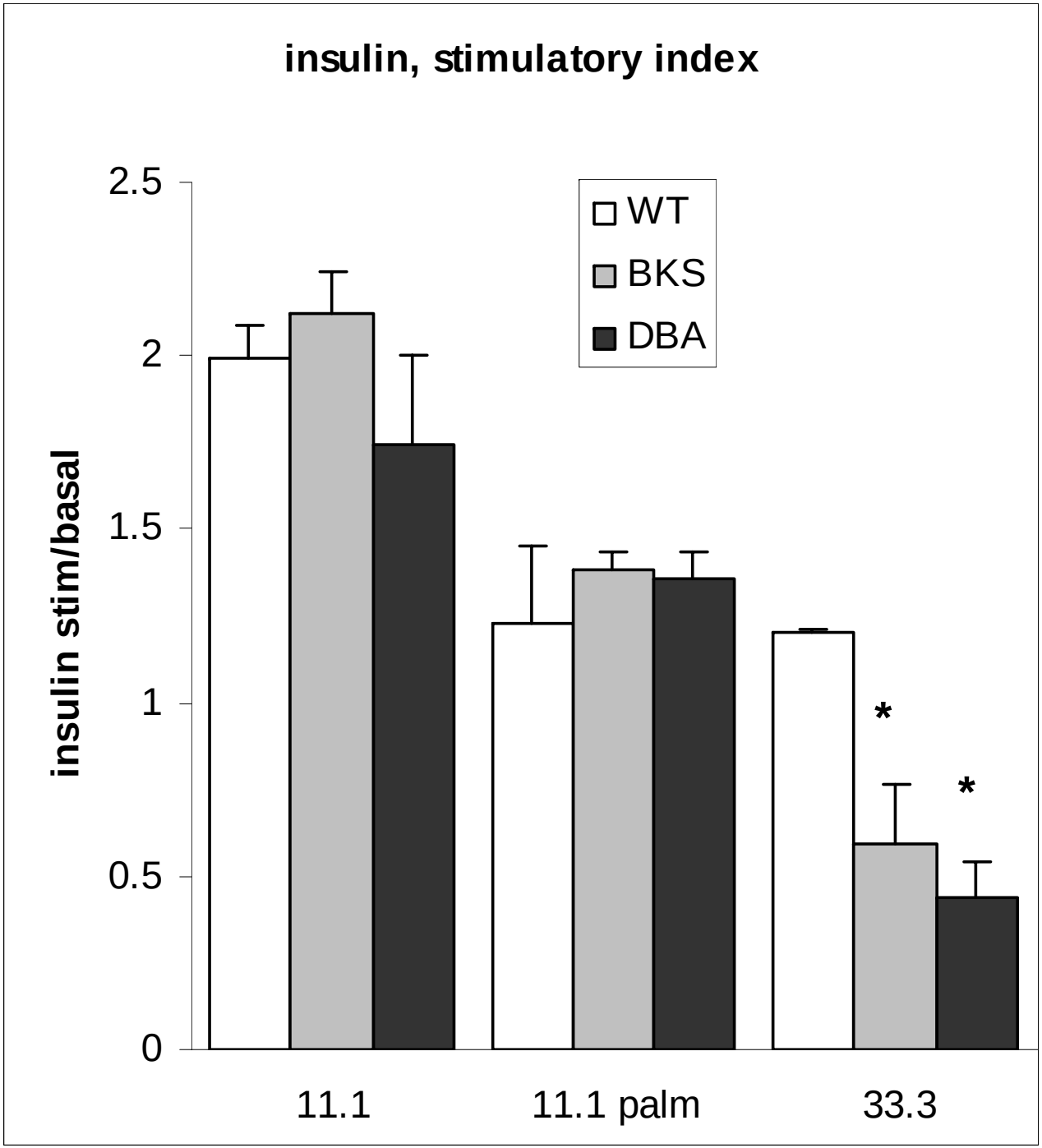
Working Hypotheses:

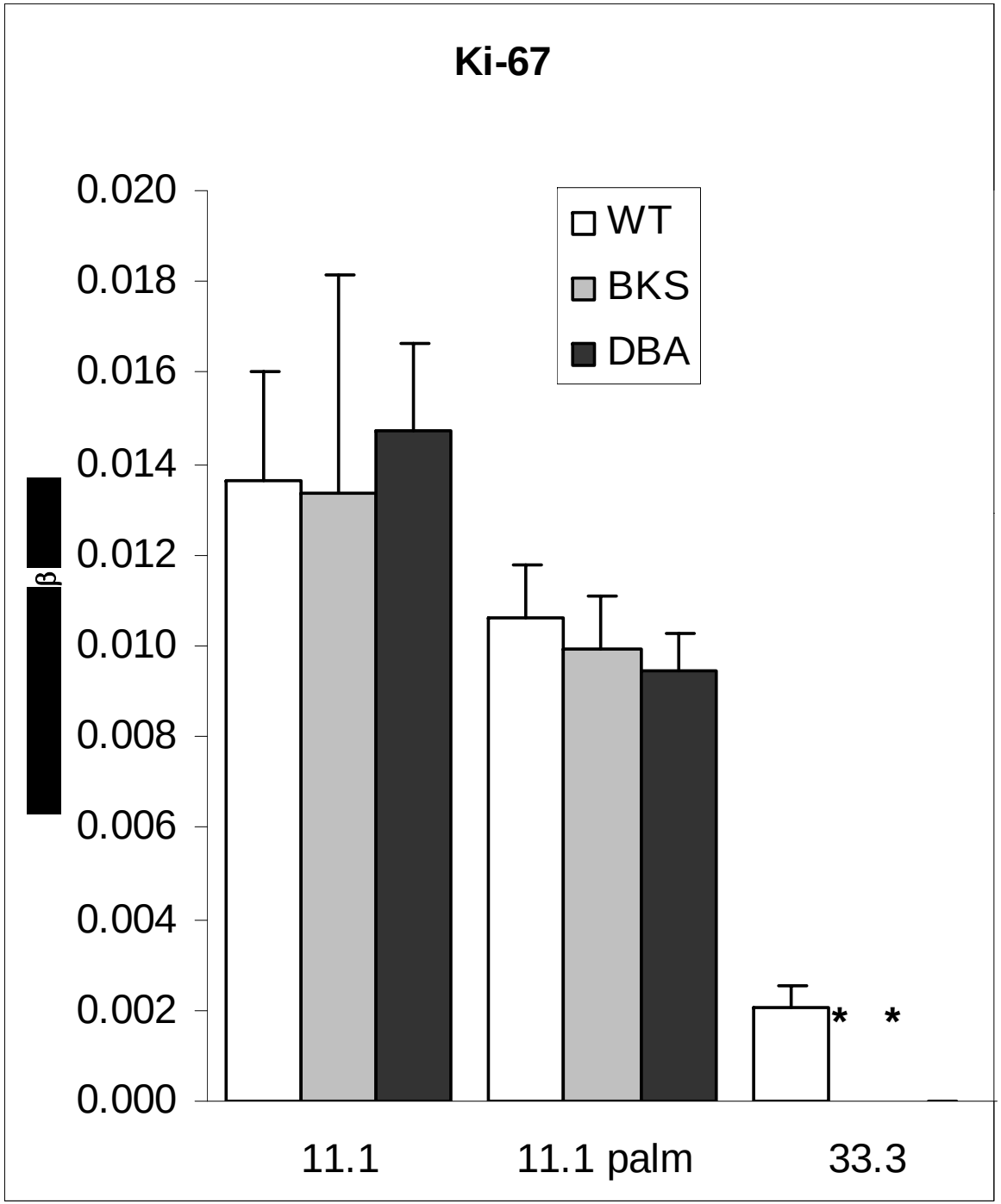
- C57BLKS db/db mice have increased insulin resistance that precedes beta cell failure and diabetes

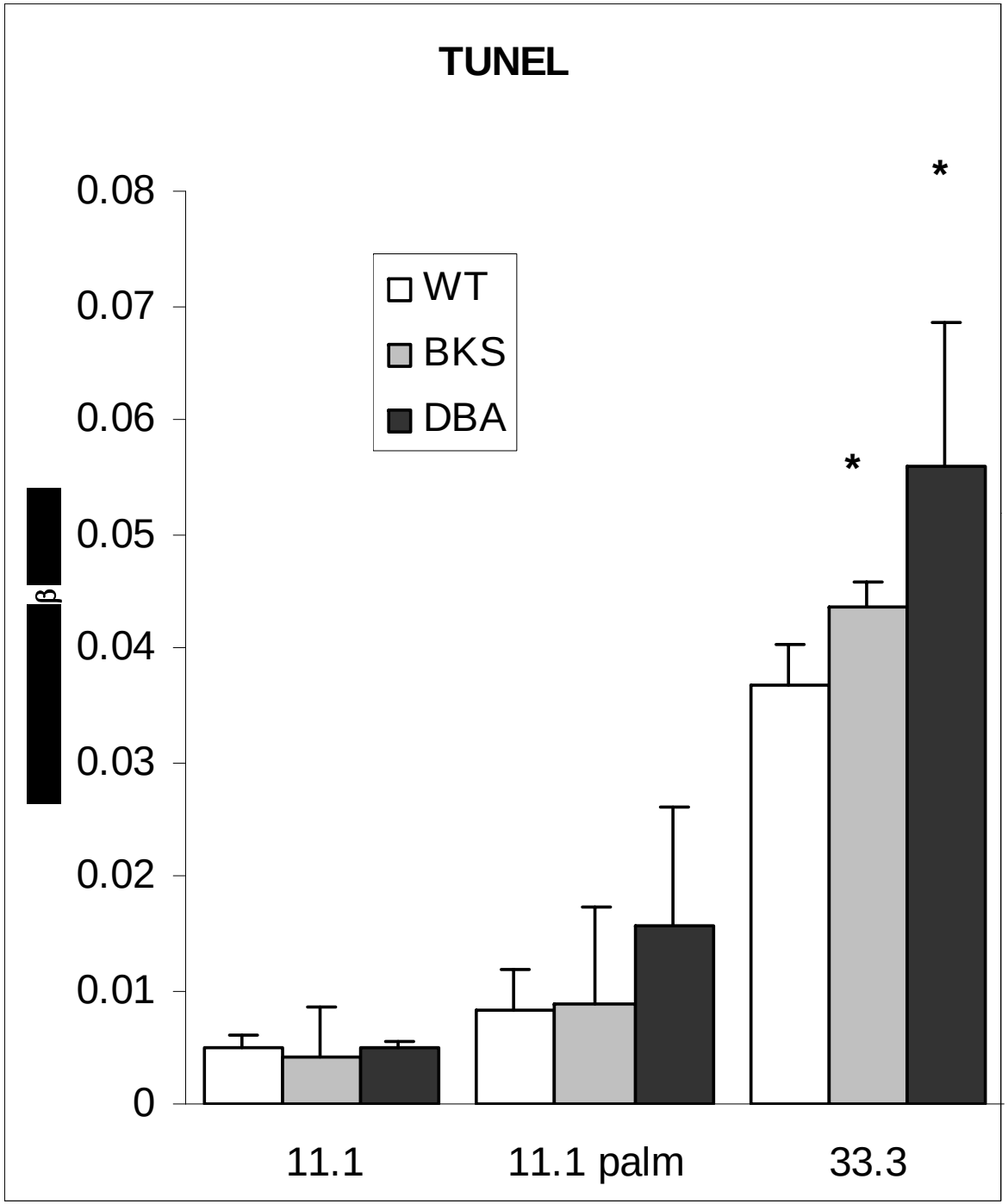
- C57BLKS db/db mice may also have increased sensitivity of beta cells to hyperglycemia

Pancreatic function in C57BLKS mice

- Goal: Compare C57BLKS with C57BL/6 islets to glucose induced insulin secretion, proliferation and apoptosis
  - Note: These experiments were done in the absence of the db/db mutation


insulin, stimulatory index
insulin stim/basal
2.5
2
1.5
1
0.5
0
WT
BKS
DBA
*
*
11.1
11.1 palm
33.3


Ki-67
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
β
WT
BKS
DBA
*
*
11.1
11.1 palm
33.3


TUNEL
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
WT
BKS
DBA
*
*
11.1
11.1 palm
33.3

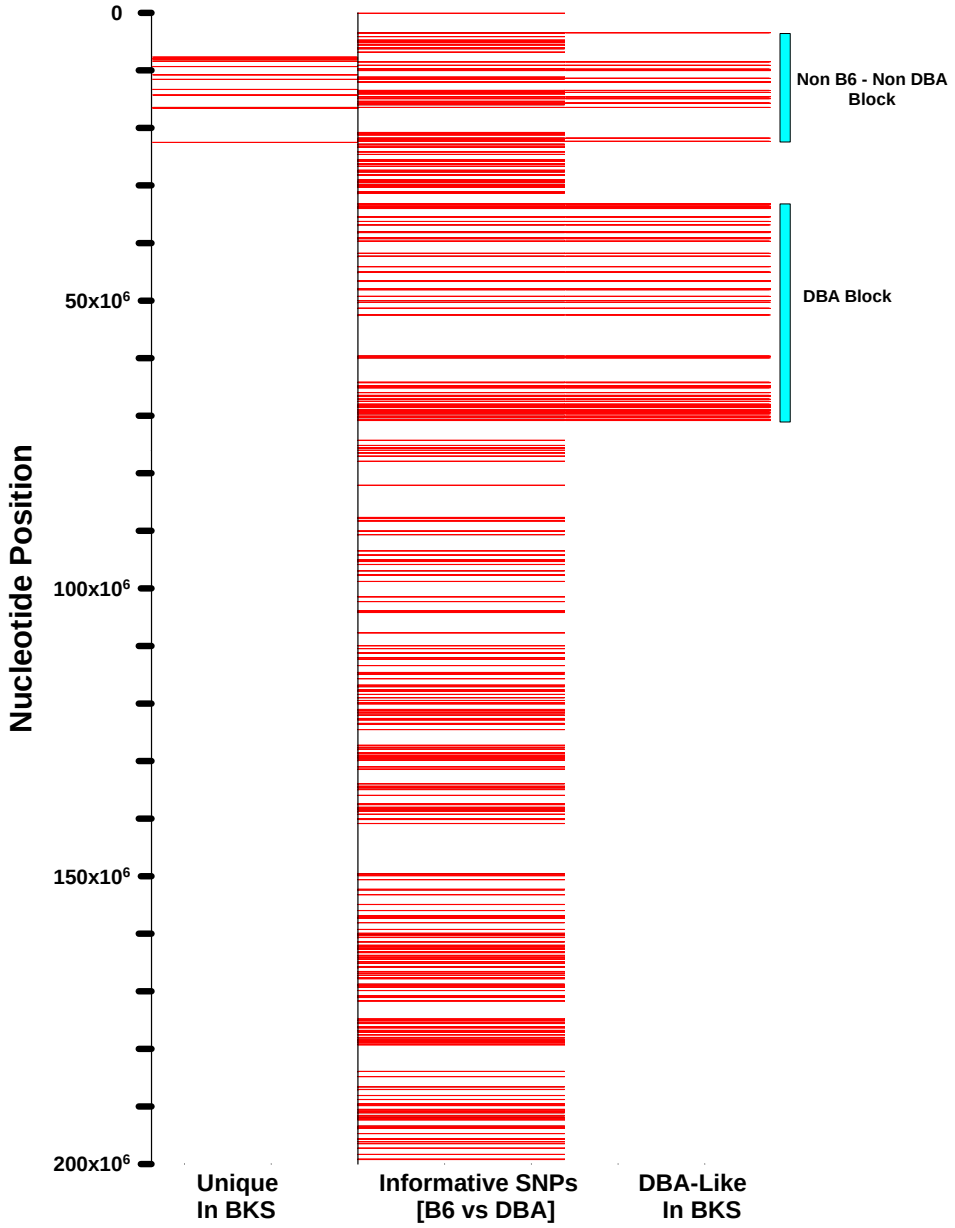
Conclusions:

- C57BLKS islets have inherently
  - Decreased insulin secretion
  - Decreased proliferation
  - Increased Apoptosis

  In response to hyperglycemia compared with C57BL/6

  Why DBA?

BKS SNP Map of DBA Regions and NonB6-NonDBA Regions



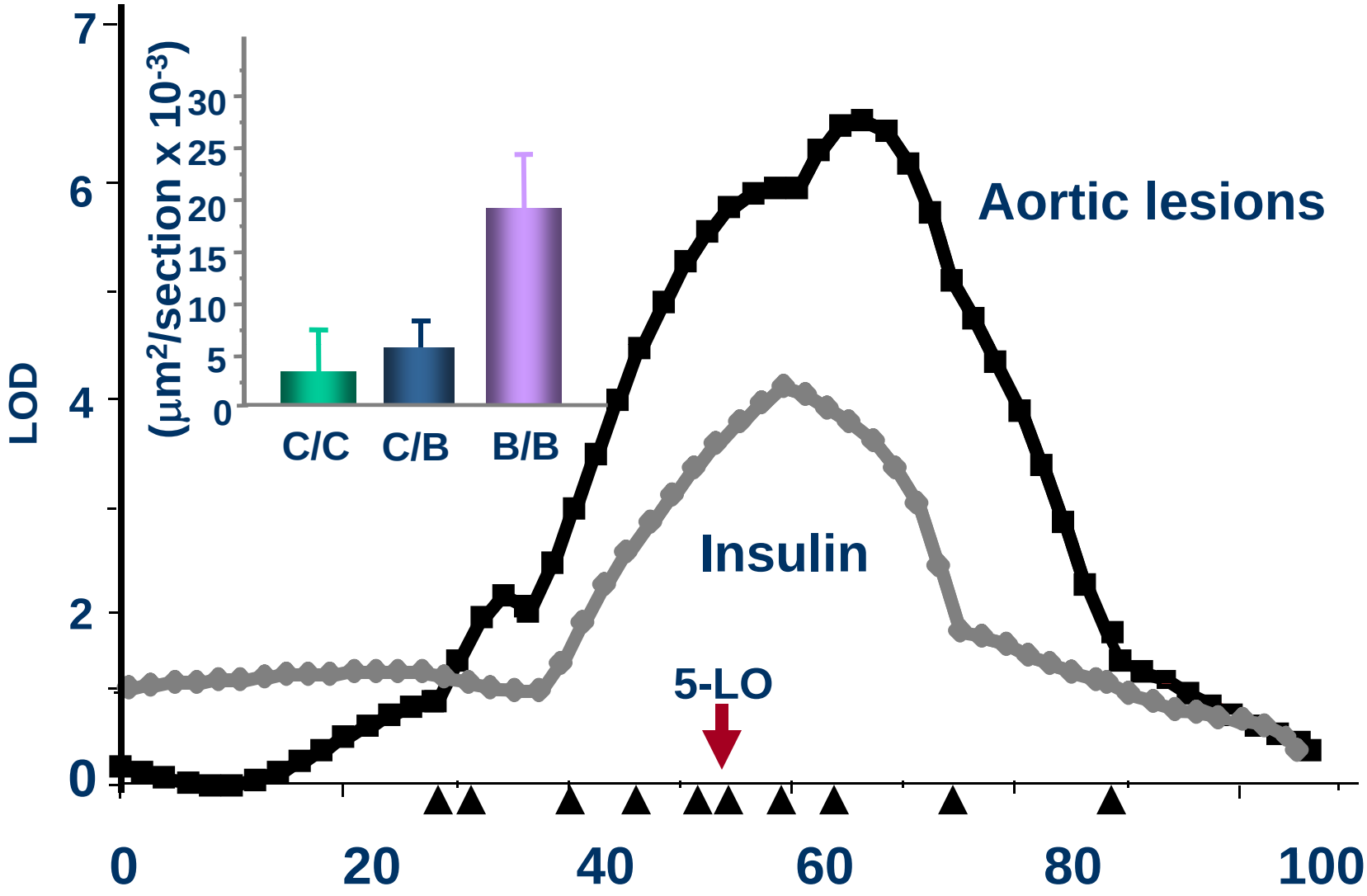
DBA Regions in C57BLKS/J

Chromosome	Proximal Marker	Prox Loc		Distal Marker	Distal Loc		Length
1	rs3707642	33,196,011		rs3659932	70,842,743		37,646,732
3	rs3675845	101,223,086		rs6212614	124,871,942		23,648,856
3	rs3679440	133,792,362		rs3676561	140,122,486		6,330,124
4	rs3674982	6,820,703		rs6196764	11,429,647		4,608,944
4	rs6340721	137,737,289		rs3023025	142,099,818		4,362,529
5	rs3674947	3,948,243		rs3706626	23,176,602		19,228,359
6	rs3709206	116,095,844		rs3659280	150,258,862		34,163,018
7	rs3726501	103,897,899		rs6216320	137,246,632		33,348,733
8	rs3657344	57,043,519		rs3678433	84,810,857		27,767,338
9	rs3690992	116,472,929		rs8254378	126,510,050		10,037,121
10	rs3664101	9,328,585		rs3667141	19,690,873		10,362,288
11	rs6263230	62,788,840		rs8241202	76,539,840		13,751,000
12	rs3688676	10,414,102		rs4229284	19,543,114		9,129,012
12	rs3023209	32,074,405		rs3699539	84,067,744		51,993,339
13	rs6209128	52,537,356		rs6391937	57,247,520		4,710,164
14	rs3710916	3,304,248		rs6396413	27,667,687		24,363,439
14	rs3707560	45,838,734		rs3700859	56,390,403		10,551,669
14	rs3722416	109,136,607		rs6384035	114,245,366		5,108,759
16	rs4153455	5,150,049		rs4158669	7,343,699		2,193,650
17	rs6293022	75,019		rs3703891	51,996,460		51,921,441
18	rs3724798	16,988,430		rs6167189	25,484,137		8,495,707
19	rs3681194	18,417,981		rs3669236	25,953,209		7,535,228
X	rs8276255	39,431,783		rs3693969	155,140,520		115,708,737
					Total Length		516,966,187
					Percent of Genome		19.9%
					(% of $2.6X10^9$ bp)		

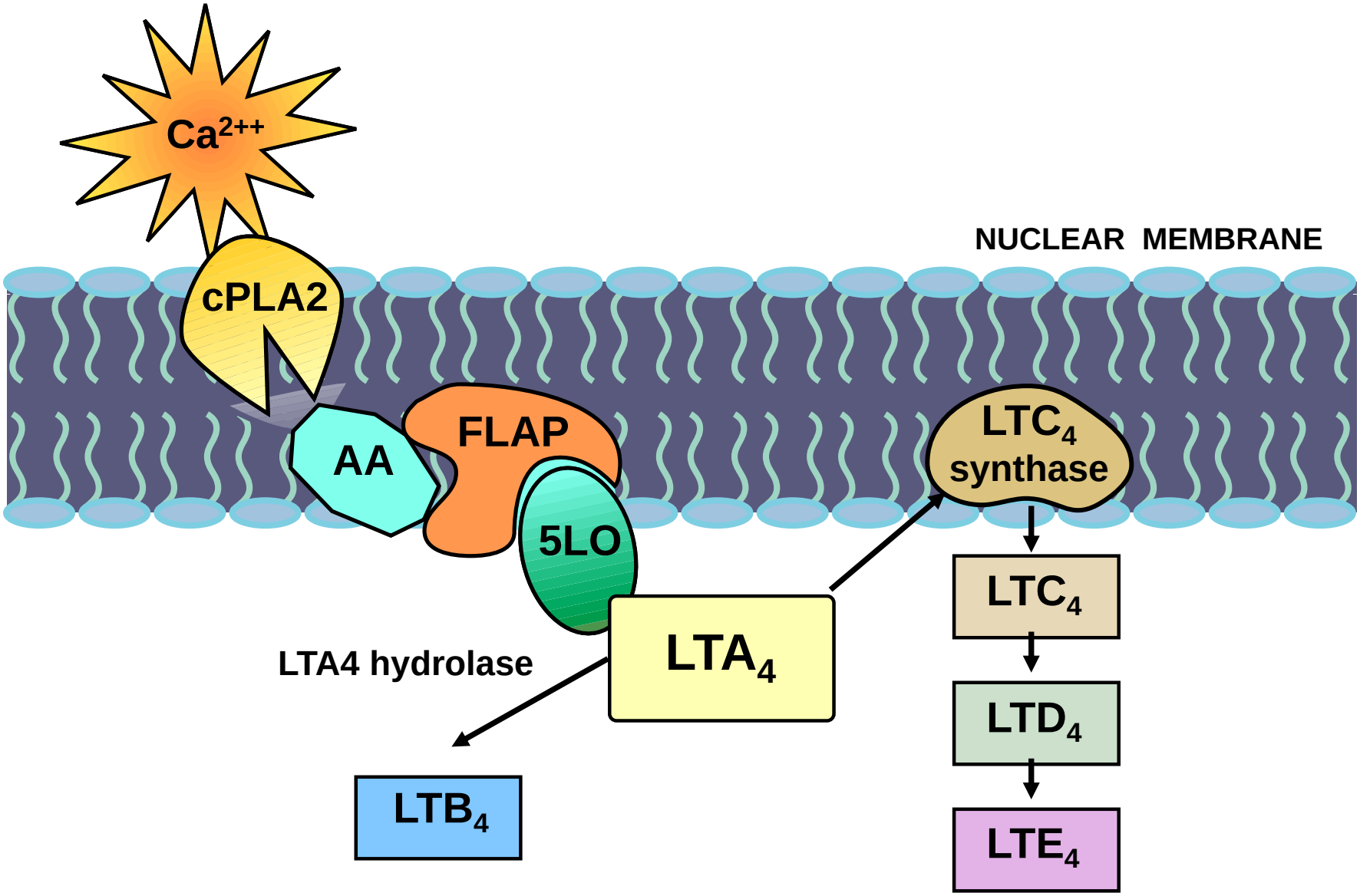
5LO Candidate Gene

- Lies on Chromosome 6 in one of the regions of C57BLKS derived from DBA
- Contributes to a number of diabetes and atherosclerosis related phenotypes.

Identification of 5-Lipoxygenase using Candidate Gene Approach

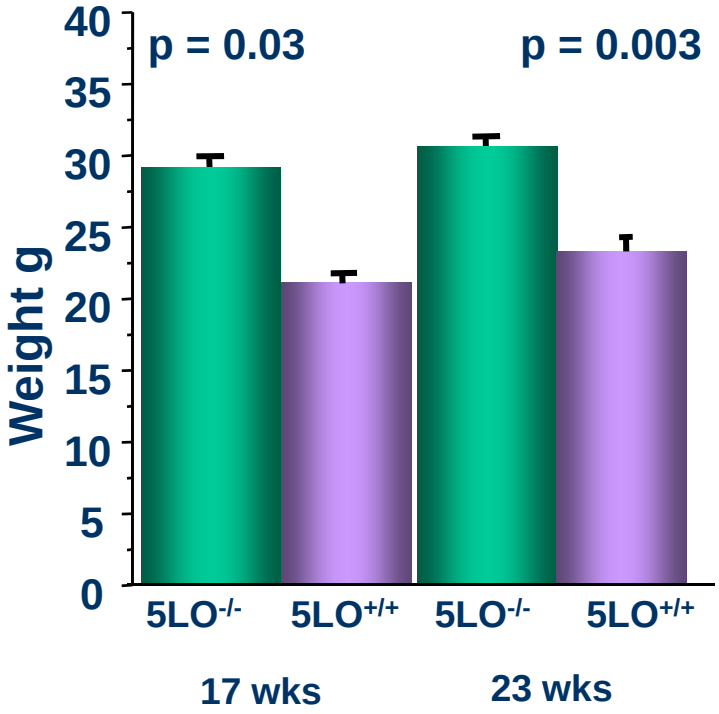


Leukotrienes are Well Known Mediators of the Acute Inflammatory Response



Weight of $5LO^{-/-}$ and $5LO^{+/+}$ Mice

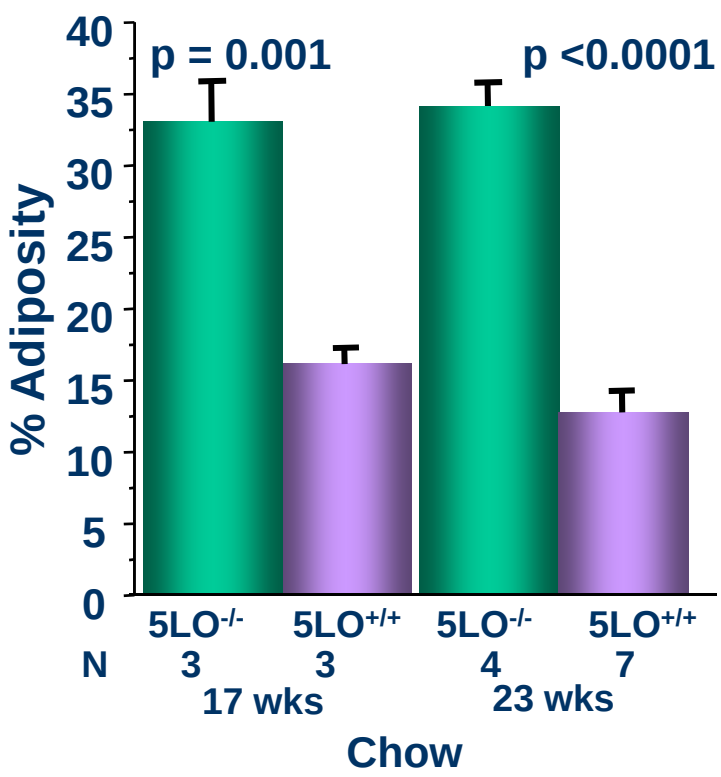
Females

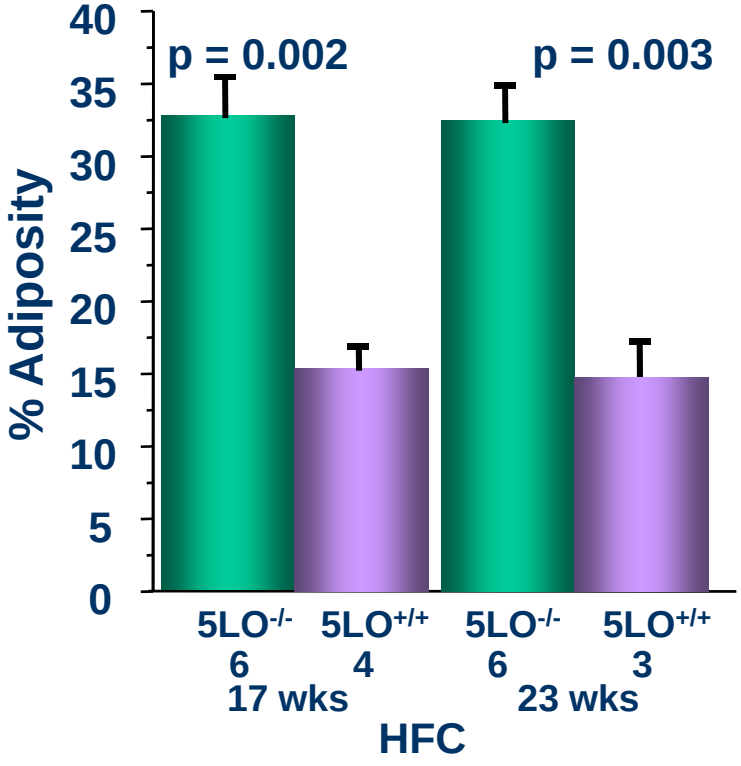


Mehrabian *et al*. Nature Genetics 10/05

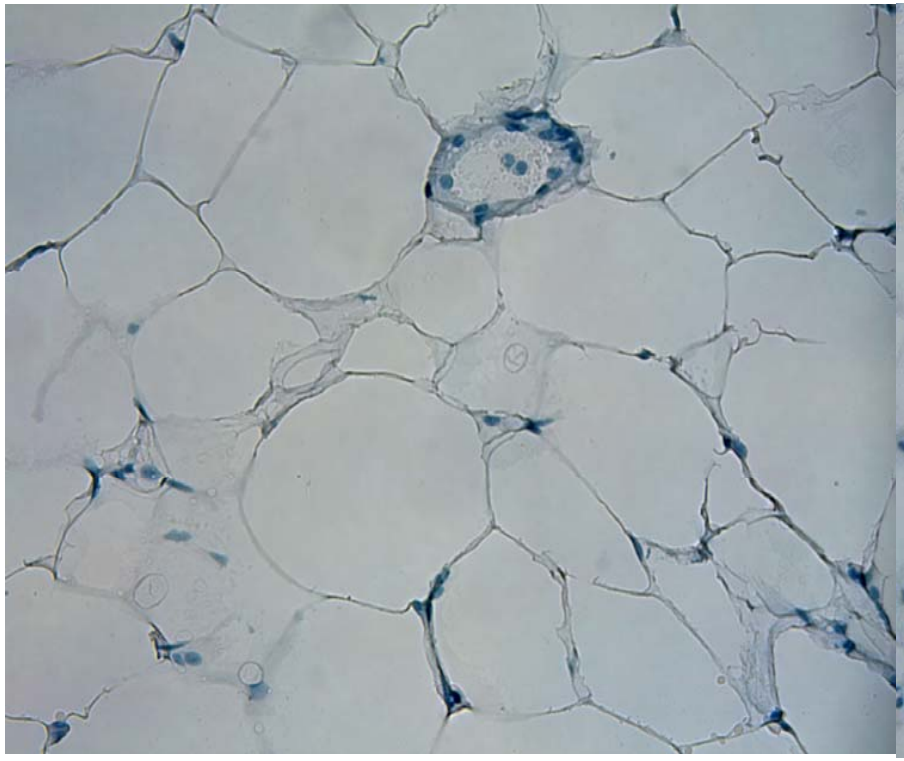
% Adiposity (total body fat/weight)

Females

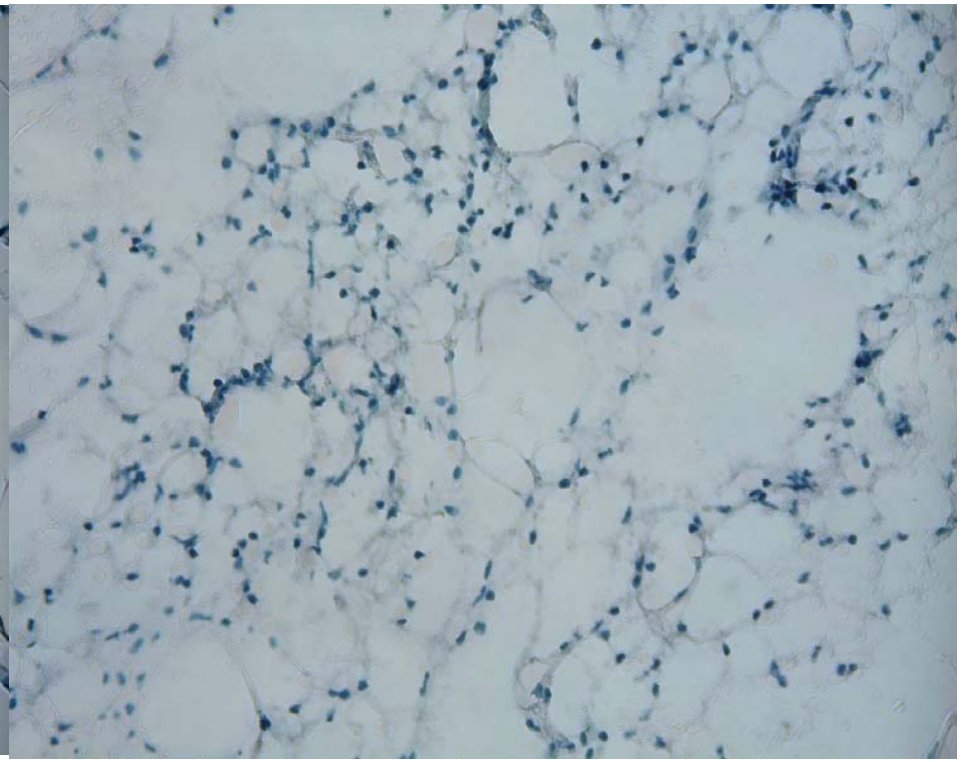




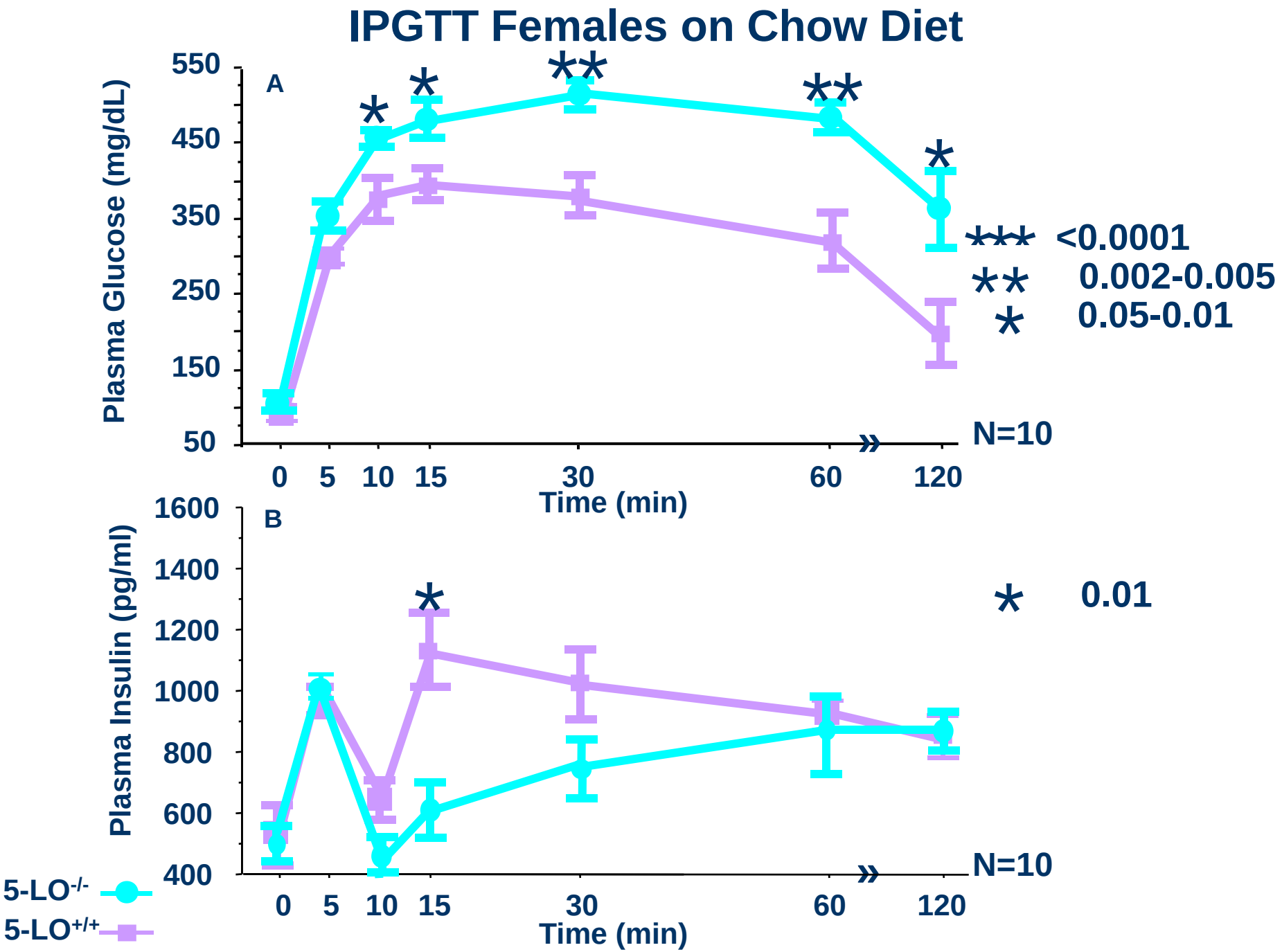
Adipose Tissue Hypertrophy



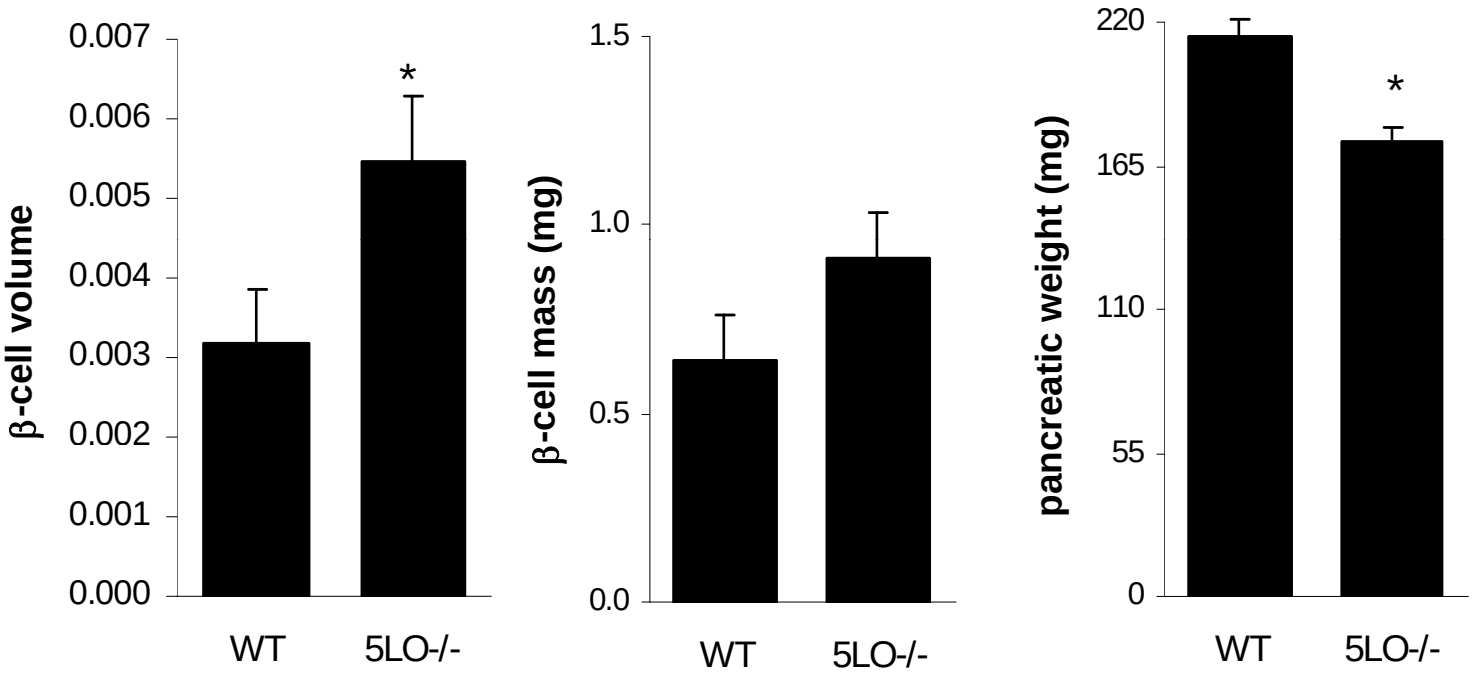
5LO$^{-/-}$



Wildtype C57BL6/J


IPGTT Females on Chow Diet
A
Plasma Glucose (mg/dL)
550
450
350
250
150
50
0
5
10
15
30
60
120
Time (min)
*
*
**
**
*
*** <0.0001
** 0.002-0.005
* 0.05-0.01
N=10
B
Plasma Insulin (pg/ml)
1600
1400
1200
1000
800
600
400
*
* 0.01
N=10
5-LO-/-
5-LO+/+

β-cell Volume and Mass is Increased in 5LO-/- Mice as an Adaptive Response



β-cell insulin secretion is significantly impaired in the 5LO knockout mice

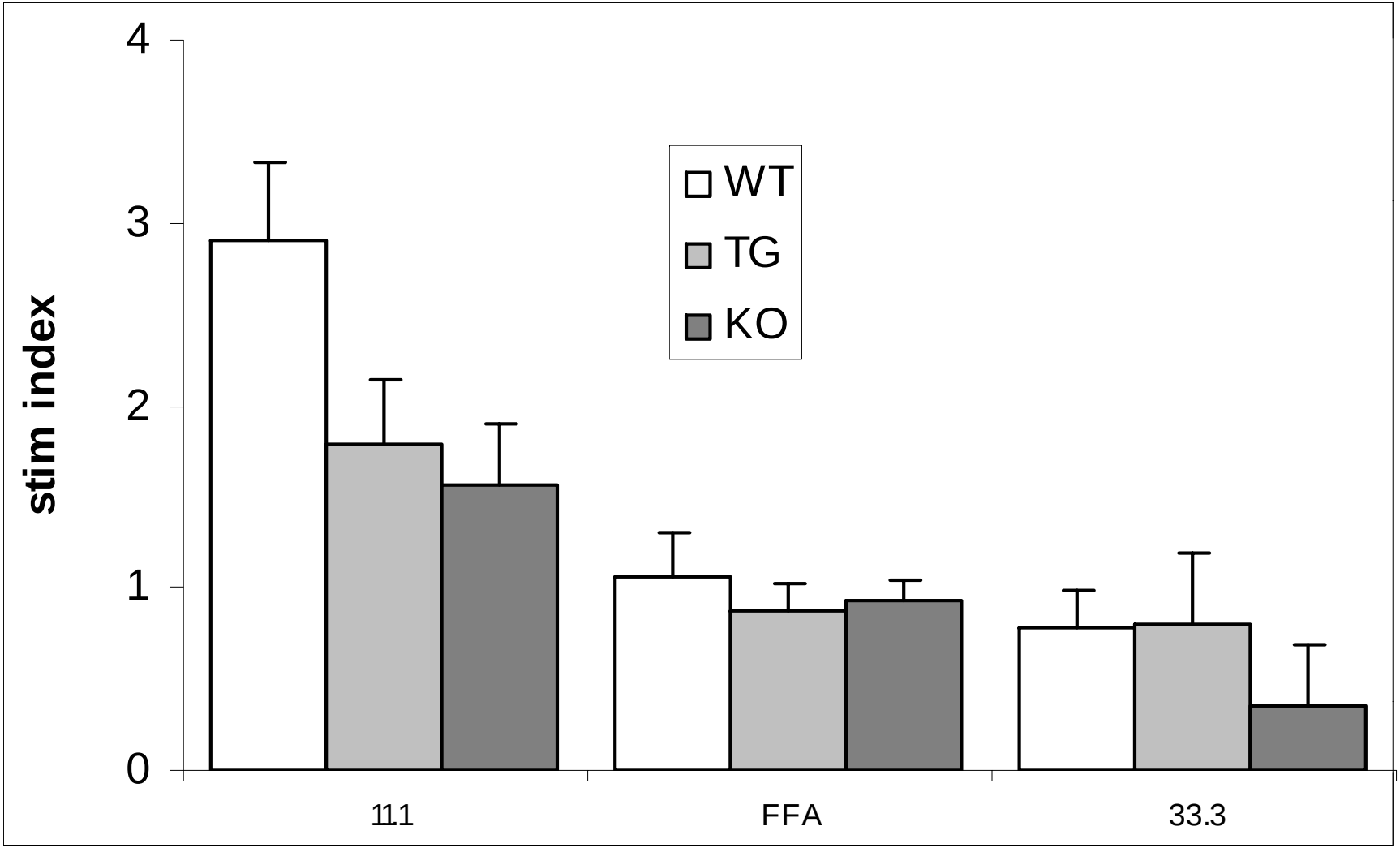
Kathrin Maedler: UCLA

5LO Directly impacts Islet Function

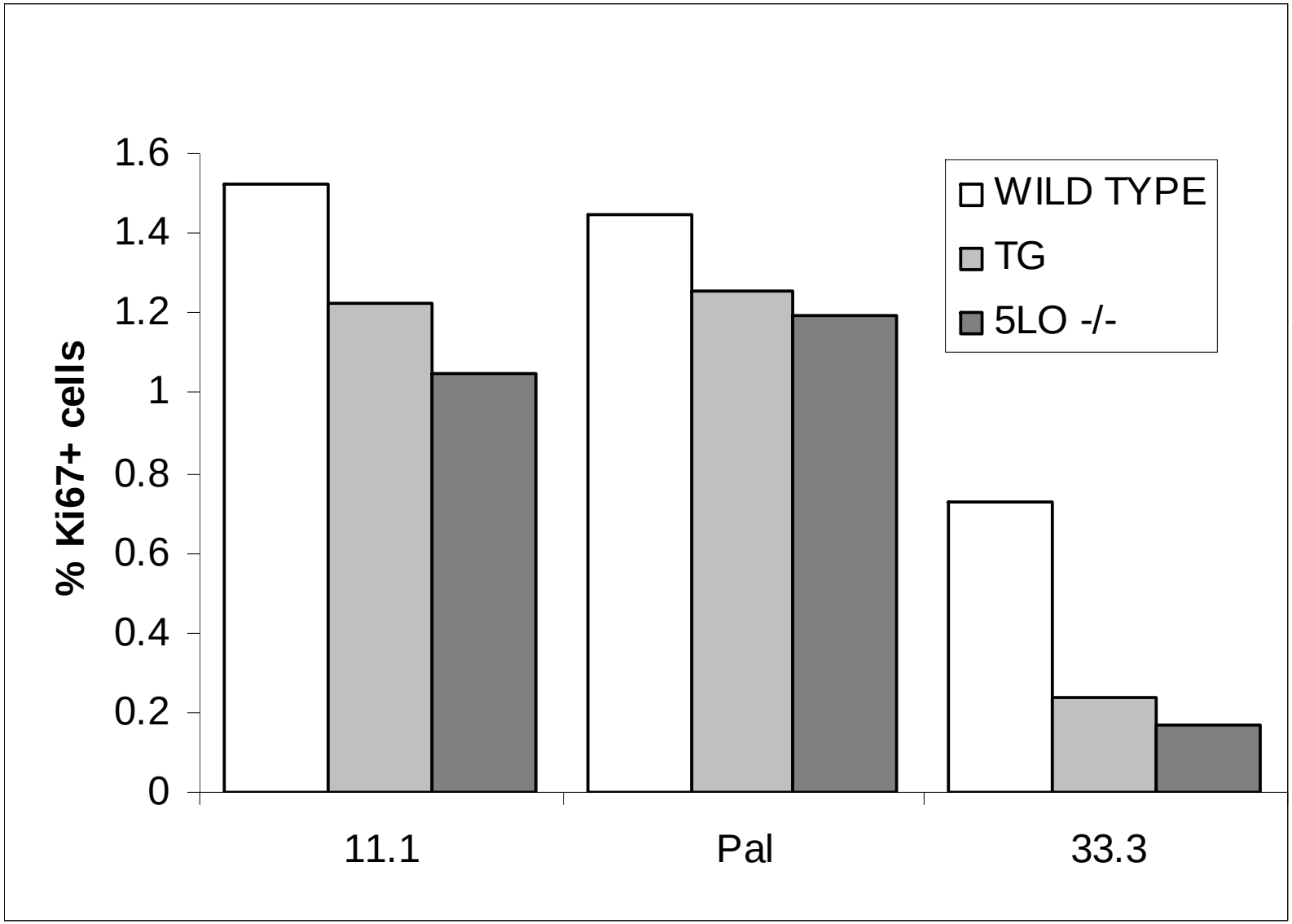
- Goal:
  Measure impact of 5LO on pancreatic function

- Isolated islet cells from BKS, B6 or DBA:
- Treat with high or low levels of glucose
- Measure response:
  - Glucose stimulated insulin secretion
  - Glucose stimulated islet proliferation
  - Glucose induced apoptosis

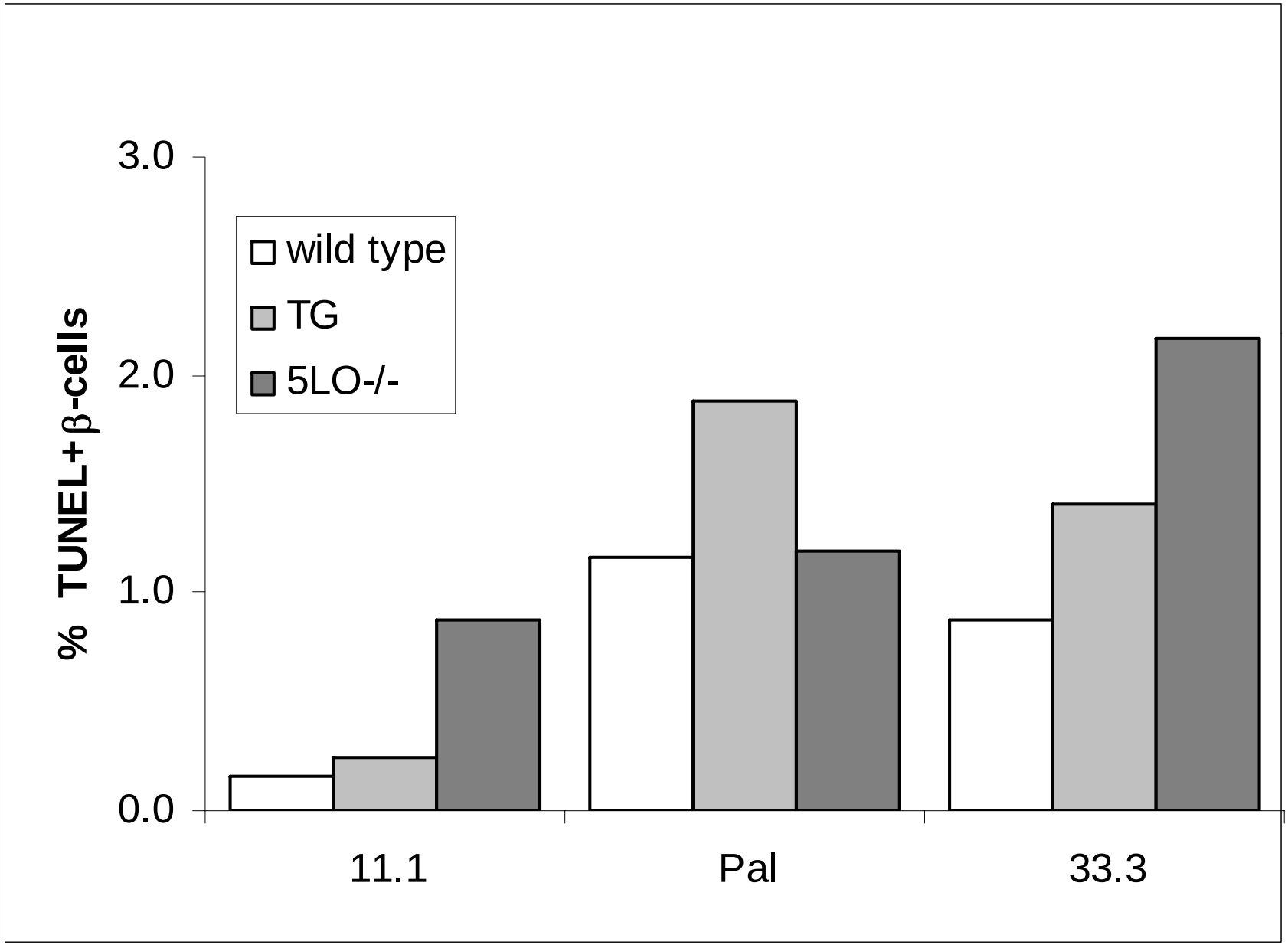
Reduced Glucose Stimulated Insulin Secretion



Proliferative Response Diminished



Apoptosis Increased



Conclusions:

- 5LO impacts islet response to glucose
  - Decreased insulin secretion
  - Decreased proliferation
  - Increased Apoptosis

5LO ApoE db/db constructs

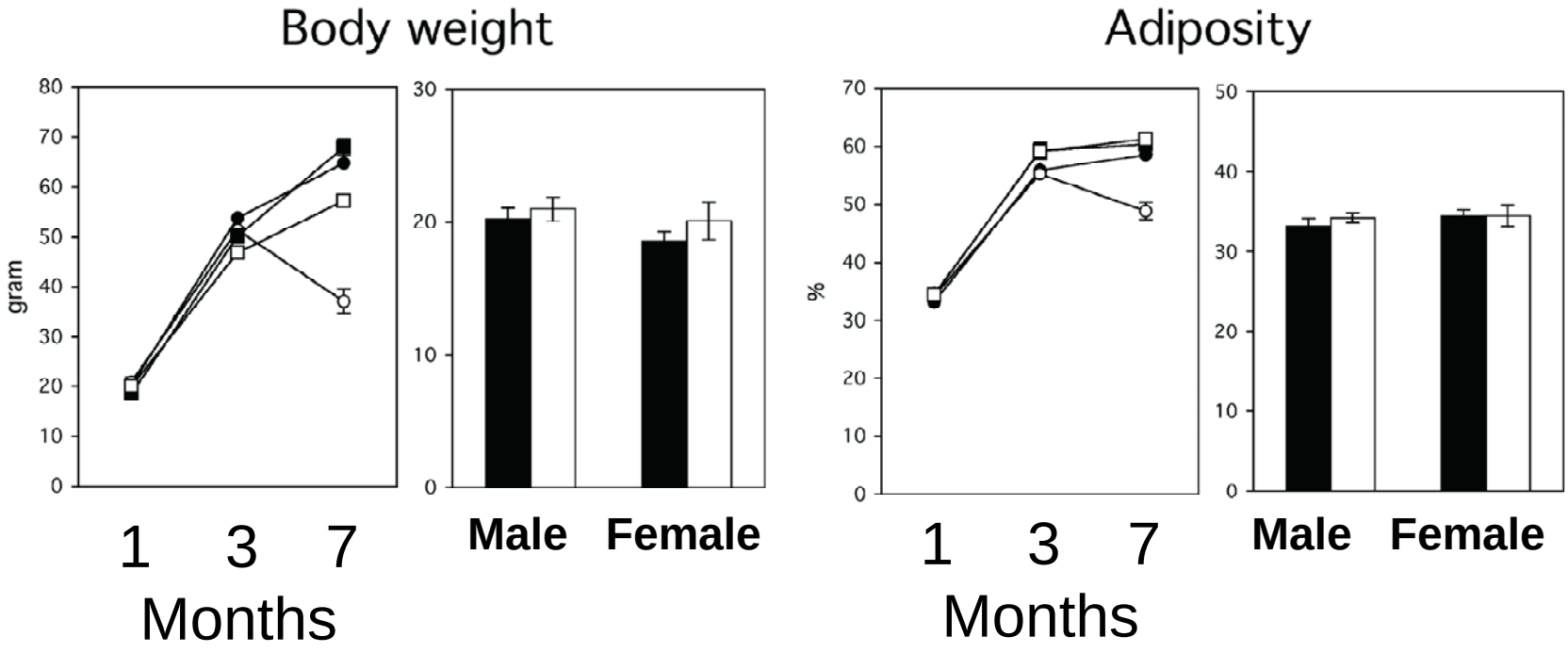
- Goal: Construction of 5-LO transgenic and knockout on C57BL/6 ApoE db/db background
  - Construction of 5LO tg ApoE db+/- and
    5LO knockout ApoE-/- db+/-complete
    Currently building breeding stocks for comparison with C57BL/6 ApoE db/db for atherosclerosis and diabetes susceptibility

Gene Expression in B6 and BKS

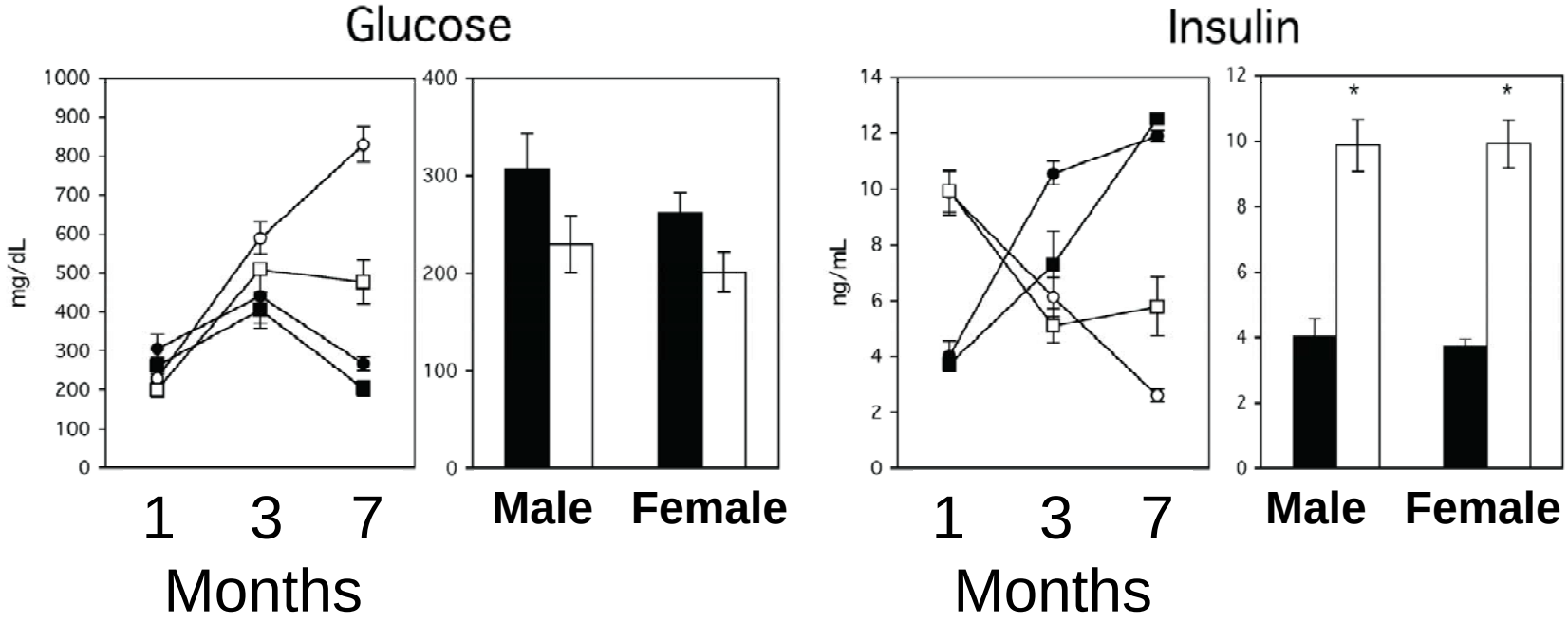
- Goal: Compare gene expression in C57BL6 db/db vs C57BLKS db/db mice
- RNA from 1 month old male mice (n=4)
  - Muscle
  - Liver
  - Adipose
- Agilent arrays (47K)
  - Each sample compared to a pool of all 8 animals in reverse-fluor pairs(24 arrays)
  - Analysis underway

Hepatic insulin resistance precedes beta cell failure in C57BLKS-db/db

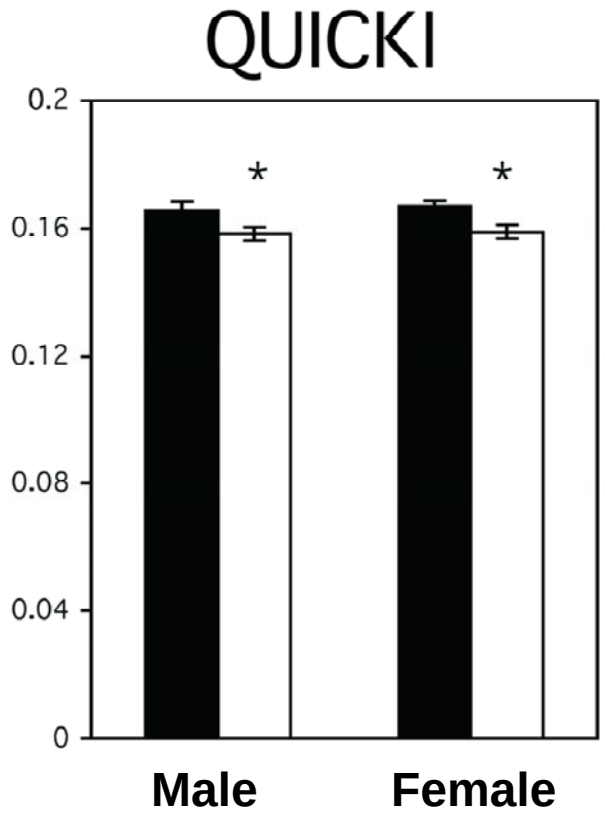
Body Weight and Adiposity BKS-db and B6-db



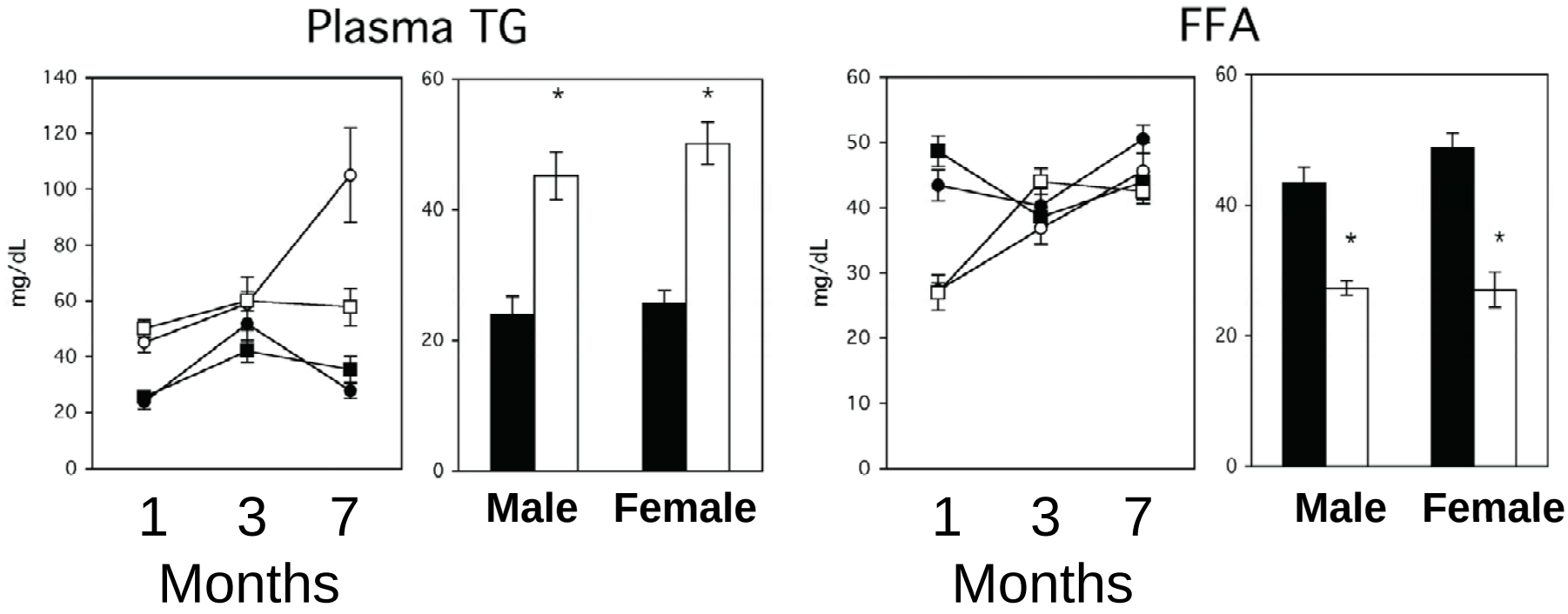
Glucose and Insulin
BKS-db and B6-db



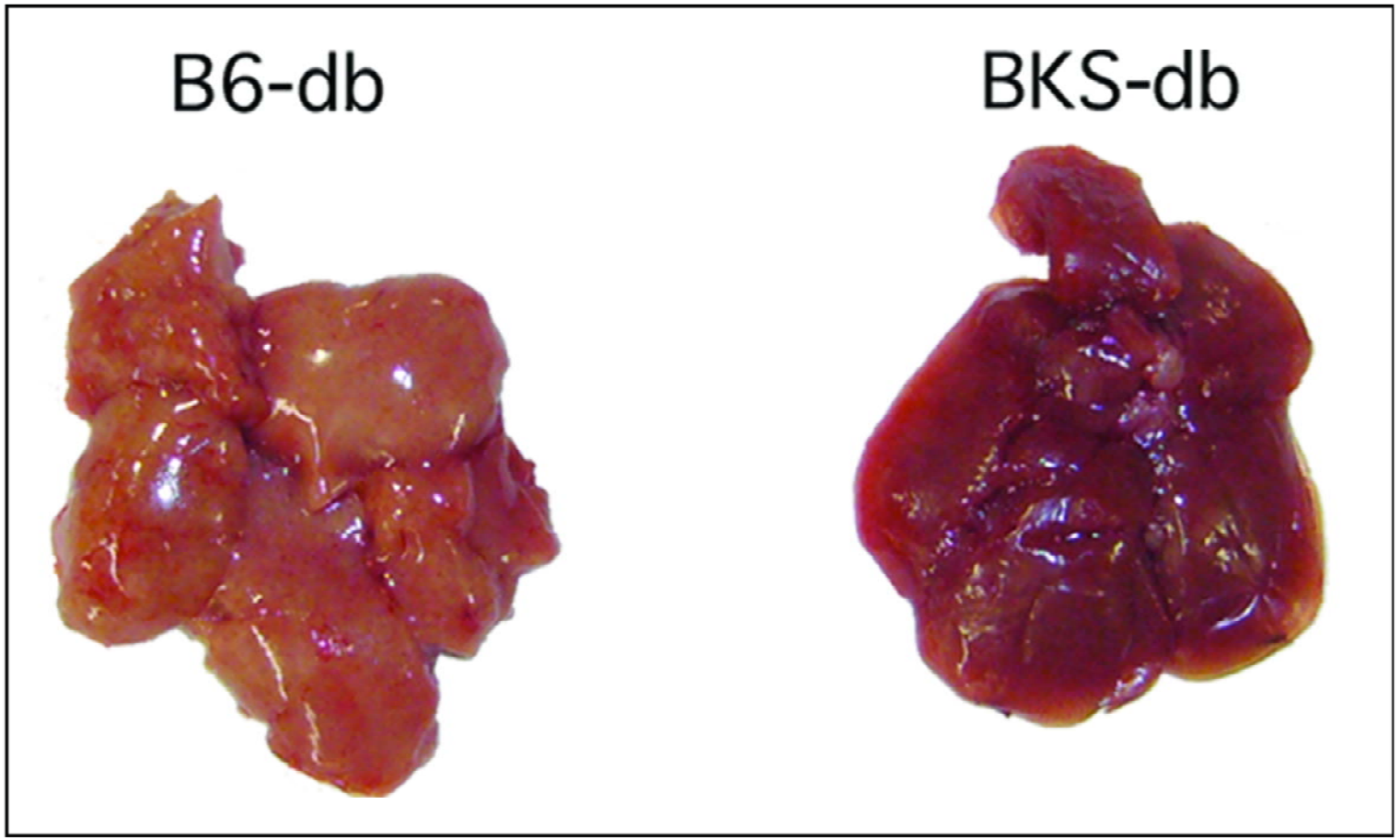
Insulin Sensitivity
BKS-db and B6-db



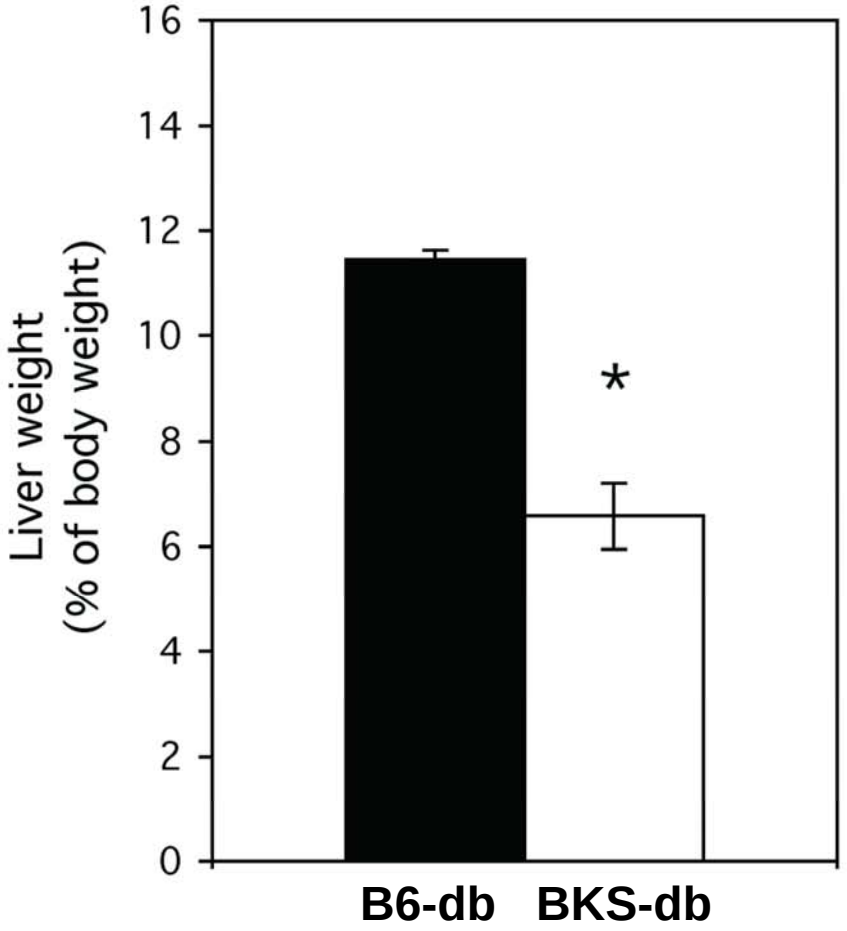
Plasma TG and FFA BKS-db and B6-db

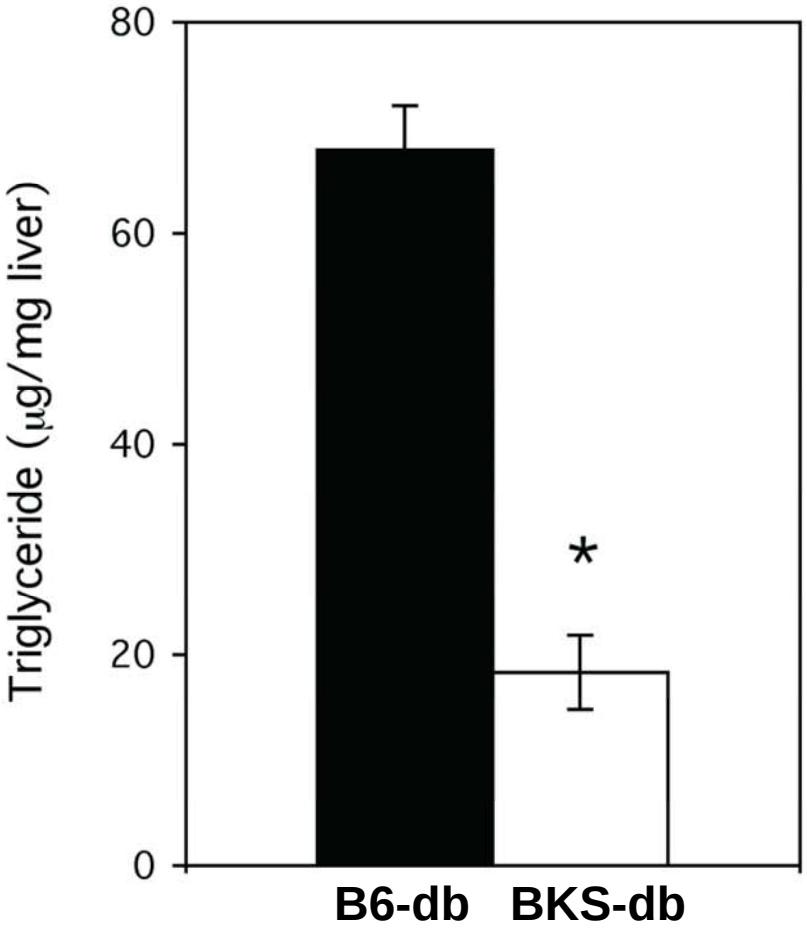


Fat Deposition in the Liver

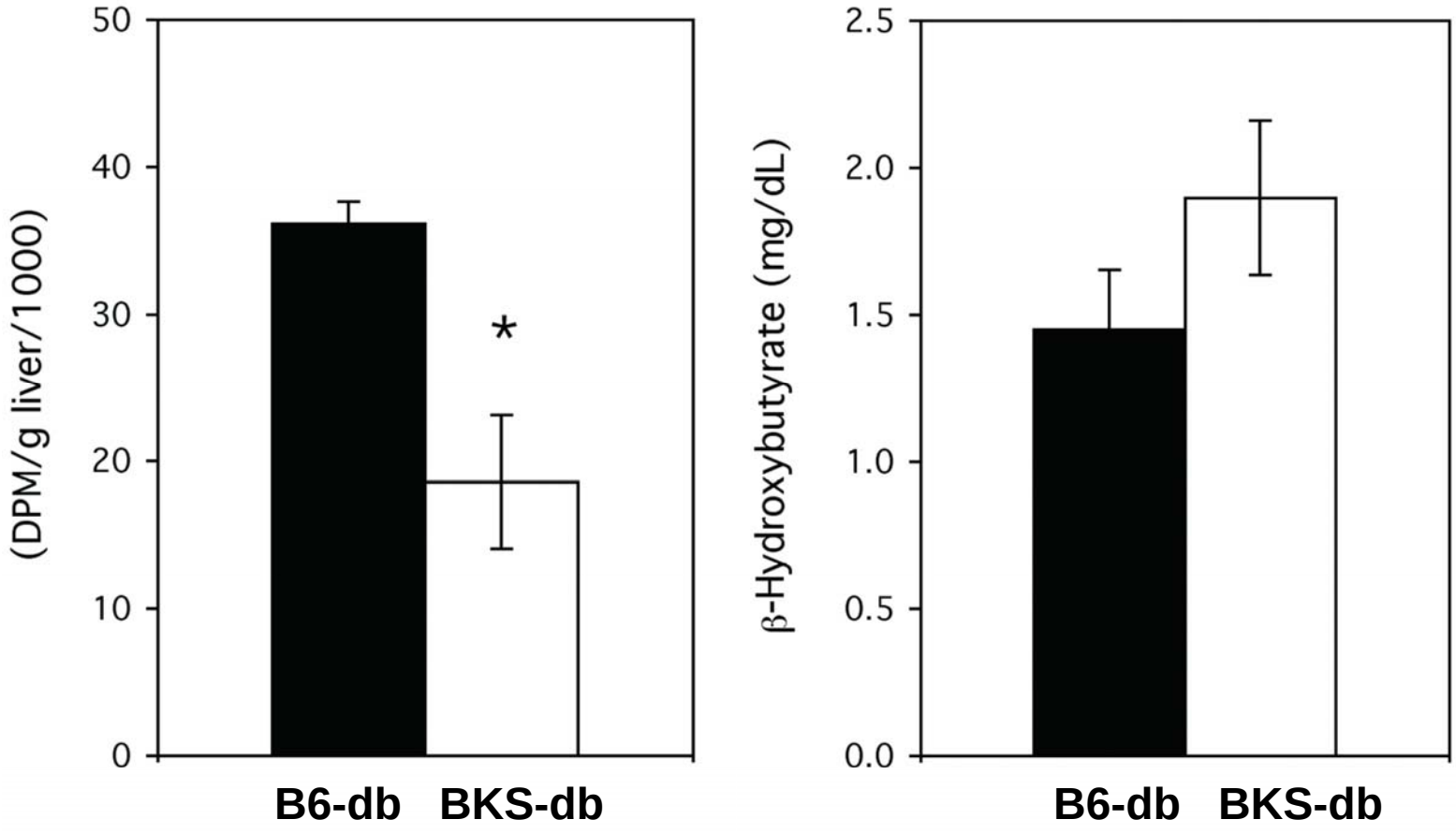


Liver Weight and Triglycerides B6-db and BKS-db

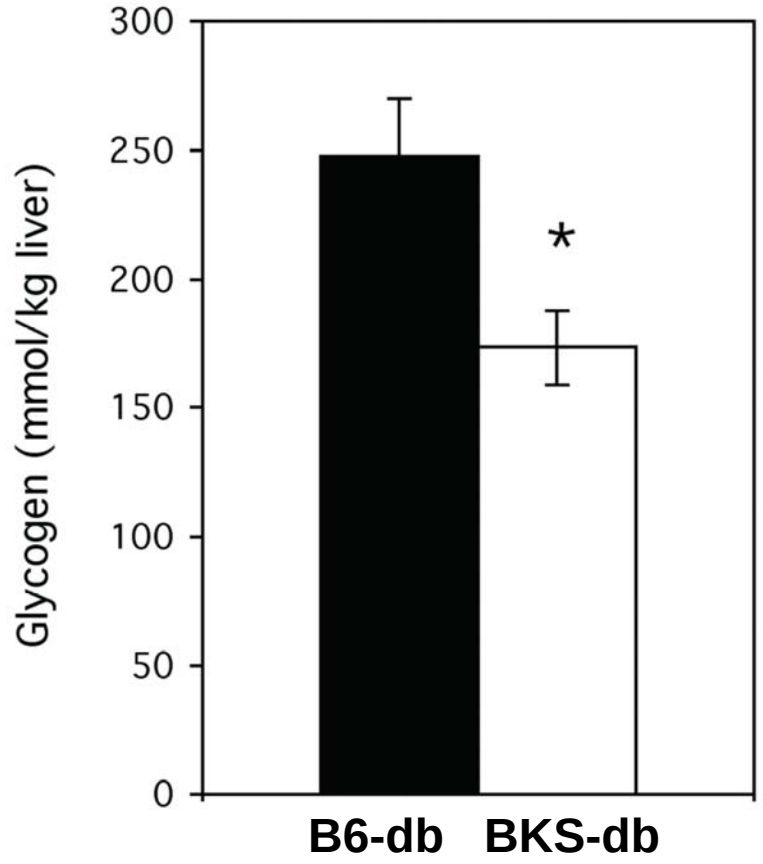




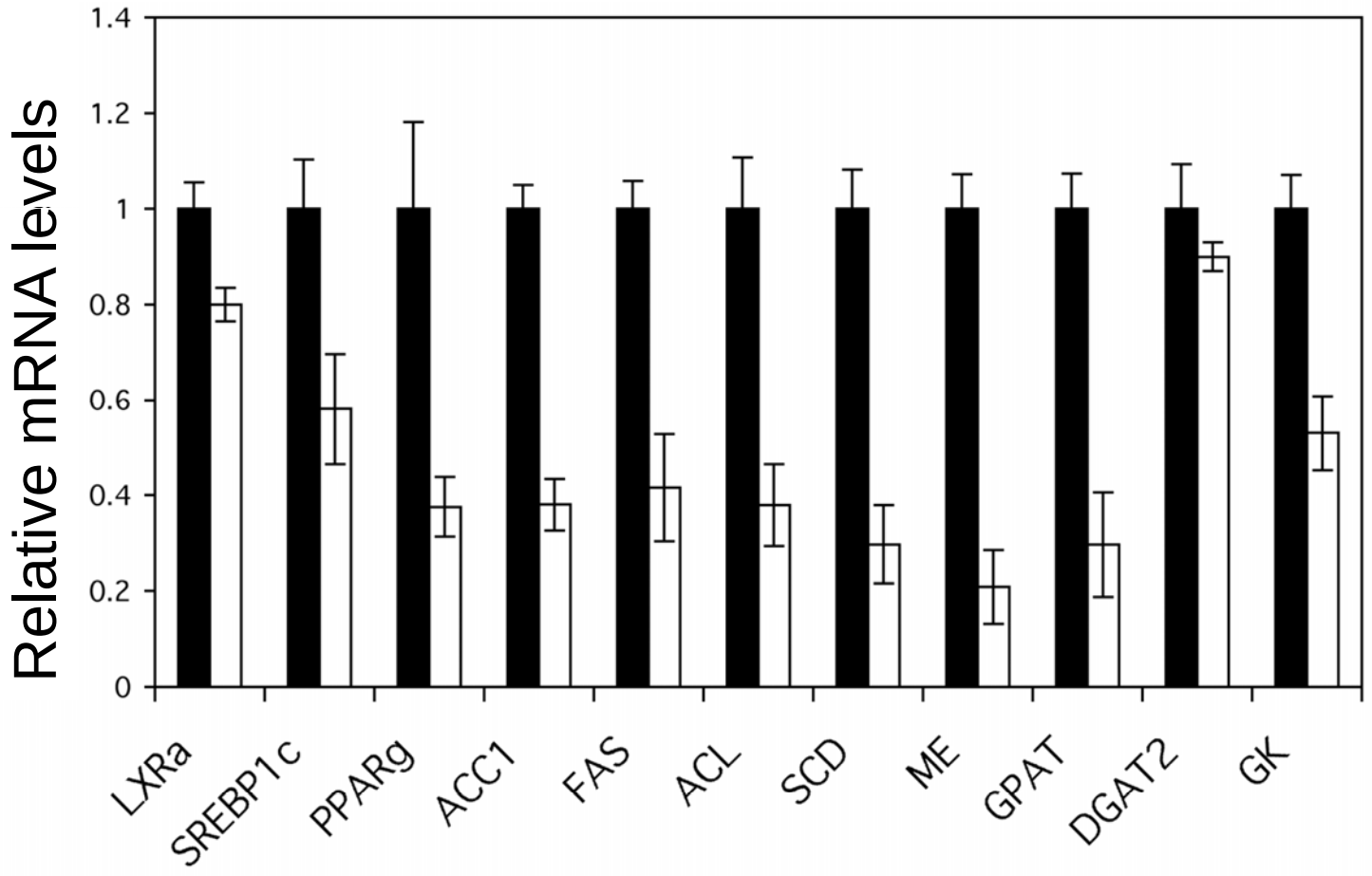
Fatty Acid Synthesis and Oxidation



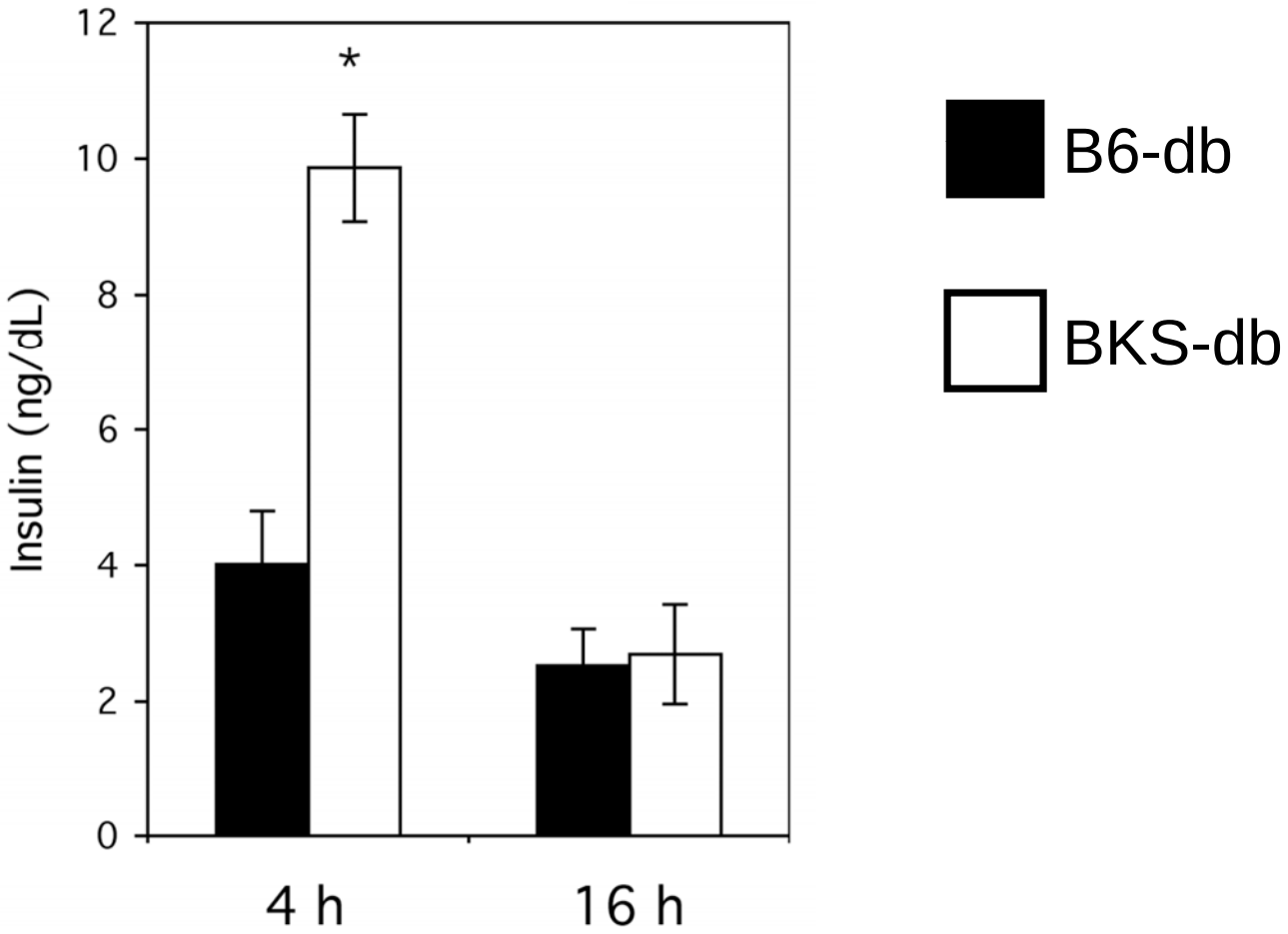
Suppressed Glycogen Storage in BKS-db



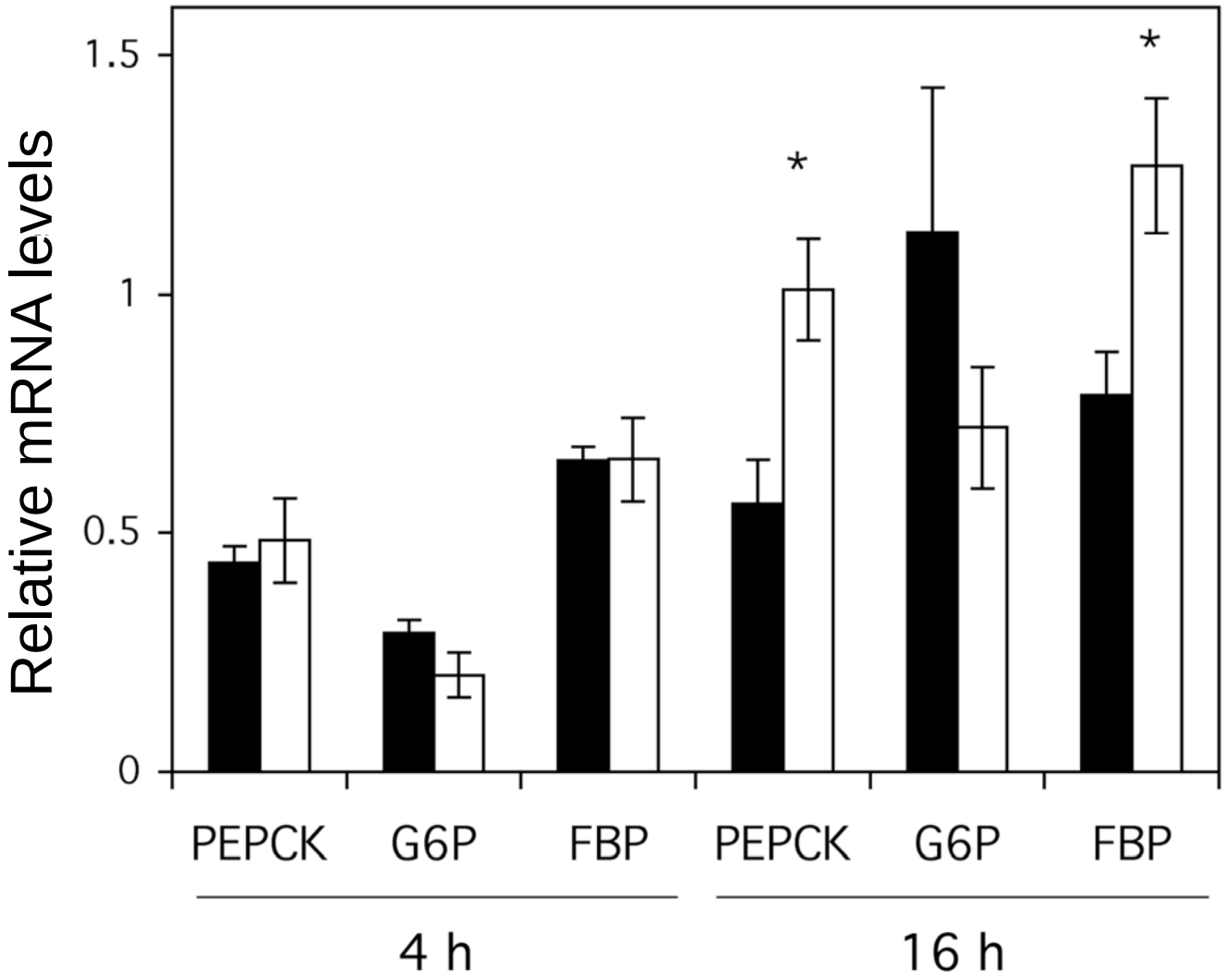
Lipogenesis and Fatty Acid Synthesis in BKS



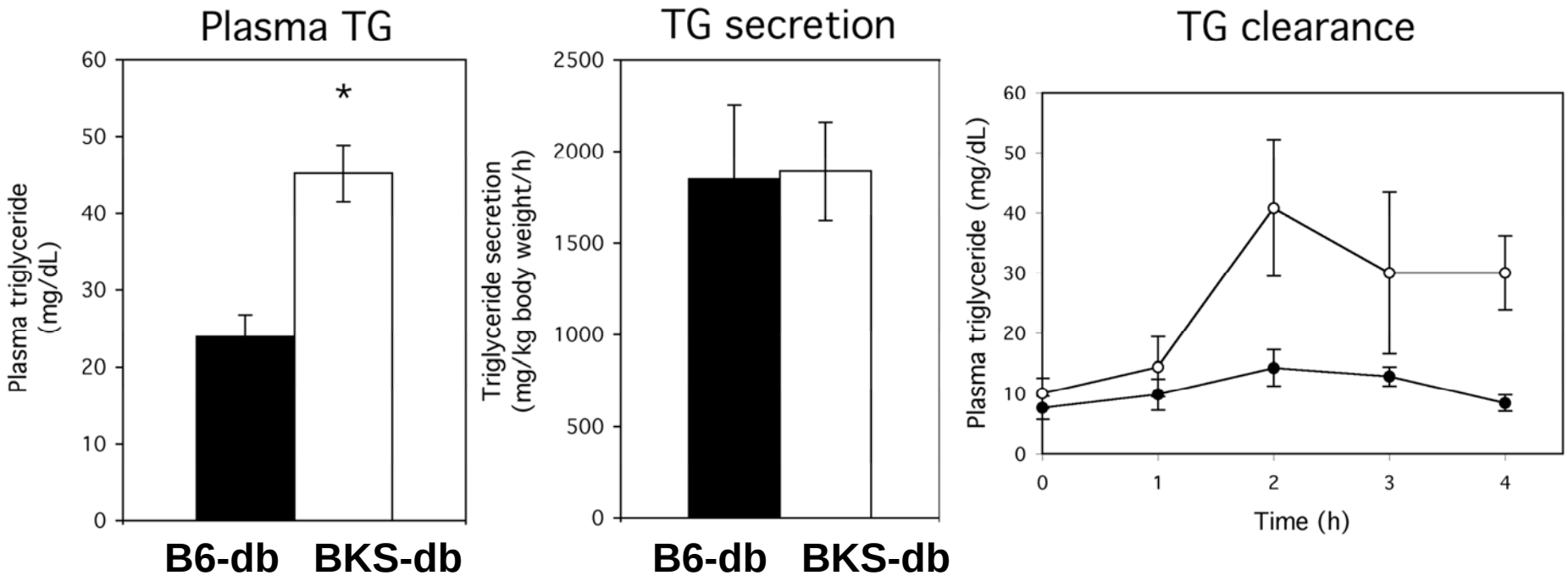
Insulin in B6-db and BKS-db



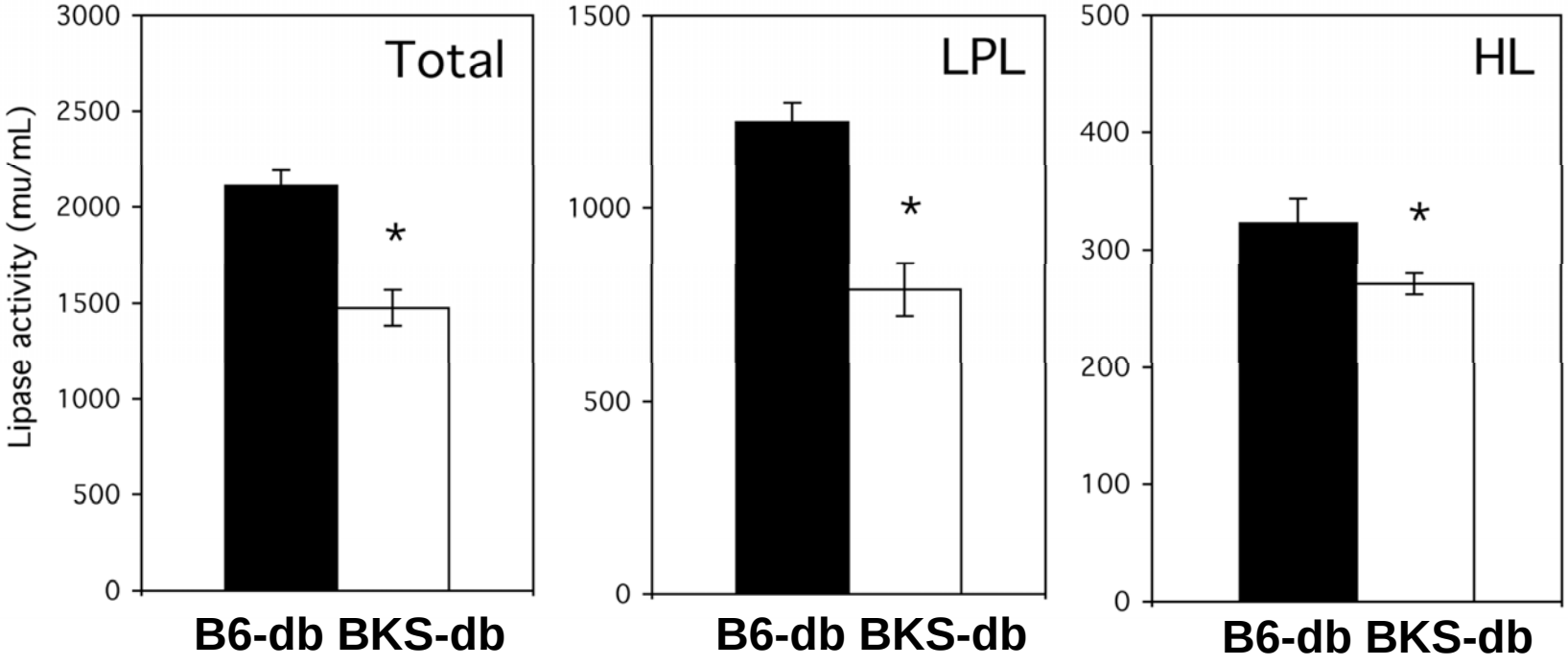
Genes for Gluconeogenesis in BKS-db



Plasma Triglycerides in BKS-db



Post-Heparin Plasma Lipase in BKS-db



Tissue Lipase in BKS-db