University of Cambridge

Lipotoxicity; the garbage in and out hypothesis

Obesity as a protective mechanism against the deleterious effects of positive energy balance

PPARγ2 prevents lipotoxicity by facilitating adipose tissue expandability and regulating lipid metabolism in peripheral organs

Toni Vidal-Puig, Cordoba,2008

Ingestion – Energy expenditure = Fat Deposition

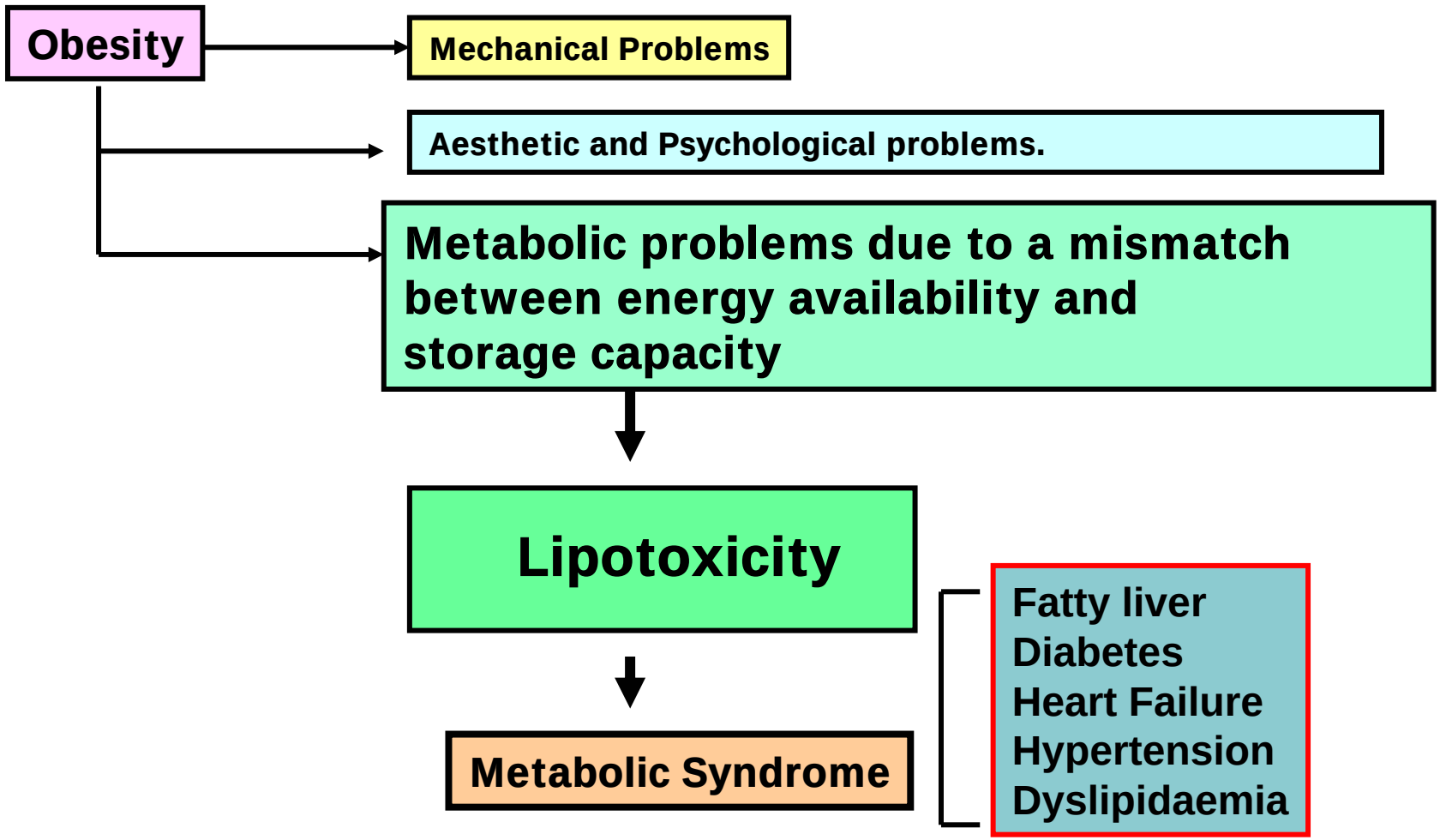
Overnutrition / Excess of energy = Obesity

Increased demands to adipose tissue expandability

The development of obesity requires a state of positive energy balance

What is not that clear is why expansion of the adipose tissue causes metabolic problems.

Adipocentric view of the Metabolic Syndrome



Overview of our Programme

LIPOTOXICITY: Inappropriate lipid storage in tissues other than adipose is the major underlying factor linking obesity and insulin resistance

Hypothesis 1: Improving the capacity for lipid storage in adipose will protect against insulin resistance and diabetes
-PPARγ and adipose tissue expandability-.

Hypothesis 2: In the advent of a failure to store lipid appropriately in adipose tissue then mitochondrial oxidation of lipids will protect against diabetes
- PGC1b as an antilipotoxic strategy

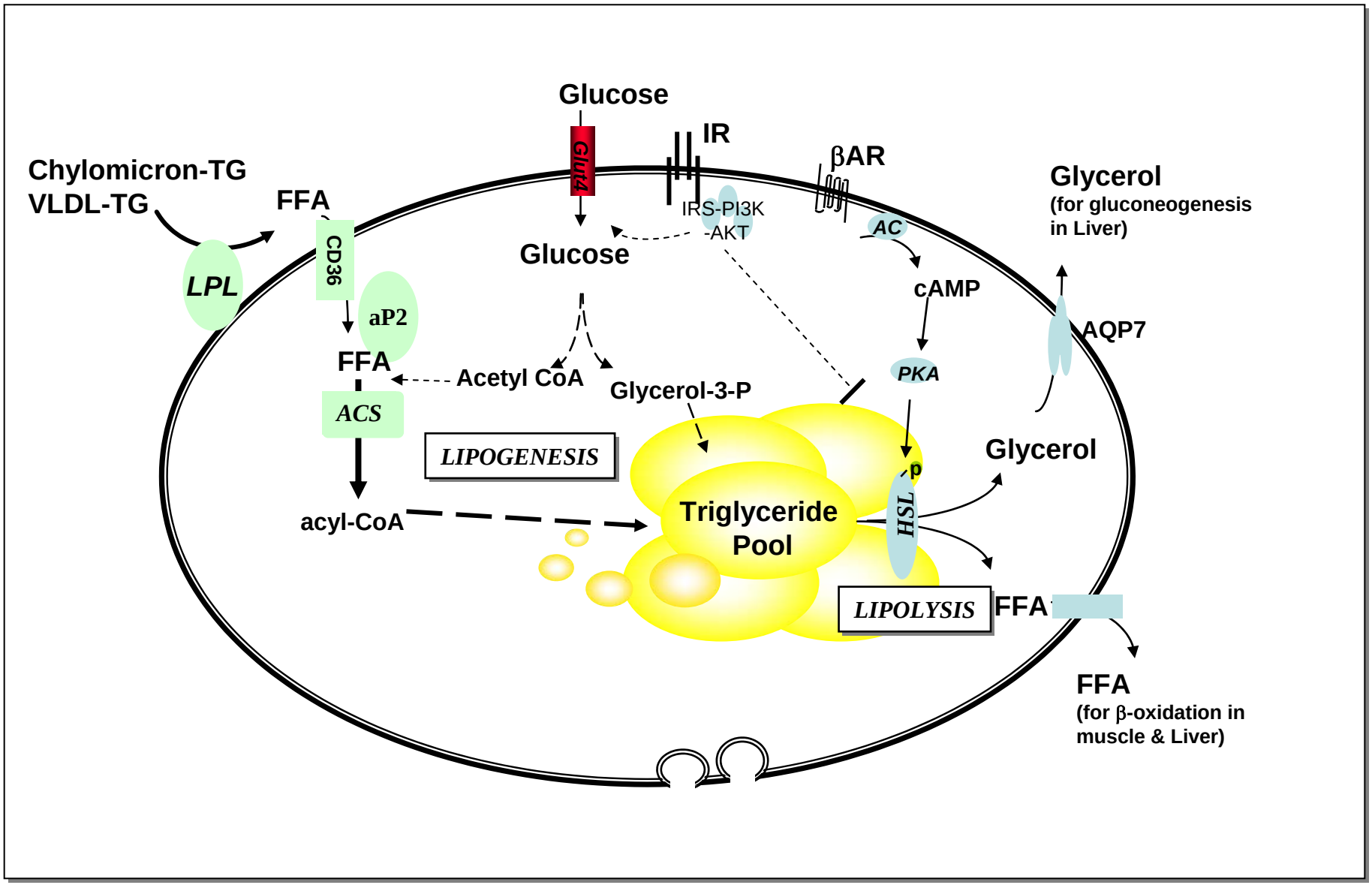
Hypothesis 3: When adipose storage and oxidation fail to prevent inappropriate deposition of lipid in other tissues, the type of lipid deposited is more important than the amount of lipid stored.
- Lipid related pathways and lipidomics

Biochemical Characteristics of Adipocytes

What is an adipocyte?

The adipocyte is the major cell component of adipose tissue in which fats (triglycerides) are stored. Adipocytes contains enzymes "lipases" that can break down fat into glycerol and fatty acids, which can be transported in the blood to the liver, where they are used in fatty-acid oxidation" *Oxford Dictionary of Biology ('96)*

Figure 1


Glucose
IR
Glut4
βAR
Chylomicron-TG
VLDL-TG
FFA
CD36
LPL
aP2
FFA
ACS
acyl-CoA
Glucose
IRS-PI3K
-AKT
AC
cAMP
PKA
Acetyl CoA
Glycerol-3-P
LIPOGENESIS
Triglyceride
Pool
HSL
p
LIPOLYSIS
Glycerol
(for gluconeogenesis
in Liver)
AQP7
Glycerol
FFA
FFA
(for β-oxidation in
muscle & Liver)

Some Substances Secreted by Adipose Tissue

Metabolic modulators
Lipoprotein lipase (LPL)
Fatty acids*
glycerol
Apoprotein E

Vasoactive factors
Monobutyrin
Angiotensinogen/
Angiotensin II*
Atrial natriuretic peptide

Others
Cholesterol ester transfer protein
Plasminogen activator-inhibitor 1*
Acrp30/AdipoQ*
LPA, lysophosphatidic acid.
Resistin*
Visfatin/PBEF*
Fasting induced adipose factor
Metallothionen
Apelin

Steroid Hormones
Oestrone
Oestradiol
Testosterone

Eicosanoids
Prostaglandins E2 (PGE2)
Prostaglandins F2a (PGF2a)
Prostacyclin (Prostaglandin I2/ PGI2)

Growth factors & Cytokines
IGF-1,
VEGF
Leptin*
Interleukin-6 (IL6)*
Tumour necrosis factor α (TNF α)*

Complement system
Factor B
Factor C, C3, C1q
Factor D (adipsin/
Acylation-stimulating protein
(ASPC3desARg)*

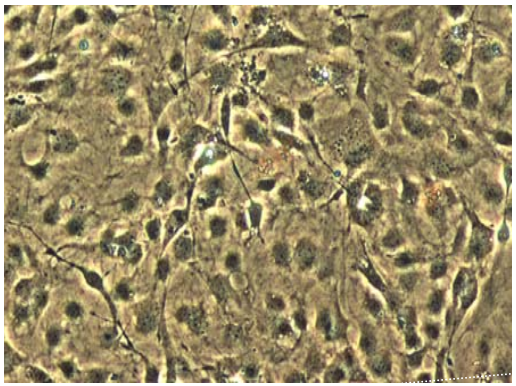
Binding proteins
IGF-BPs
Retinol BP

Extracellular matrix proteins
MCP-1

adapted from Vernon RG etal. Domestic Animal Endocrinology 21:197-214 (2001)

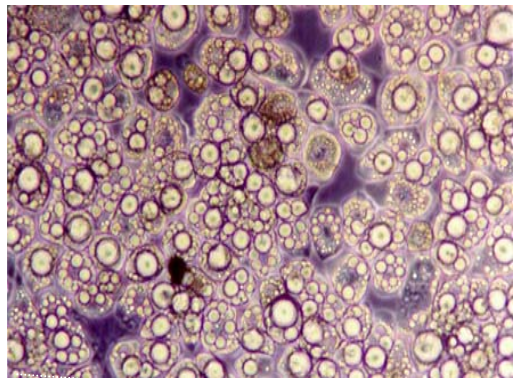
COMPLEX Tissue: Transcriptional regulation of adipogenesis

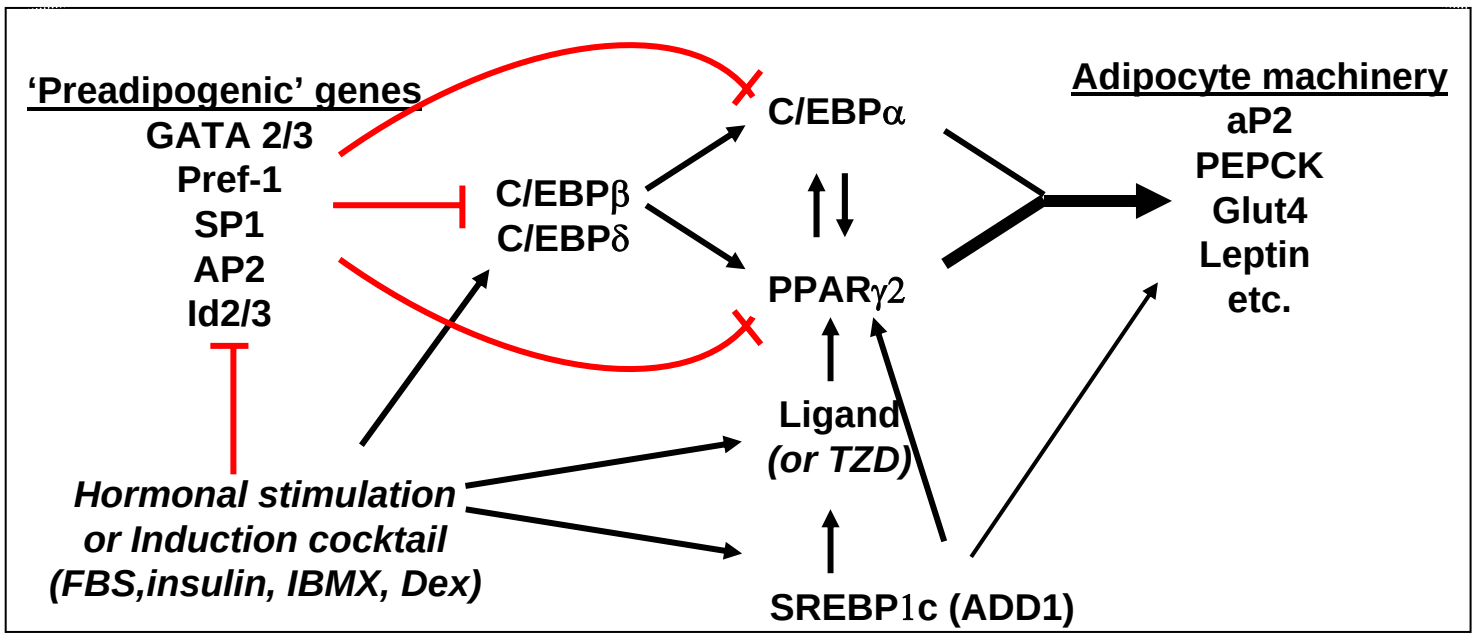
Preadipocytes



differentiation

Adipocytes





Some ideas

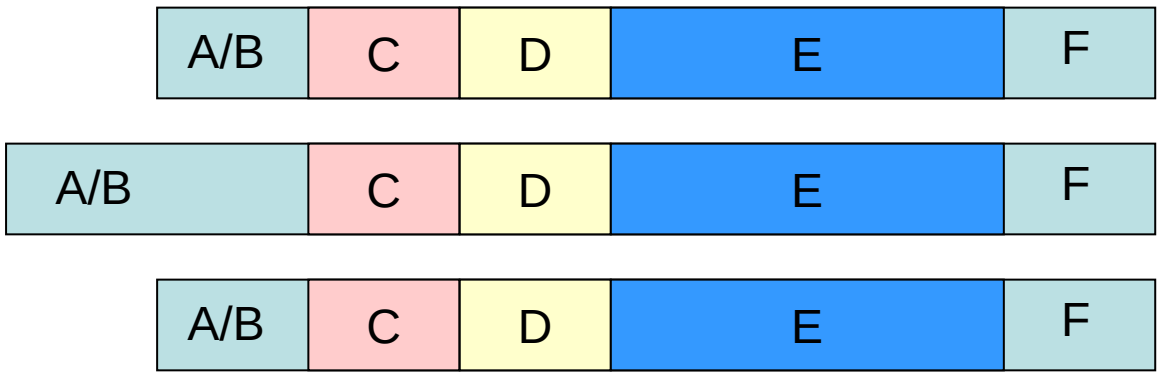
In the context of positive energy balance, accommodation of excess of energy in adipose tissue poses an unprecedented challenge to adipose tissue expandability.

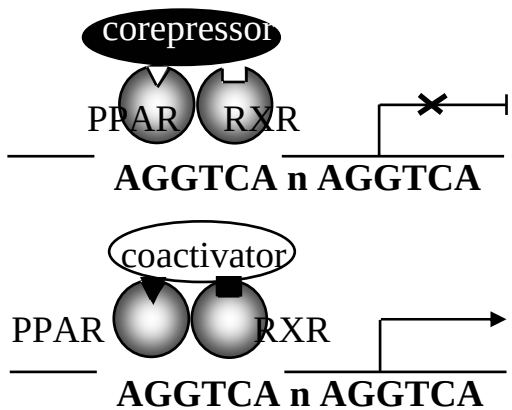
Given its intrinsic complexity, it is not unlikely that adipose tissue expandability may be limited.

PPARs

- Insulin resistance
- Obesity
- Diabetes
- Blood pressure
- Dyslipidaemia

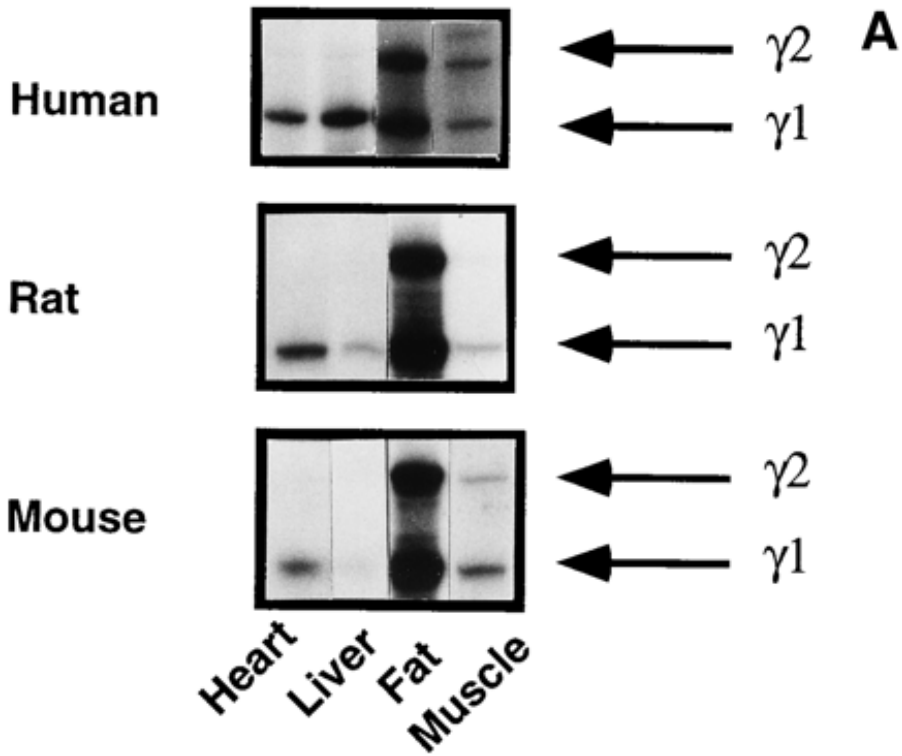
PPARγ: Proadipogenic Gen that facilitates the expansion of the adipose tissue



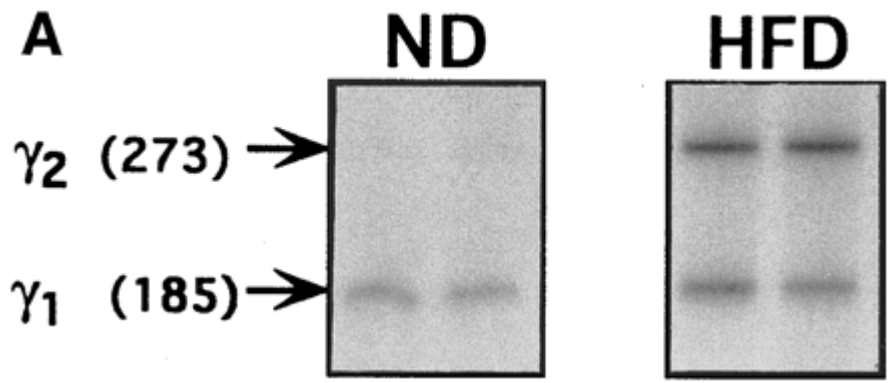


A/B	**N-terminal A/B domain**
C	**DNA Binding Domain**
D	**Hinge**
E	**Ligand Binding Domain**
F	**C- terminal region**

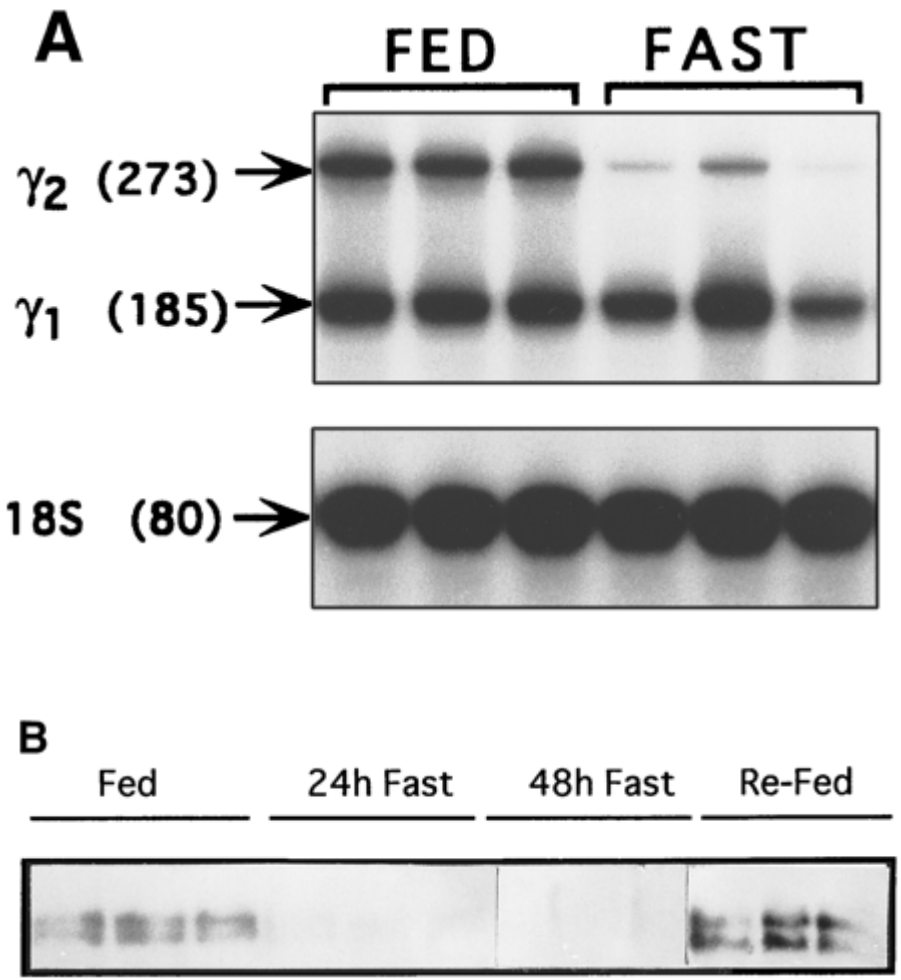
PPARγ isoform tissue distribution



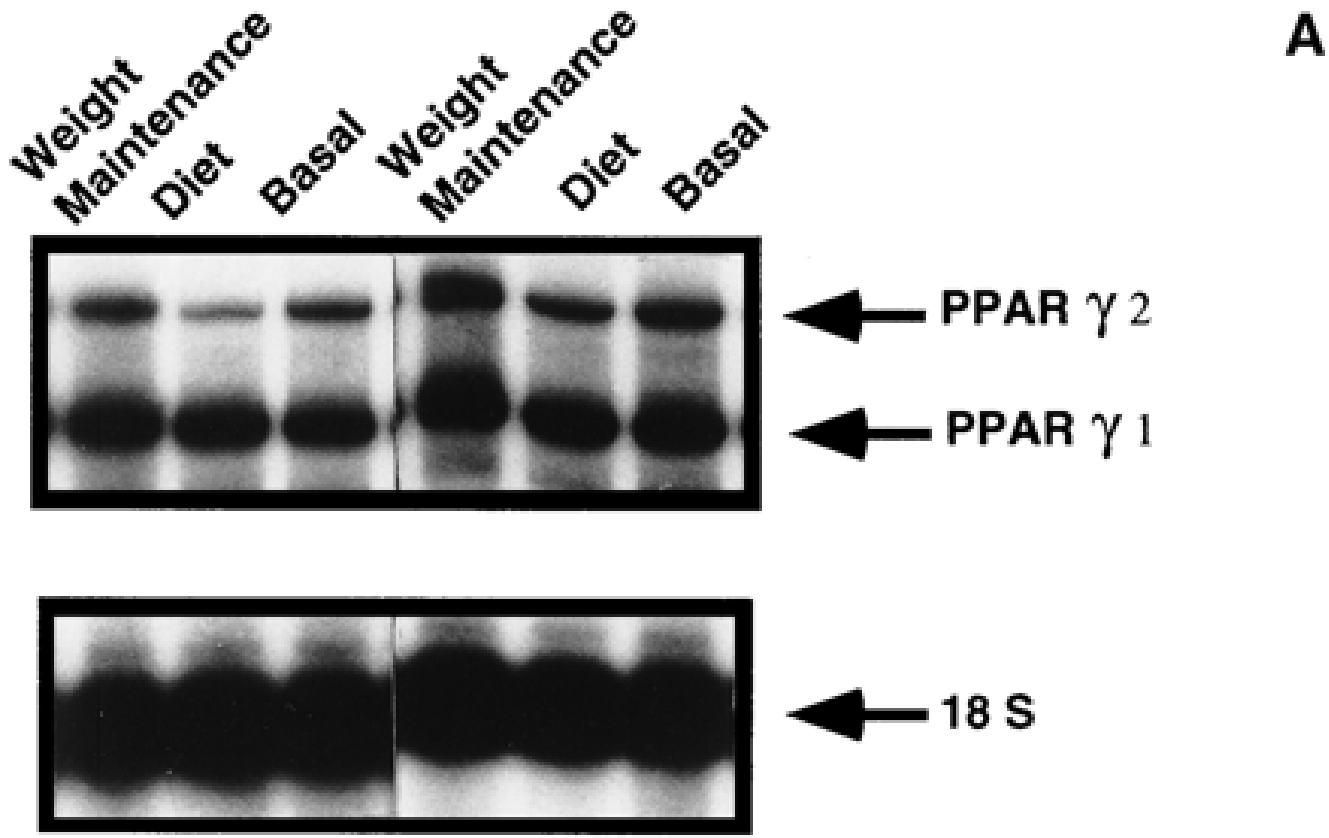
HFD induces PPARγ2 isoform in liver and muscle of the BATless and ob/ob mouse



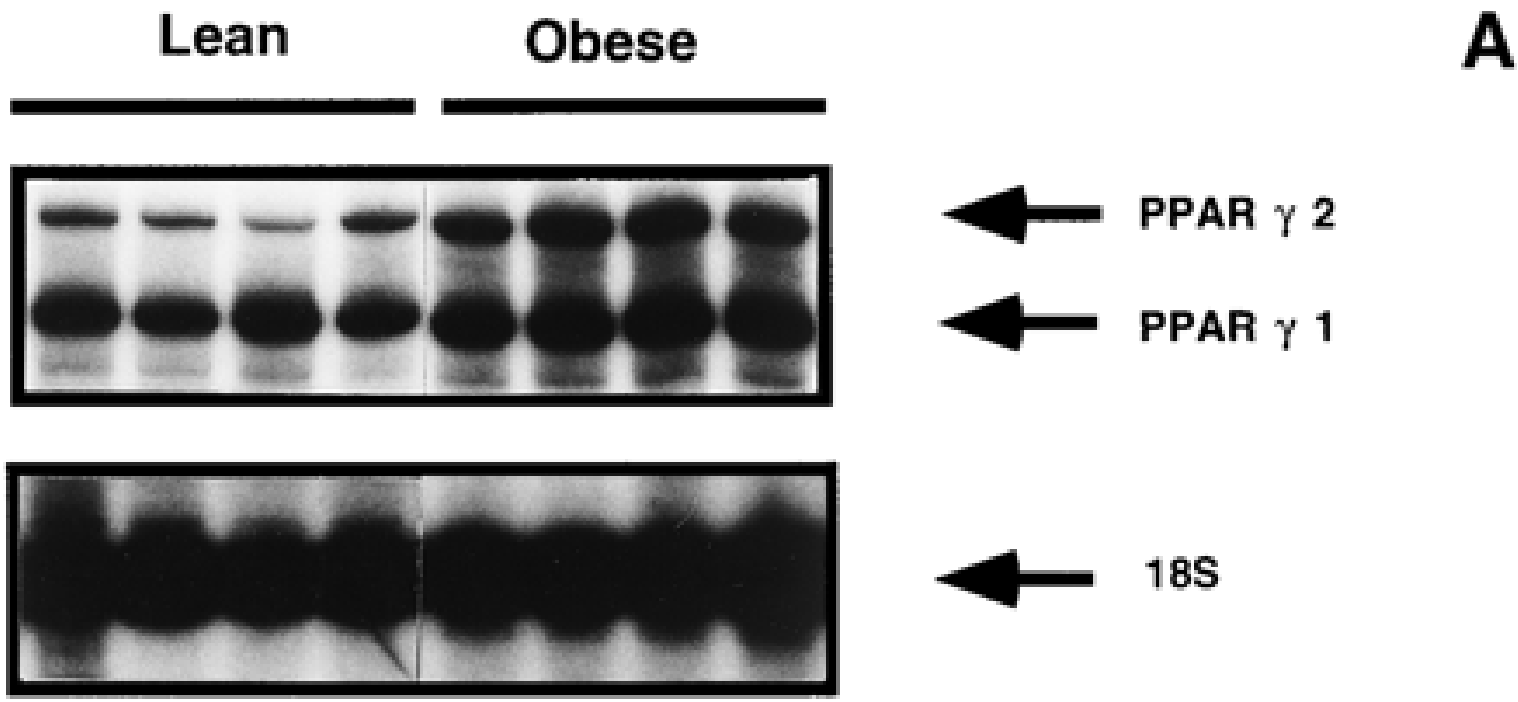
PPARγ2 mRNA and protein are regulated in adipose tissue by fasting



PPARγ2 gene expression is regulated in human adipose tissue during weight loss.

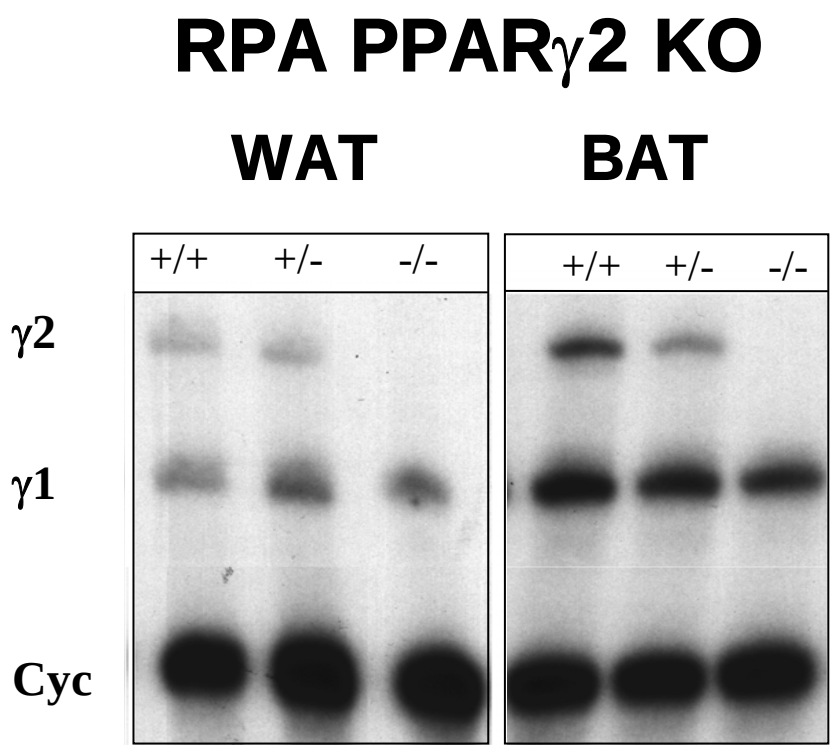


PPARγ2 is upregulated in adipose tissue of human normoglycemic morbid obese individuals.

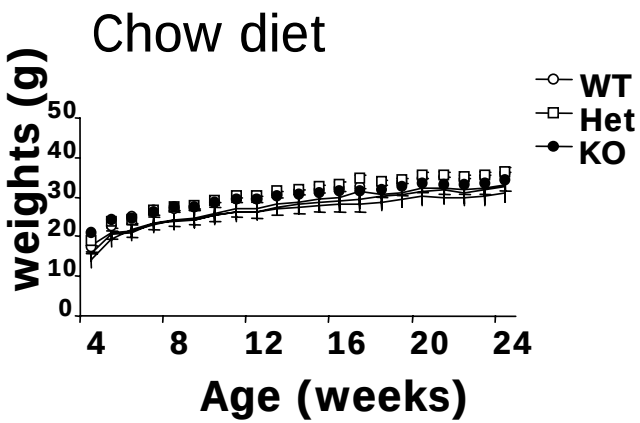


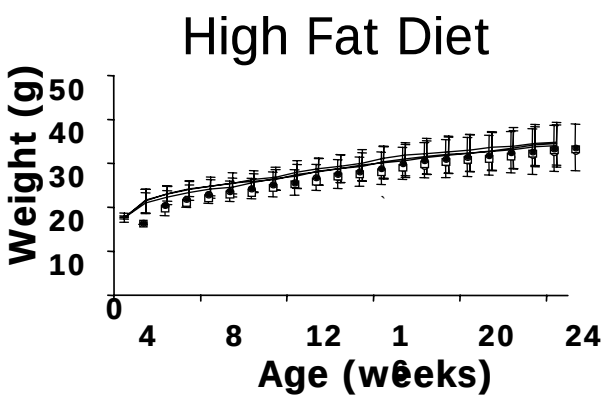
What are the metabolic alterations
in a rodent model with neutral energy
Balance (lean)
and defective adipose tissue
Expandability?

PPARγ2 KO MOUSE

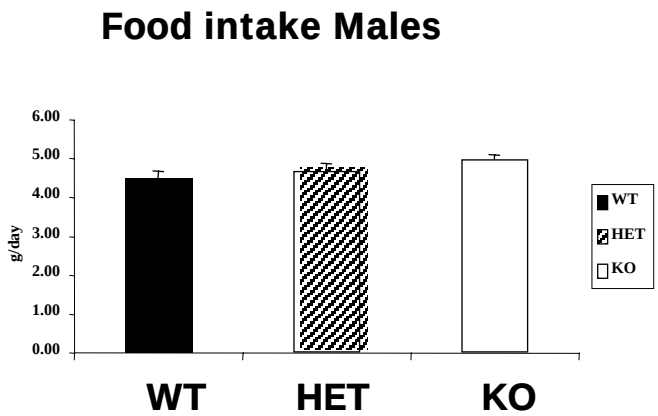


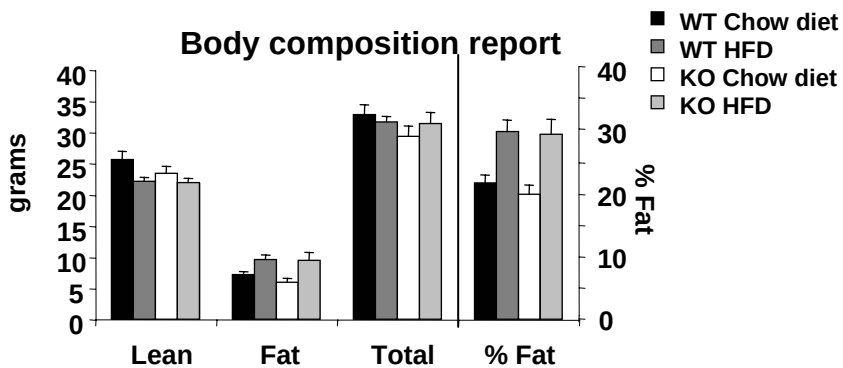
Our PPARγ2 on a 129 background had Normal Body weight, Food intake, Energy expenditure and body Composition.

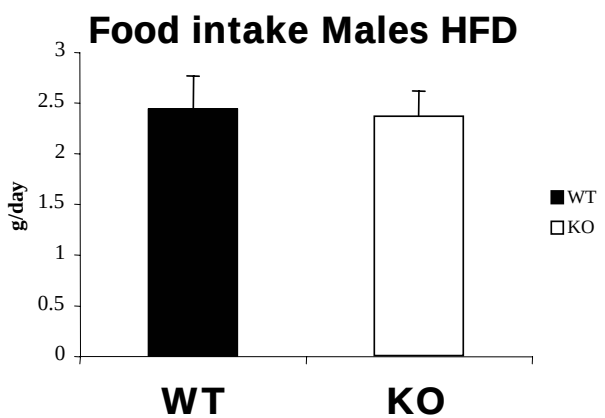




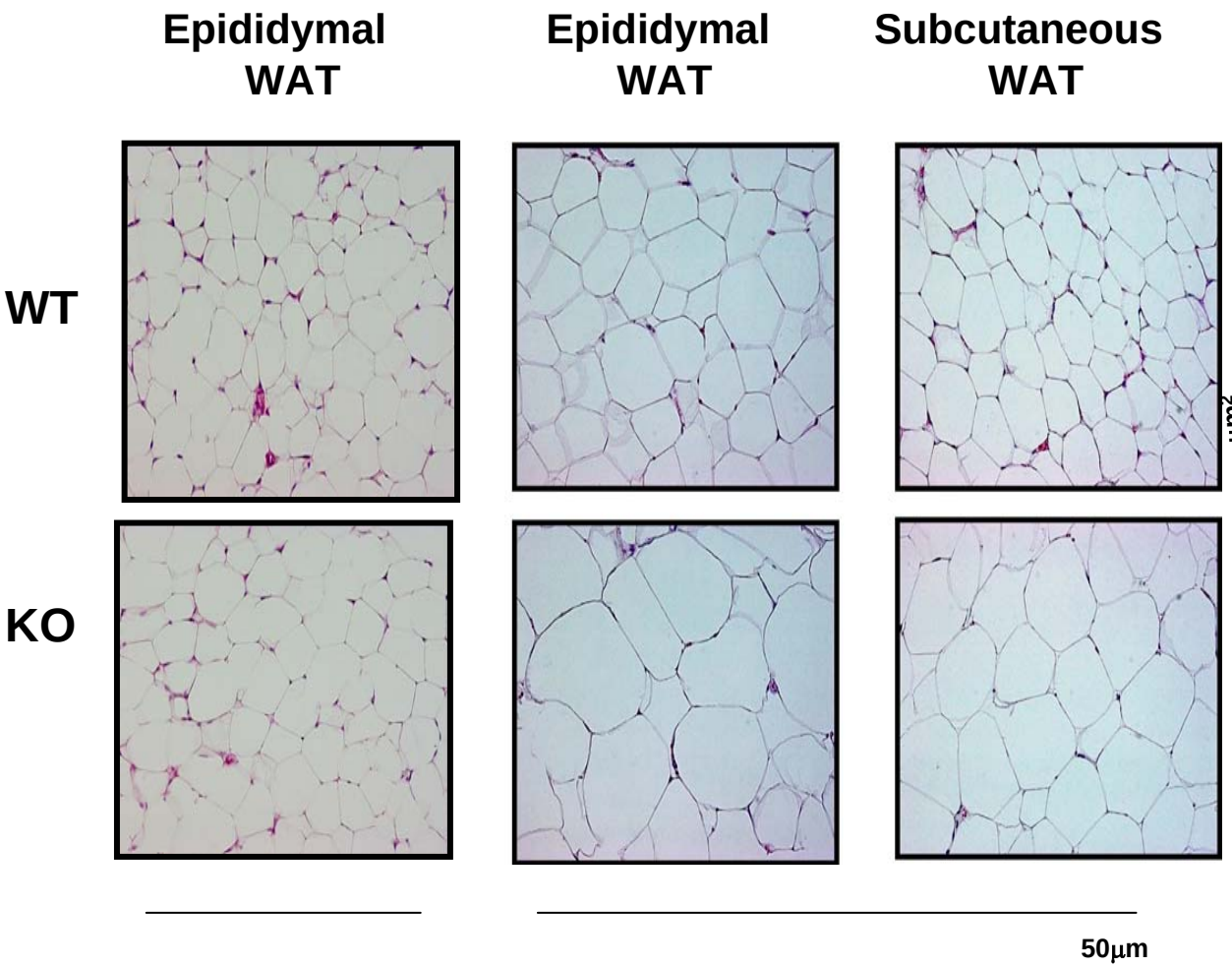
n=20

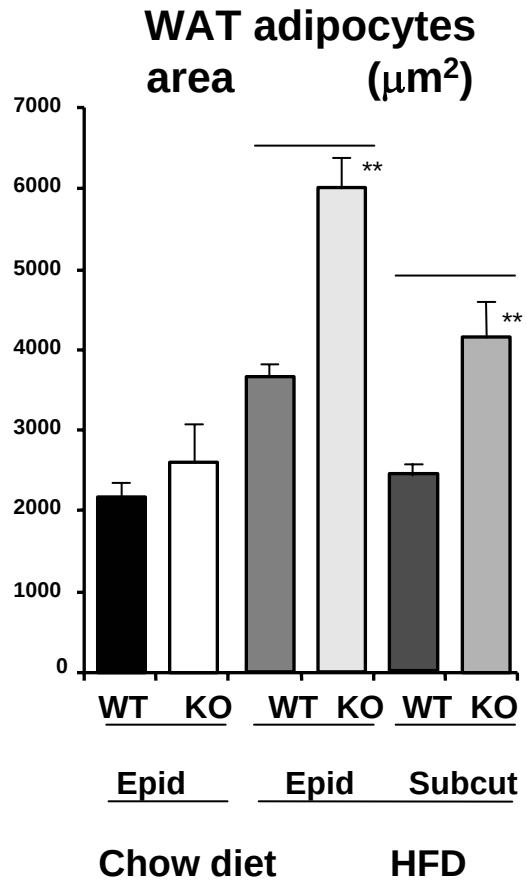




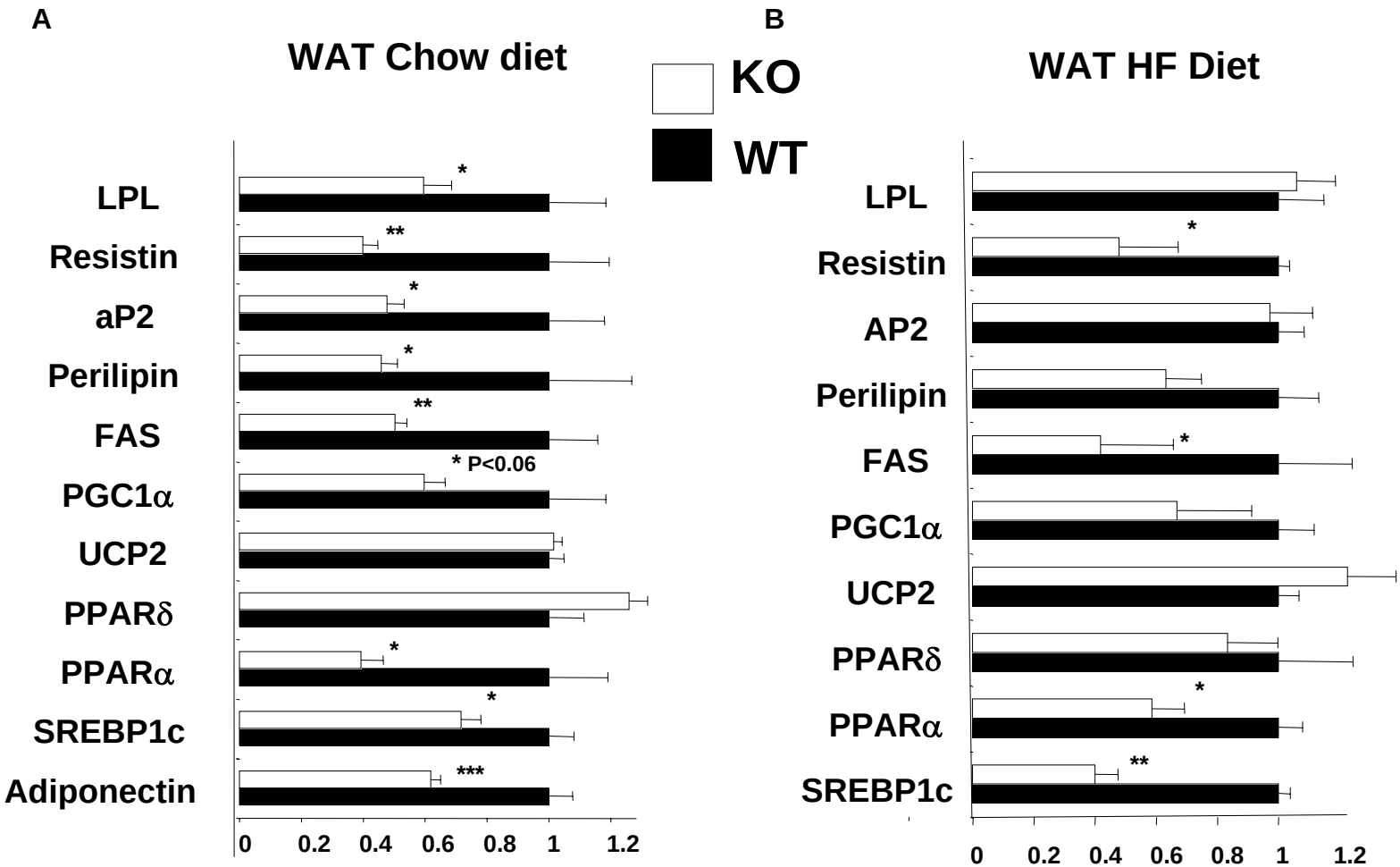


High fat diet induces adipocyte hypertrophy in PPARγ2KO mouse



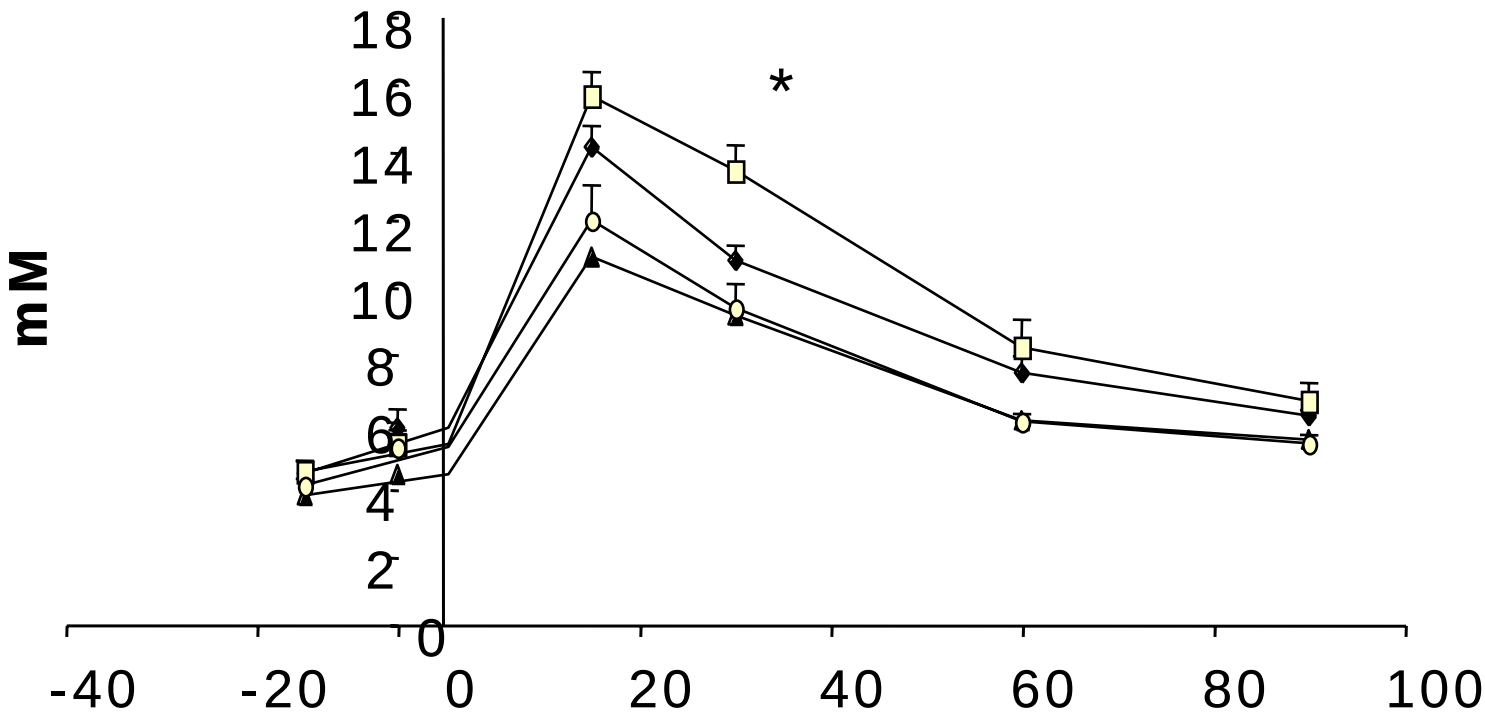


Gene expression analysis in Adipose Tissue reveals expected profile



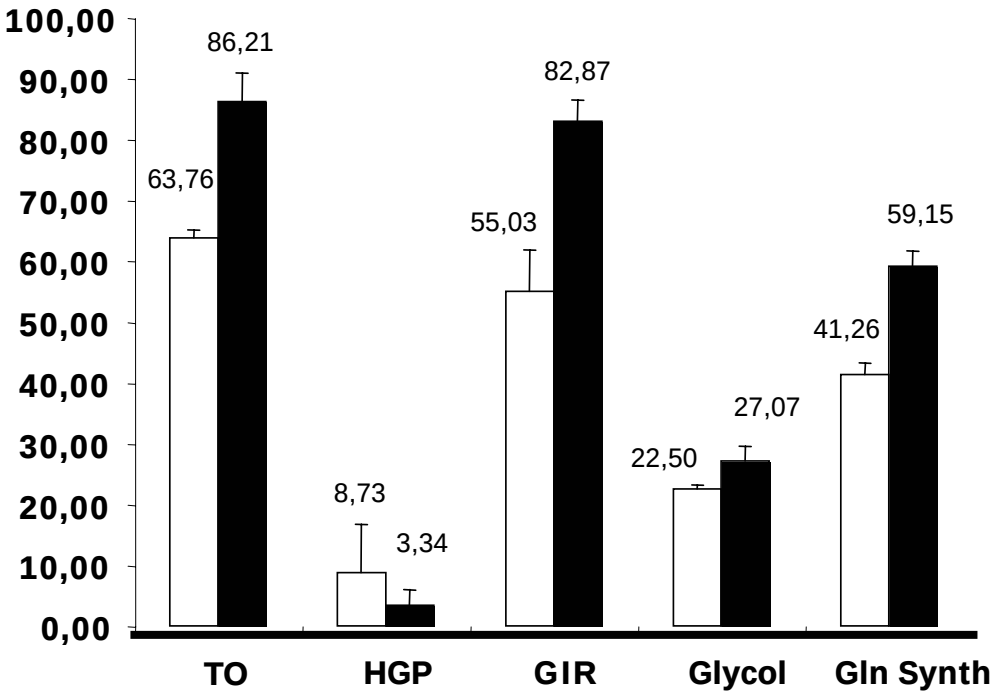
Microarray analysis + Pathway analysis

Mild abnormal GTT in male PPARγ2 ko mice on chow diet



Glucose turnover rates are lower in male PPARγ2 KO mice in chow diet

Insulin resistant phenotype



PPARγ 2 - / -

PPAR γ2 + / +

TO Total Glucose Output
HGP Hepatic Glucose production
GIR Glucose infusion rate
Glycolysis
Glycogen synthesis

Table 1. Metabolic parameters of 16 week old PPARγ2 KO and WT mice

	Males Chow diet		Males 12 weeks on HFD	
	WT	KO	WT	KO
Glucose (mg/dl)	130±9.0	147±5.9	236±15.5	238±11.1
Glucose (mg/dl) fasting	63±4.1	88±5.1 **	106.9±12.	113.0±14.
Triglycerides (mmol/L)	0.93±0.12	0.87±0.15	0.68±0.17	0.78±0.09
Free Fatty Acids (µmol/L)	295±63	231±17	250±32	219±22
Insulin (µg/L)	0.49±0.10	0.34±0.05	0.98±0.20	1.31±0.13
Insulin fasting (µg/L)	0.14±0.03	0.35±0.11		
Leptin (ng/ml)	2.77±0.44	4.13±0.56*	8.76±1.1	16.3±1.4
Adiponectin (µg/ml)	15.2±1.2	7.9±1.2***	11.3±1.0	7.46±1.3*

Which are the metabolic alterations in a murine model with positive energy balance and defective adipose tissue expandability?

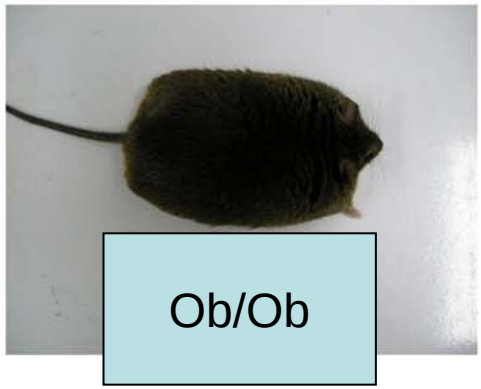
PPARγ2 KO MOUSE: defect in adipose tissue expandability

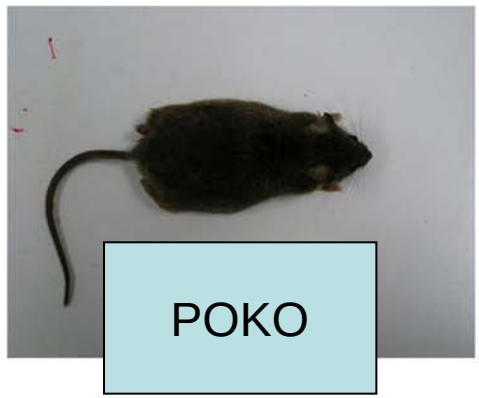
X

Crossed with genetically Obese leptin deficient Ob/Ob mouse

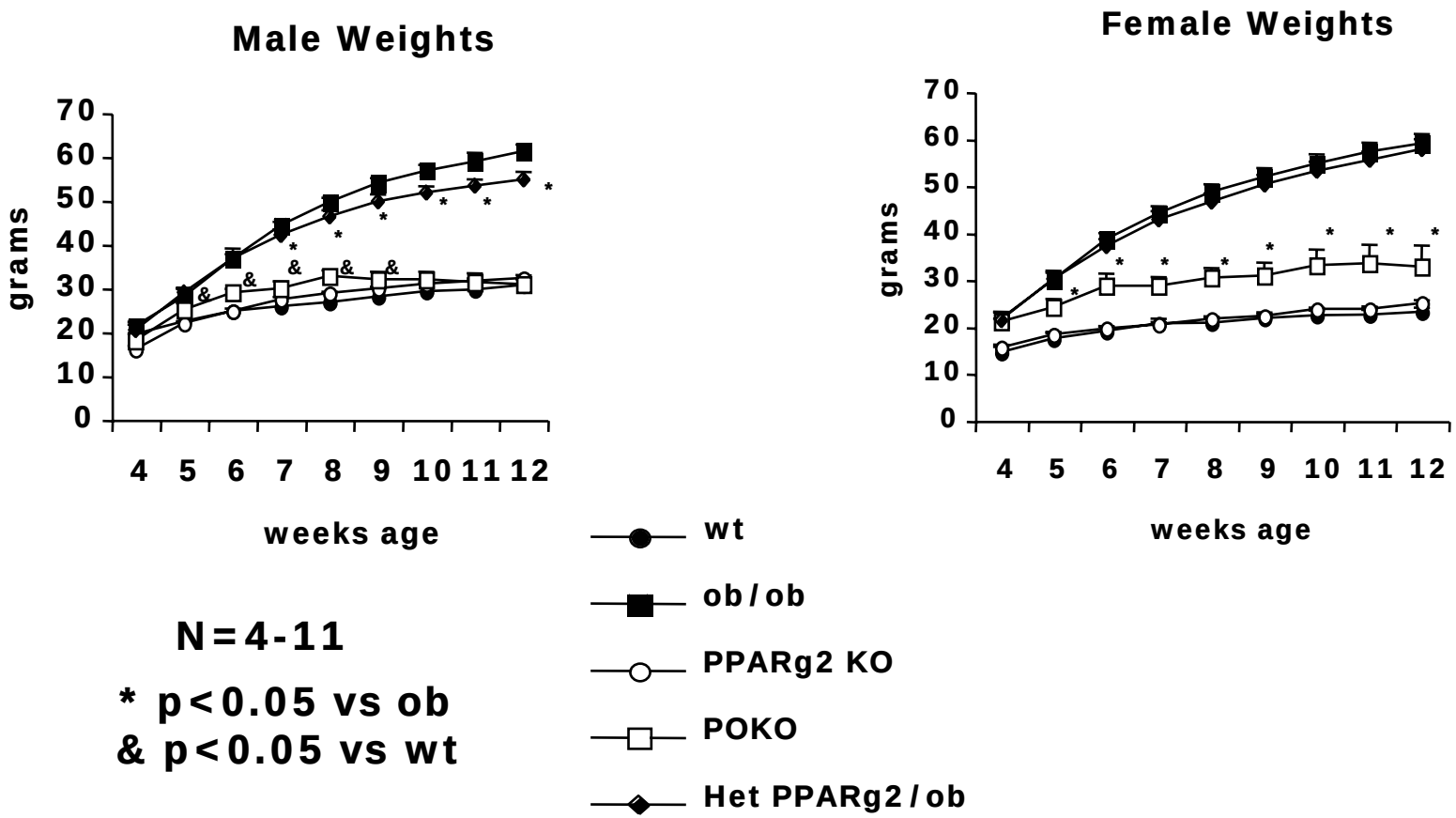
POKO Mouse


WT


Ob/Ob


POKO

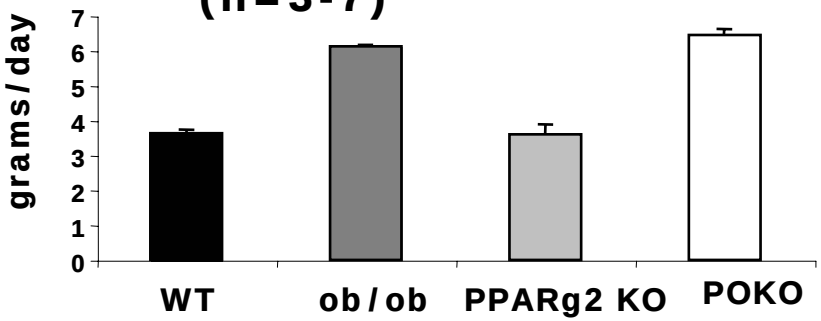
Growth curves from **PPARγ2KOxob/ob** mice



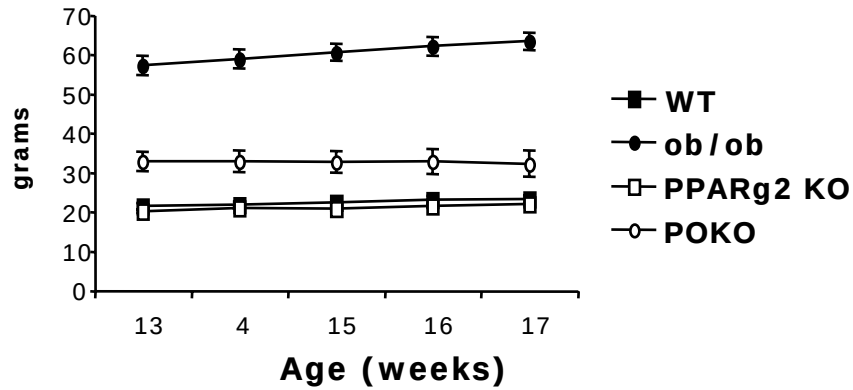
Ob/Ob and POKO mice have similar energy balance

Adult studies (16-week old mice)

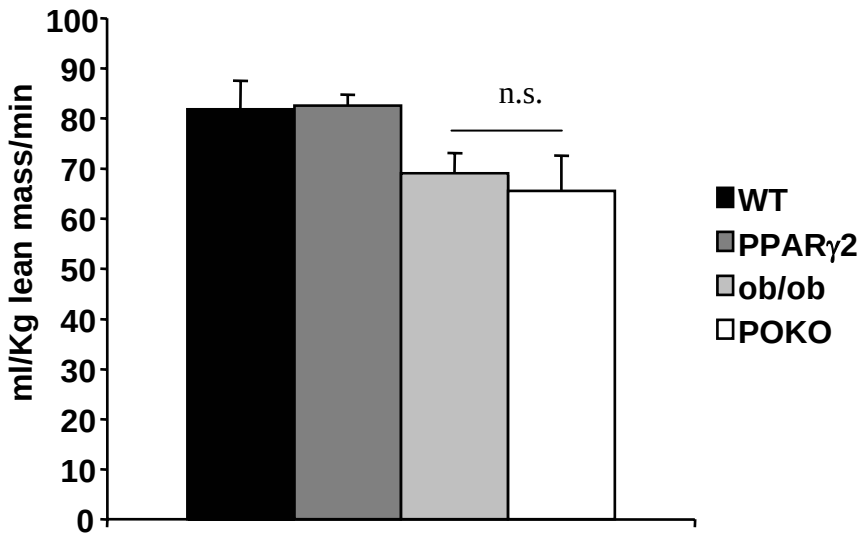
Food consumption Females (n=3-7)



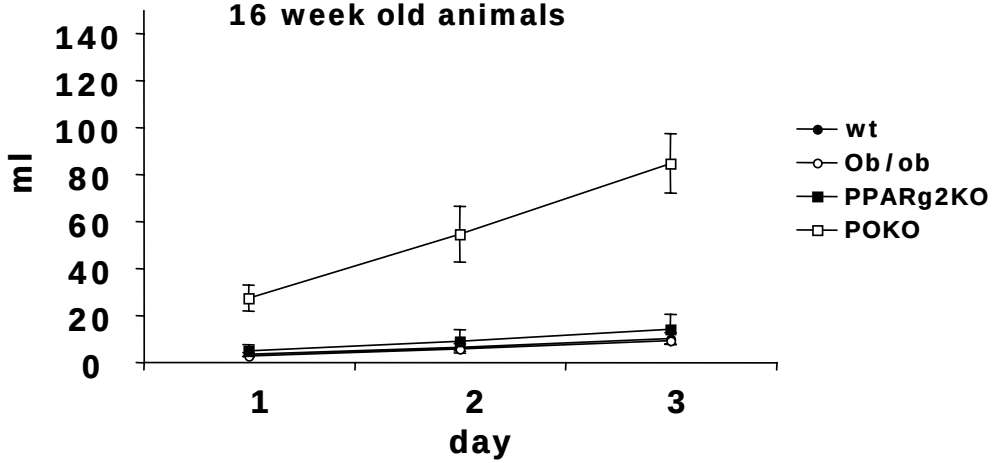
Weights during Food consumption



Oxygen consumption VO2 POKO 6wo females



Accumulative water intake/72 h in 16 week old animals



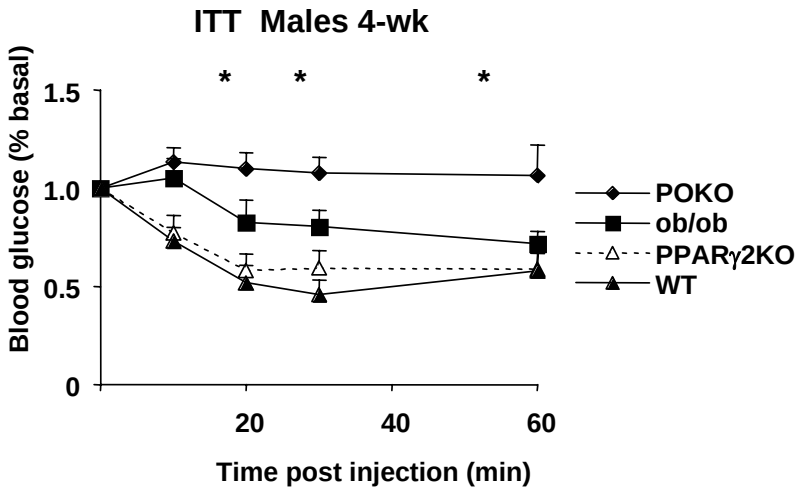
		Males	16 weeks
	WT	Ob/ob	POKO
Weight (g)	36.02±1.61	75.76±4.56	40.47±8.57
Glucose fed (mmol/L)	10.93±1.45	15.27±2.47	hi
Glucose fasted (mmol/L)	5.46±0.52	10.74±1.78	22.64±3.98
Ins fed (ug/L)	3.38±0.41	39.08±10.72	13.2±1.25
Ins fasted (ug/L)	0.21±0.04	4.04±0.80	
FFA (umol/L)	718.5±103.75	883.57±68.27	995.66±105.65
Chol (umol/L)	3.3±0.05	6.4±0.55	5.2±0.69
Trig (umol/L)	2.05±0.07	3.17±0.97	6.9±1.8
Leptin (ng/L)	17.30±2.33		
Adiponectin (ug/ mL)	22.69±0.82	11.84±1.22	4.96±2.05

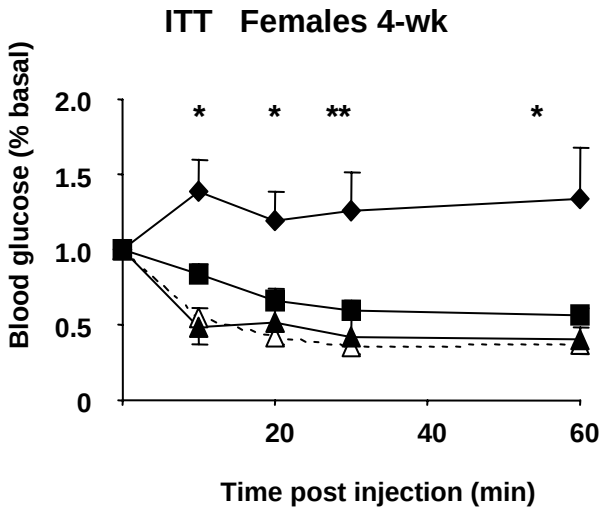
POKO Mouse develops early hyperglycemia compared to ob/ob

A

	Females					
	Week 3		Week 4		Week 5	
	Weight (g)	Glucose (nmol/L)	Weight (g)	Glucose (nmol/L)	Weight (g)	Glucose (nmol/L)
WT	8.6±0.2	7.8±0.6	15.6±0.7	9.0±0.3	19.3±0.7	8.6±0.3
ob/ob	10.9±0.9	9.6±1.0	22.78±1.9	11.0±1.0	36.7±0.9	18.7±2.5
PPARγ2 KO	8.0±0.8	9.3±0.4	15.8±1.3	9.3±0.8	18.4±0.4	8.9±0.5
POKO	8.9±0.7	10.3±1.5	17.9±1.61	**20.9±3.3****	27.8±1.0***	**28.65±1.2***

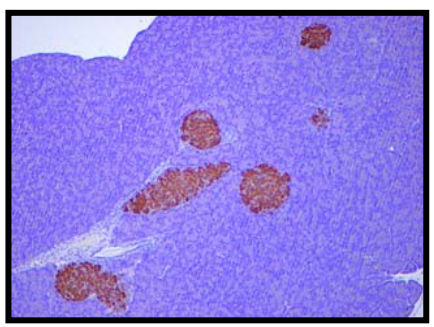
POKO Mice develop earlier insulin resistance compared to the ob/ob mice



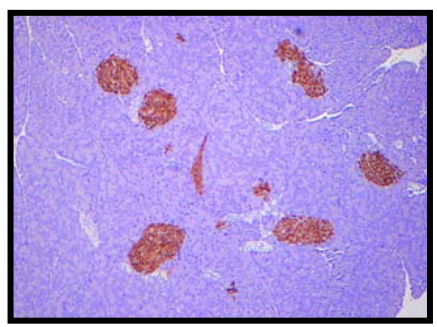


C

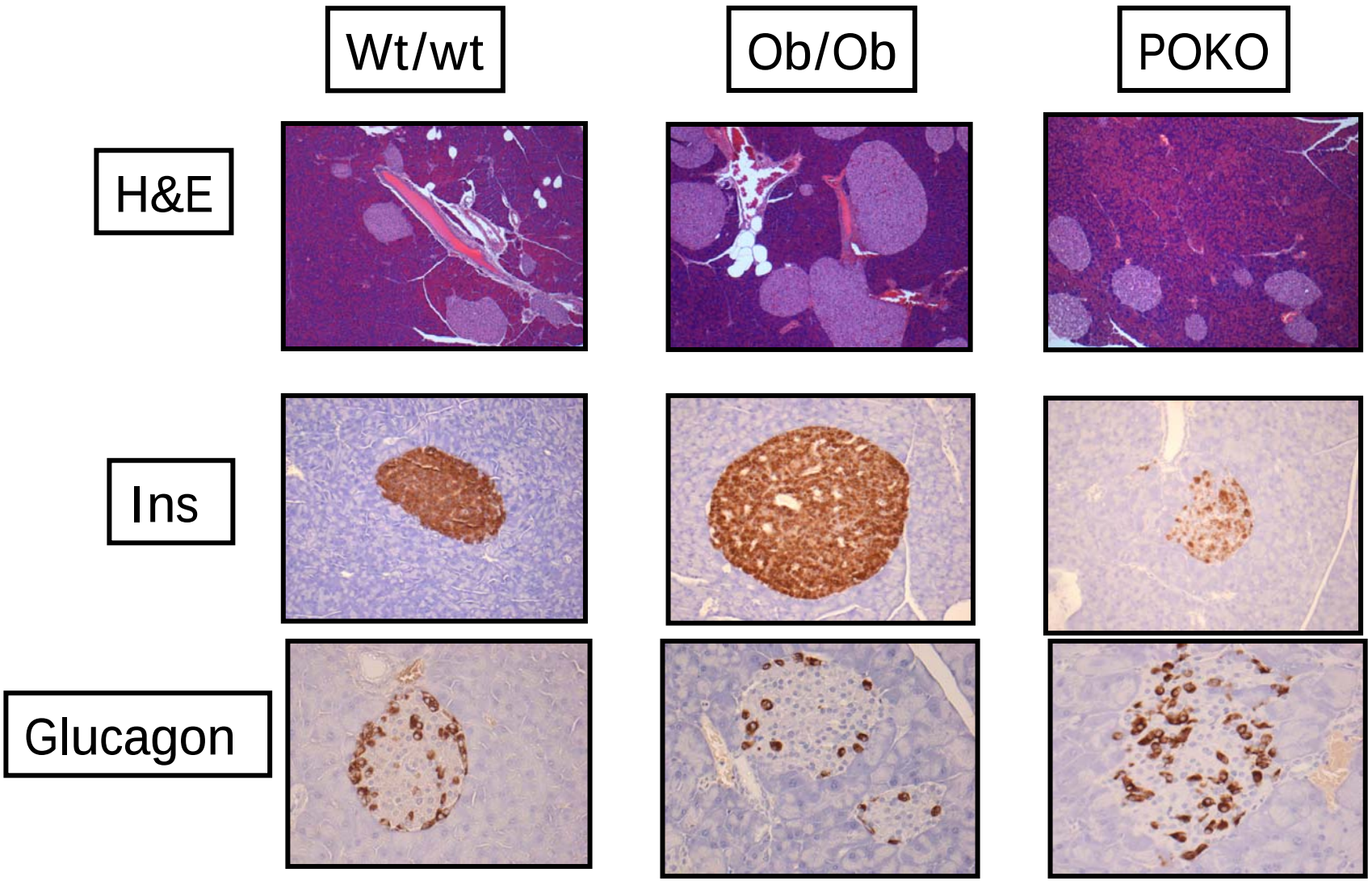
ob/ob islets-4-wk



POKO islets-4-wk

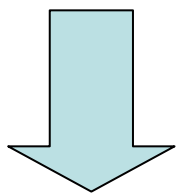


By the age of 16 weeks the POKO Mouse shows beta cell failure



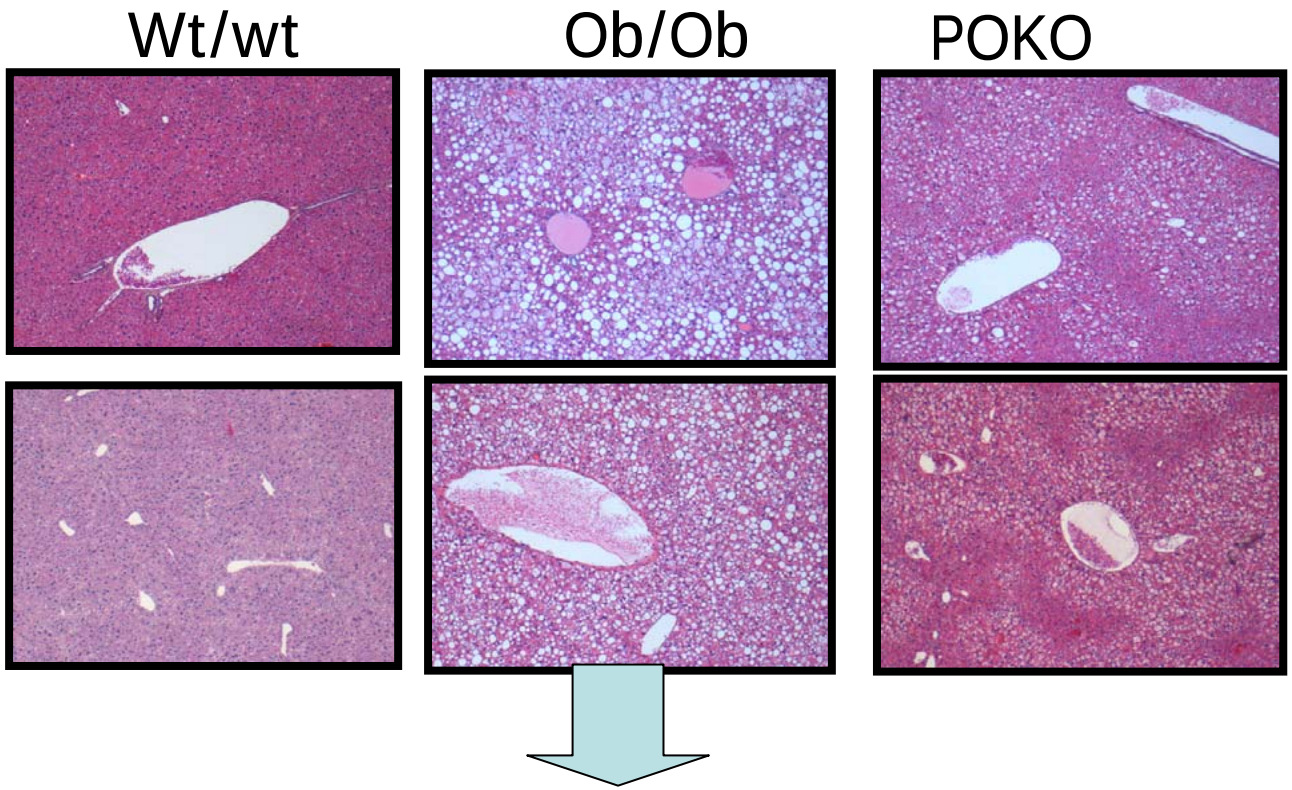
Normal adaptive response of beta cells to insulin resistance did not occur in POKO mouse:

- **Lack of hypertrophy**
- **Pancreatic islets remained similar size to WT and PPARγ2KO**



PPARγ2 may be required for beta cell mass adaptive response to Insulin resistance.

Paradoxically the POKO Mouse accumulates less fat in the liver than ob/ob mice

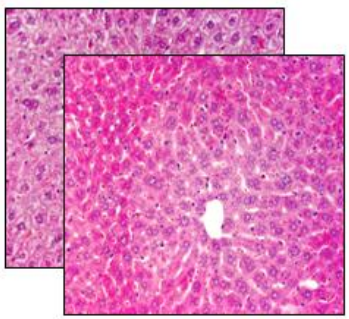


PPARg2 isoform in the liver may contribute of Ob/ob mice may contribute to deposition of triacylglycerols

Hypothesised that lipotoxicity may be the common pathogenic mechanism for the severe metabolic phenotype of the POKO Mice.

Metabolomics platform
Experiment design + Analytical chemistry + Chemometrics + Bioinformatics

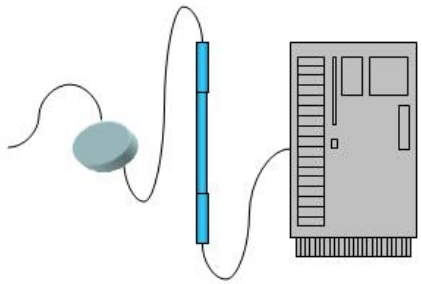
Samples
(cells, tissues, biofluids)

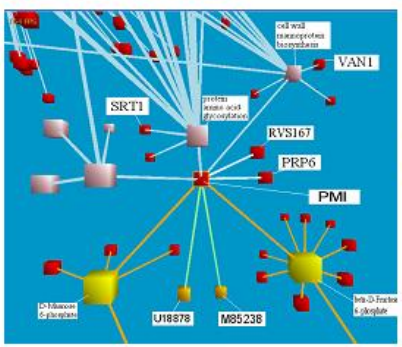


Metabolite extraction methods
and analytical platforms

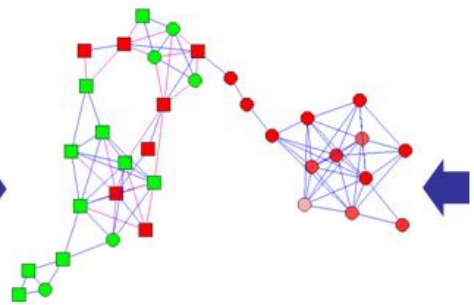
- Global screening (H_2O soluble)
- Global screening (Lipids)
- Targeted lipidomics platforms (eicosanoids, sterols, …)
- Primary metabolites platforms (TCA metabolites, nucleotides, …)

Profiling experiments
(LC/MS, GC/MS, NMR)





Bio-/chemo-informatics
knowledge mining



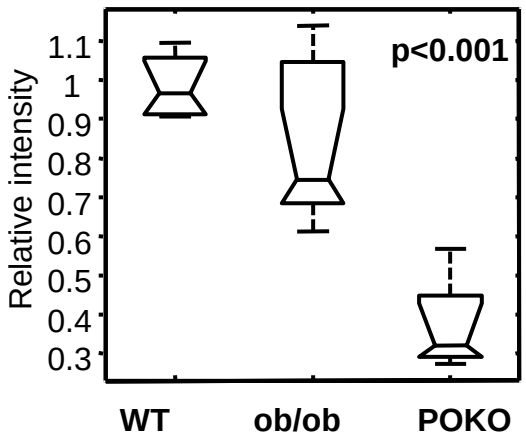
Multivariate statistical analyses
Data-driven integration

Data processing
Identification

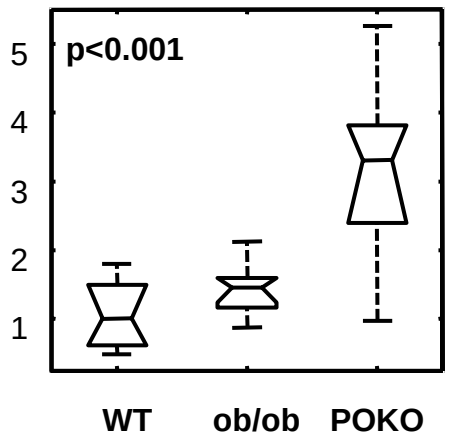
Biological insight

LIPIDOMIC ANALYSIS of WAT REVEALS IMPAIRED TGL DEPOSITION AND INCREASED REACTIVE LIPID SPECIES

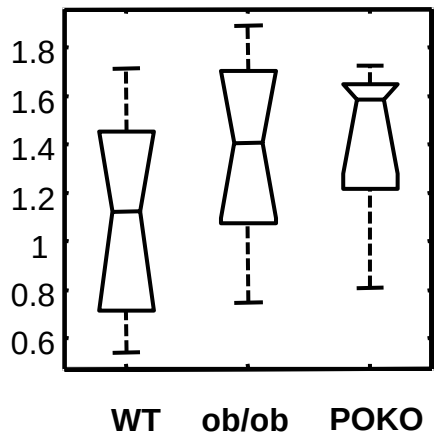
Triacylglycerol (48:2)



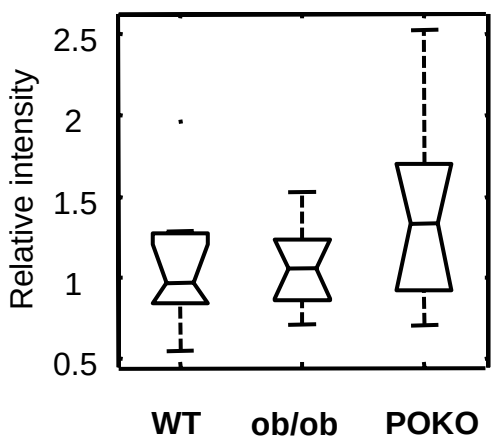
Diacylglycerol (36:4)



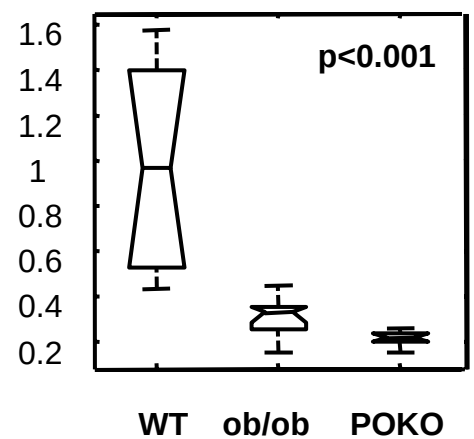
Ceramide (d18:1/16:0)



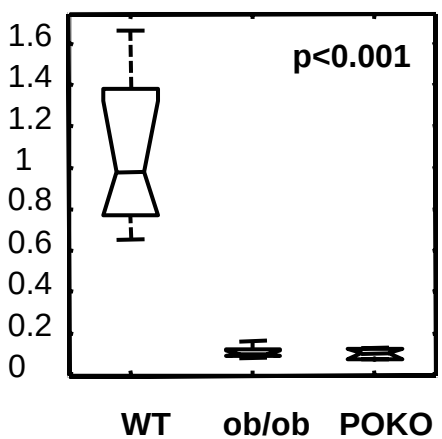
Lysophosphatidylcholine (18:0)



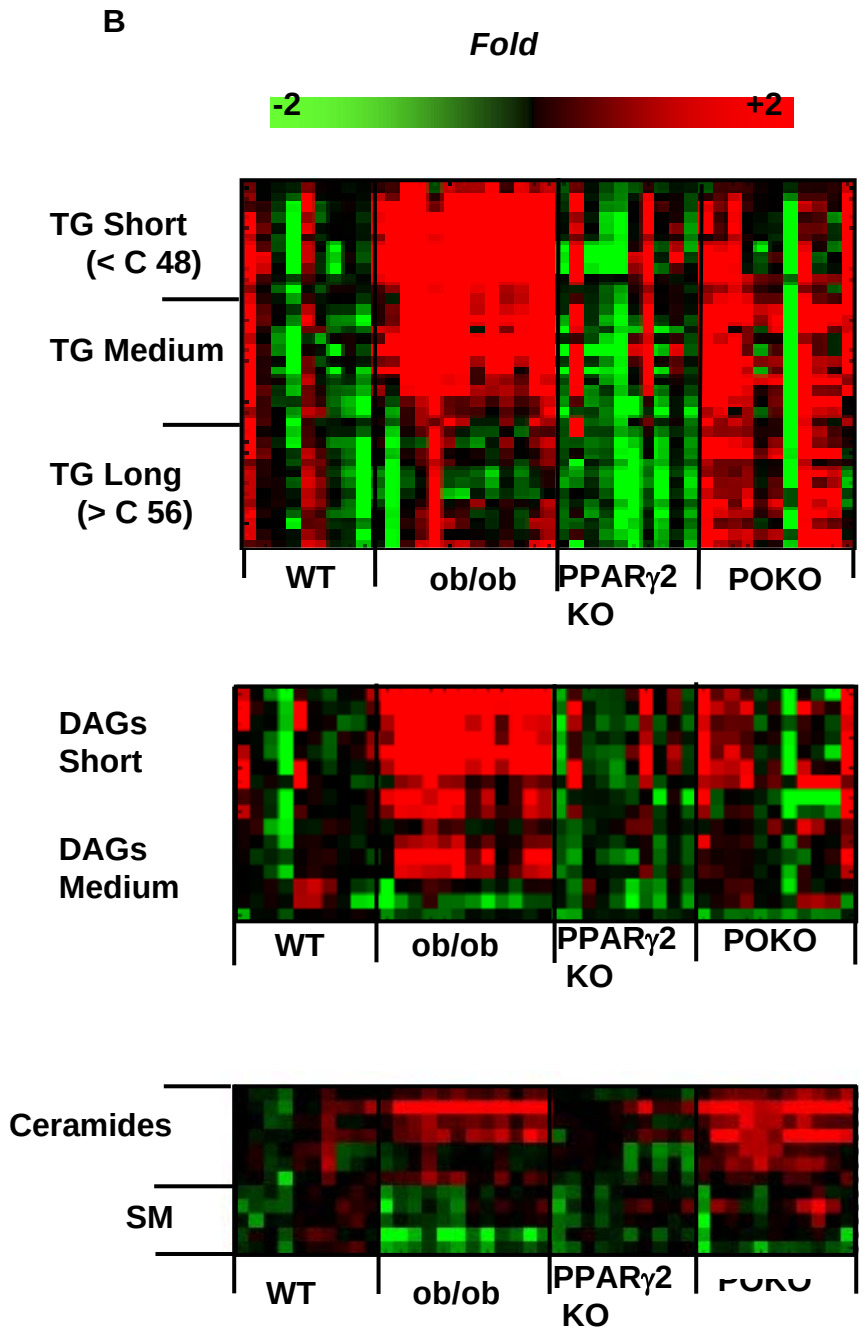
Sphingomyelin (d18:1/16:0)



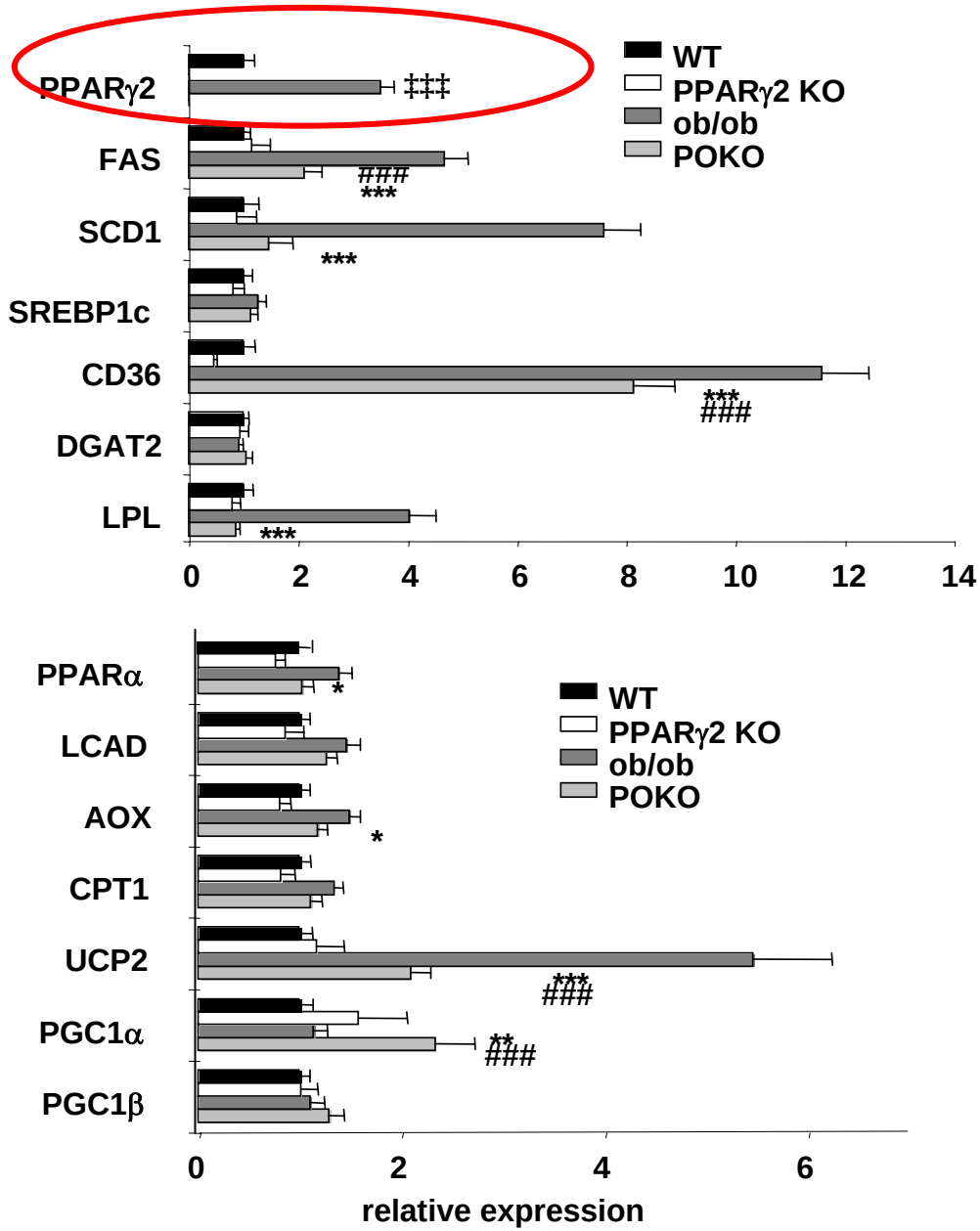
Ethanolamine plasmalogen (36:1)



Lipidomic analysis in Liver reveals POKO mouse accumulate less TGLs and More reactive lipid species in the liver than Ob/ob mouse



Transcriptomic Analysis of liver from 16 week old POKO mouse reveals impaired expression of genes involved in fat deposition compared to ob/ob mouse.

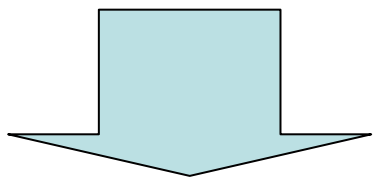


Overall, our **lipidomic studies** identify a remarkable similar pattern of changes in lipid species in adipose tissue liver, skeletal muscle and pancreatic islets characterised by:

A. Decreased Triacylglycerols levels and Plasmalogens

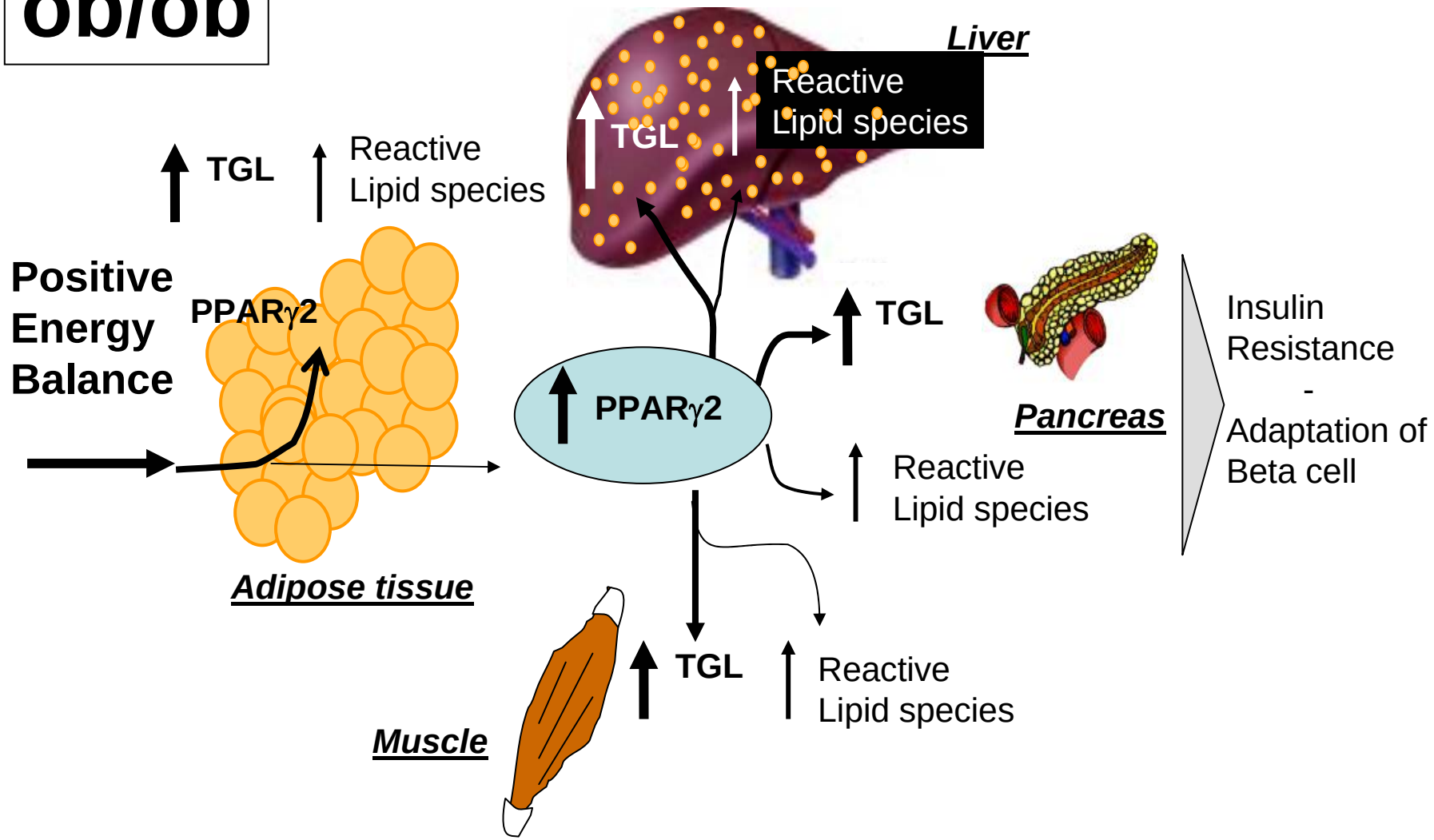
B. Increased reactive lipid species such as ceramides and Lysophosphatidylcholines.

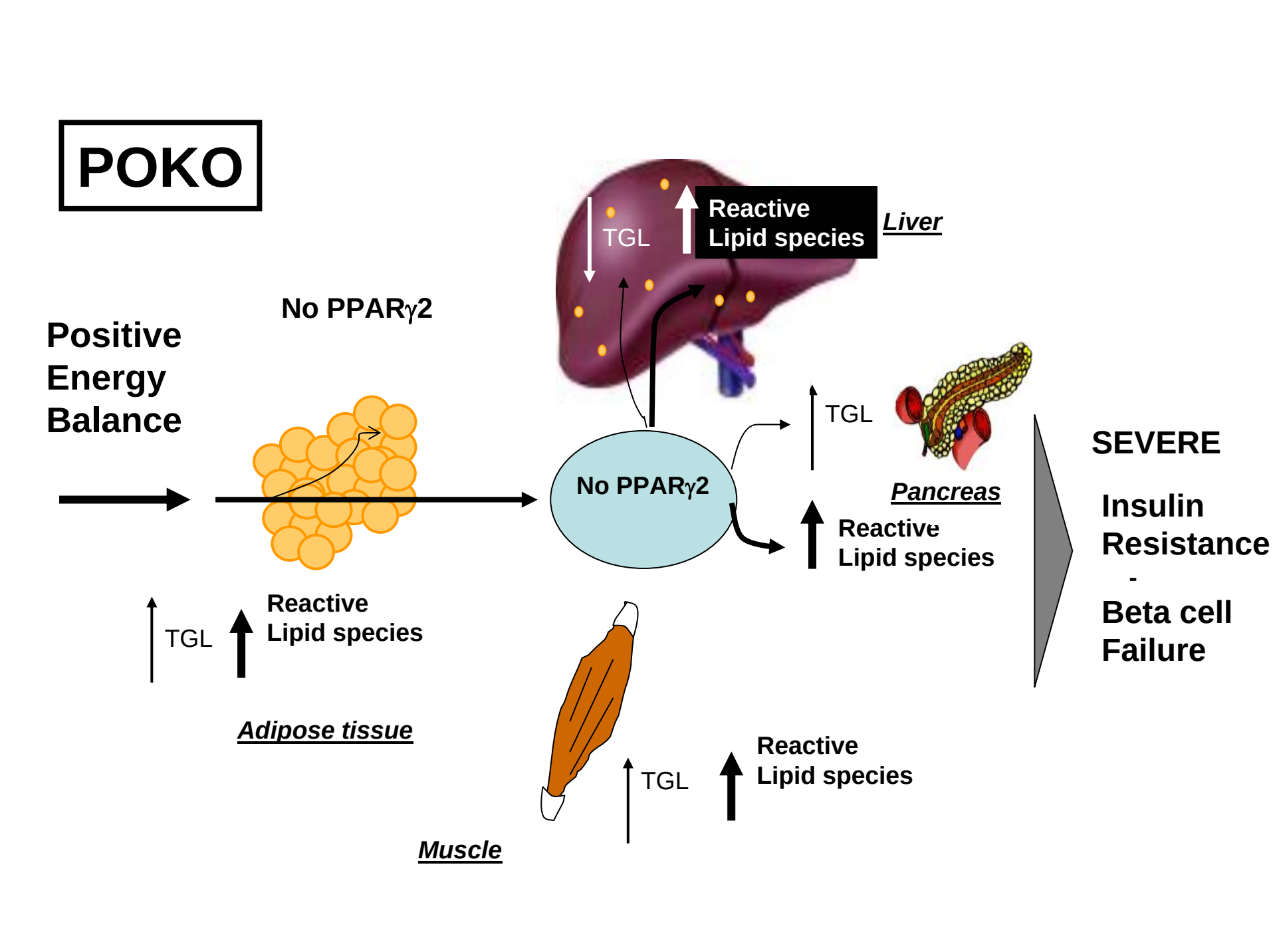
in POKO mouse compared to Ob/Ob mouse.



Under conditions of positive energy balance ectopic expression of PPARγ2 facilitates deposition of fat In the form of harmless TGLs

ob/ob


Liver
TGL
Reactive
Lipid species
TGL
Reactive
Lipid species
Positive
Energy
Balance
PPARγ2
Adipose tissue
PPARγ2
TGL
Reactive
Lipid species
Pancreas
Insulin
Resistance
-
Adaptation of
Beta cell
Muscle
TGL
Reactive
Lipid species


POKO
TGL
Reactive
Lipid species
Liver
No PPARγ2
Positive
Energy
Balance
TGL
Pancreas
No PPARγ2
Reactive
Lipid species
SEVERE
Insulin
Resistance
-
Beta cell
Failure
TGL
Reactive
Lipid species
Adipose tissue
TGL
Reactive
Lipid species
Muscle

PPARγ2 prevents lipotoxicity by

a.Promoting adipose tissue expansion

b. Increasing lipid buffering capacity in peripheral tissues.

c. Facilitating adaptive proliferative Response of beta cells to insulin resistance

Some thoughts

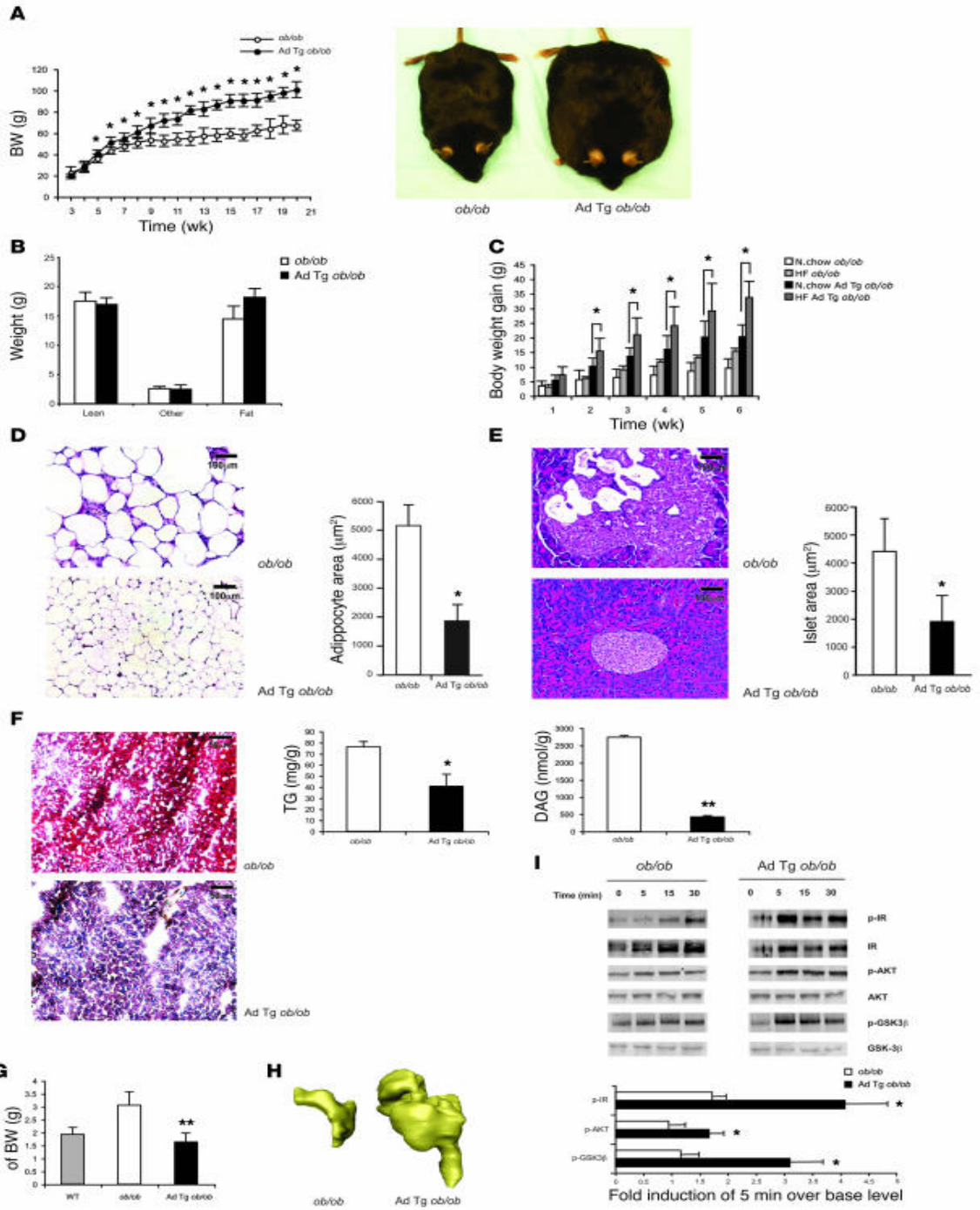
•PPARγ2 isoform is metabolically **important** particularly under conditions of positive energy balance since ablation of PPARγ2 results in massive metabolic failure.

•PPARγ2 exerts a **protective role** when expressed *de novo* in peripheral organs by increasing their capacity to buffer toxic lipids.

•**Adipose tissue expandability** as an important determinant of obesity associated metabolic complications.

•**Mismatch** between energy availability and storage capacity key to understanding obesity associated complications.

Obesity-associated improvements in metabolic profile through expansion of adipose tissue
Ja-Young Kim, and Philipp E. Scherer. J Clin Invest. 2007 September 4; 117(9): 2621–2637.



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