Lipotoxicity; the garbage in and out hypothesis

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

$\text{PPAR}_\gamma^2$ 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

- Obesity
  - Mechanical Problems
  - Aesthetic and Psychological problems.
  - Metabolic problems due to a mismatch between energy availability and storage capacity
    - Lipotoxicity
      - Fatty liver
      - Diabetes
      - Heart Failure
      - Hypertension
      - Dyslipidaemia
    - 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.
- PPAR$\gamma$ 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.
What is an adipocyte?

The adipocyte is the major cell component of adipose tissue in which fats (triglycerides) are stored. Adipocytes contain 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
Some Substances Secreted by Adipose Tissue

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

**Steroid Hormones**
- Oestrone
- Oestradiol
- Testosterone

**Vasoactive factors**
- Monobutyrin
- Angiotensinogen/
- Angiotensin II*
- Atrial natriuretic peptide

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

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

**Binding proteins**
- IGF-BPs
- Retinol BP

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

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

**Extracellular matrix proteins**
- MCP-1

COMPLEX Tissue: Transcriptional regulation of adipogenesis

Preadipocytes

Adipocytes

differentiation

‘Preadipogenic’ genes
- GATA 2/3
- Pref-1
- SP1
- AP2
- Id2/3

Hormonal stimulation or induction cocktail (FBS, insulin, IBMX, Dex)

C/EBPα

C/EBPβ

C/EBPδ

PPARγ2

Ligand (or TZD)

SREBP1c (ADD1)

Adipocyte machinery
- aP2
- PEPCK
- Glut4
- Leptin etc.

Regulatory factors:
- C/EBPβ
- C/EBPδ
- PPARγ2

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

- PPARγ1
- PPARγ2
- PPARγ3

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

RPA PPARγ2 KO

WAT       BAT

<table>
<thead>
<tr>
<th>γ2</th>
<th>γ1</th>
<th>Cyc</th>
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Our PPARγ2 on a 129 background had Normal Body weight, Food intake, Energy expenditure and body Composition.
High fat diet induces adipocyte hypertrophy in PPARγ2KO mouse

**Epididymal WAT**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epididymal WAT</strong></td>
<td>Chow diet (20x)</td>
<td>HFD (20x)</td>
</tr>
<tr>
<td><strong>Subcutaneous WAT</strong></td>
<td>Chow diet (20x)</td>
<td>HFD (20x)</td>
</tr>
</tbody>
</table>

**WAT adipocytes area (µm²)**

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<td>Chow diet</td>
<td>HFD</td>
</tr>
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**Legend**

- WT: Wild Type
- KO: Knockout
- Chow diet: Regular diet
- HFD: High Fat Diet

**Significance**

- **: p < 0.05
- ***: p < 0.01

**Figure Description**

- The images show histological sections of adipose tissue from WT and KO mice fed either a Chow diet or HFD.
- The bars represent the mean adipocyte area ± SEM for each group, with significant differences indicated.
- The scale bar represents 50 µm.
Gene expression analysis in Adipose Tissue reveals expected profile

**Microarray analysis + Pathway analysis**
Mild abnormal GTT in male PPARγ2 ko mice on chow diet

Cont males  KO males  Cont females  KO females
Glucose turnover rates are lower in male PPARγ2 KO mice in chow diet.

Insulin resistant phenotype

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

<table>
<thead>
<tr>
<th></th>
<th>Males Chow diet</th>
<th>Males 12 weeks on HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>KO</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>130±9.0</td>
<td>147±5.9</td>
</tr>
<tr>
<td>Glucose (mg/dl) fasting</td>
<td>63±4.1</td>
<td>88±5.1 **</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.93±0.12</td>
<td>0.87±0.15</td>
</tr>
<tr>
<td>Free Fatty Acids (µmol/L)</td>
<td>295±63</td>
<td>231±17</td>
</tr>
<tr>
<td>Insulin (µg/L)</td>
<td>0.49±0.10</td>
<td>0.34±0.05</td>
</tr>
<tr>
<td>Insulin fasting (µg/L)</td>
<td>0.14±0.03</td>
<td>0.35±0.11</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>2.77±0.44</td>
<td>4.13±0.56*</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>15.2±1.2</td>
<td>7.9±1.2***</td>
</tr>
</tbody>
</table>
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

Crossed with genetically Obese leptin deficient Ob/Ob mouse

POKO Mouse
Growth curves from **PPARγ2KOxob/ob** mice

**Male Weights**

- wt
- ob/ob
- PPARg2 KO
- POKO
- Het PPARg2/ob

**Female Weights**

- wt
- ob/ob
- PPARg2 KO
- POKO
- Het PPARg2/ob

*N=4-11*

* p<0.05 vs ob
& p<0.05 vs wt
Ob/Ob and POKO mice have similar energy balance
Adult studies (16-week old mice)

**Food consumption Females (n=3-7)**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ob/ob</th>
<th>PPARg2 KO</th>
<th>POKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>grams/day</td>
<td></td>
<td></td>
<td></td>
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</table>

**Weights during Food consumption**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ob/ob</th>
<th>PPARg2 KO</th>
<th>POKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>grams</td>
<td></td>
<td></td>
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</table>

**Oxygen consumption VO2** POKO 6wo females

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>PPARγ2</th>
<th>ob/ob</th>
<th>POKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml/kg lean mass/min</td>
<td></td>
<td></td>
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</table>

**Accumulative water intake/72 h in 16 week old animals**

<table>
<thead>
<tr>
<th></th>
<th>wt</th>
<th>Ob/ob</th>
<th>PPARg2KO</th>
<th>POKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td></td>
<td></td>
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<td></td>
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</table>

n.s. = not significant
<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>16 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Ob/ob</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>36.02±1.61</td>
<td>75.76±4.56</td>
</tr>
<tr>
<td>Glucose fed (mmol/L)</td>
<td>10.93±1.45</td>
<td>15.27±2.47</td>
</tr>
<tr>
<td>Glucose fasted (mmol/L)</td>
<td>5.46±0.52</td>
<td>10.74±1.78</td>
</tr>
<tr>
<td>Ins fed (ug/L)</td>
<td>3.38±0.41</td>
<td>39.08±10.72</td>
</tr>
<tr>
<td>Ins fasted (ug/L)</td>
<td>0.21±0.04</td>
<td>4.04±0.80</td>
</tr>
<tr>
<td>FFA (umol/L)</td>
<td>718.5±103.75</td>
<td>883.57±68.27</td>
</tr>
<tr>
<td>Chol (umol/L)</td>
<td>3.3±0.05</td>
<td>6.4±0.55</td>
</tr>
<tr>
<td>Trig (umol/L)</td>
<td>2.05±0.07</td>
<td>3.17±0.97</td>
</tr>
<tr>
<td>Leptin (ng/L)</td>
<td>17.30±2.33</td>
<td></td>
</tr>
<tr>
<td>Adiponectin (ug/mL)</td>
<td>22.69±0.82</td>
<td>11.84±1.22</td>
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</table>
POKO Mouse develops early hyperglycemia compared to ob/ob

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>Week 3</td>
</tr>
<tr>
<td></td>
<td>Weight (g)</td>
</tr>
<tr>
<td>WT</td>
<td>8.6±0.2</td>
</tr>
<tr>
<td>ob/ob</td>
<td>10.9±0.9</td>
</tr>
<tr>
<td>PPARγ2 KO</td>
<td>8.0±0.8</td>
</tr>
<tr>
<td>POKO</td>
<td>8.9±0.7</td>
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POKO Mice develop earlier insulin resistance compared to the ob/ob mice.
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 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
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
Reactive Lipid species

Liver

Muscle

Adipose tissue

PPARγ2

TGL

Positive Energy Balance

Pancreas

Insulin Resistance - Adaptation of Beta cell
POKO

Positive Energy Balance

No PPAR\(\gamma\)2

Reactive Lipid species

Liver

Pancreas

Adipose tissue

Muscle

SEVERE

Insulin Resistance - Beta cell Failure

TGL

Reactive Lipid species
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
Gema Medina   Sergio Rodguez   Claire Lagathu   Marc Slawick   Adrienn Kis

Sam Virtue   Rachel Hagen   Andy Whittle

Mark Campbell   Martin   Agnes Lukasic   Margaret Blount   Janice Carter

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