

UNIVERSITY OF CAMBRIDGE

**Following the role of PPARs in controlling metabolism by metabolomics**

Jules Griffin  
[jlq40@mole.bio.cam.ac.uk](mailto:jlq40@mole.bio.cam.ac.uk);  
 Department of Biochemistry,  
 University of Cambridge

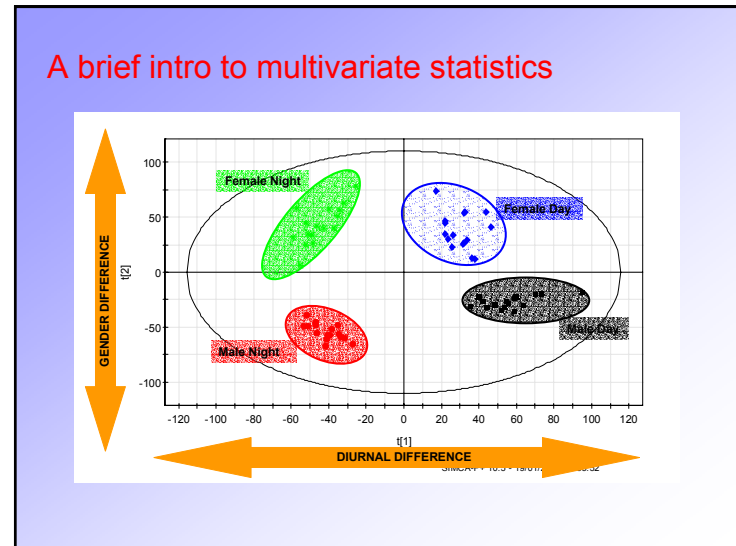
HDR, Helsinki  
 June 2008

## Overview

- What is metabolomics and why might it be useful?
- Metabolomics and animal models of diabetes
  - PPAR- $\alpha$  – a regulator between the fed and fasted state
  - The ob/ob mouse and PPAR- $\delta$  agonists
- Metabolomics and safety assessment of pesticides
  - Non-genotox carcinogens
- Future directions

## The basis of metabolomics

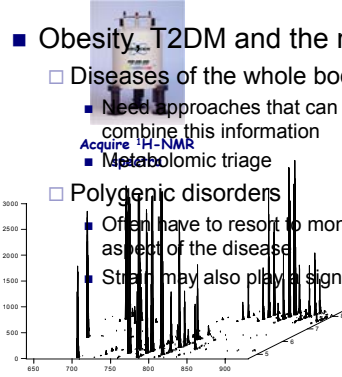
- Metabolomics/metabonomics
  - the quantitative measurement of metabolic responses to pathophysiological stimuli or genetic modification
- Measure small molecule concentrations through a global approach
- Use pattern recognition to define metabolism in a multidimensional space
  - metabolic phenotype
  - metabolite
- Use this information to determine an end point (e.g. drug toxicity, disease state) or to data mine another – omic technology.



## Metabolomics and the metabolic syndrome

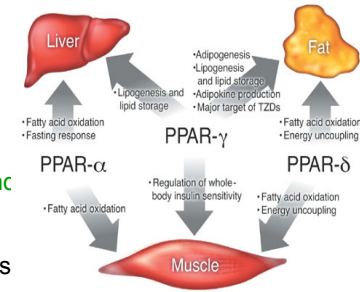
### Obesity, T2DM and the metabolic syndrome are:

- Diseases of the whole body
- 1H, 13C, HRMAS
- Need approaches that can analyse a large range of tissues and combine this information
- GC-MS
- Aq. Metabolites, total FAs, FFAs, SPE lipidomics
- Polygenic disorders
- Often have to resort to monogenic models that only model an aspect of the disease
- Shotgun lipidomics
- Start may also play a significant role
- Targetted chromatography analysis



## The PPAR receptors:

- **PPAR $\alpha$**  is expressed in metabolically active tissues including: liver, kidney, heart, skeletal muscle, and brown fat.
- **PPAR $\gamma$**  is found in adipose tissue, colon and macrophages
- **PPAR $\delta$**  is expressed ubiquitously in numerous tissues, with highest levels found in brain, adipose tissue, and skin.



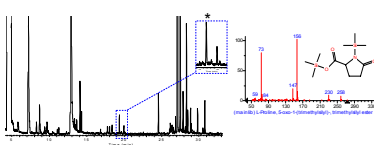
### Soluble PPAR $\alpha$ of liver tissue viable

- Only display a phenotype under stressed conditions
- Overnight fast

### HRMAS NMR of liver tissue

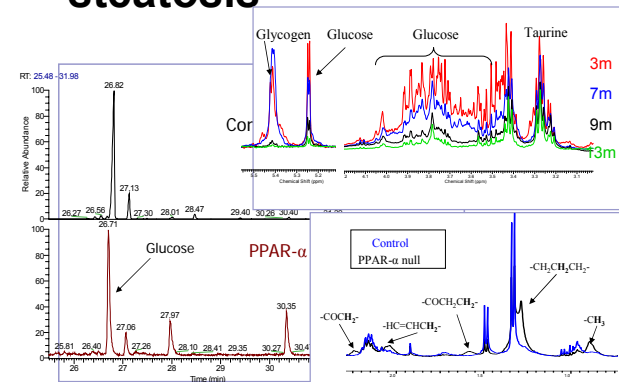
- Have been shown to have decreased gluconeogenesis capability
- Showing stable isotope work

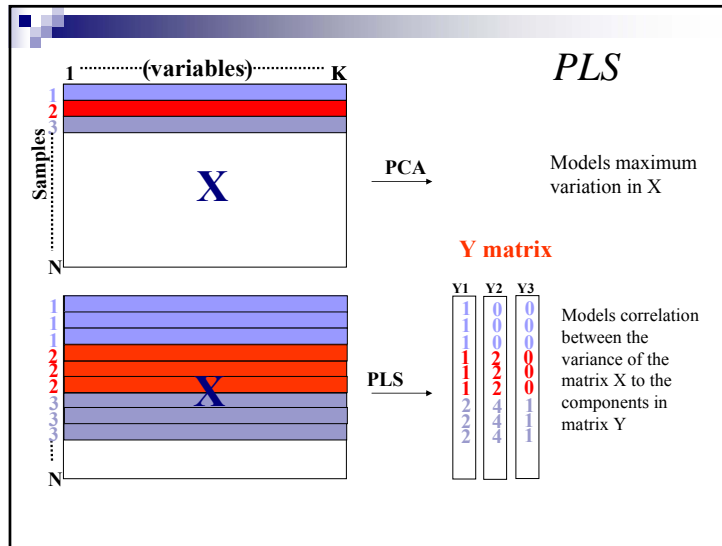
### GC-MS of liver tissue



- Mice were selected at 6 age points between 1-13 months (n=5)
- Examined blood plasma, liver, heart, skeletal (soleus & gastroc), adipose
- <sup>1</sup>H NMR – 20-30 metabolites in tissues (aq. extract)
- HRMAS – unique insight into metabolism in intact tissues
- GC-MS – 100-150 metabolites in tissues (aq) and ~30 fatty acids from lipid fraction

## Glycolysis and hepatic steatosis





### The interaction between age and disease

- Ageing trends can readily be identified by PCA and PLS
  - Does disease interact with ageing?
- PLS models can be used to predict age
  - PPAR- $\alpha$  mice age at a faster rate?
  - Driven by glucose and glycogen decreases

### Analysis of lipid content & age

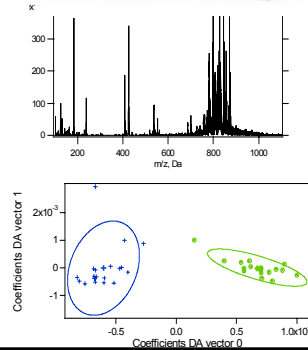
- Changes include increased concentrations of linoleic acid and decreased concentrations of arachidonic acid

### Fatty Acid Metabolism

- PPAR- $\alpha$  important in controlling expression of Stearoyl-CoA Desaturase (SCD)
- Key enzyme in lipogenesis
  - Saturated fats  $\rightarrow$  Monounsaturated Fats
- Metabolism even altered in adipose tissue

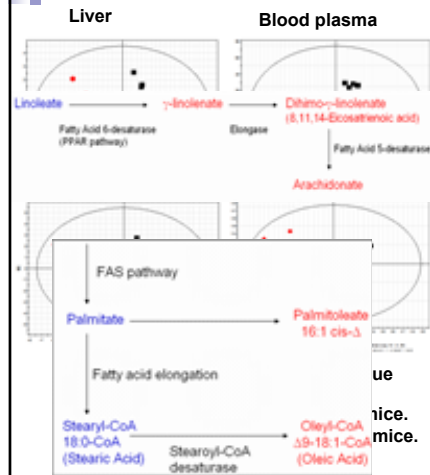
## LC-MS

- Advantage: able to see larger molecules: lipids and proteins
- Direct infusion of chloroform/methanol cardiac lipid extracts
- Differentiation of control mice and PPAR- $\alpha$  knock outs through phospholipid composition at a range of ages (3 and 13m)
- PC changes show further evidence of differential expression of elongases and desaturases



## PPAR- $\delta$ - a neglected target

- PPAR- $\delta$  has been much less researched compared with the other PPARs
  - In part reflecting the only recent production of good selective ligands
- But agonists target a number of problems associated with T2DM and obesity
  - E.g. Increases  $\beta$ -oxidation in skeletal muscle and adipose tissue
- Examining the effect on systemic metabolism in the ob/ob mouse
  - Liver, adipose tissue, skeletal muscle and blood serum



- GC-MS of fatty acids distinguishes drug exposed from 'normal' ob/ob mice
- Many of the metabolic changes are the reverse seen in the PPAR- $\alpha$  ko mouse
- Switch to oxidative metabolism in muscle?
  - Increased amino acid metabolism

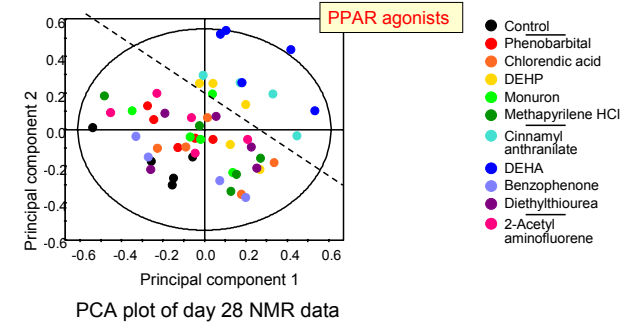
## Non-genotoxic carcinogens

Metabolomics and transcriptomics

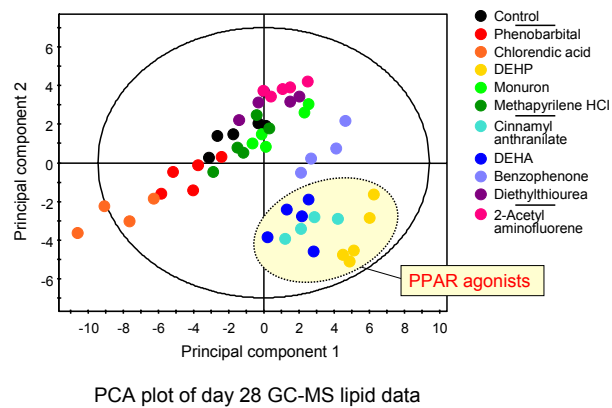
## Non-genotoxic carcinogens

- Aim to identify early stage biomarkers of non-genotoxic carcinogenesis by metabolomics, transcriptomics & histopathology
  - Difficult to screen for
  - only detected in long term *in vivo* assays.
  - Mechanisms poorly understood
- 10 hepatotoxic cmpds
  - 90 day study
  - Single dose of each compound. Dose is NTP carcinogenic dose.

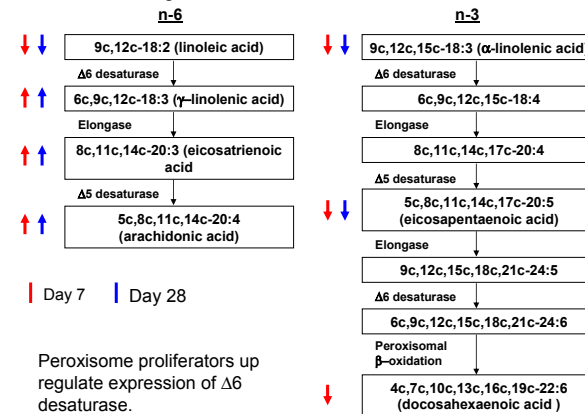
## PPAR agonists and non-genotoxicity



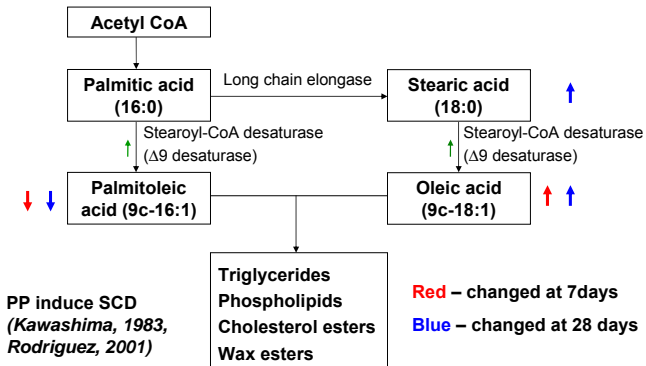
## 10 compound study



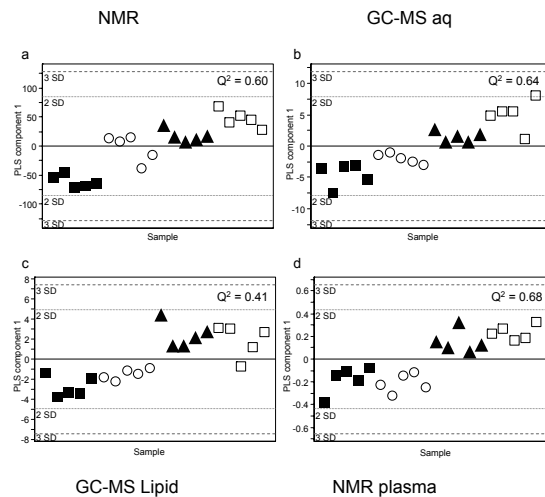
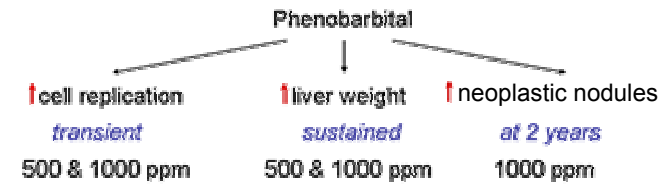
## HUFA synthesis



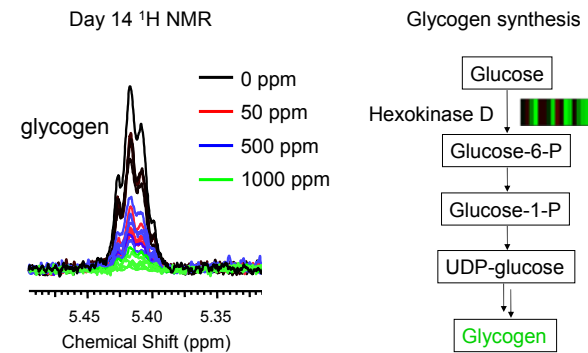
## Monounsaturated Fatty Acid Synthesis

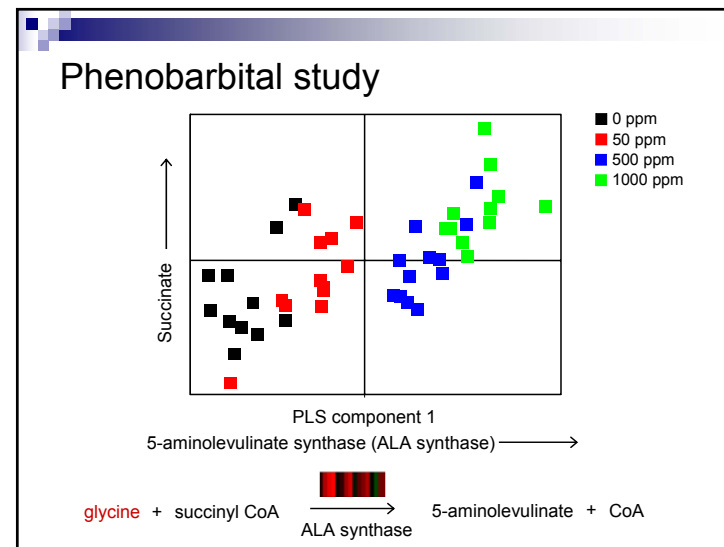
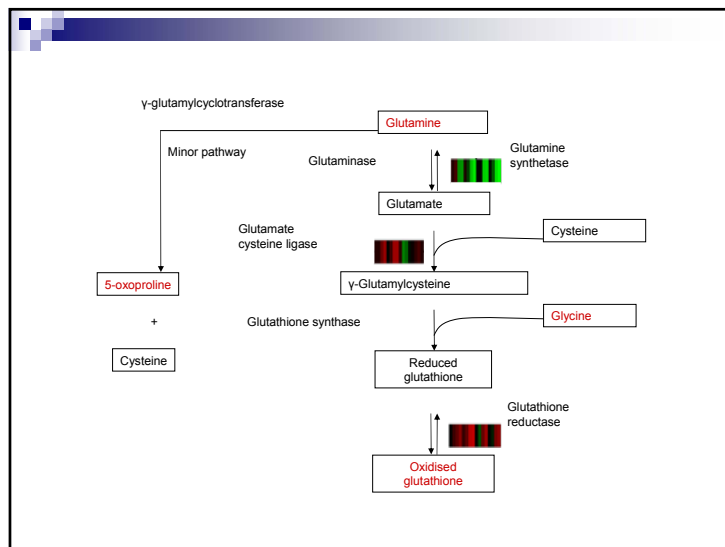


- Examined
  - Blood plasma (NMR; LC-MS)
  - Urine (NMR, LC-MS)
  - Liver (NMR, GC-MS, Affymetrix, histology)
- 0, 50, 500 & 1000 ppm continuous dietary dosing.
- 5 animals per group (9 week old male F344 rats).
- Animals killed after 1, 3, 7, and 14 days.



## Used PLS to examine correlates between metabolomics and transcriptomics





## Conclusions

- Metabolomics distinguishes a range of cmpds and provides a dose response behaviour for PB
- The metabolic profiles can readily be correlated with transcriptions
- For PB we identify some classic changes:
  - Decrease in glycogen
  - Increases in glutathione
  - Synthesis of P450s

## Future Directions

- For diabetes and obesity we now/will have good coverage of the metabolome in tissues and biofluids
  - Can profile a range of mouse models
  - Metabolomic databases
- We can use metabolomics as molecular triage for transcriptomics and proteomics

