Understanding the impact of metabolic syndrome on the heart using metabolomics

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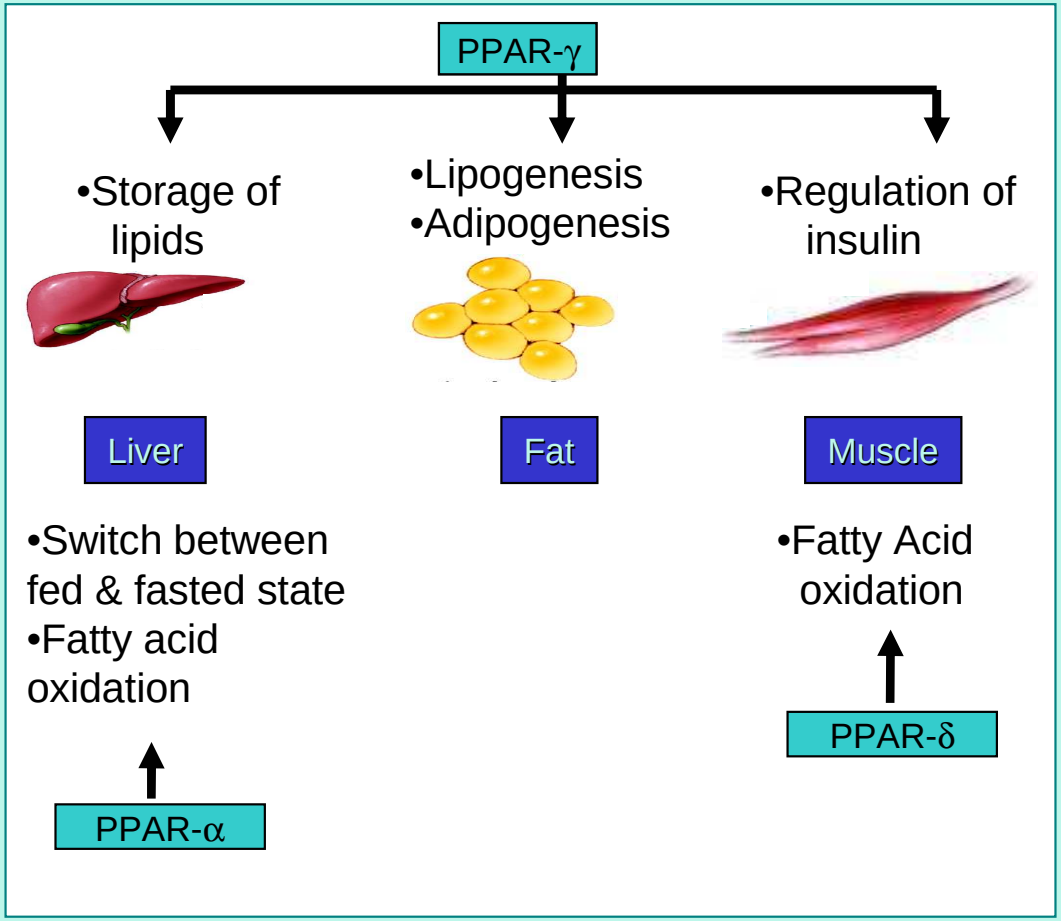
Obesity

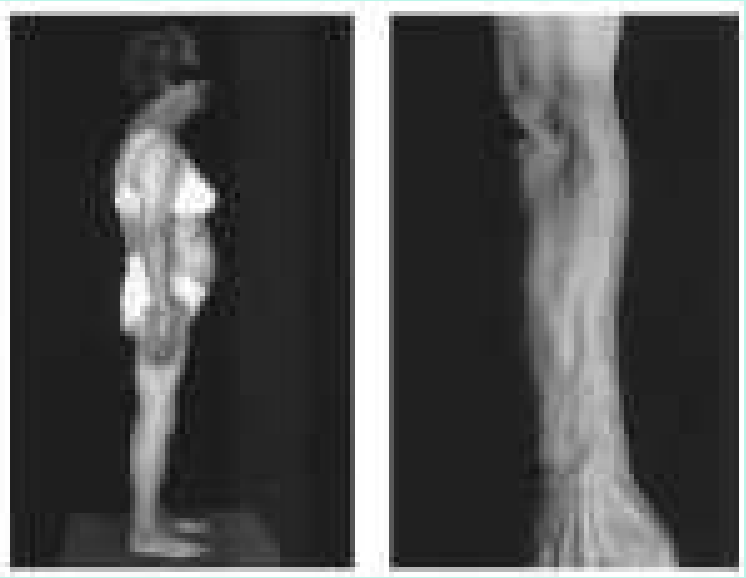
- Obesity is a growing problem in the Western world- UK: 17% males and 21% females obese
- Obesity is often associated with hypertension, dyslipidaemia, hyperuricaemia and type 2 diabetes mellitus
  - All are known risk factors for heart disease
- Thiazolidinediones (TZDs; anti-diabetic agents) and fibrates (dyslipidaemia treatment) are effective treatments
  - Found to be PPAR agonists
- Exact mechanism of action of these drugs remains elusive

PPARs



- Group of transcription factors belonging to the nuclear receptor superfamily discovered in early 1990's
- Numerous fatty acids & derivatives (e.g. eicosanoids and prostaglandins) are natural ligands
- Crucial in genetic regulation of complex pathways of mammalian metabolism inc. fatty acid oxidation and lipogenesis
- To date three isoforms have been identified; **PPAR-α**, **PPAR-δ** (/-β) and **PPAR-γ**, each with different tissue distribution

Metabolic Syndrome



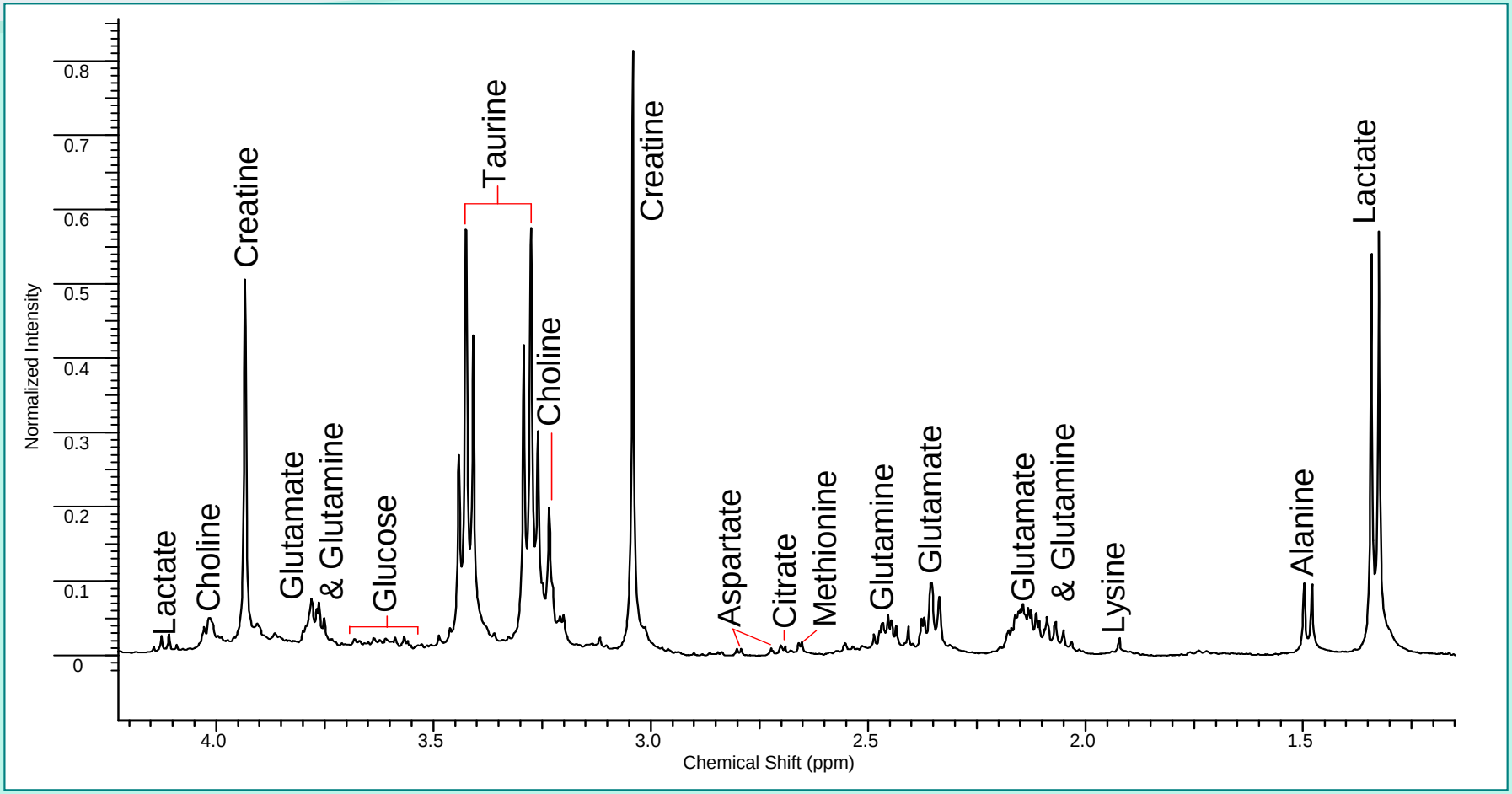
Taken from
Savage et al., Diabetes. 2003 Apr;52(4):910-7.

- Dominant negative PPAR-γ mutations in humans (heterozygous proline-467-leucine and valine-290-methionine)
- Causes resistance to insulin regulation of muscle glucose uptake and lipolysis in adipose tissue
- 'Metabolic Syndrome'
- Subjects examined by MRI reveal consistent paucity of limb & gluteal fat
- Remaining adipose tissue has an impaired ability to increase lipolysis during fasting, & is unable to suppress the mobilisation of fatty acids

Models of disease

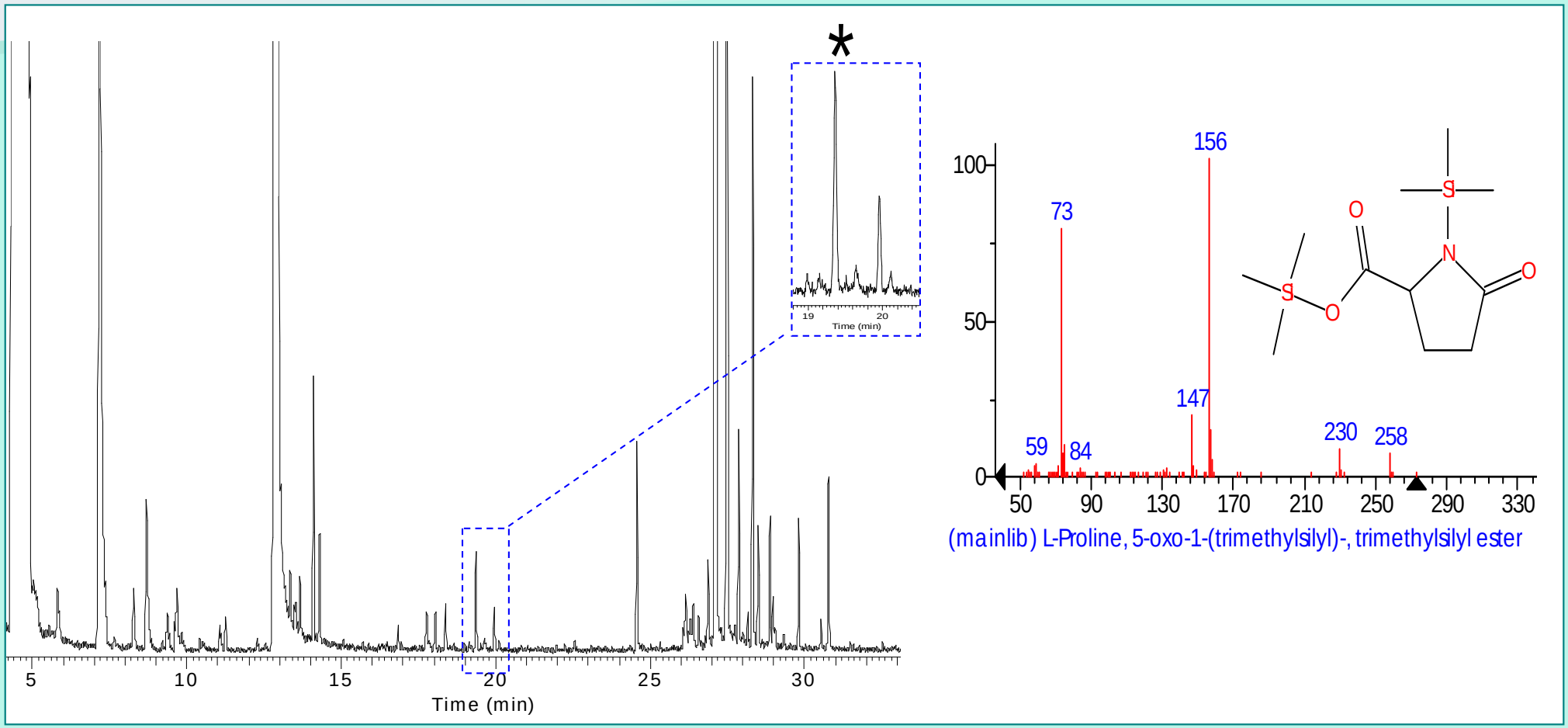
- PPAR-α null mouse
  - Lower expression of several mitochondrial fatty acid metabolising enzymes
- Analysing 6 tissues: adipose, diaphragm, gastrocnemius and soleus skeletal muscle, liver and heart
- Analysing time course of disease progression: 1-13 months
- Using both ^{1}H-NMR spectroscopy and GC-MS
- Looking for time points important in disease progression
  - Application of other "-omic" technologies

Metabolic fingerprinting



- **Cardiac Tissue, 400MHz, 128 scans (~8 minute analysis time)**
- **Quantified and identified approximately 15 metabolites**

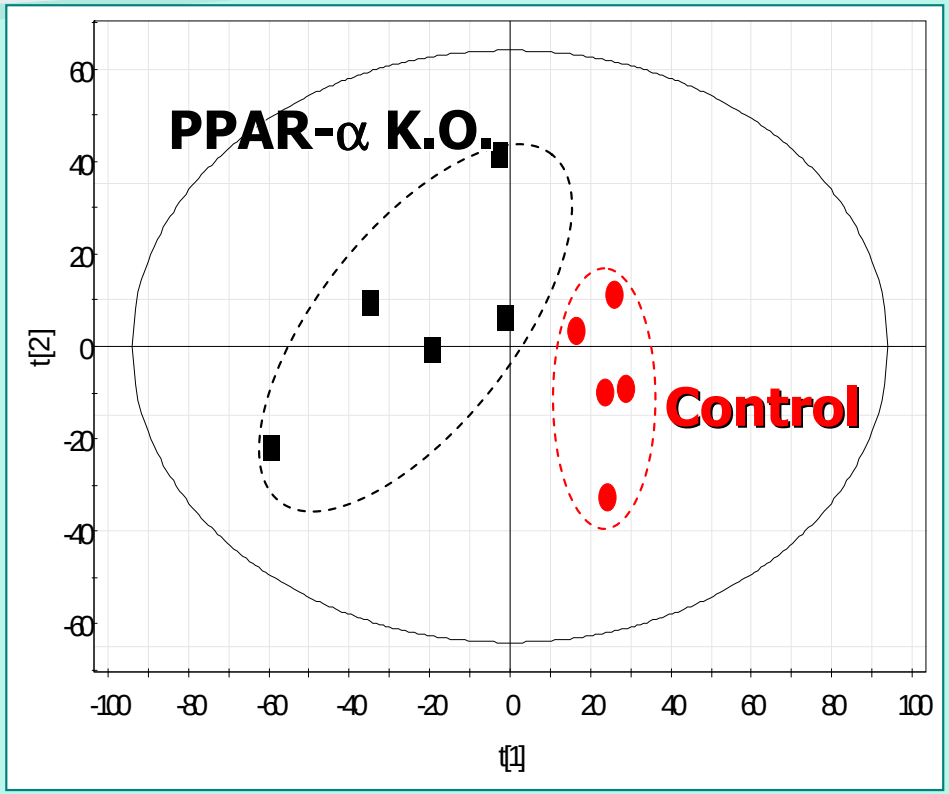
Metabolic Profiling



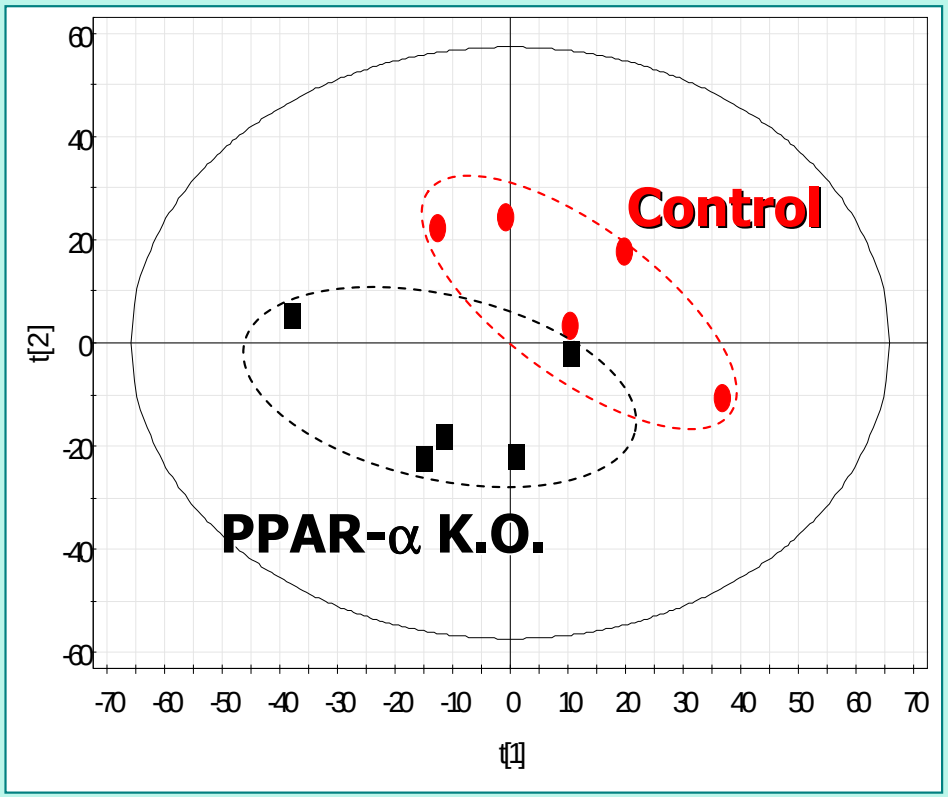
- **Cardiac Tissue, 30m x 25 mm x 25 mm column, 70 minute analysis**
- **Quantified ~120 metabolites, structure assigned to 75 of these**
- **FAME analysis of lipids may quantify a further 20 metabolites**

Analysis of Cardiac Tissue

PCA- 1m

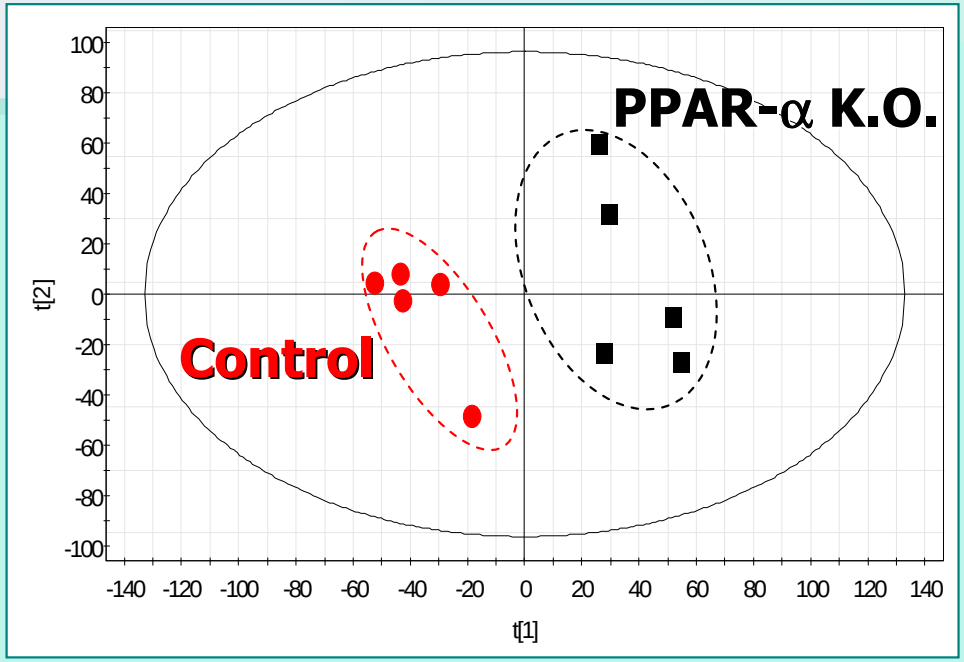


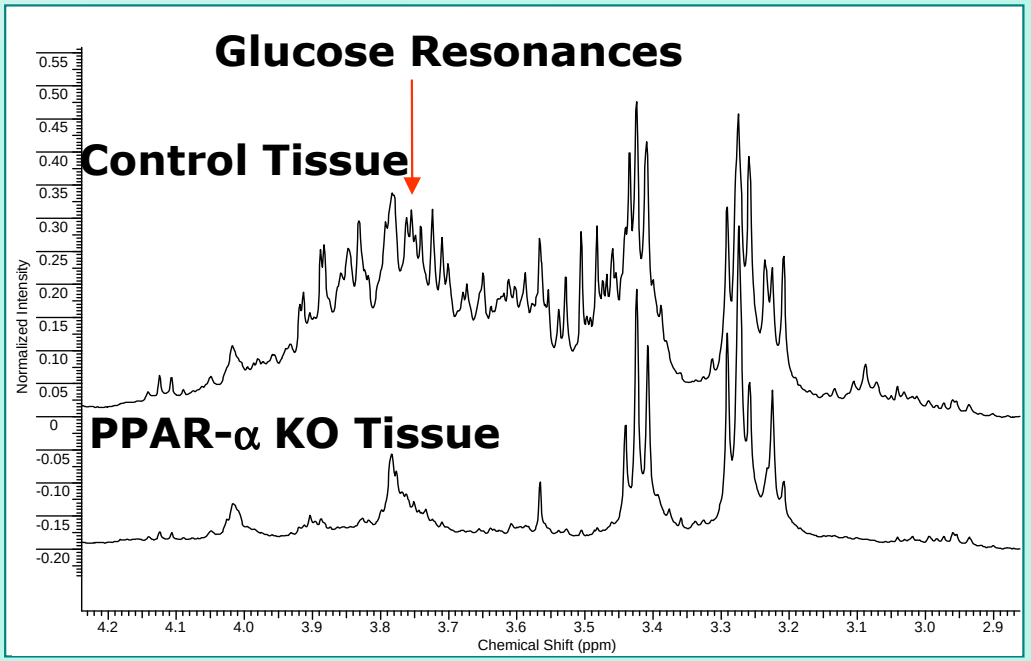
PCA- 13m



- 1m- PPAR-α KO shows increased lactate and glycerol, and decreased creatine and choline
- 13m- PPAR-α KO shows increased taurine and glutamate, and decreased creatine, and alanine

Analysis of Liver Tissue

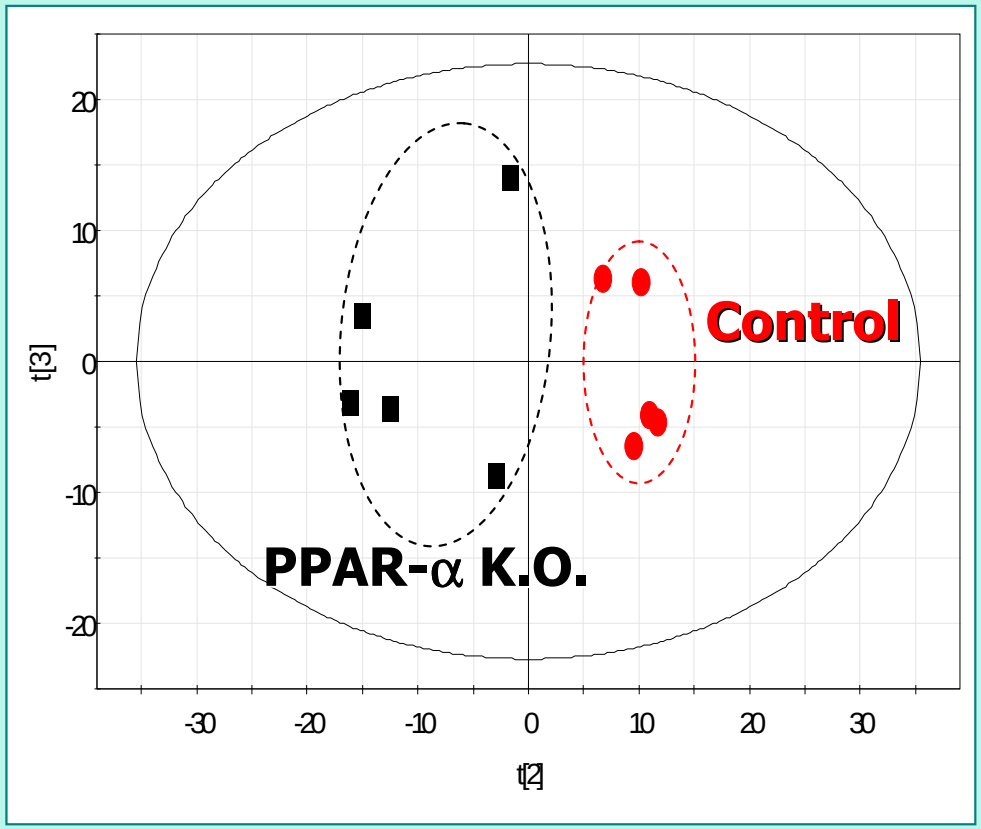




- Largest differences seen in the liver
  - As expected knowing the tissue distribution of PPAR-α
- Major perturbation in sugar concentrations, especially at later time points
  - 7, 9, and 13 months
- Possibly an indication of hepatic steatosis?

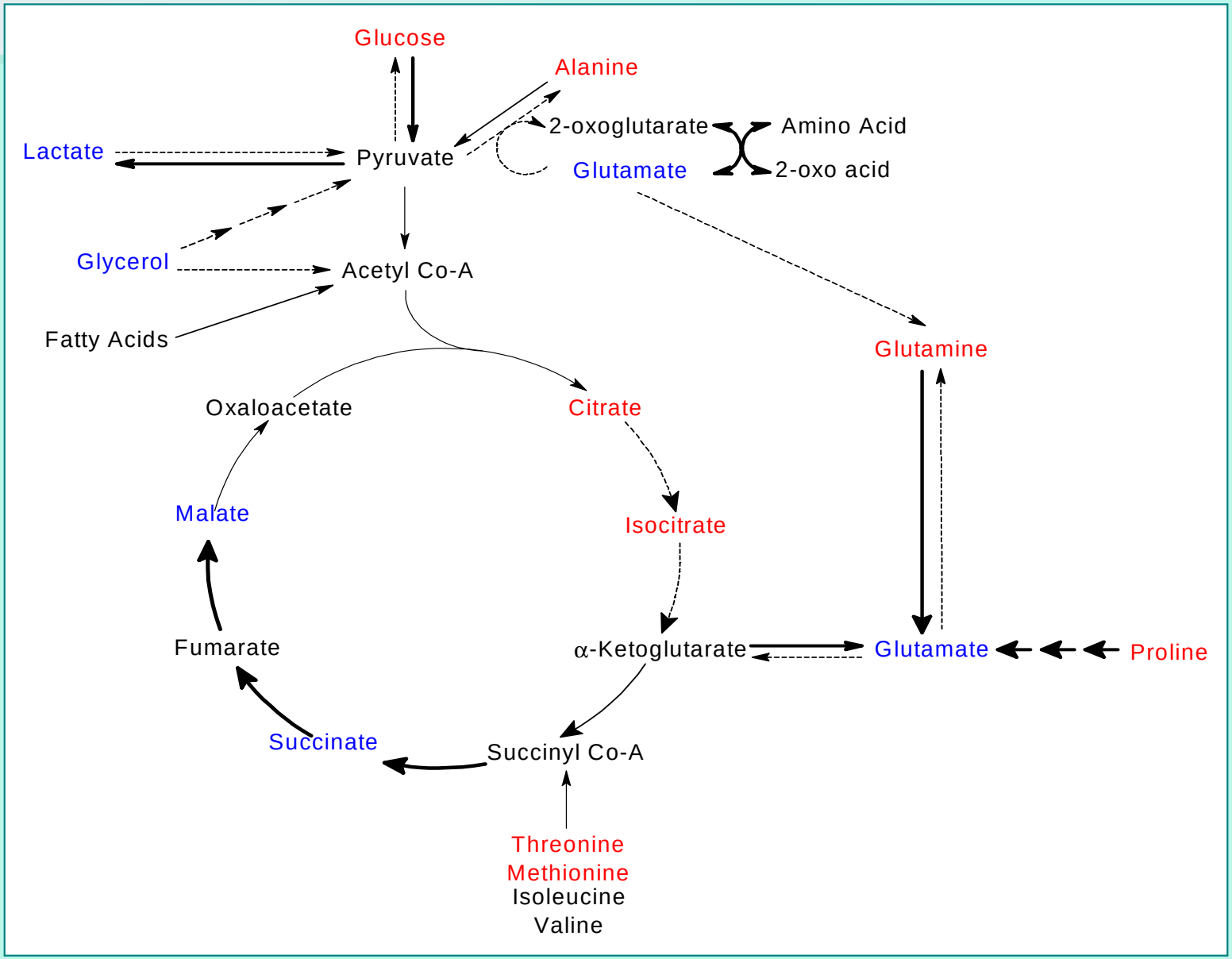
Analysis of Adipose Tissue

PCA- 1m (MAS)



- PPAR-α K.O.: increased alanine, glutamate, proline, saturated lipids, decreased choline, glucose, creatine.
- Differences seen here at 1m
  - Low PPAR-α expression
  - Before any apparent overt pathological changes
- Indicative of tight metabolic control between tissues
  - Hormones &/or substrate availability
- Metabolic crosstalk between pathways controlled by PPAR-α & -γ

Metabolic Perturbations



Alternative Models

- PPAR-γ_2 K.O. mouse
  - PPAR-$\gamma^{/-}$ embryonically lethal
  - Insulin sensitivity decreased
  - Reduction in amount of adipose tissue
- P465L mouse
  - Dominant negative mutation in the *pparg* gene (encodes PPAR-γ)
  - Symptoms less severe than in KO
- NHR-49 KO in *C. elegans*
  - Influences the expression of 13 genes involved in lipid metabolism
  - Key regulator of fat usage
  - Biological activities similar to mammalian PPARs
  - ^{13}C label tracer studies

Conclusions

- A combination of NMR and GC-MS allows the characterisation of ~100-120 metabolites
  - Now can quantify 140-150 with additional analysis of FAs
- Use of metabolomics via the combination of ^{1}H-NMR spectroscopy, GC-MS and PR techniques can be used to differentiate PPAR-α KO mice from controls at ages between 1 and 13 months
- Differences consistent with profound perturbations in glycolysis, gluconeogenesis and amino acid metabolism
- Can detect changes in tissues with very faint PPAR-α expression indicating a degree of metabolic crosstalk between pathways controlled by the PPARs

Future Work

- Analyse more closely disease progression in the PPAR-α null mouse to identify important metabolic perturbations and time points
  - GC-MS
  - Univariate Statistics
  - Transcriptomics/ Proteomics
  - Repeat with a 24h fast
- Metabolically profile the PPAR-γ mouse models
  - Look for similarities/differences to changes seen in the PPAR-α null mouse
  - Look at the effect of a high fat diet
- Metabolically profile the NHR-49 K.O. C.elegans
  - Conduct ^{13}C label tracer studies to analyse which pathways are perturbed

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