Syngene

Putting Science to Work

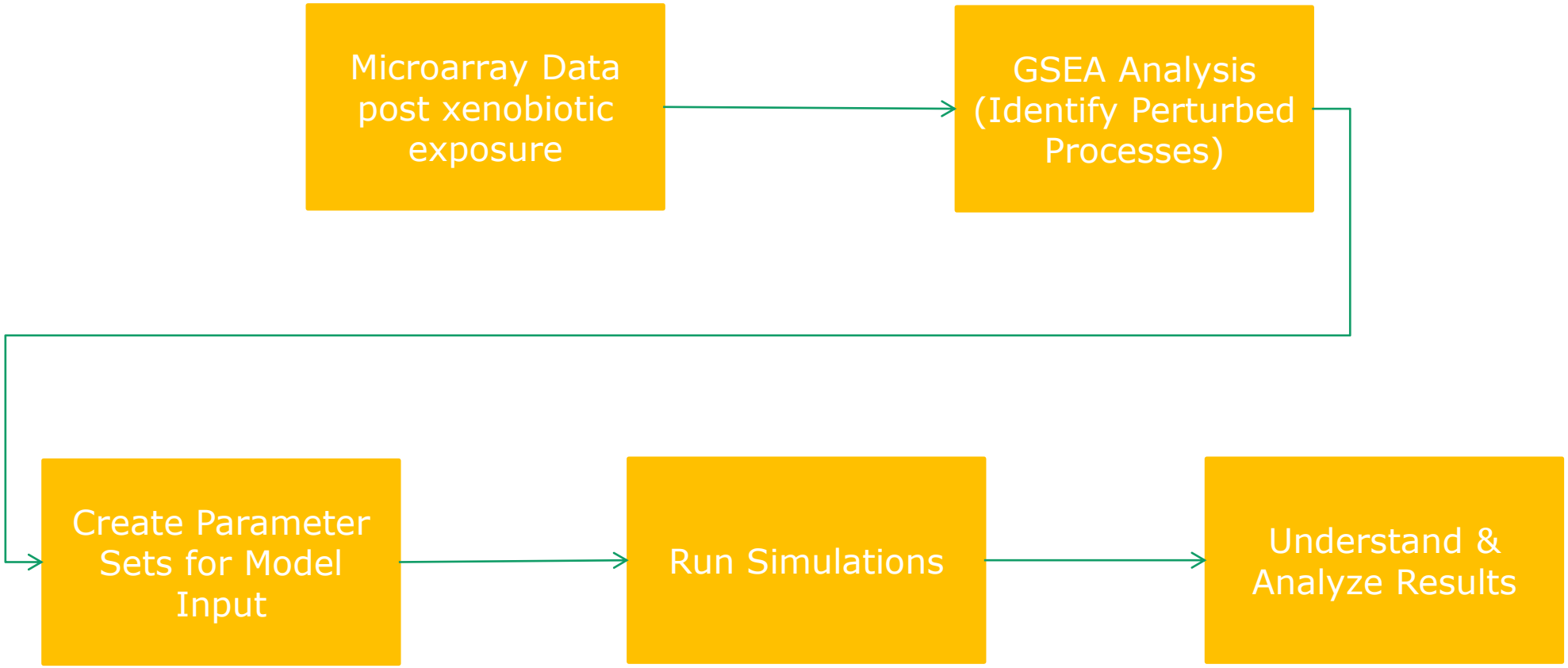
Integrating Multiple Data using Syngene's Virtual Liver

Objective

- Use microarray results as input to the virtual liver model for prediction of toxicity

- Combine multiple experimental information for toxicity prediction

Flow Chart of Data Handling



Gene sets used for GSEA analysis

Insulin signalling

Glycolysis

Glycogen synthesis

Glycogen depletion

Gluconeogenesis

TCA cycle

Oxidative phosphorylation

ROS pathway

ROS pathway (Broad)

Lipogenesis

Fatty acid transport and activation

Triglyceride synthesis

Beta-oxidation

Ketone body synthesis

Phopsholipid synthesis

VLDL assembly and secretion

Cholesterol synthesis

Bile acid synthesis

Canalicular bile tranport

Sinusoidal bile transport

Cytoskeleton network

Apoptosis

Inflammation (Kegg)

Aspartate-glutamate network (Kegg)

Cysteine-methionine network (Kegg)

Unfolded protein response (Reactome)

Ubiquitin mediated proteolysis

GSH depletion by conjugation

GSH depletion by enzyme inhibition

Phase 1 drug metabolism enzymes

Phase 2 drug metabolism enzymes

Understanding Direct and Indirect Effects in Microarray

- Down regulation of mRNA (Direct Effect)
  - Gene product is inhibited due to transcriptional inhibition of a master regulator

- Up regulation of mRNA (Indirect Effect)
  - Activity of an enzyme is inhibited due to the drug. Synthesis of either the same enzyme or another increases in compensation
    - e.g. increased glucose concentration in plasma can up-regulate GLUT 2 to increase the entry of glucose in liver
    - e.g. Valproate treatment is known to inhibit beta oxidation and gluconeogenesis. Increase in mRNA of the relevant enzymes are observed

Analysis of Cyclosporine treatment - Microarray results

- Experimental details:

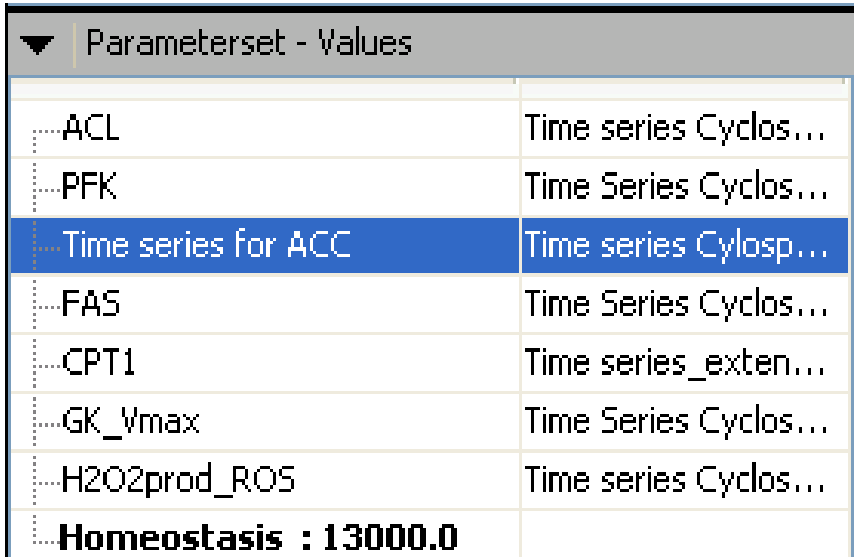
  - 350 mg/kg/day
  - Frequency of dosing: daily, Oral gavage
  - Animals were sacrificed and samples are collected at day 1, day 3 and day 5
  - Sample are analysed using Agilent 2100 BioAnalyzer
  - GEO dataset : GSE8858

List of affected biological processes by cyclosporine treatment

Processes	1 day	3 days	5 days
Glycolysis	Down	**Down***	Up
Betaoxidation	Up	**Down***	**Down***
Canalicular bile transporters	Down	Up	**Down***
Sinusoidal bile transporters	Up	**Up***	Up
Cytoskeleton network	Down	Down	**Up***
ROS synthesis	Up	Up	**Up***
Lipogenesis	Down	**Down***	Down

Altered biological processes are input in the model

- Each process change is represented by a set a parameters in the model
- The altered parameters are input into the model and simulations performed
- The results of the simulations are analyzed

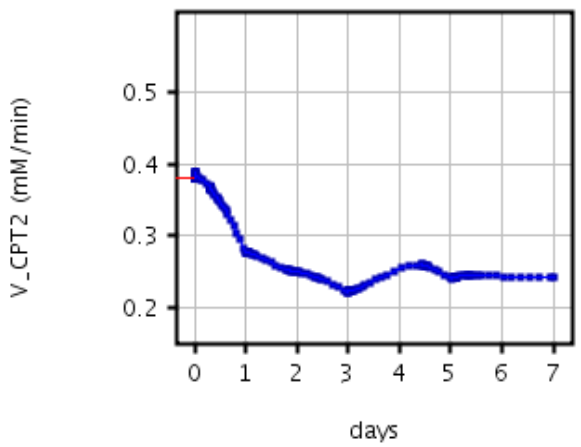


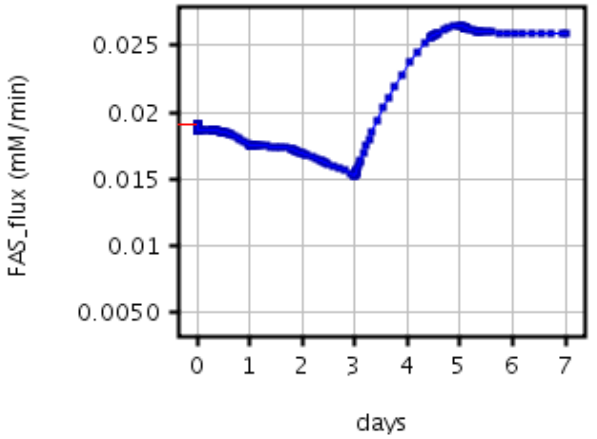
Effect of Cyclosporine Simulation results summary

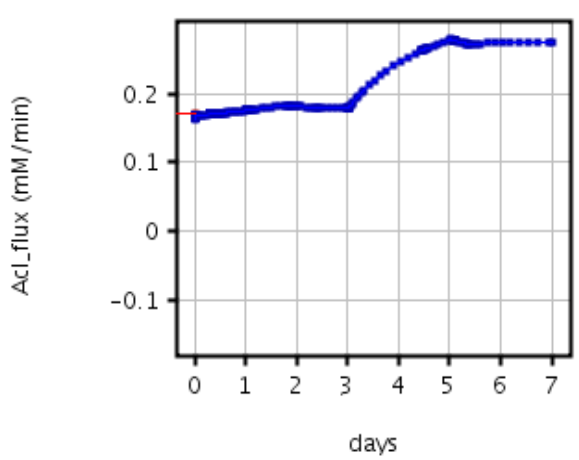
Observation	Cause
TG accumulation	Increased β oxidation, *denovo* lipogenesis
Decreased Redox potential	Increased oxidative stress
Minor increase in blood TCA	Changed transporter activity
25% reduction in ATP	Reduced glycolytic activity

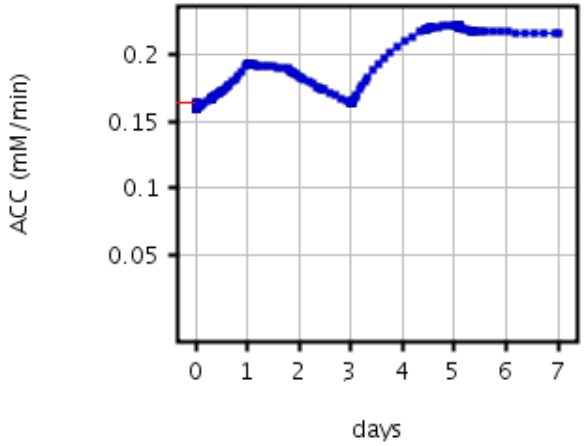
TG accumulation occurs due to multiple mechanisms

- Initially there is an inhibition of CPT1 (lowered β-oxidation)
- At later time points there is an increase in denovo-lipogeneis
- Both lead to TG accumulation

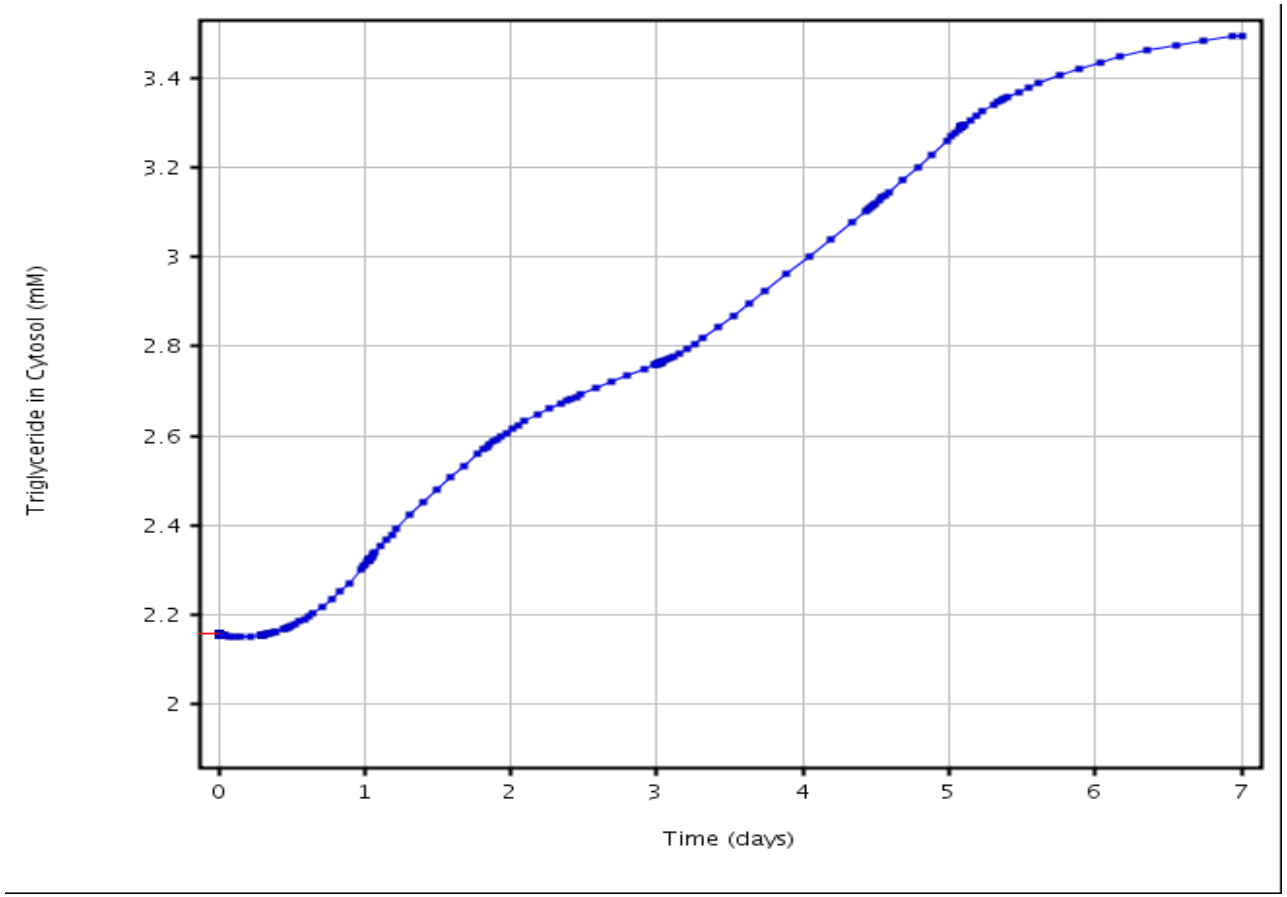








TG accumulation due to Cyclosporine treatment

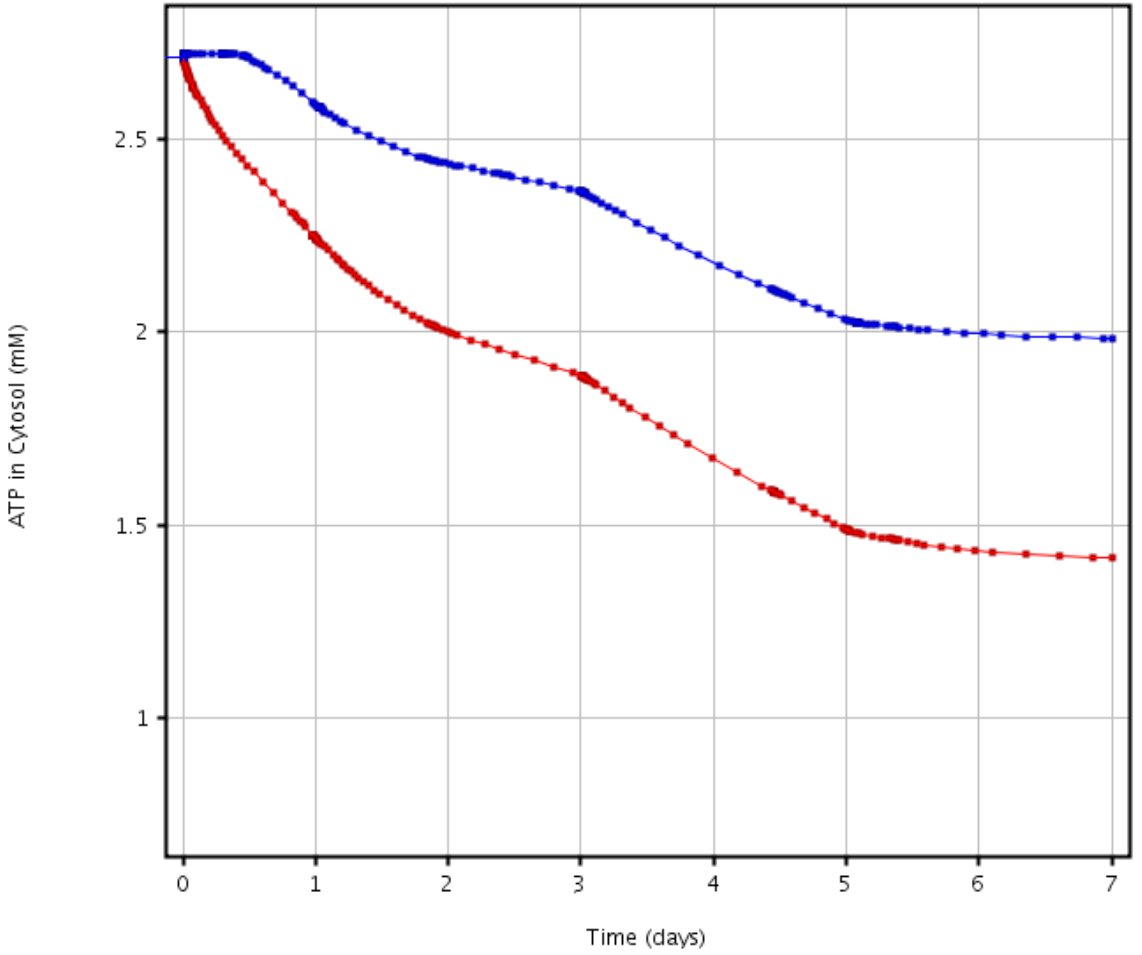


Adding other experimental inputs

- To the microarray data, we added other experimental inputs to see whether this platform could "integrate" data from multiple sources
- For cyclosporine, we added two measurements
  - Membrane potential change in the presence of drug
  - BSEP inhibition by cyclosporine
- We then performed the simulations again to look at the effect on the predictions

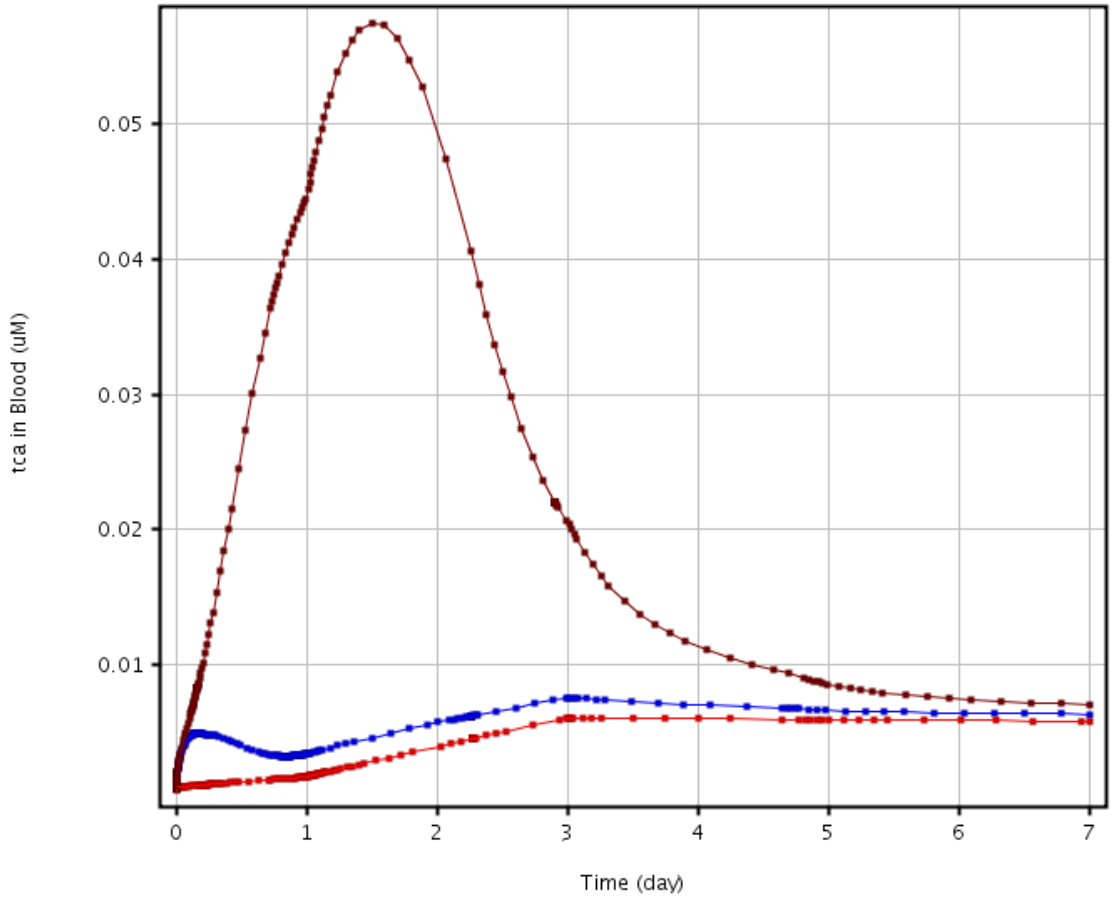
Inputting Membrane potential allows a more realistic simulation of ATP effect

- The reduction in membrane potential causes a impact on the mitochondrial energy production
- This affects the overall energetics in the cell and reproduces the cyclosporine impact on ATP more realistically



Synergistic Effect of ATP & BSEP inhibition

- The synergistic impact of ATP reduction and BSEP suppression is able to predict cyclosporine cholestatic potential correctly
- Note that merely adding the BSEP inhibition effect does not capture this



Effect of Cyclosporine – Integrated simulations summary

Observation (Microarray alone, Microarray & other measurements)	Cause
TG accumulation	Increased β oxidation, denovo lipogenesis
Decreased Redox potential	Increased oxidative stress Increased oxidative stress due to mitochondrial dysfunction
Minor increase in blood bile salts Profound increase in blood bile salts	Changed transporter activity Synergistic Effects of transporter & energetics
25% reduction in ATP 40-50% reduction in cellular ATP	Reduced glycolytic activity Impaired mitochondrial function leading to lowered oxidative phosphorylation


Thank you