



Workshop Training Series

Metabolic Research Services at BORC

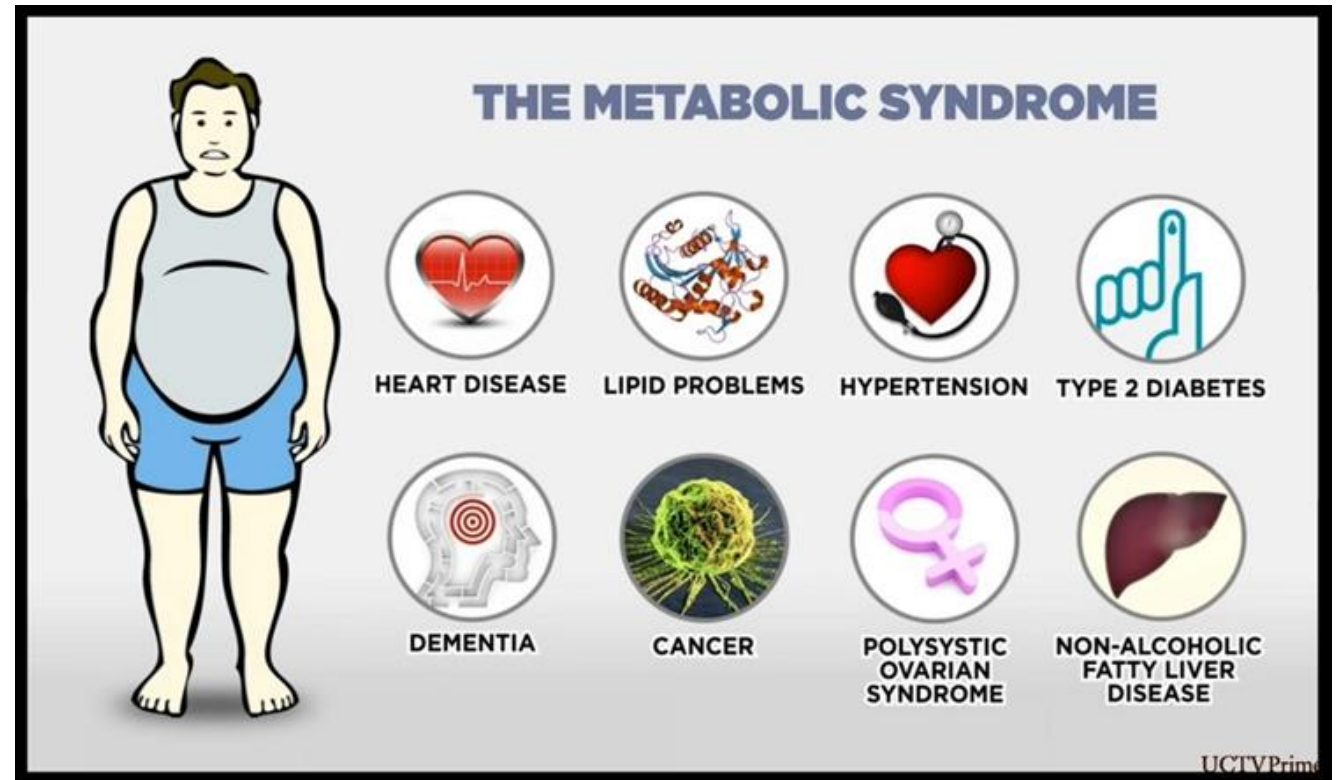
Yongjun Wang Ph.D.

Director of Biomedical and Obesity Research Core

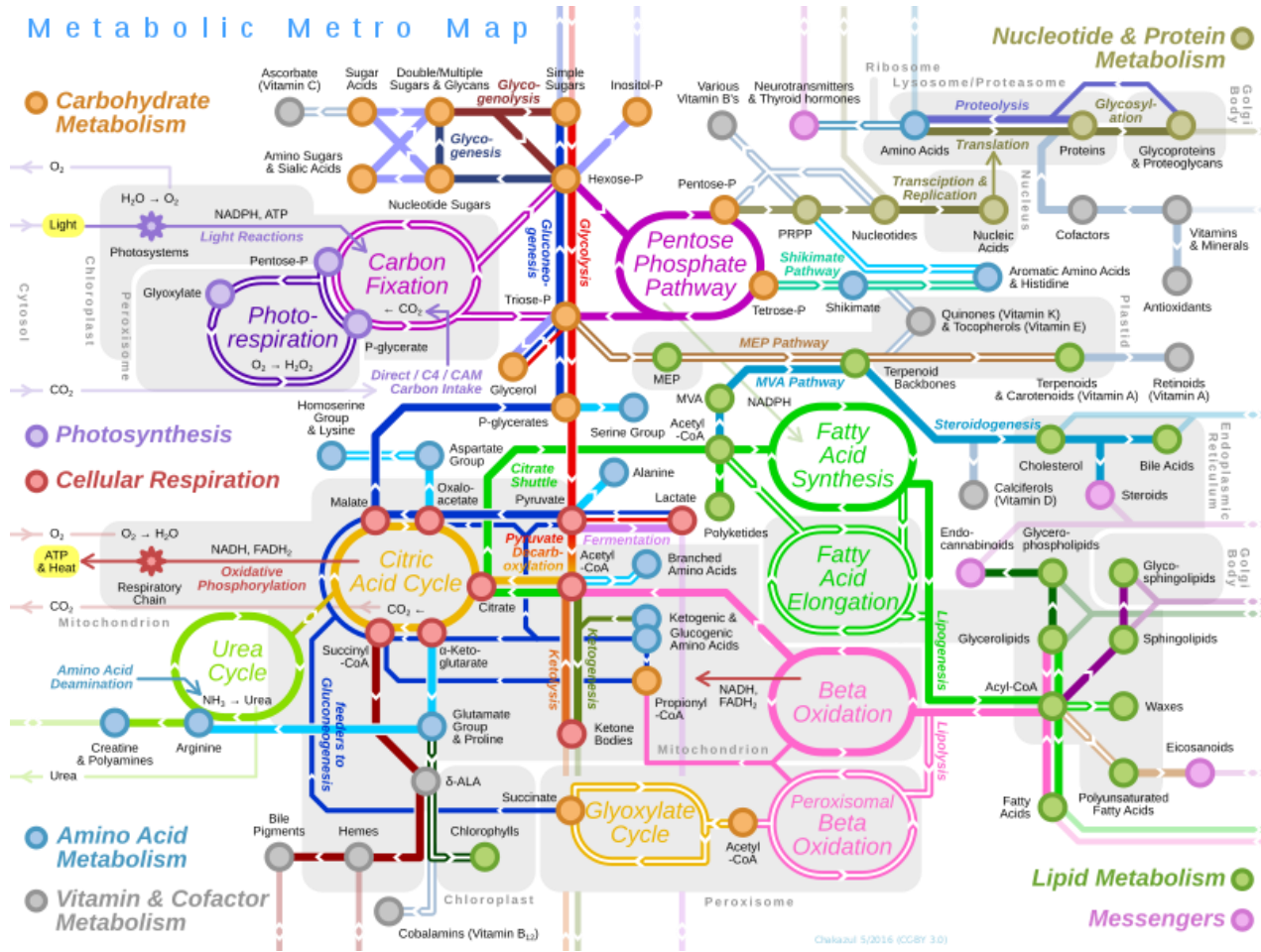
**Nebraska Center for the Prevention of Obesity
Diseases through Dietary Molecules**

Obesity and Metabolic Syndrom

- Obesity is a condition characterized by excessive accumulation of fat by the growth of hypertrophy and / or hyperplasia of the adipocytes.
- This growth occurs not only in the subcutaneous adipose tissue, but also in internal organs
- Obesity particularly in the abdominal cavity, always leads to several coexisting clinical conditions collectively named as metabolic syndrome.



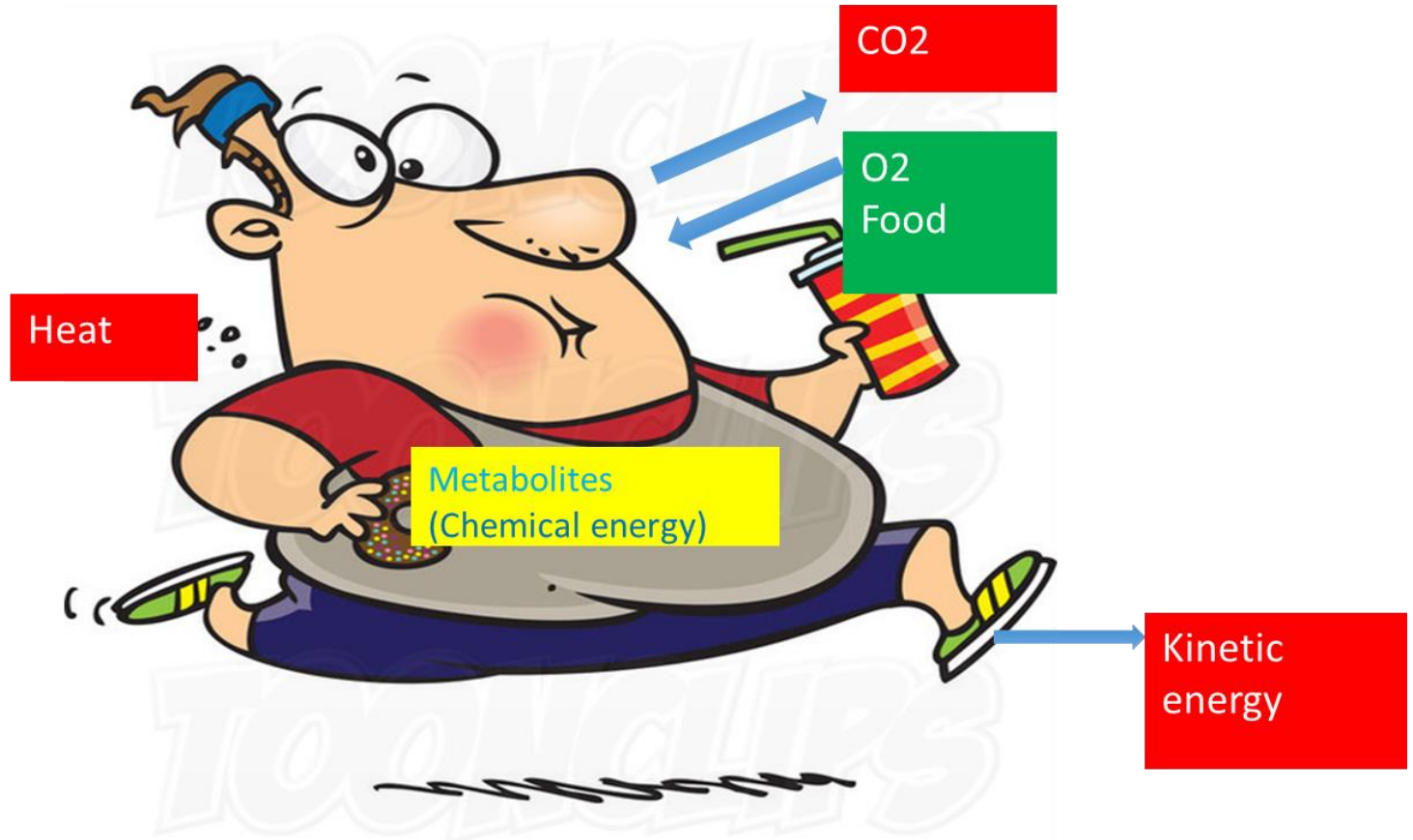
What is Metabolism?



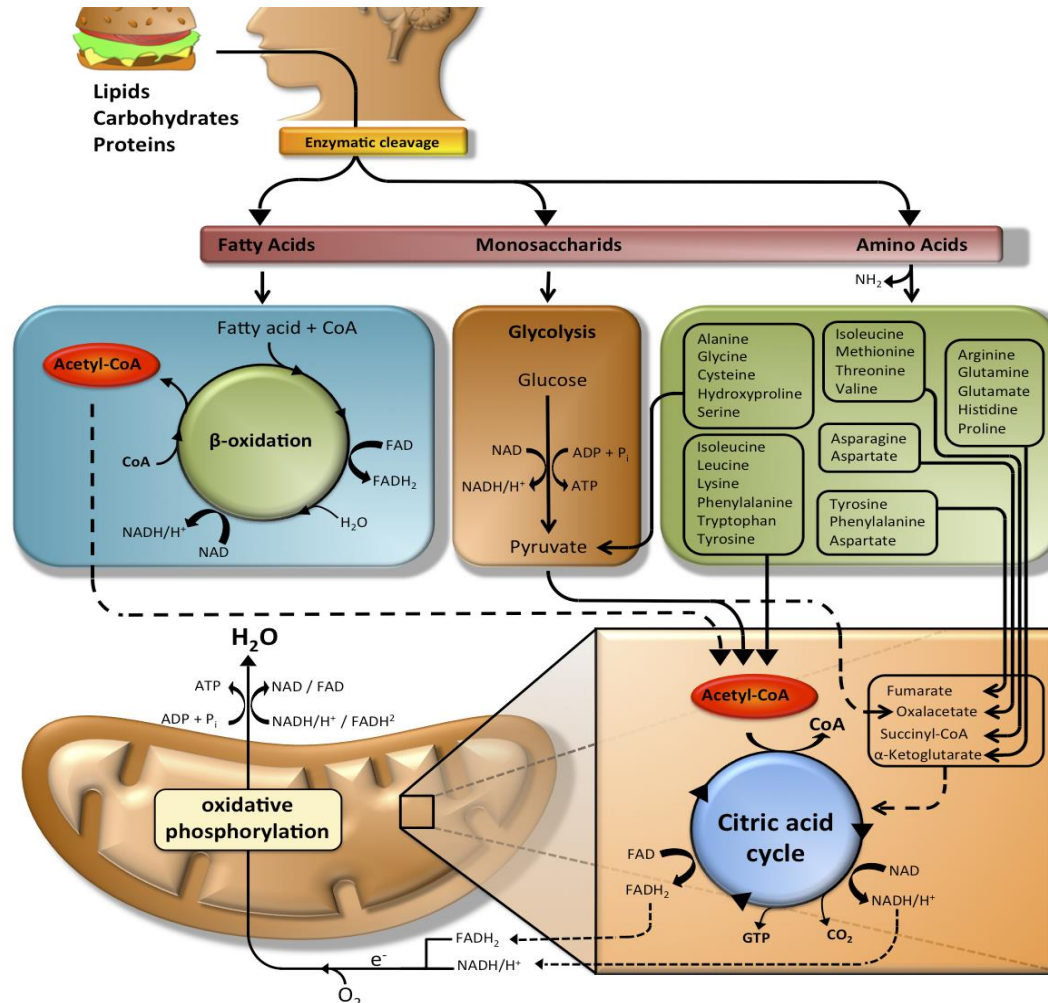
Metabolism is a general term representing all chemical reactions occurring in living organisms. Metabolic pathways describes series of chemical reactions that either break down a large compound into smaller units (catabolism) or build more complex molecules from smaller ones (anabolism), along with energy production or consumption

Energy Metabolism-Whole body Level

- Body weight
- Body Composition
- Food intake
- Energy expenditure
- Activity

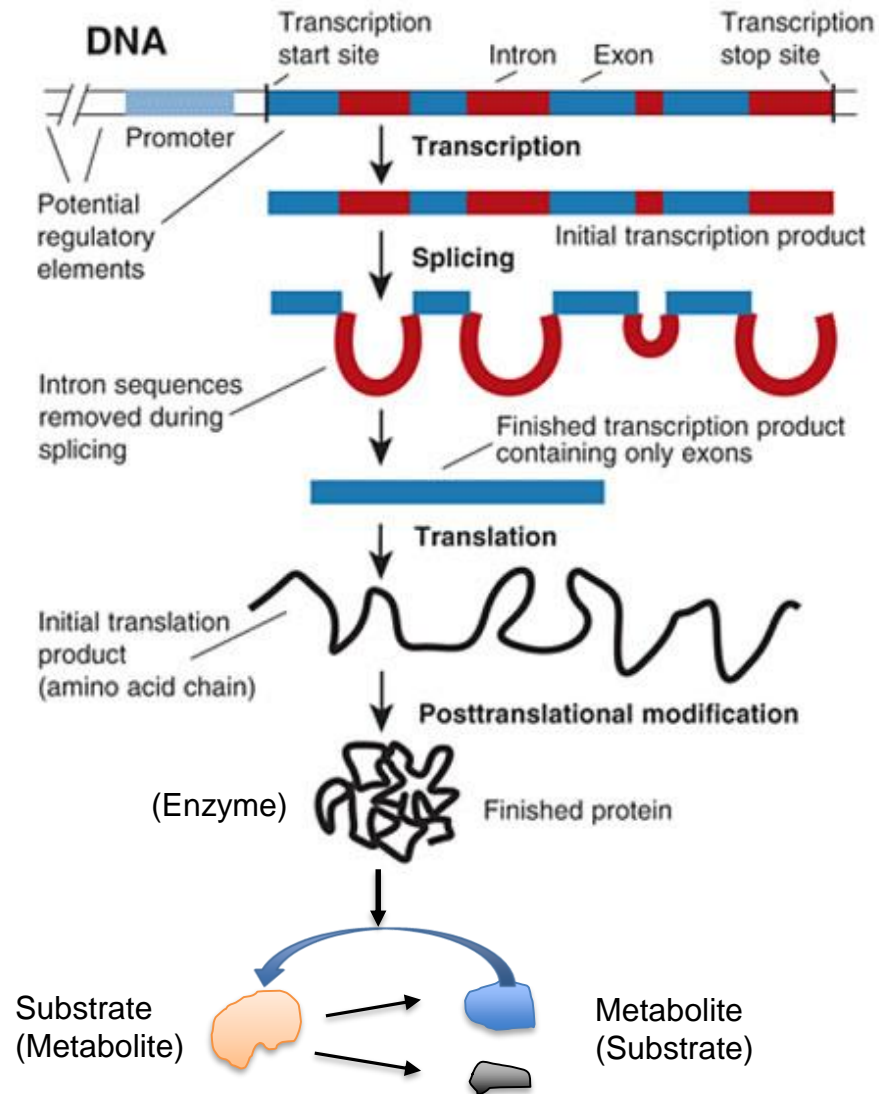


Energy Metabolism – Cell Level



- Glycolysis
- b-Oxidation
- Mitochondrial respiration

Regulation of Metabolism at Molecular Level



Genomics

Transcriptomics

Proteomics

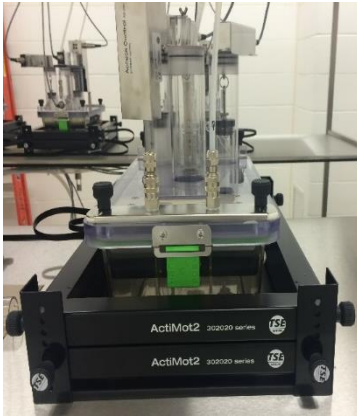
Metabolomics

Metabolic Study Resource in BORC

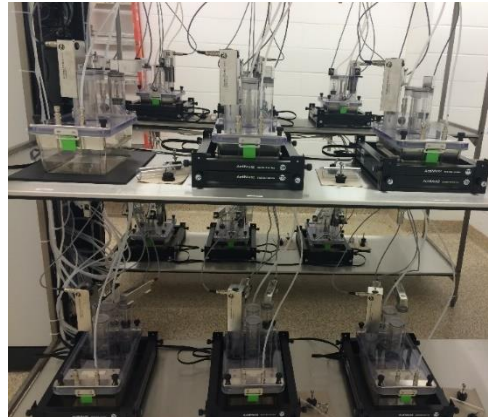
- [Metabolic cages \(TSE Systems\)](#)
- [XFe-24 Extracellular Flux Analyzer \(Seahorse Bioscience\)](#)
- [Vitros 250 Chemistry Analyzer](#)
- [Agilent GC-MSD](#)

TSE PhenoMaster Metabolic Cages

A multi-modular platform that allows researchers to carry out metabolic, behavioral and physiological analysis of mice in an automated and synchronized manner. Currently our system has 12 cages with modules to measure metabolic performance, activity, as well as feeding and drinking behavior.



Metabolic cage



Metabolic cages



Monitoring control



The components measured by the system

- **Calorimetry**
- **Activity**
- **Drinking and feeding behavior**

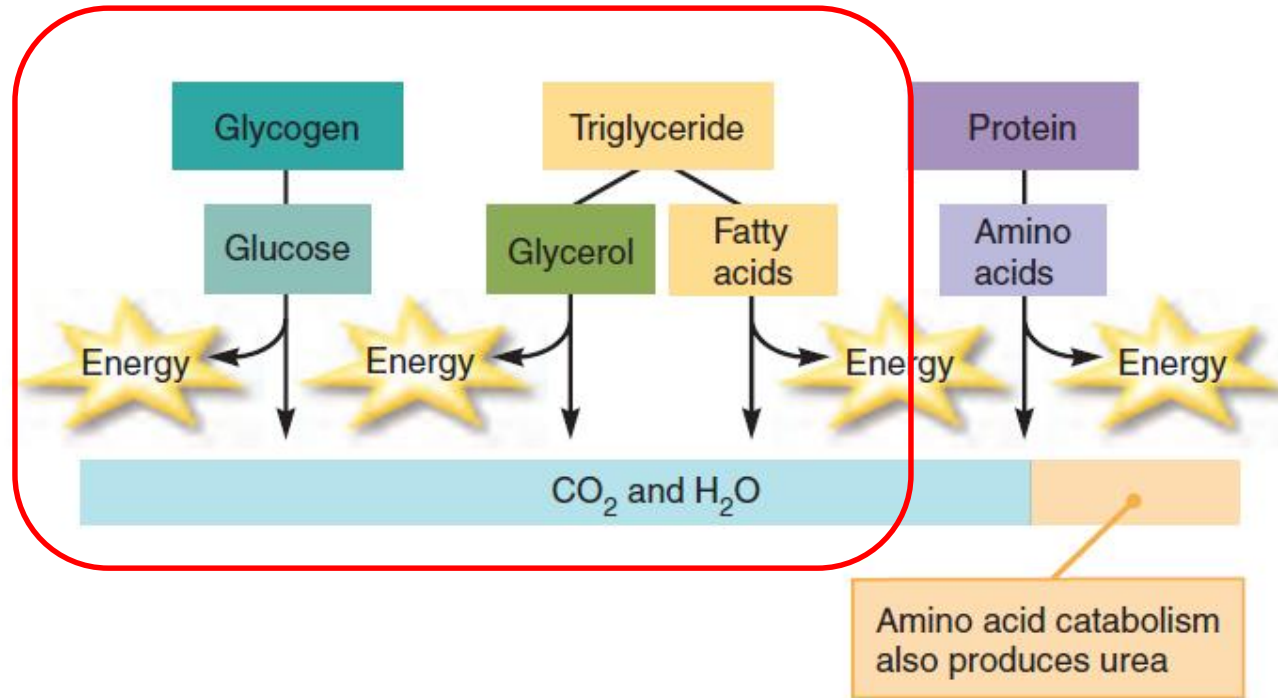
Calorimetry Results Parameter

Parameter	Description	Unit	Remarks
Flow	Flow	l/min	
Temp	Temperature	°C	Measurement in the box.
O2	Concentration	%	Reference and per box.
CO2	Concentration	%	Reference and per box.
dO2	Difference	%	Reference O2 - Box O2.
dCO2	Difference	%	Reference CO2 - Box CO2.
VO2	O2 consumption	ml/(kg x h) or ml/h	
VCO2	CO2 production	ml/(kg x h) or ml/h	
RER	Respiratory Exchange Rate		VCO2/VO2
H	Heat	kcal/(kg*h) or Kcal/h	Also possible in W/kg

Calorimetry Results Parameter

Parameter	Description
XT, YT	Breaks X-beam total (is equivalent to XA + XF) Breaks Y-beam total (is equivalent to YA + YF)
XF, YF	Breaks X-beam, fine movements Breaks Y-beam, fine movements
XA, YA	Breaks X-beam, ambulatory movements Breaks Y-beam, ambulatory movements
Z	Breaks Z-beam, rearing
Z2	Breaks Z2-beam, rearing
CenT, PerT	Sum central and peripheral ambulatory and fine movement
CenA, CenF	Central ambulatory and fine movement
PerA, PerF	Peripheral ambulatory and fine movement

The Major Source of Energy

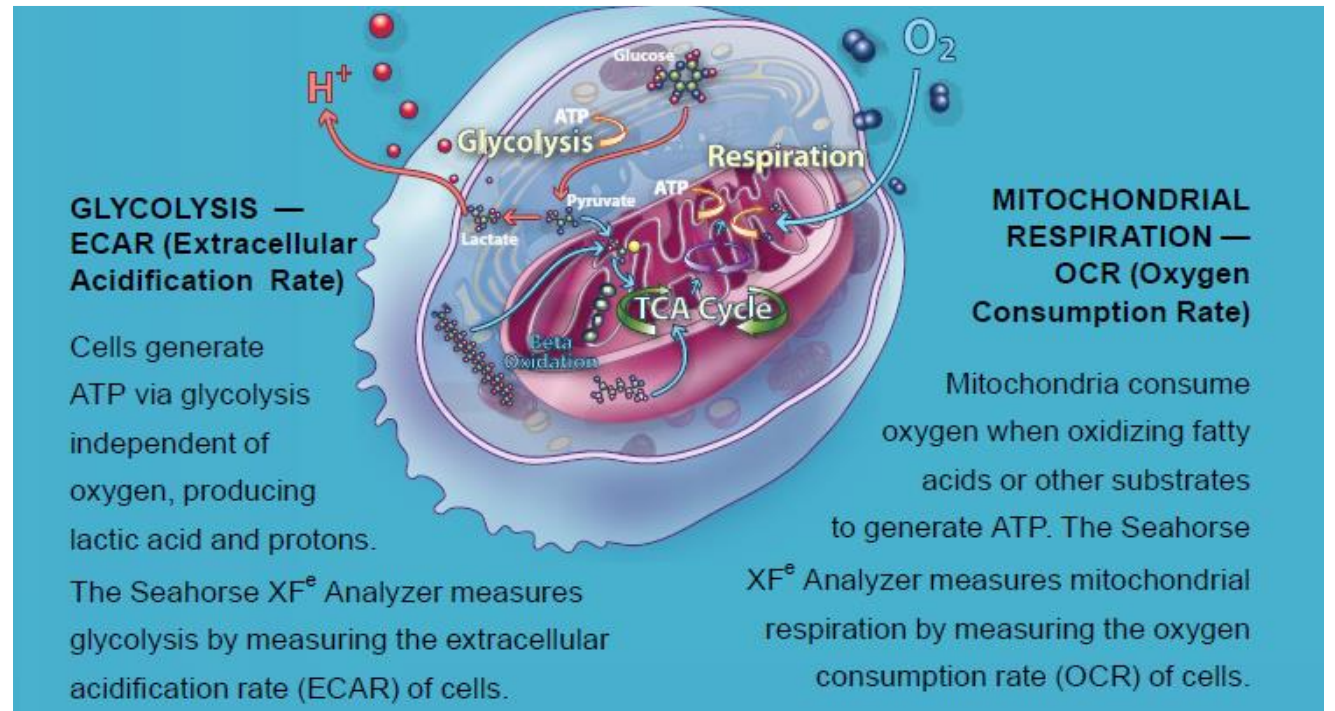


The majority of proteins is used for biosynthesis of new proteins and accounts for a small proportion of energy source in normal physiological condition.

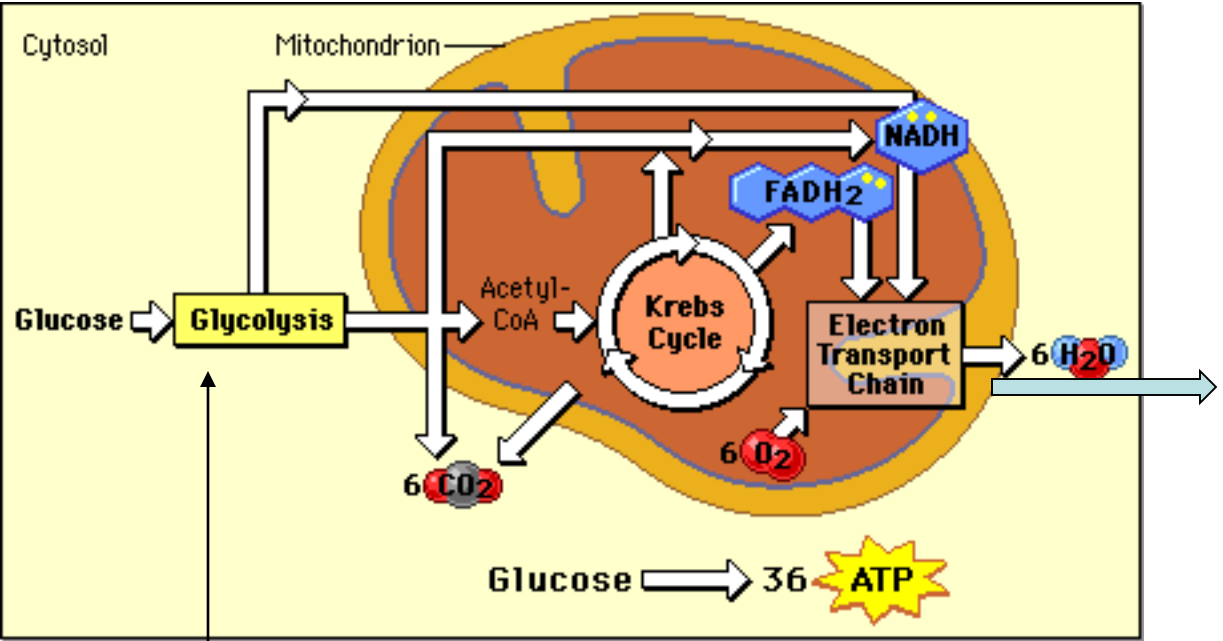
Parameters Affecting Energy Expenditure

- **Genetic impacts**
- **Circadian rhythm**
- **Locomotor activity**
- **Group size (thermal conduction)**
- **Food intake (thermic effect of food)**
- **Body weight**
- **Body composition (muscles)**
- **Body size (Bergman's rule)**
- **Environmental temperature**

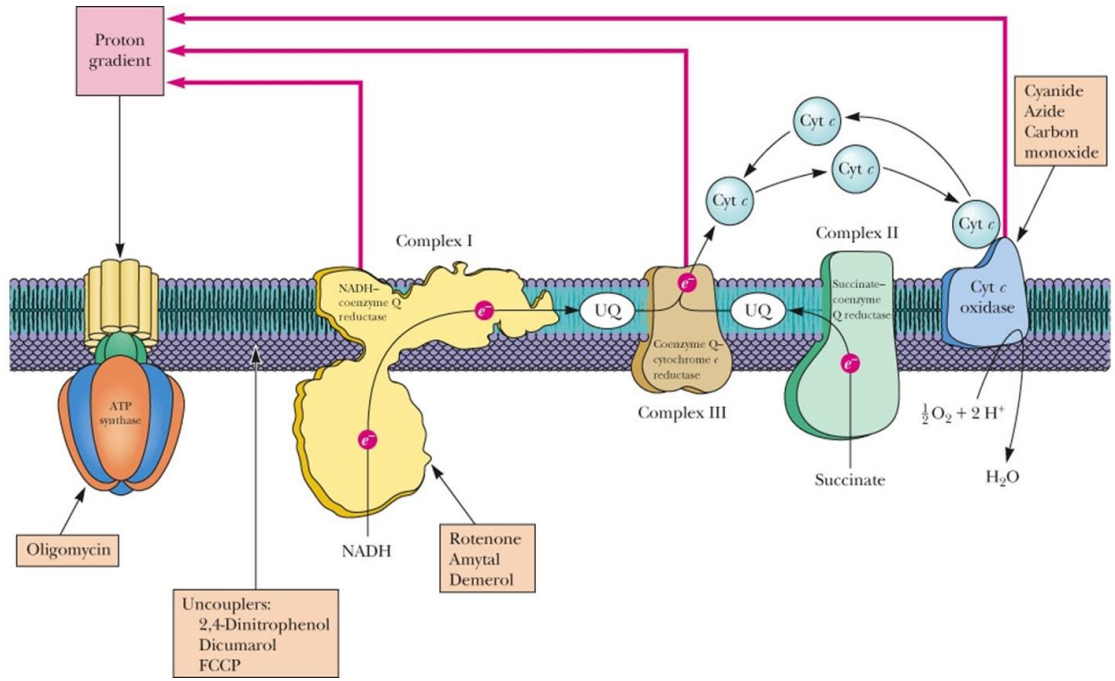
Seahorse XFe Extracellular Flux Analyzer



Glycolysis and Oxidative Phosphorylation



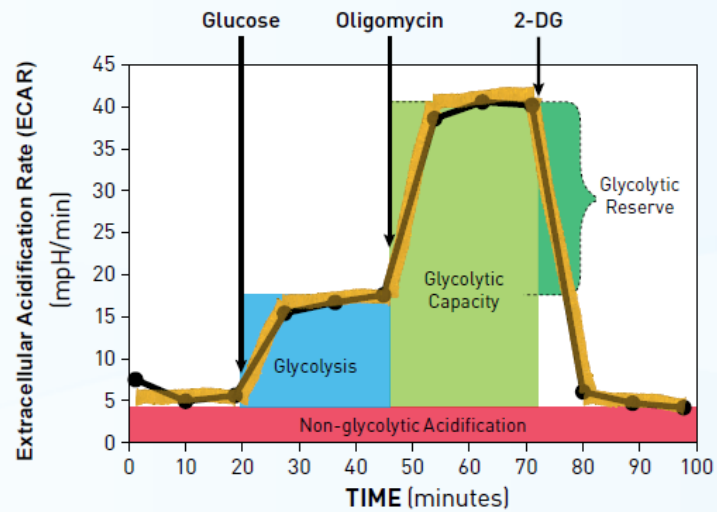
2-Deoxy-D-glucose



Glycolysis Stress Test

Seahorse XF Glycolysis Stress Test

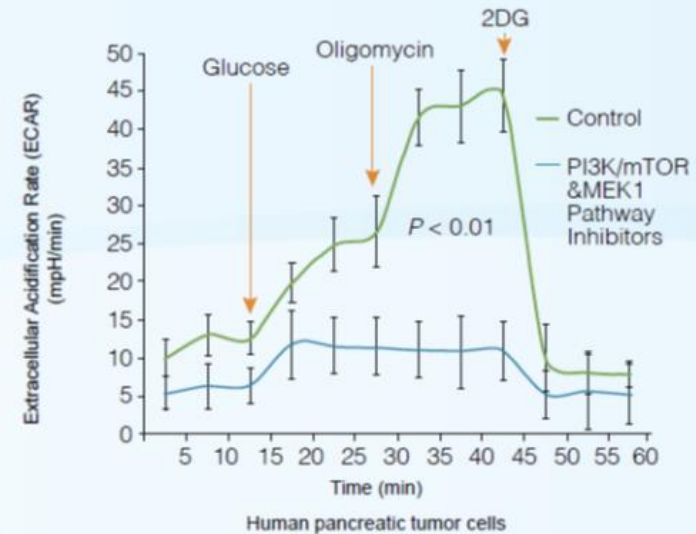
Glycolytic Function



The Seahorse XF Glycolysis Stress Test reports three key parameters of glycolytic function: glycolysis, glycolytic capacity, and glycolytic reserve.

Cancer

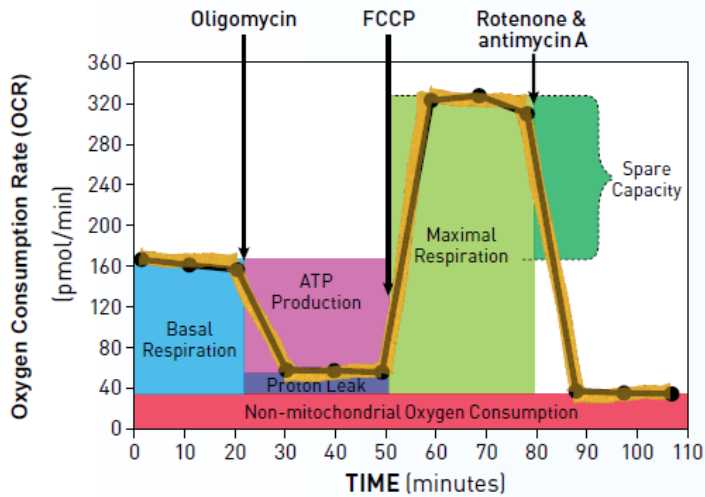
Seahorse XF Glycolysis Stress Test reveals mechanism of action of cancer pathway inhibition.



Viale *et al.*, (2014) Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature*. 514(7524):628-32.

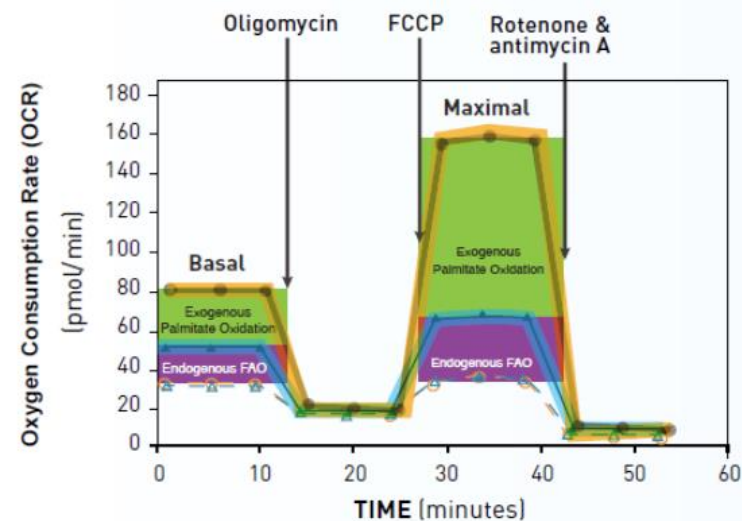
Mitochondrial Stress Test

Seahorse XF Cell Mito Stress Test Mitochondrial Respiration



The Seahorse XF Cell Mito Stress Test measures the key parameters of mitochondrial function: basal respiration, ATP production, proton leak, maximal respiration, and spare respiratory capacity.

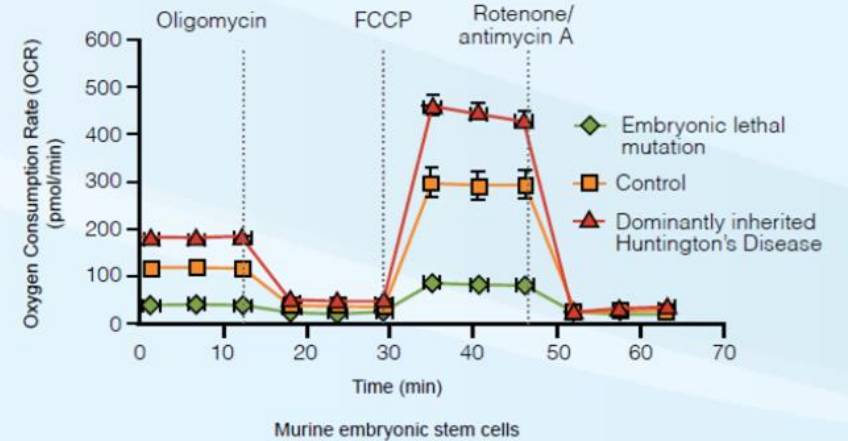
Seahorse XF Fatty Acid Oxidation Assay Exogenous & Endogenous Fatty Acid Oxidation



The Seahorse XF Fatty Acid Oxidation assay measures both exogenous palmitate oxidation and endogenous fatty acid oxidation.

Neurodegeneration

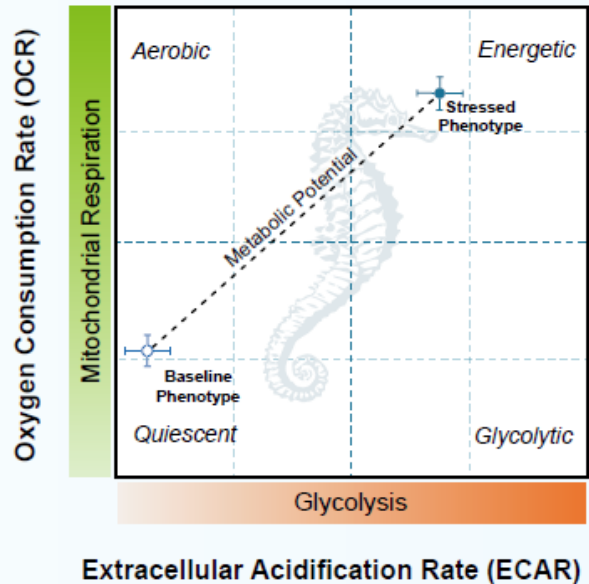
Seahorse XF Cell Mito Stress Test reveals metabolic signatures of embryonic stem cells with Huntington mutations.



Ismailoglu *et al.*, (2014). Huntingtin protein is essential for mitochondrial metabolism, bioenergetics and structure in murine embryonic stem cells. *Dev. Biol.* 391(2): 230-40.

Energy Phenotype Test

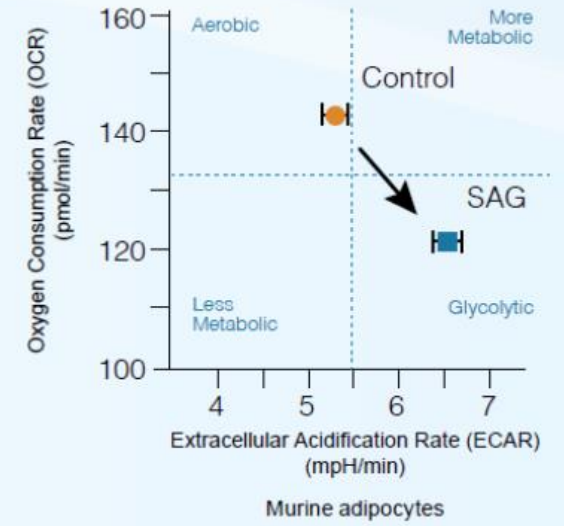
Seahorse XF Cell Energy Phenotype Test Metabolic Phenotype & Potential



The Seahorse XF Cell Energy Phenotype Test measures the key parameters used to determine the energy phenotype of a cell: baseline phenotype, stressed phenotype, and metabolic potential.

Obesity/Diabetes

Seahorse XF technology reveals Hedgehog pathway-induced switch to a glycolytic phenotype in adipocytes.



Teperino *et al.*, (2012) Hedgehog partial agonism drives Warburg-like metabolism in muscle and brown fat. *Cell*. 151(2): 414-26.

Seahorse Application

Obesity/Diabetes

Cancer Biology

Immunology

Neurodegeneration

**Other pathways involve changes of
glycolysis and mitochondria respiration**

UltraFocus DXA

UltraFocusDXA is a fully shielded x-ray cabinet designed for ultra high-resolution imaging and dual-energy x-ray absorptiometry (DXA) analysis.



The system automatically calculates bone mineral density (BMD), bone mineral content (BMC), and body composition in under 3 minutes.

Both whole body and region of interest (ROI) data can be selected, and separate bone, lean and fat tissue maps are presented to simplify ROI analysis.

Vitros 250 Dry Chemistry Analyzer

The Ortho Clinical Vitros 250 Chemistry System is chemical analyzer for Serum, Plasma, Urine, Cerebrospinal fluid, and cell culture medium (no color)

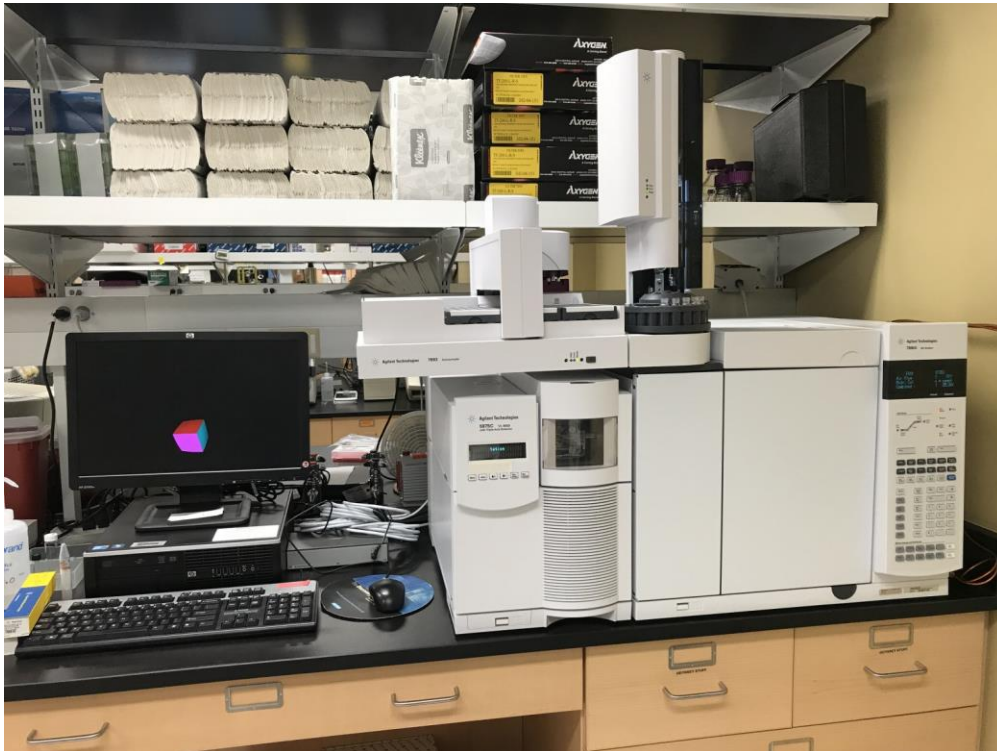


- **The VITROS 250 uses dry slides that have multilayered analytical elements coated on polyester supports.**
- **The analyte in the sample catalyzes the reaction sequence to yield products which absorb light at wavelengths in various regions (340 – 680nm).**
- **Depending on the analytes, the test types can be colorimetric, enzymatic end point, two-point or multi-point rate, or potentiometric.**

Metabolite Panels

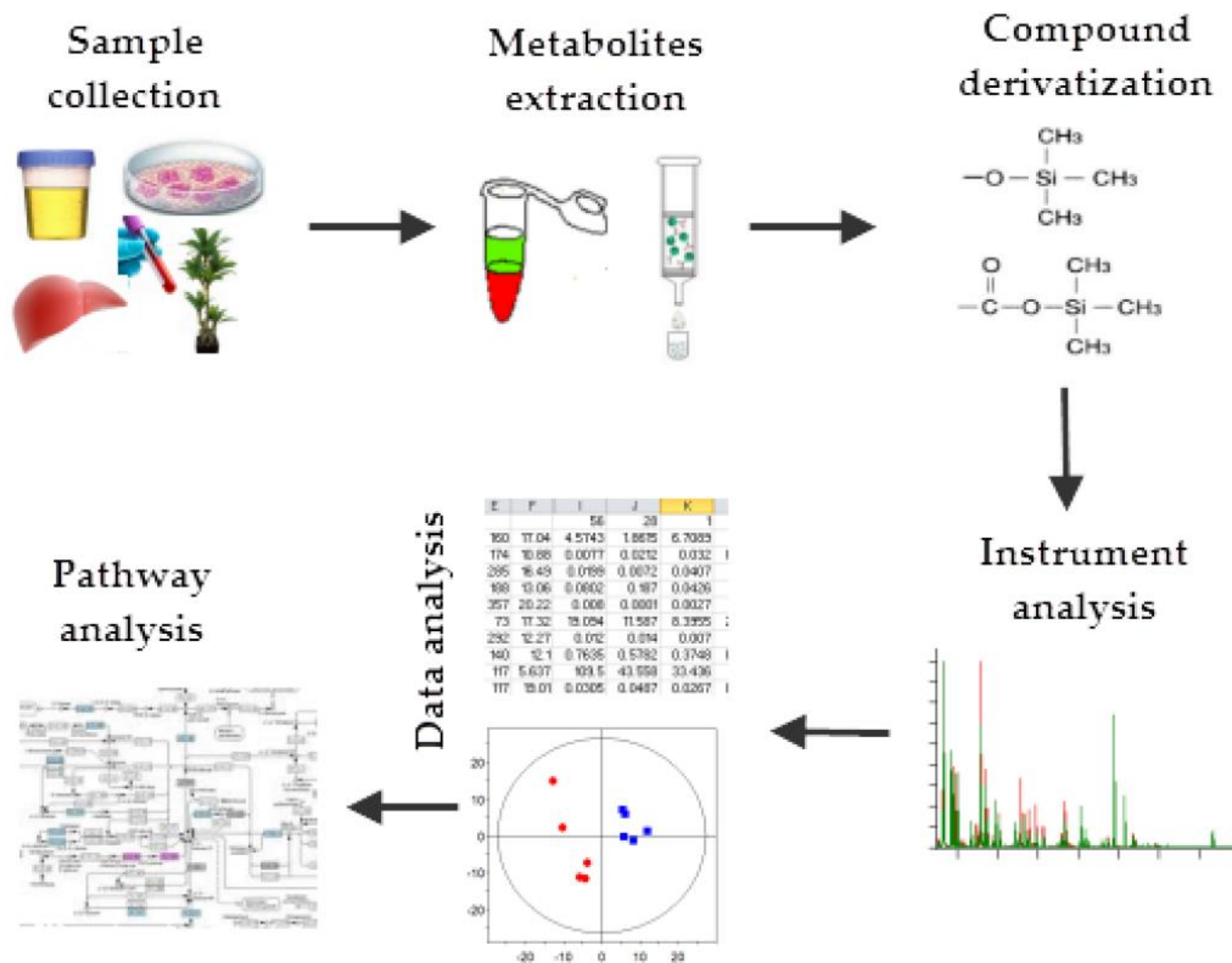
<u>Basic Metabolic Panel</u>	<u>Comprehensive Metabolic Panel</u>	<u>Lipid Panel</u>
Glucose	Glucose	Triglycerides
Calcium	Total Protein	Total Cholesterol
BUN	Albumin	HDL Cholesterol
Creatinine	Globulin	CHOL/HDLC
BUN/Creatinine ratio	A/G Ratio	LDL
Sodium	Total Bilirubin	VLDL
Potassium	ALT (SGOT)	
Chloride	AST (SGPT)	
Bicarbonate (ECO ₂)	Alkaline Phosphatase	<u>Hepatic Function Panel</u>
	Calcium	
<u>Electrolyte Panel</u>	BUN	
	Creatinine	Albumin
	BUN/Creatinine	Total Bilirubin
Sodium	Sodium	Direct Bilirubin
Potassium	Potassium	Alkaline Phosphatase
Chloride	Chloride	AST (SGPT)
Bicarbonate (ECO ₂)	Bicarbonate (ECO ₂)	ALT (SGPT)

Agilent GC/MS



- Gas chromatography–mass spectrometry (GC-MS) has many advantages to analyze small and volatile molecules such as steroids, fatty acids, and hormones.
- It can separate complex samples, quantify analytes, and determine trace levels of organic contamination.
- The technique can be applied towards the study of liquid, gaseous and solid samples.
- GC-MS becomes one of the key technologies for metabolite profiling and increasingly contributes to our understanding of the metabolic pathway.

The procedure for GC-MS-based metabolomics



Thank you!