



# Phenotypic profiling by mass spectrometry technology

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AND PERSONALIZED MEDICINE

Emerging pathways in Personalized Medicine:  
breaking barriers and moving forward



# From Genotype to Phenotype

Genotype: the genetic makeup of a cell, an organism, or an individual

Phenotype: the composite of an organism's observable characteristics or traits

Phenotype changes in response to genotype-environment interactions

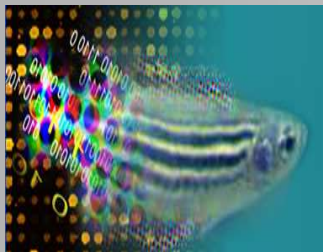


# Beyond genomics: Phenotype characterization



Genomics: risk class prediction

Bioinformatics: evaluation of  
genotype/environment interactions:  
refine assessment of risk



**Phenomics:** phenotype  
characterization fills the gap between  
genotype-based predictions and real  
physiological condition

# Phenotypic change relies on metabolic change

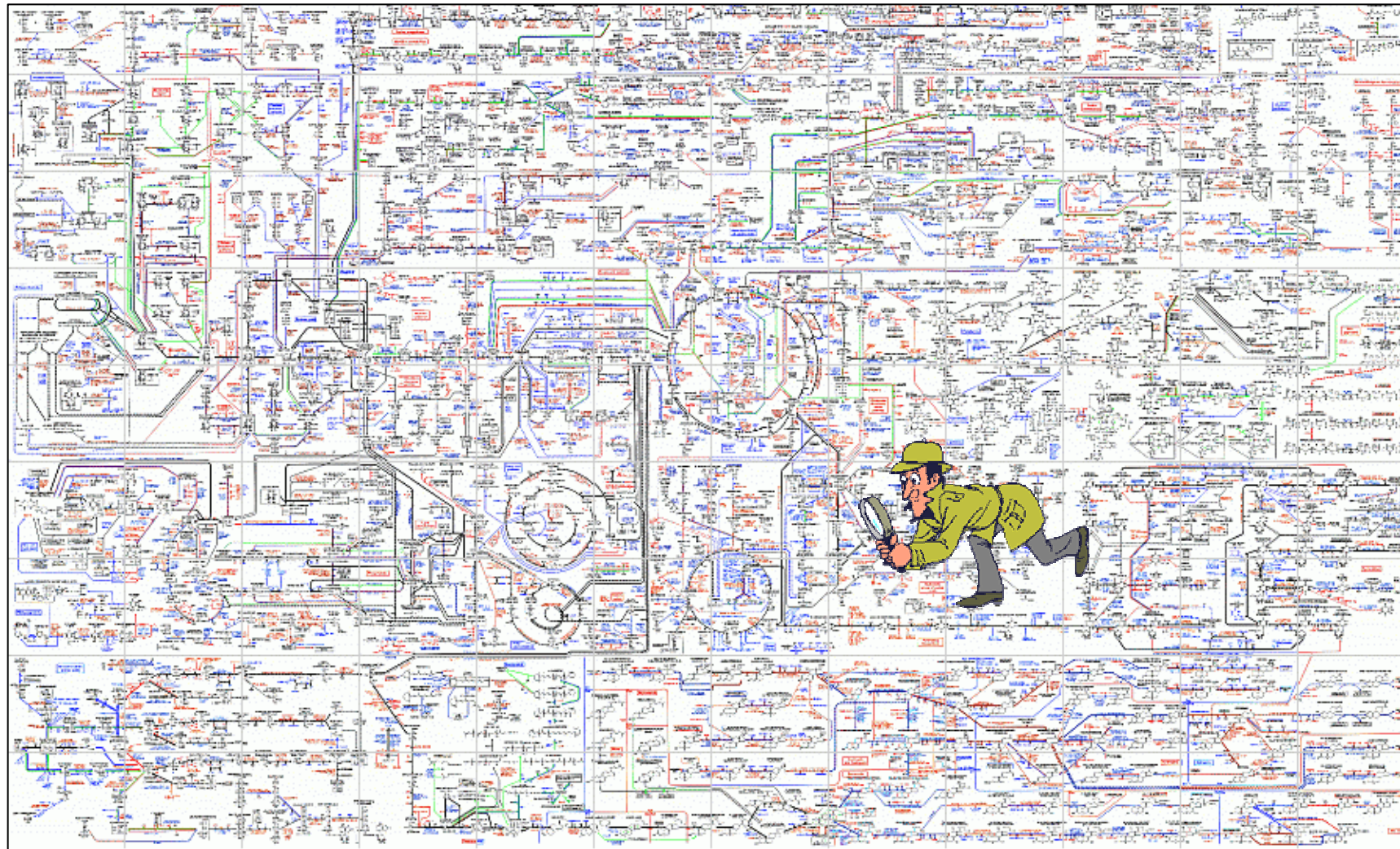
Cell metabolism (μεταβολή, "change" or μεταβολισμός, "outthrow" is the set of chemical reactions that happen in the cells of living organisms to sustain life.

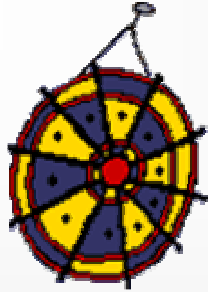


Metabolic imbalance affects wellness, fitness and sport performance...



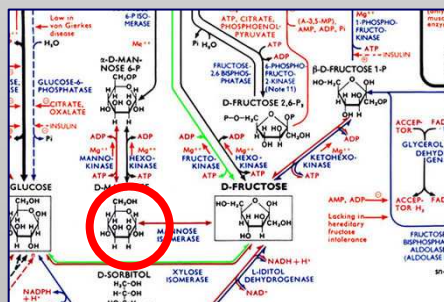
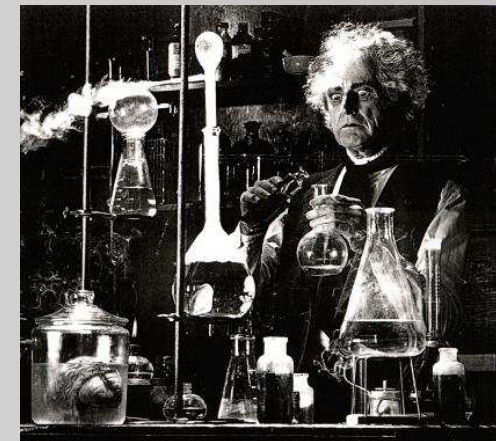
# The cell metabolism





Identification of the imbalanced biochemical pathway prompts to the actuation of tailored intervention to reset the right metabolic flow.

As the scientific knowledge increases, more and more molecular markers of metabolism imbalance come available to design a deep view of the individual metabolic competence



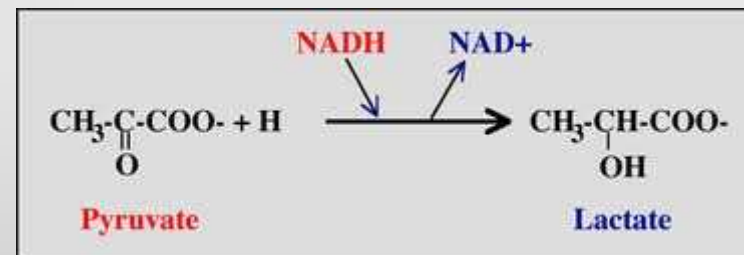
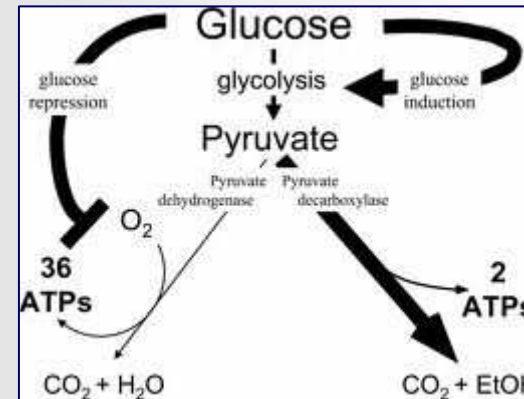
Altered levels of specific metabolites represent landmarks of a deficit in a specific cellular process.



# Metabolism of carbohydrates



Pyruvate and lactate are direct metabolic markers of the efficiency and function of dietary carbohydrate ingestion



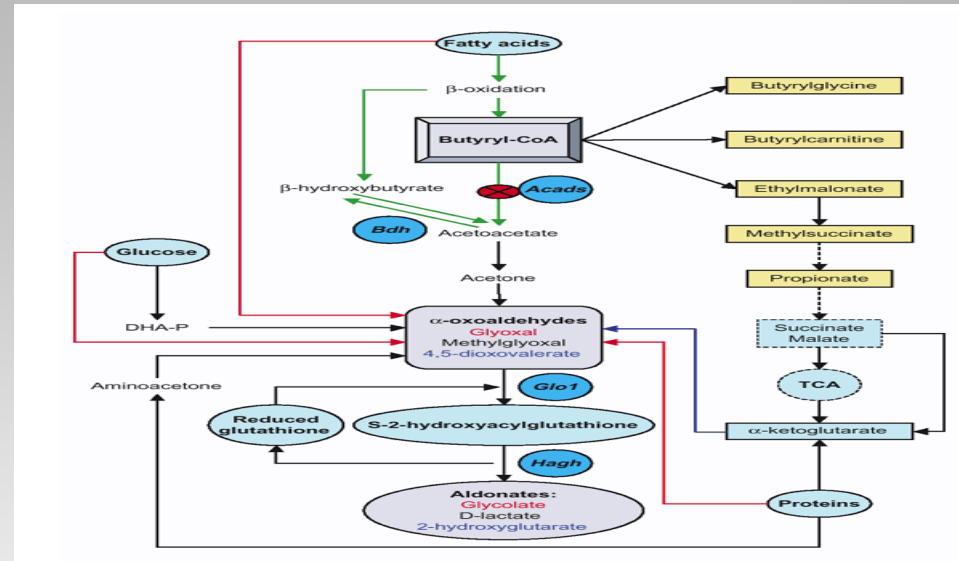
High levels of pyruvate are related to malnutrition and anorexia and can point to a B vitamin and lipoic acid deficiency

High levels of lactate are related to chronic infectious diseases, alcohol overuse, blood sugar dysregulation and can point to a CoQ10, biotin, thiamine and lipoic acid deficiency.

# Metabolism of fatty acids



Ethylmalonate and methylsuccinate are direct metabolic markers of the efficiency and function of fatty acids metabolism.



Elevated levels of **ethylmalonate**, a fatty acid metabolite indicates carnitine and riboflavin deficiency states. This can lead to the inability to oxidize long-chain fatty acids and amino acids.

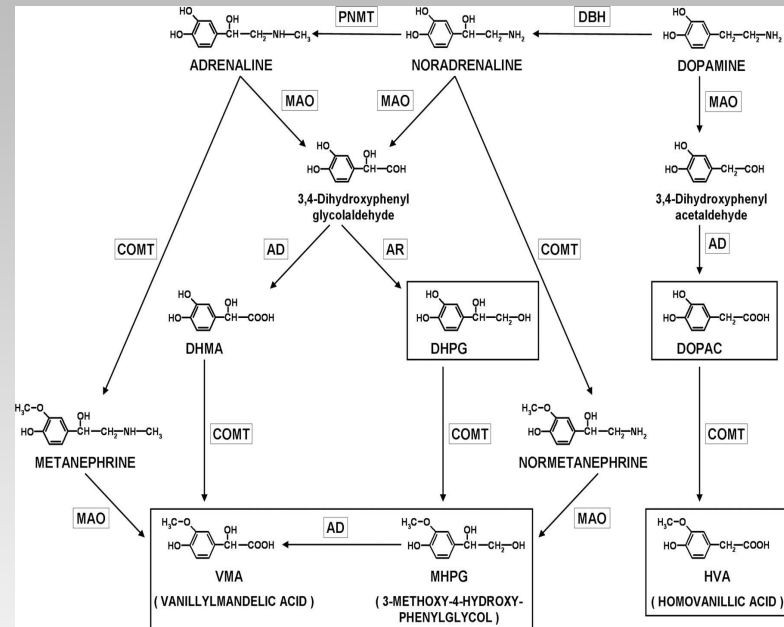
When elevated in the presence of high levels of adipate, a severe fatty acid oxidation impairment is potentially present. Intervention options: carnitine, vitB2.

High levels of **methylsuccinate** can point to ketosis, hypoglycemia, lactic acidosis, liver dysfunction, malnutrition, impaired beta-oxidation, weakness, nausea and fatigue.



# Neurotransmitter Metabolism

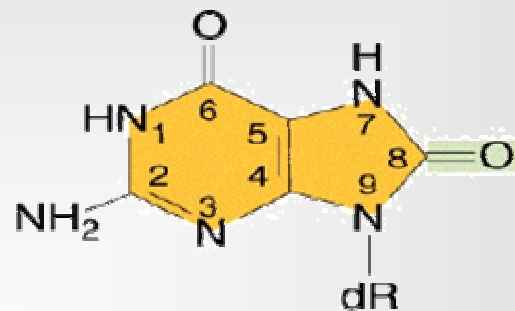
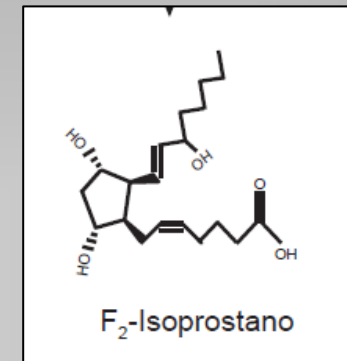
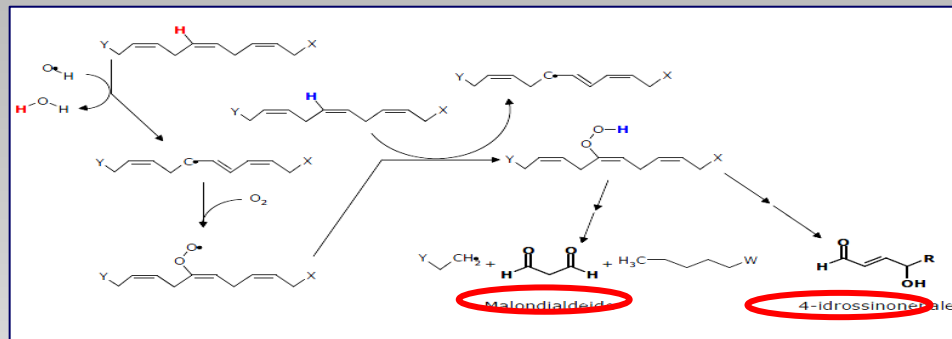
Homovanillate and 5-hydroxyindoleacetate are direct markers of the efficiency and function of neurotransmitter metabolism.



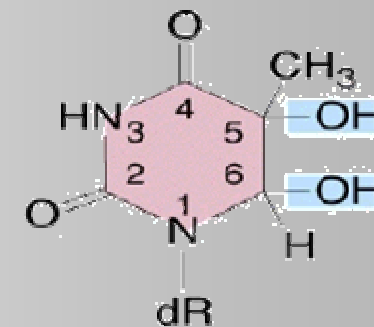
Low levels of homovanillate correlate with low central nervous system levels of epinephrine and norepinephrine, associated with signs of depression, insomnia, fatigue and inability to cope with stress.

5-hydroxyindoleacetate, a serotonin metabolite, can point to a higher need for tryptophan. Clinical signs can include depression, fatigue, insomnia, and other behavior imbalances.

# Oxidative stress markers



8-Oxo-7-hydrodeoxyguanosine  
(8-oxodG)



Thymidine glycol

# Mass spectrometry and phenotype profiling

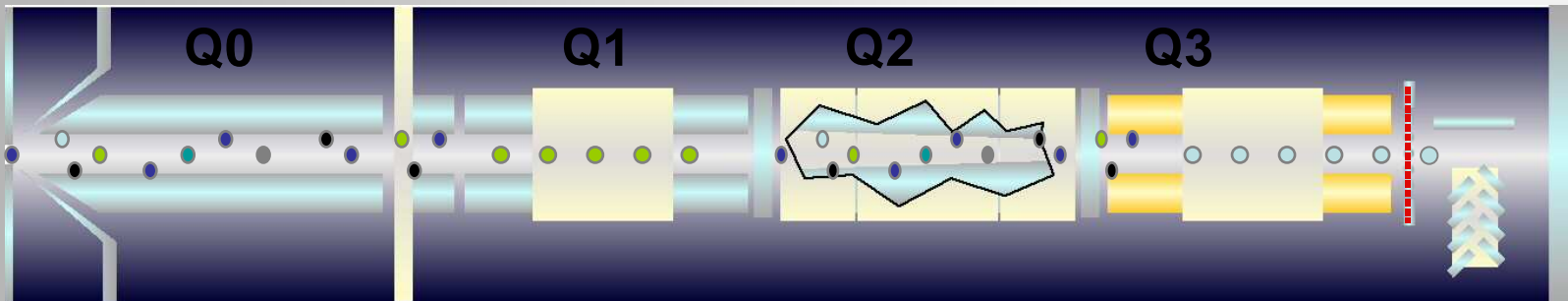
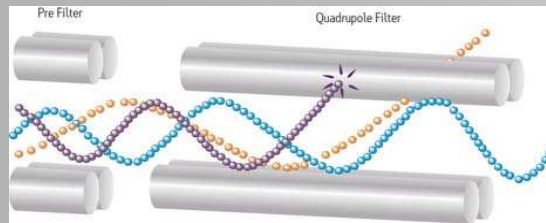


Mass Spectrometry differentiates molecular ions using the mass-to-charge ratio  $m/z$

## Advantages:

- Specificity
- Sensitivity
- Accuracy
- Automation
- Simple sample preparation
- Low-cost/sample
- Open system

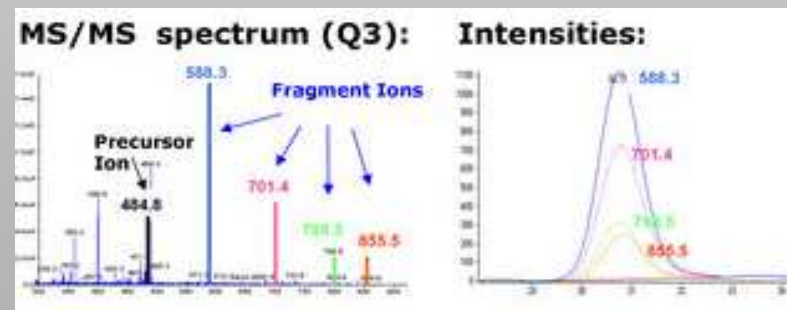
# How mass spectrometer works?



*Precursor ion selection*

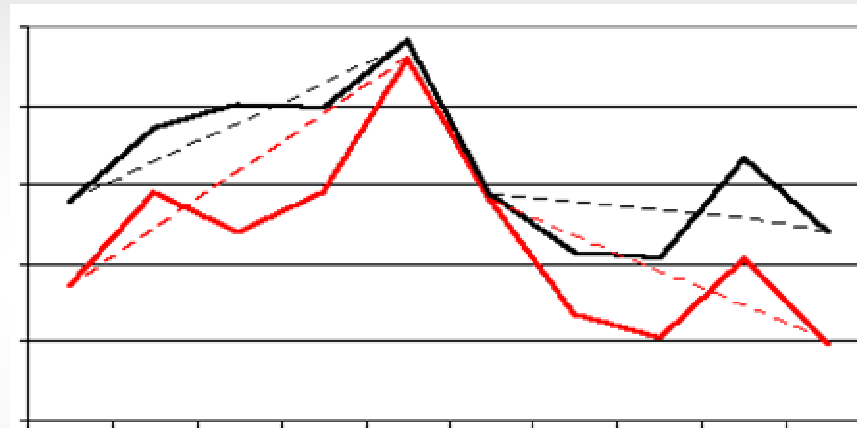
*Fragmentation*  
N<sub>2</sub> CAD Gas

- Q1 Selects an [M+H]<sup>+</sup>
  - Q2 fragments the selected ion.
  - Q3 selects only daughter ions.
- Only the daughter ions reach the detector.



# Genotype-phenotype relations: integrated strategy

Gene polymorphisms related to oxidative stress	
GENE	POLIMORFISMO
CYBA	C242T, A640G
NOS3	Glu298Asp
MPO	G-463A
GSTP1	A313G
GSTM1	delezione
GSTT1	delezione
SOD2	Ex2+24T>C
GPX1	Pro198Leu
CAT	C-262T
EPHX	C337T, A416G
NQO1	C609T
PON1	A575G, L55M
CFH	Y402H
XRCC1	G28152A
OGG1	C315>T
ERCC1	T19007C, C8092T
ERCC2	2251A>C
XPC	Lys939Glu



**A new era incoming:  
Therapeutic Metabolite Monitoring**



***“A state of complete physical, mental and social well-being and not merely the absence of disease or infirmity”***



# Credits

Maurizio Simmaco

Giovanna Gentile

Marina Borro

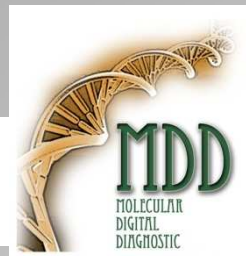
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