



Knowledge that will change your world

1st UAB Metabolomics Workshop
July 22-25, 2013

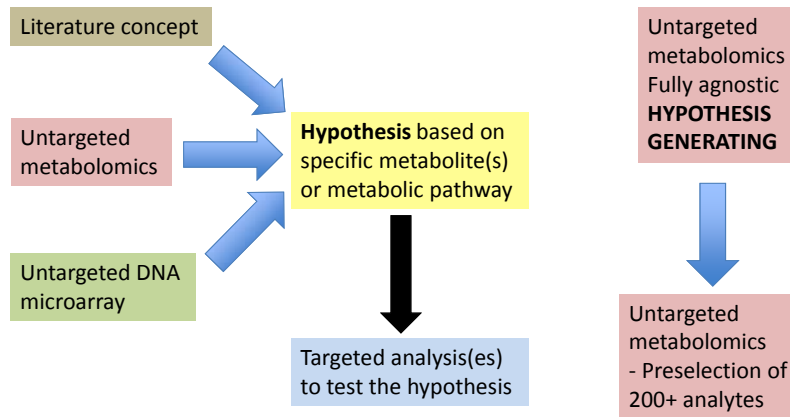
Untargeted and Translational Metabolomics

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Rationale for untargeted metabolomics

- **“Metabolites are canaries of the genome”**
- **Human body consists of two classes of genome – a supra-organism**
 - Human cells of different types
 - Multitude of microorganisms in different microbiomes
- **Metabolites are not predictable**
- **Cannot limit analysis of the metabolome**

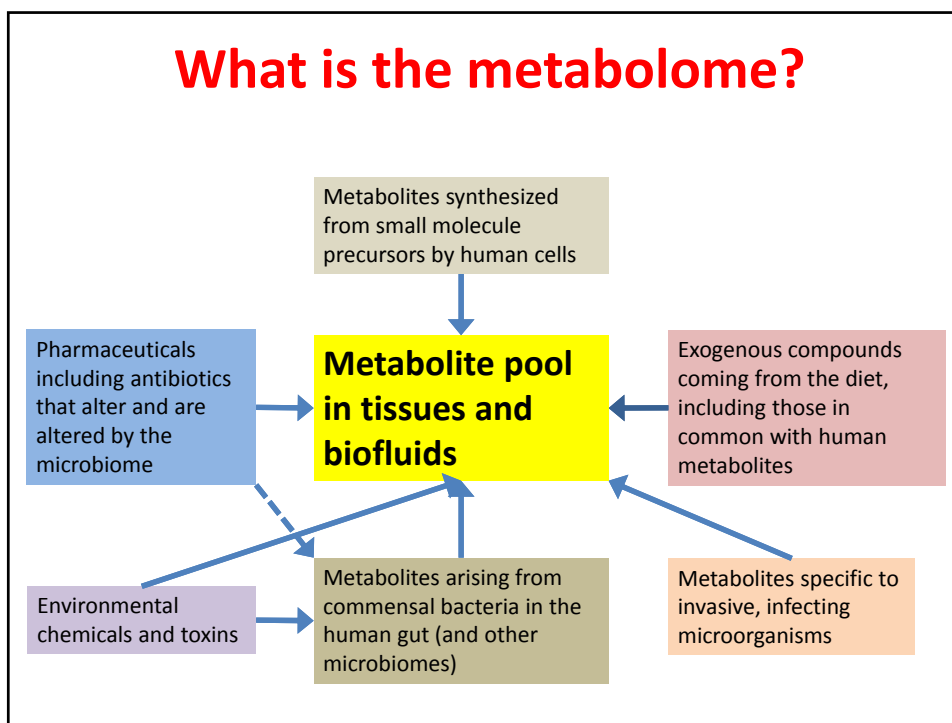
Targeted vs Untargeted metabolomics



Untargeted metabolomics

- The body contains myriads of small molecules
- Some come from known pathways, whereas others are consumed, made by good and bad microorganisms, or taken as therapeutics

What is the metabolome?



Measuring the untargeted metabolome

- The “metabolome” is too large to be measured in one analysis
 - Gas/volatiles
 - Water soluble
 - Positively charged
 - Negatively charged
 - Neutral
 - Lipid
 - Solvent extractable

The water-soluble metabolome

- **Ultrafilter through 0.22 μm membrane**
 - This removes any particles and protects the column
 - Very important for nanoLC-MS
 - Could adsorb metabolites selectively
 - Allows very hydrophilic metabolites to be studied
- **Treat with β -glucuronidase/sulfatase**
 - This allows extraction of compounds that partition into an immiscible solvent (ethyl acetate, diethyl ether or dichloromethane)

The water-soluble metabolome

- **Use of ion exchange media**
 - **Cation exchange**
 - Wash with 6 M HCl and then with double distilled, deionized water until neutral
 - Pass filtered sample over the column and wash with DD H₂O
 - this removes neutral and negatively charged metabolites
 - Elute with 2 M NH₄OH or NH₄HCO₃
 - This isolates the amino acids, peptides, amines and other amino-containing compounds
 - Excess NH₃ removed by evaporation

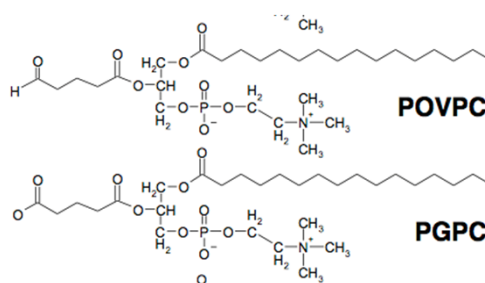
The water-soluble metabolome

- Use of ion exchange media
 - Anion exchange
 - Wash with NaCl to convert to the Cl⁻ form and then with double distilled, deionized water until neutral
 - Pass filtered sample over the column and wash with DD H₂O
 - this removes neutral and positively charged metabolites
 - Elute with 2 M formic acid or Na formate (has to be converted to formic acid with a cation exchange resin)
 - Formic acid is volatile (bp 100°C)
 - This isolates the organic acid fraction and phosphorylated intermediates

The lipid metabolome

Very complex
(>180,000)

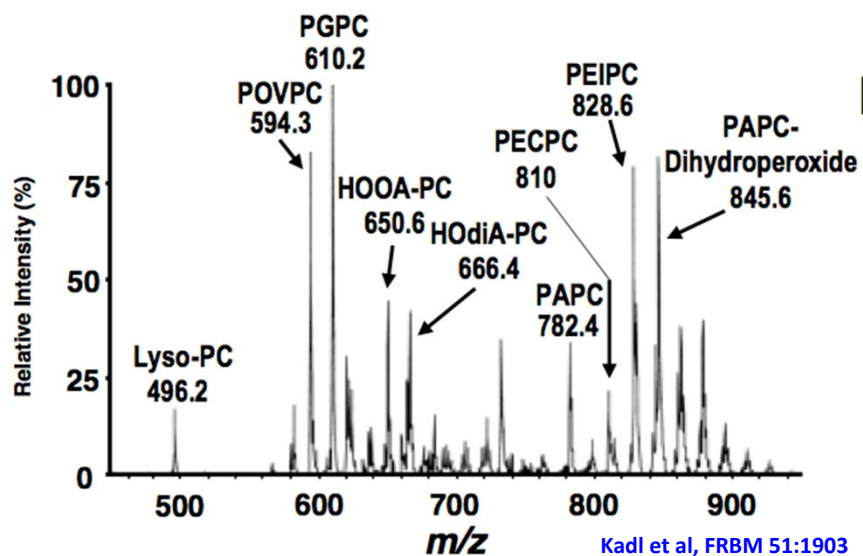
- Besides the expected lipids, lipid metabolites may may be formed under oxidative stress



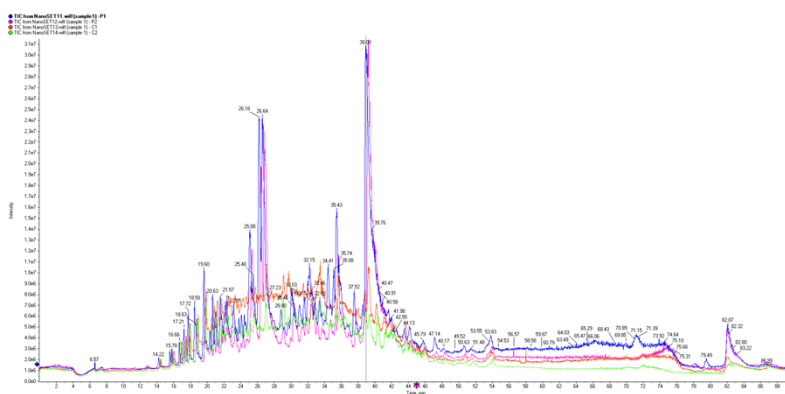
E.g., a phosphatidylcholine with a long chain unsaturated fatty acid can be oxidized at a double bond to produce a short stump. These lipids are nM ligands for TLR2.

Kadl et al, FRBM 51:1903

Oxidization of 16:0/22:6 PC



The challenge of untargeted metabolomics – too many peaks



Complex
sample
(10,000
cpds)

→ Fractionate by liquid chromatography over say 20 min, i.e., on average 500 compounds elute per min or 8 per sec

Recording ions in untargeted LC-MS

Too many to follow with a **quadrupole detector** (low mass resolution scan up to m/z 1000 takes one second),

But well suited to a **time-of-flight detector** (medium resolution mass scan in <50 msec and no upper mass limitation)

If tandem mass spectra are also collected (only possible on an instrument with a TOF or Orbitrap detector), then peaks (and their associated ions) that are significantly different can be more easily identified from interpretation of their MSMS spectrum

Huge amount of data that are collected need a visualization tool

Translational metabolomics

Nature 472:57-63, 2011

Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Zeneng Wang, Elizabeth Klipfell, Brian J. Bennett, Robert Koeth, Bruce S. Levison, Brandon DuGar, Ariel E. Feldstein, Earl B. Britt, Xiaoming Fu, Yoon-Mi Chung, Yuping Wu, Phil Schauer, Jonathan D. Smith, Hooman Allayee, W. H. Wilson Tang, Joseph A. DiDonato, Aldons J. Lusis, and Stanley L. Hazen

A bellwether paper for the development of metabolomics

Metabolites and Major Adverse Cardiovascular Events

Selection of 75 patients and 75 matched controls

Learning cohort of 50
analyzed by LC-MS

40 analytes where $\log(P) > 1.3$ and $p < 0.05$

Validation cohort of 25
analyzed by LC-MS

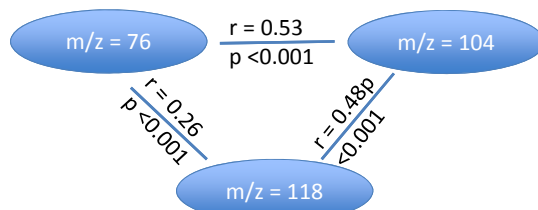
24 analytes $\log(P) > 1.3$ and $p < 0.05$

18 analytes

Wang et al., Nature (2011)

Choice of metabolites to follow up

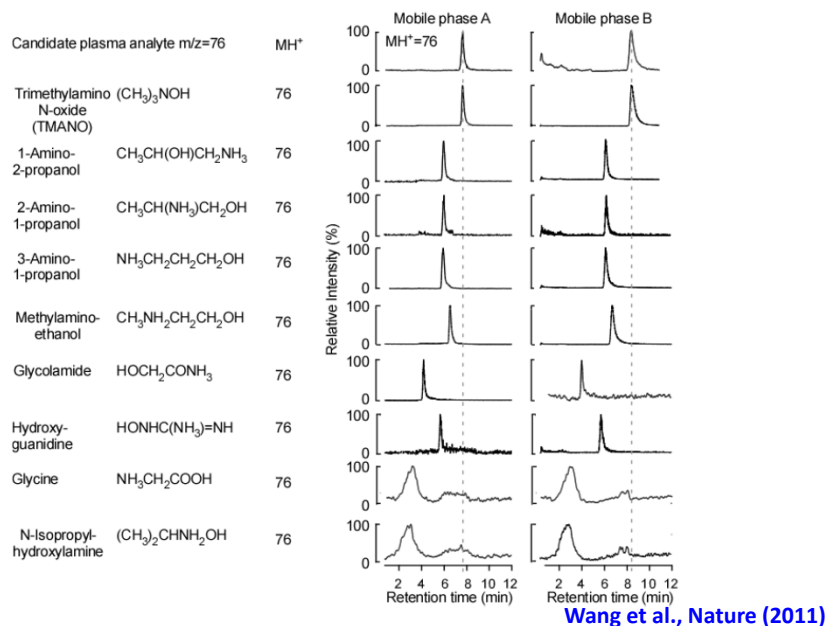
- Three metabolites stood out
 - m/z 76, 104 and 118
 - Across the different patients, these metabolites were the most intercorrelated.



?Participation in a common pathway

Wang et al., Nature (2011)

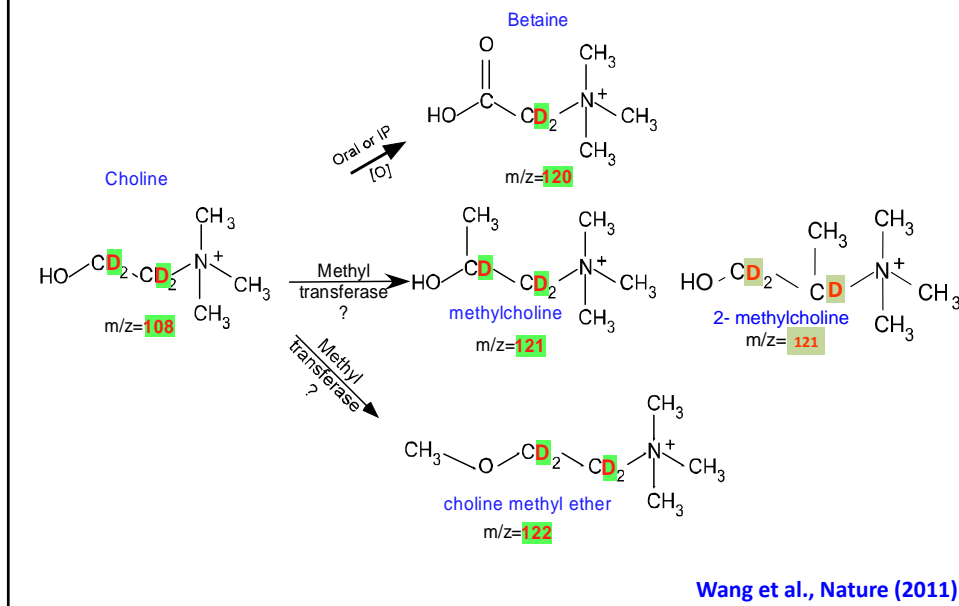
Identifying the m/z 76 ion



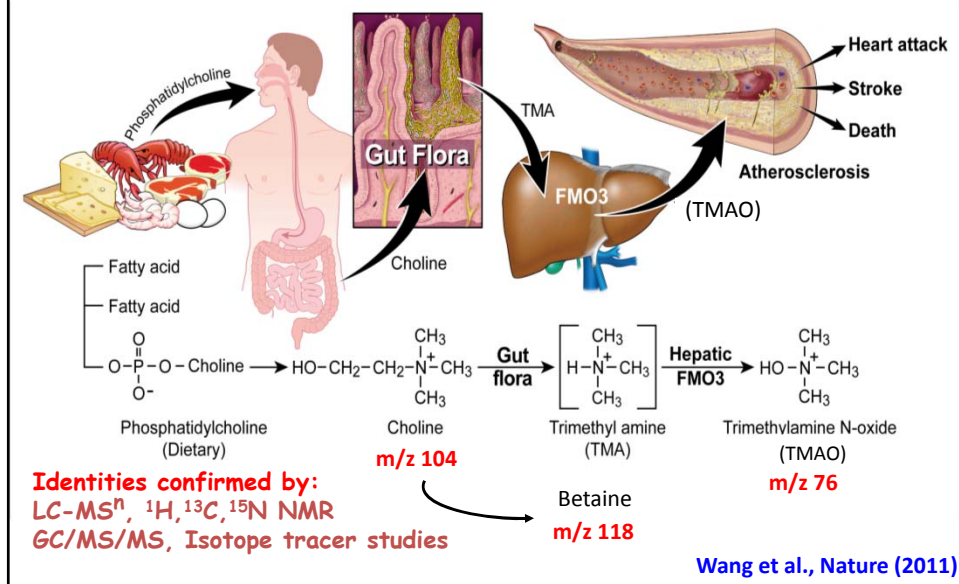
Important points to make

- The ion was noted as m/z 76
 - Must contain 1 N (or another odd number) atom
 - 3 of the 9 possibilities would have had a different m/z value
 - Glycine and glycolamide (C₂H₆NO₂) – m/z 76.076
 - Hydroxyguanidine (CH₆N₃O) – m/z 76.051
 - Others (C₃H₁₀NO) – m/z 76.039
- The chromatographic property of the metabolite is as important as the mass spec data

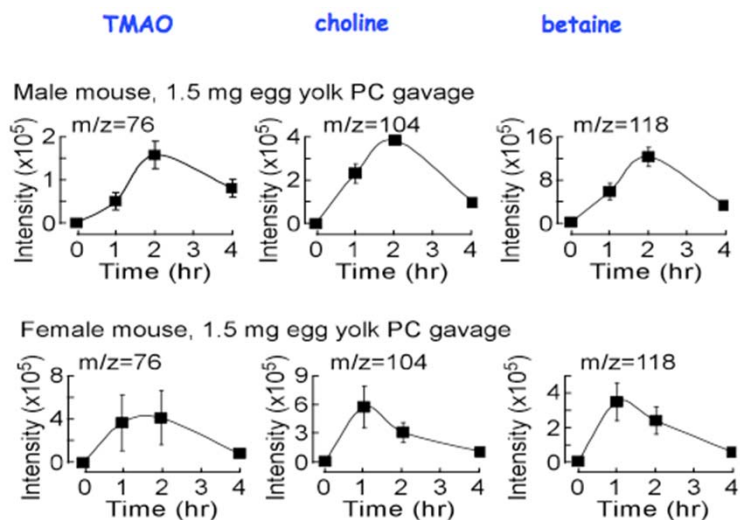
Using isotopes to identify m/z 118



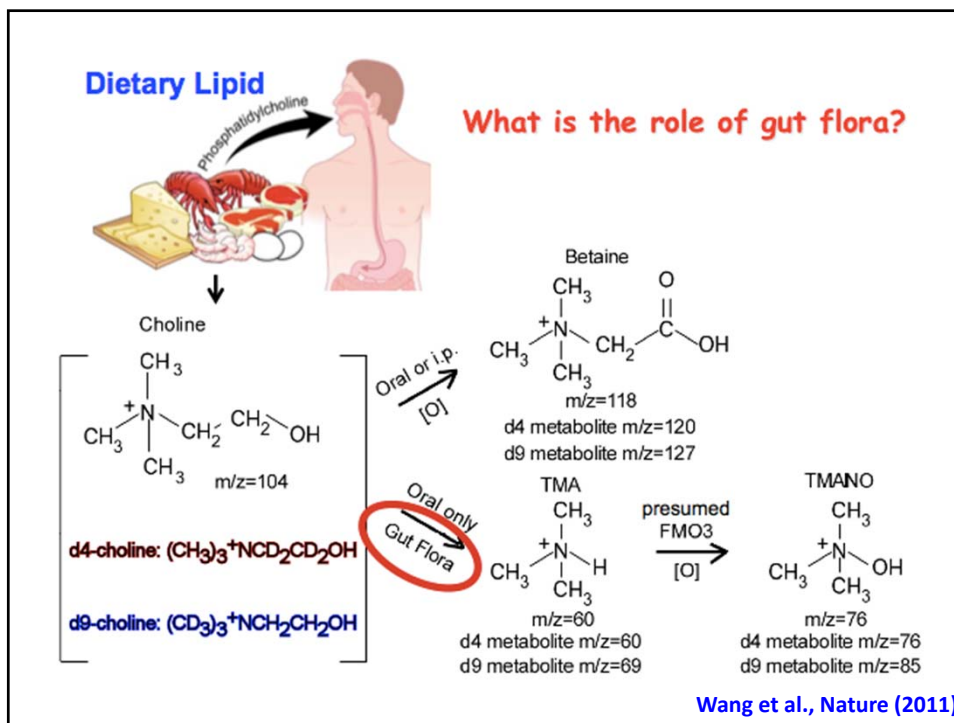
Choline, betaine and trimethylamine-N-oxide are plasma analytes associated with CVD



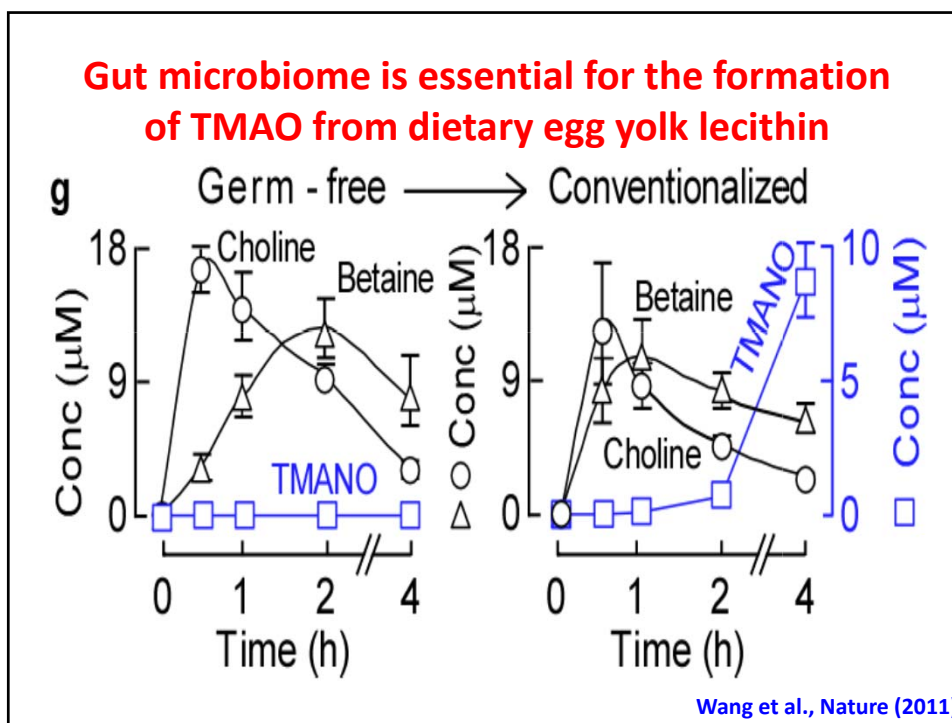
Ingestion of egg yolk PC produces increases in plasma TMAO, choline and betaine



Wang et al., Nature (2011)



Wang et al., Nature (2011)



Summary and remarks

- **Untargeted metabolomics needs careful planning**
 - Selection of the subject groups
 - Selection of the part of the metabolome to analyze
 - Instrument (LC and MS type)
- **Even the simplest looking metabolite may not be what you think it is**
 - Think out of the box (into the microbiomes)