

*Knowledge that will change your world*

## **Untargeted and Translational Metabolomics**

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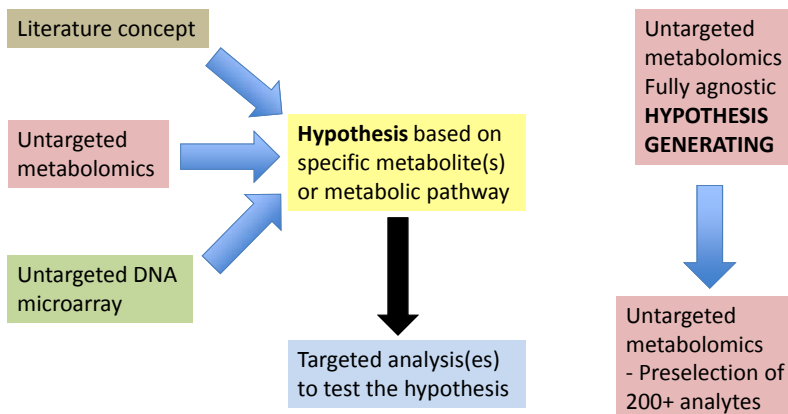
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### **Rationale for untargeted metabolomics**

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

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## Targeted vs Untargeted metabolomics



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## 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

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## 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. Lusa, and Stanley L. Hazen

A bellwether paper for the development of metabolomics

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## 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

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Wang et al., Nature (2011)

**How were the patients matched?**

**Where did the patients come from?**

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**How were the samples treated?**

**What was the analytical platform?**

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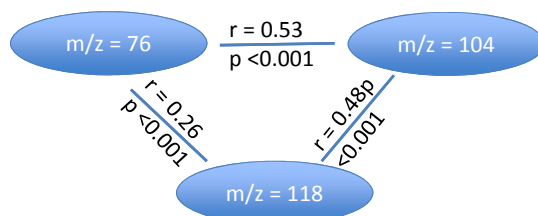
**How were the metabolite data statistically treated and validated?**

**How were the critical metabolites selected for follow up?**

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## 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.

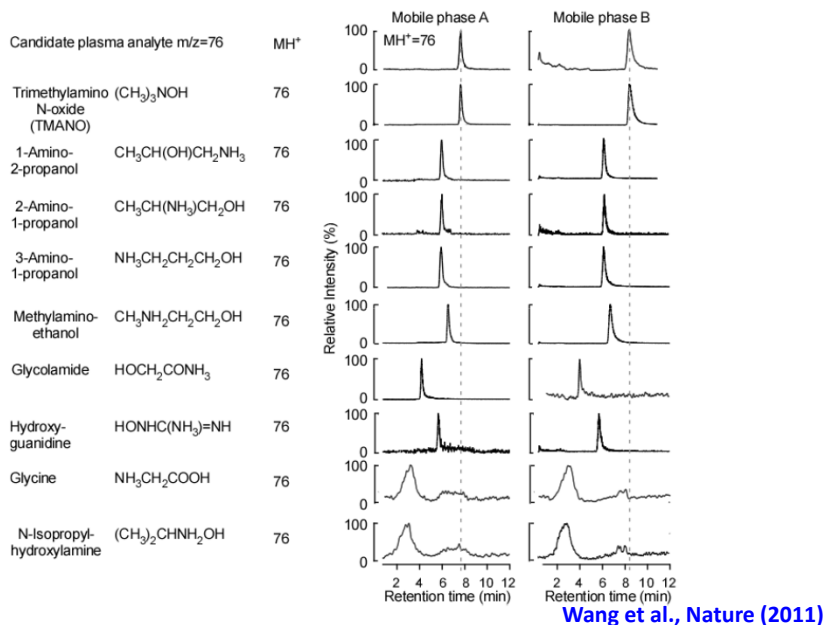


?Participation in a common pathway

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Wang et al., Nature (2011)

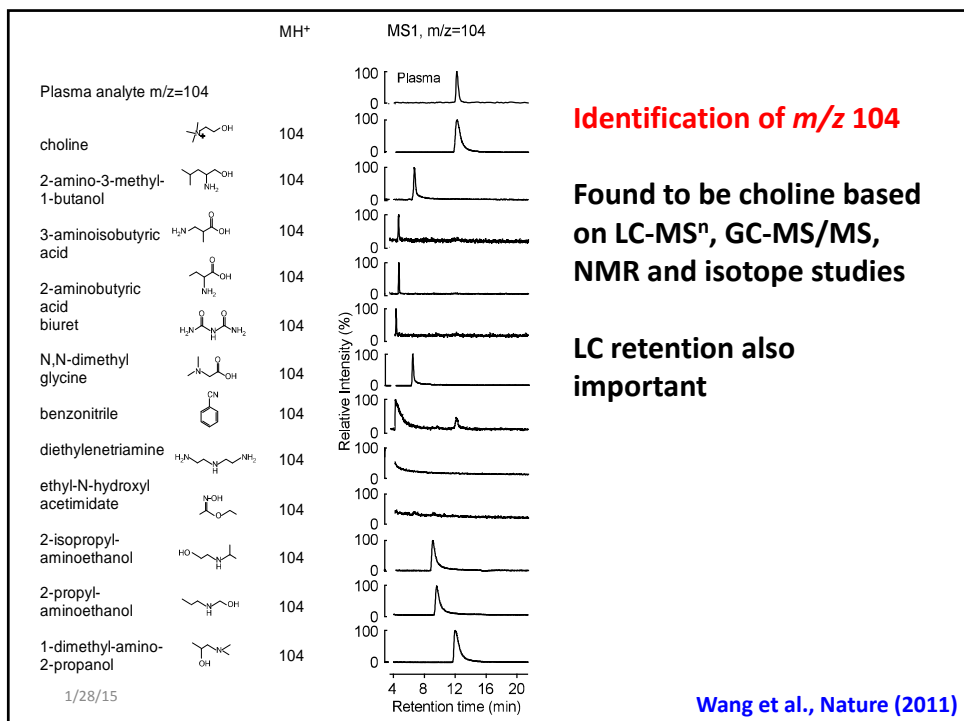
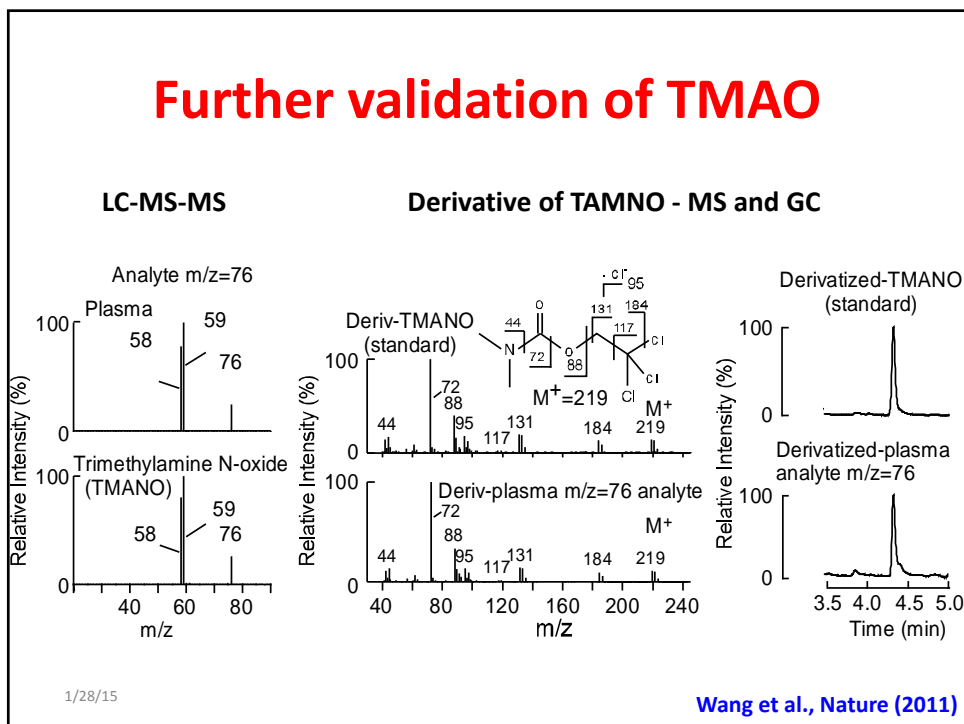
## Identifying the $m/z$ 76 ion



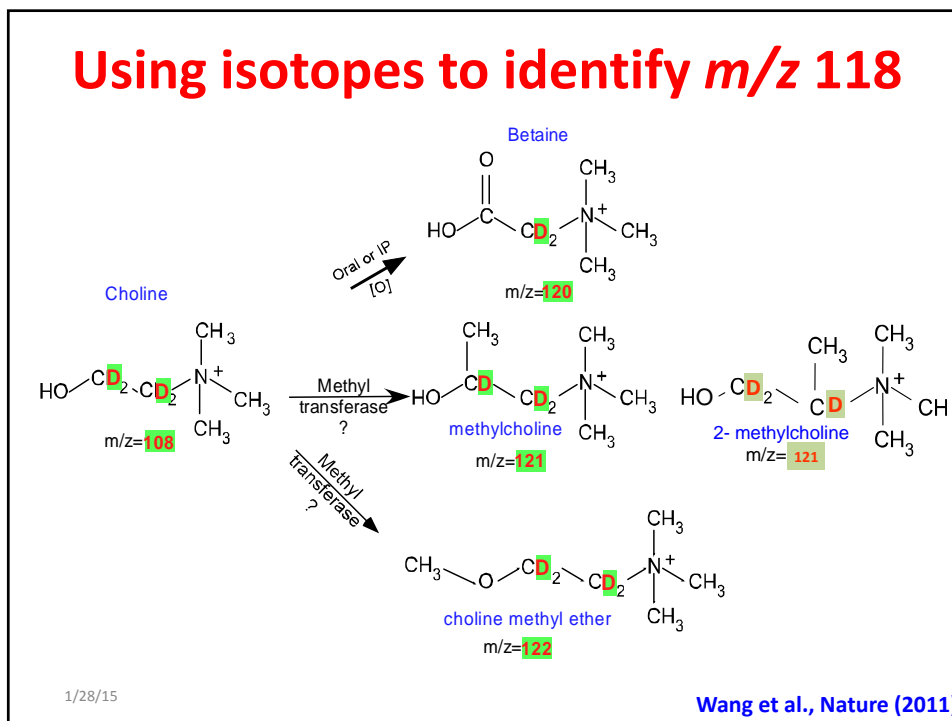
## Important points to make

- 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 ( $C_2H_6NO_2$ ) –  $m/z$  76.076
    - Hydroxyguanidine ( $CH_6N_3O$ ) –  $m/z$  76.051
    - Others ( $C_3H_{10}NO$ ) –  $m/z$  76.039
- The chromatographic property of the metabolite is as important as the mass spec data

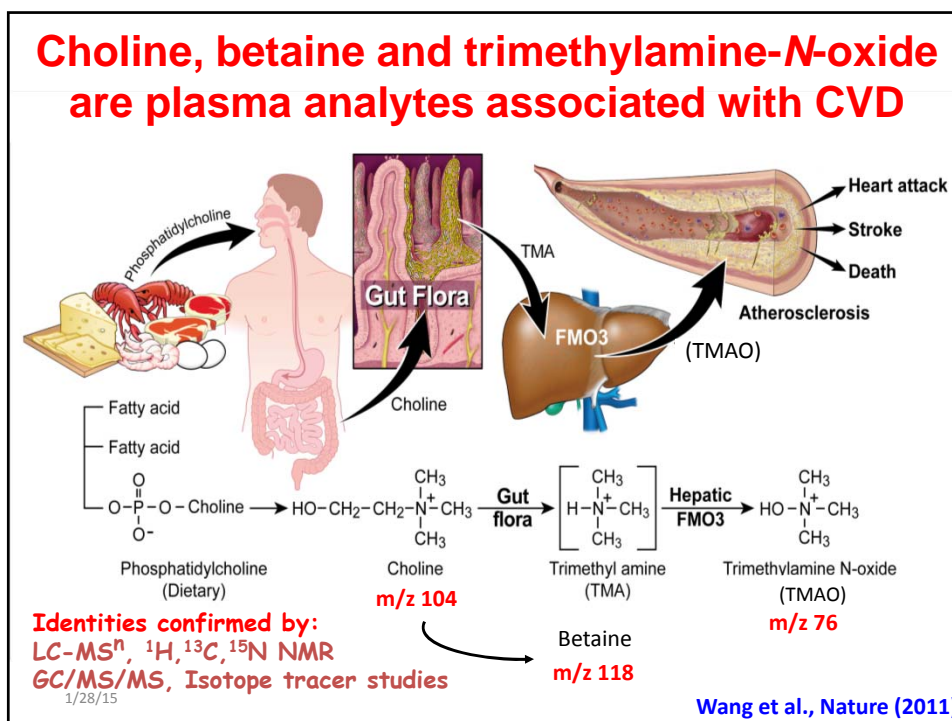
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## Using isotopes to identify $m/z$ 118

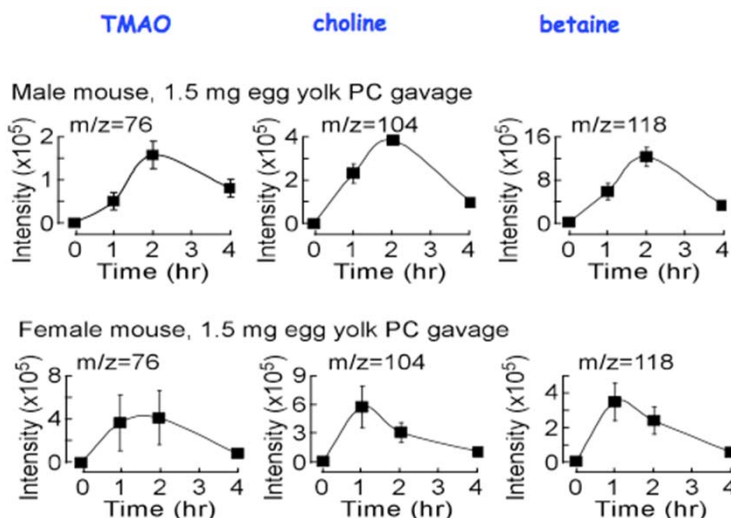


## Choline, betaine and trimethylamine-N-oxide are plasma analytes associated with CVD





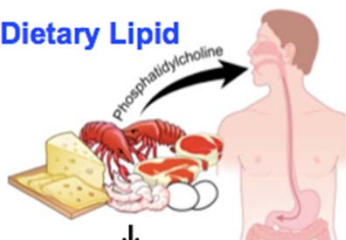
## Ingestion of egg yolk PC produces increases in plasma TMAO, choline and betaine



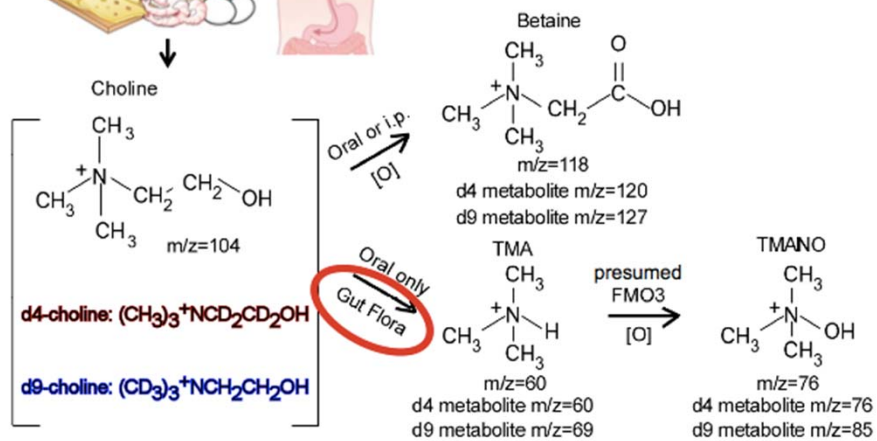
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Wang et al., Nature (2011)

### Dietary Lipid

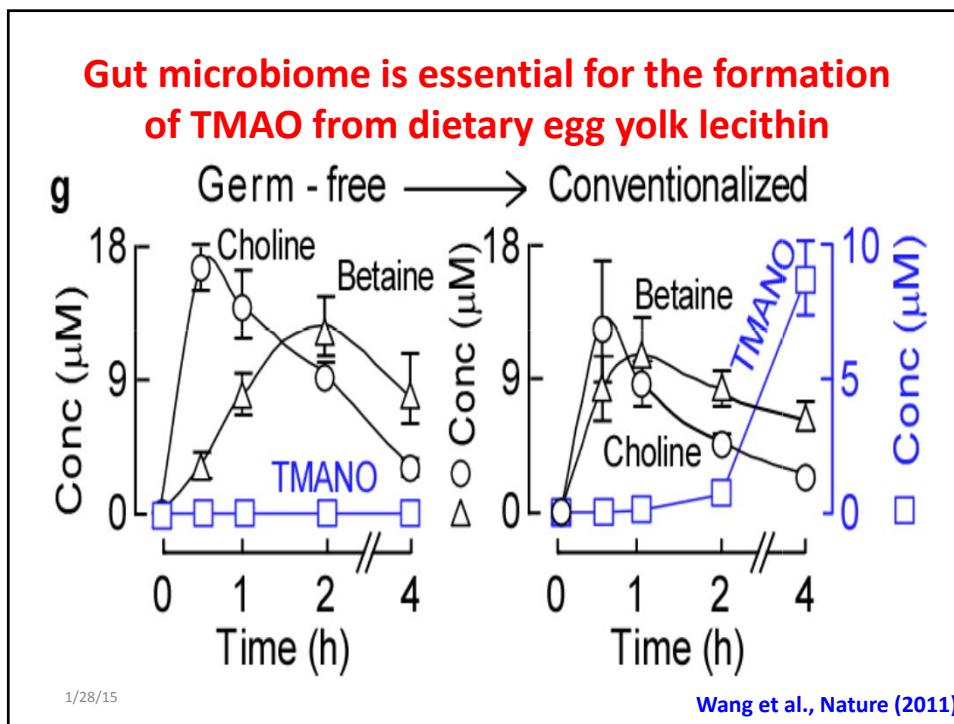


### What is the role of gut flora?



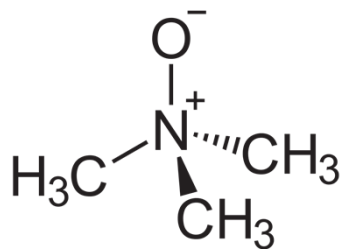
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Wang et al., Nature (2011)



## What is TMAO?

- It is an osmolyte in fish, particularly deep sea fish
- Used in the fish as protein stabilizer
- Degrades to trimethylamine in rotting fish



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**What is the fate of TMAO when eating fish or eggs?**

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## **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)

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