Knowledge that will change your world

Untargeted and Translational Metabolomics

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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
- We also eat metabolites and metabolite precursors from other genomes
- Metabolites are not predictable
- Cannot limit analysis of the metabolome

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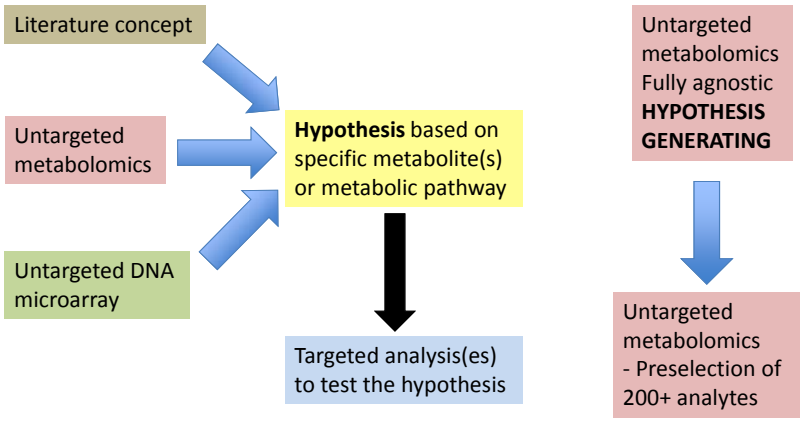
Targeted vs Untargeted metabolomics



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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**
- **Some metabolites come from common, even familiar, precursors**

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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. Lusis, and **Stanley L. Hazen**

A bellwether paper for the development of metabolomics

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Metabolites and Major Adverse Cardiovascular Events

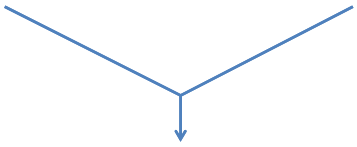
Selection of 75 patients and 75 matched controls

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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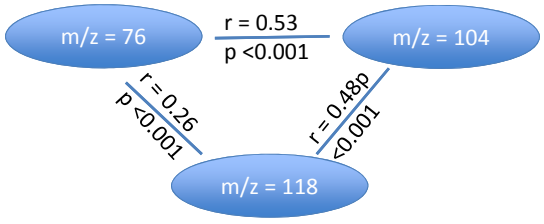
Choice of metabolites to follow up

- **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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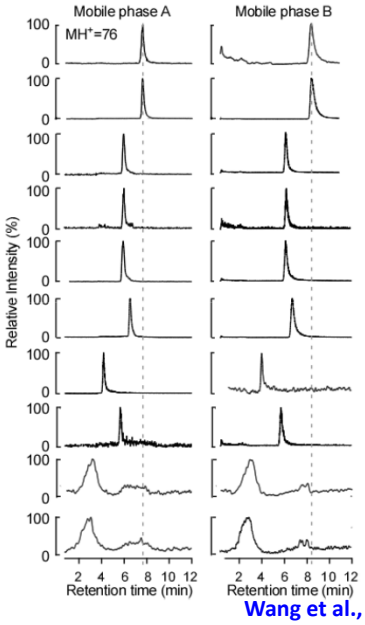
Wang et al., Nature (2011)

Identifying the *m/z* 76 ion

Candidate plasma analyte m/z=76		MH^+
Trimethylamino N-oxide (TMANO)	$(CH_3)_3NOH$	76
1-Amino-2-propanol	$CH_3CH(OH)CH_2NH_3$	76
2-Amino-1-propanol	$CH_3CH(NH_3)CH_2OH$	76
3-Amino-1-propanol	$NH_3CH_2CH_2CH_2OH$	76
Methylamino-ethanol	$CH_3NH_2CH_2CH_2OH$	76
Glycolamide	$HOCH_2CONH_3$	76
Hydroxy-guanidine	$HONHC(NH_3)=NH$	76
Glycine	NH_3CH_2COOH	76
N-Isopropyl-hydroxylamine	$(CH_3)_2CHNH_2OH$	76



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Wang et al., Nature (2011)

Important points to make

- **The ion was noted as *m/z* 76**
  - **Must contain 1 N (or another odd number) atom**
    - **Nitrogen rule**
  - **3 of the 9 possibilities would have had a different *m/z* value**
    - **Glycine and glycolamide ($C_2H_6NO_2$) – *m/z* 76.039**
    - **Hydroxyguanidine (CH_6N_3O) – *m/z* 76.051**
    - **Others ($C_3H_{10}NO$) – *m/z* 76.076**
- **The chromatographic property of the metabolite is as important as the mass spec data**

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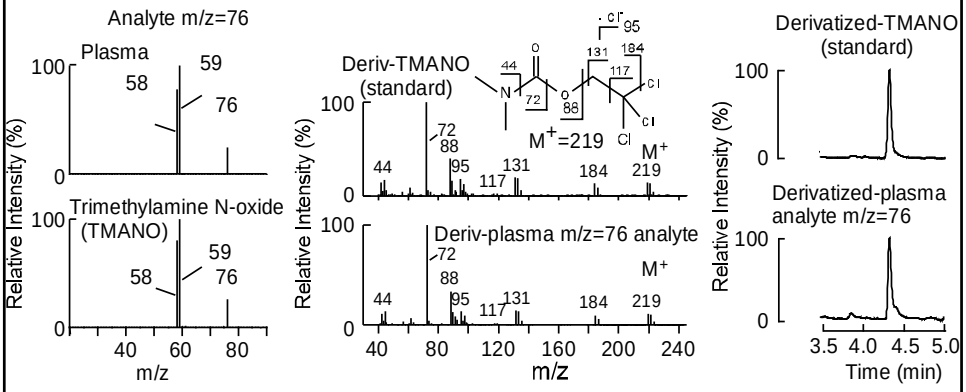
Further validation of TMAO

LC-MS-MS | **Derivative of TAMNO - MS and GC**



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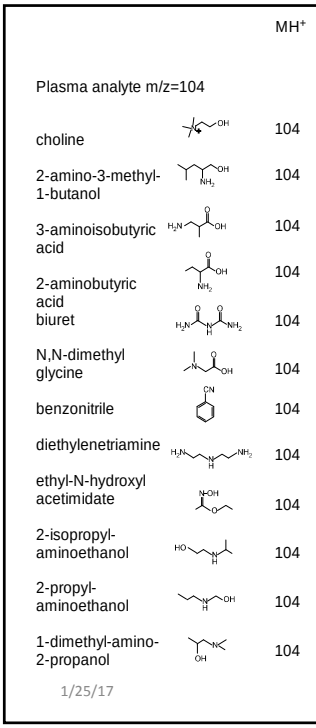
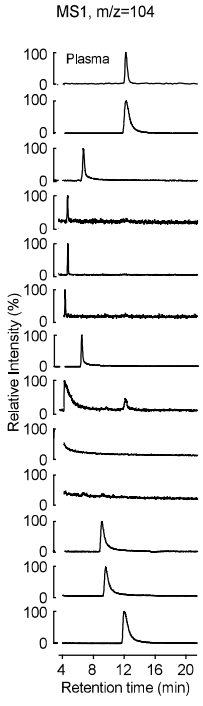
Wang et al., Nature (2011)





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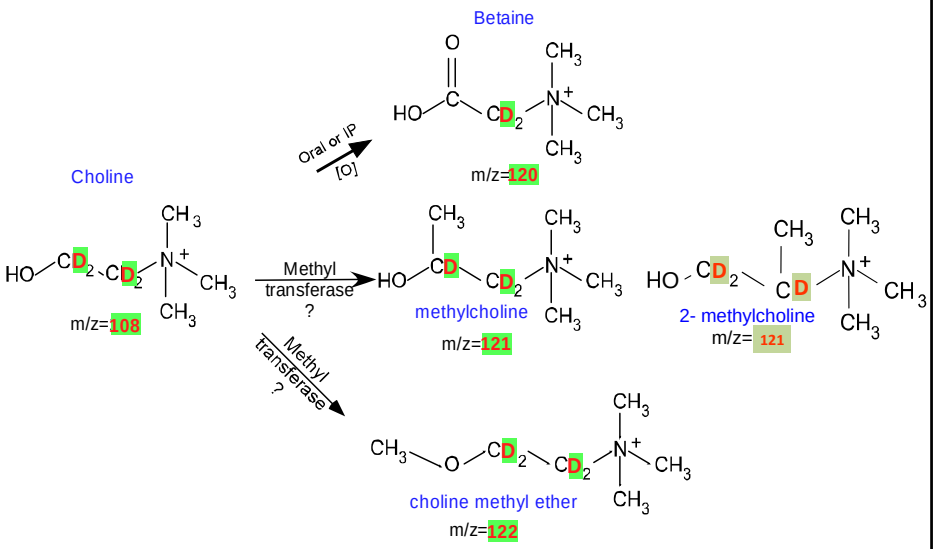
Identification of *m/z* 104

Found to be choline based on LC-MS^n, GC-MS/MS, NMR and isotope studies

LC retention also important

Wang et al., Nature (2011)

Using isotopes to identify *m/z* 118



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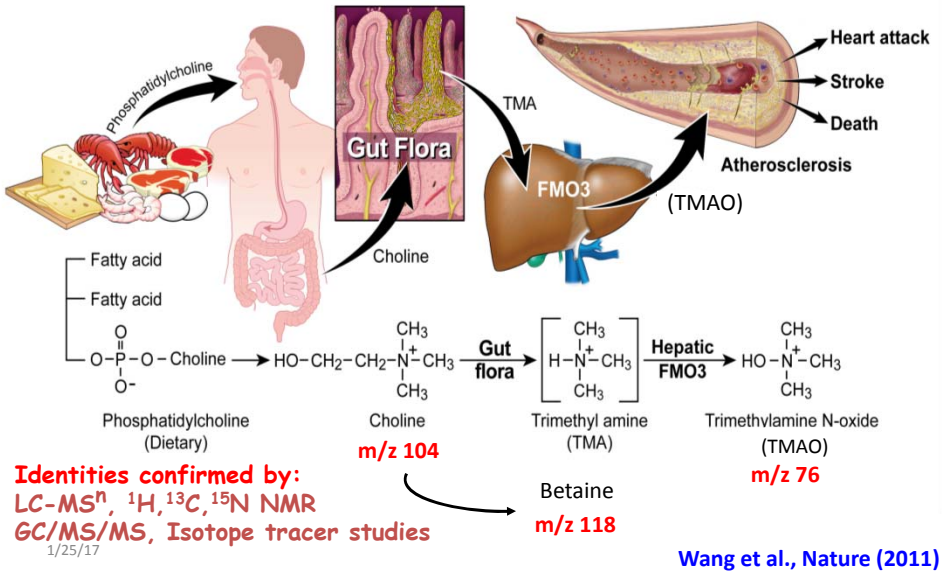
Wang et al., Nature (2011)

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



Identities confirmed by:
LC-MS^n, ^{1}H, ^{13}C, ^{15}N NMR
GC/MS/MS, Isotope tracer studies

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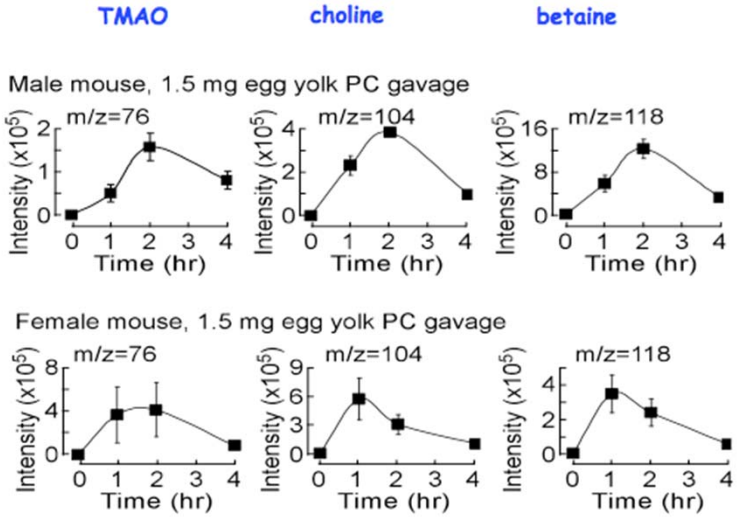
Wang et al., Nature (2011)

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



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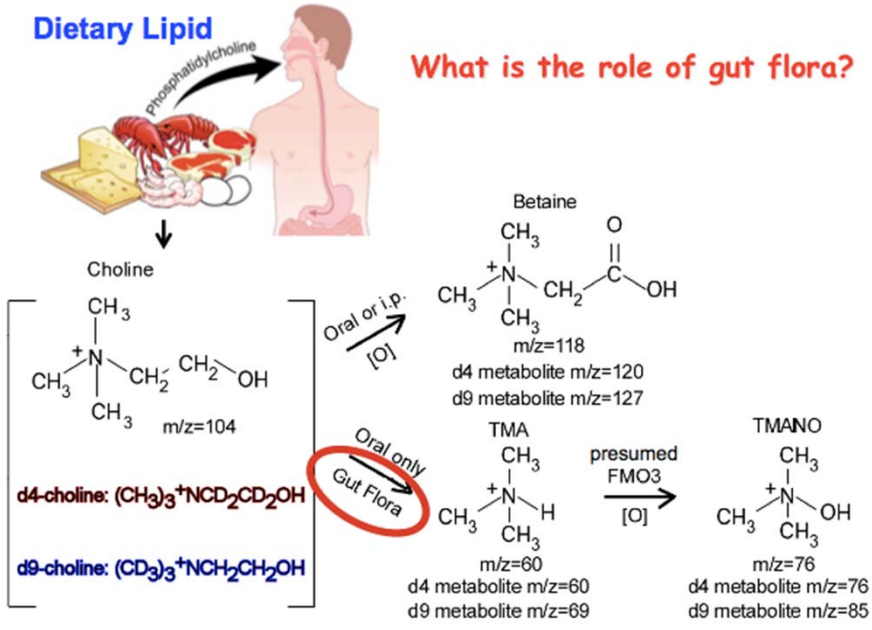
Wang et al., Nature (2011)



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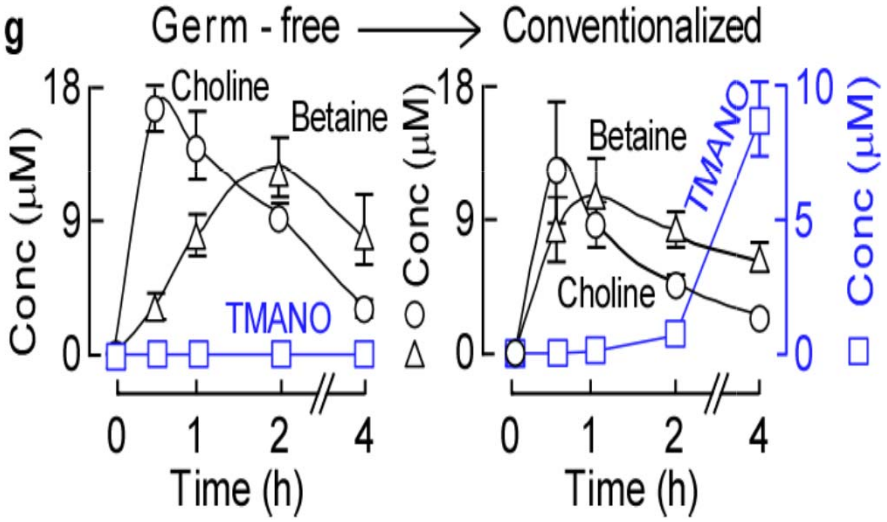
Wang et al., Nature (2011)

Gut microbiome is essential for the formation of TMAO from dietary egg yolk lecithin



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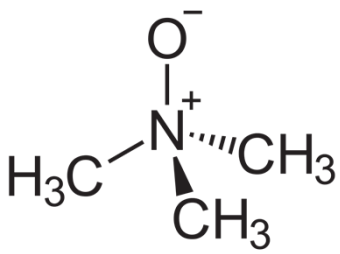
Wang et al., Nature (2011)

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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Videos of Dr. Hazen's UAB talks

- http://www.uab.edu/proteomics/metabolomics/workshop/2014/videos/hazen2_2.html
- http://www.uab.edu/proteomics/metabolomics/workshop/2014/videos/hazen_qa.html

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So, is TMAO causative, as opposed to associative, of heart disease risk?

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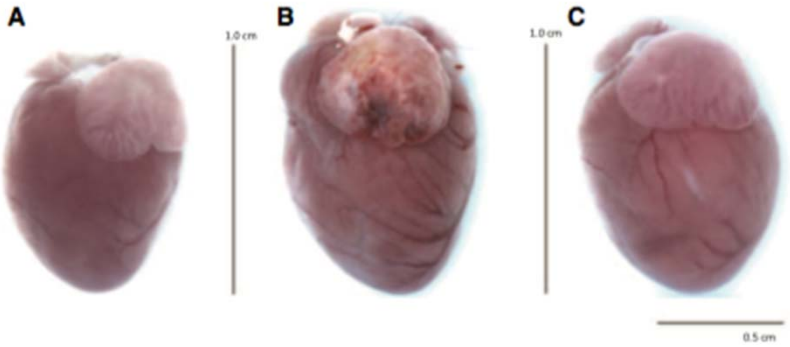
Both TMAO and choline cause cardiac hypertrophy



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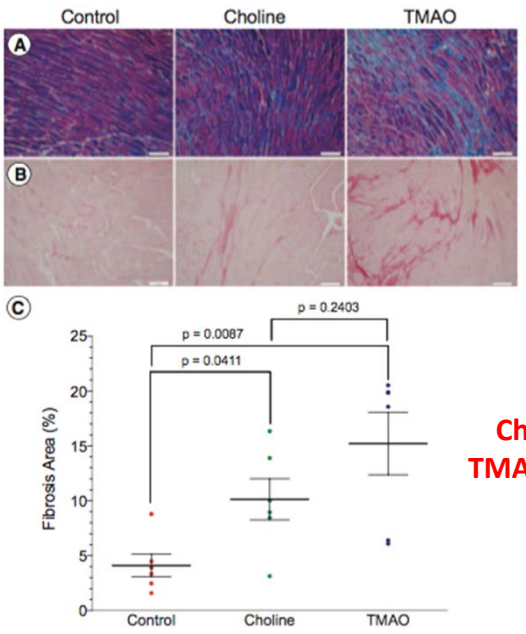
Wang et al. 2016



Choline and particularly TMAO cause cardiac fibrosis

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Wang et al. 2016

Remarks in the Discussion by Wang et al.

- **"(A) limitation of the present study is we do not know at the molecular level how TMAO exerts its adverse effects on cardiac remodeling, fibrosis, and function,**
- **and whether there is a specific TMAO receptor or is acting via its known effect on protein conformation and stability."**

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Wang et al. 2016