Metabolomics Studies Reveal New Pathways in Cardiovascular Disease with Potential for Diagnostics and Therapeutic Targeting

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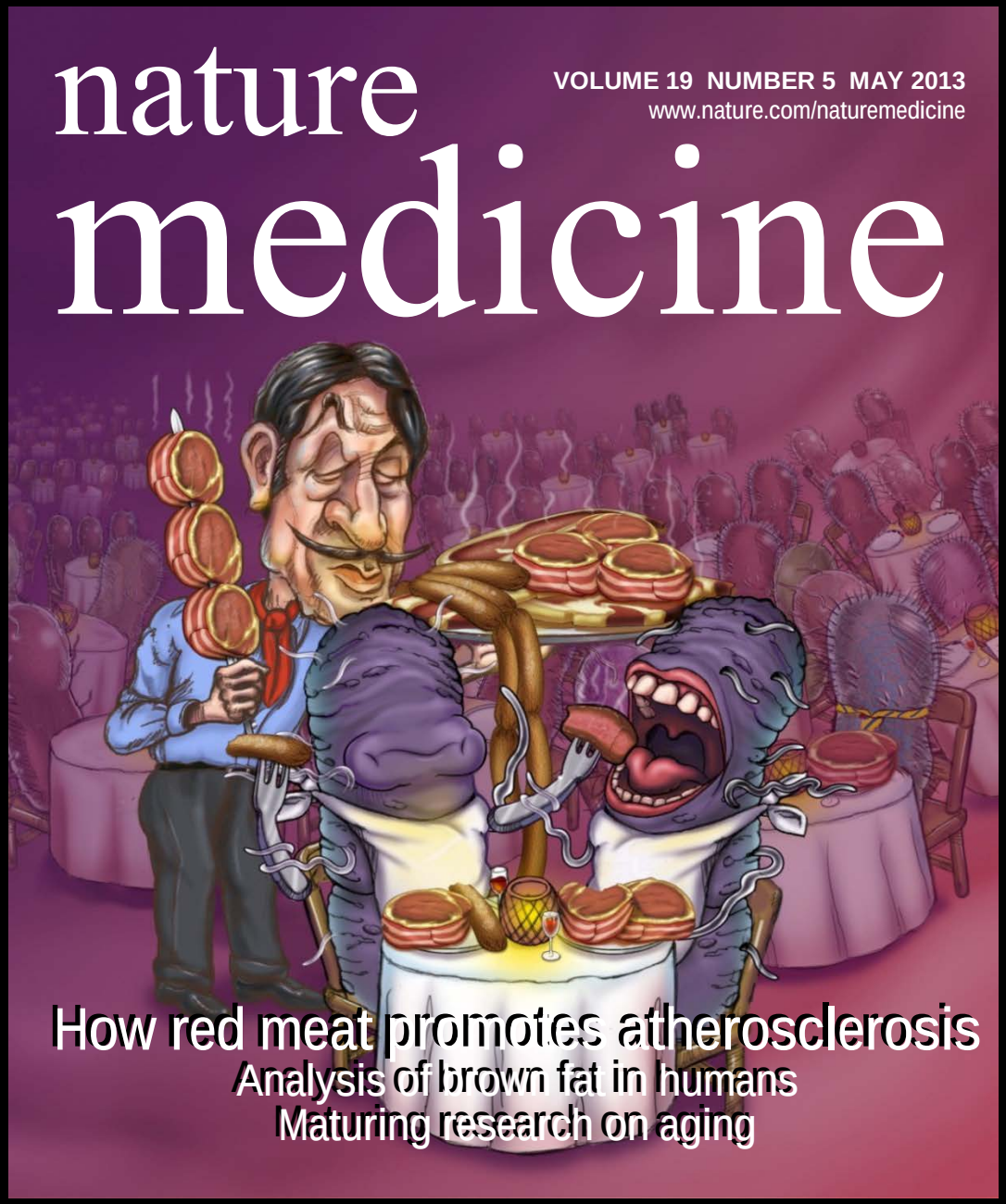
Section Head, Preventive Cardiology, Cleveland Clinic



Disclosure Information

Stanley L. Hazen, MD, PhD

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Abbott Laboratories, Cleveland Heart Lab (CHL), Esperion, and LipoScience.

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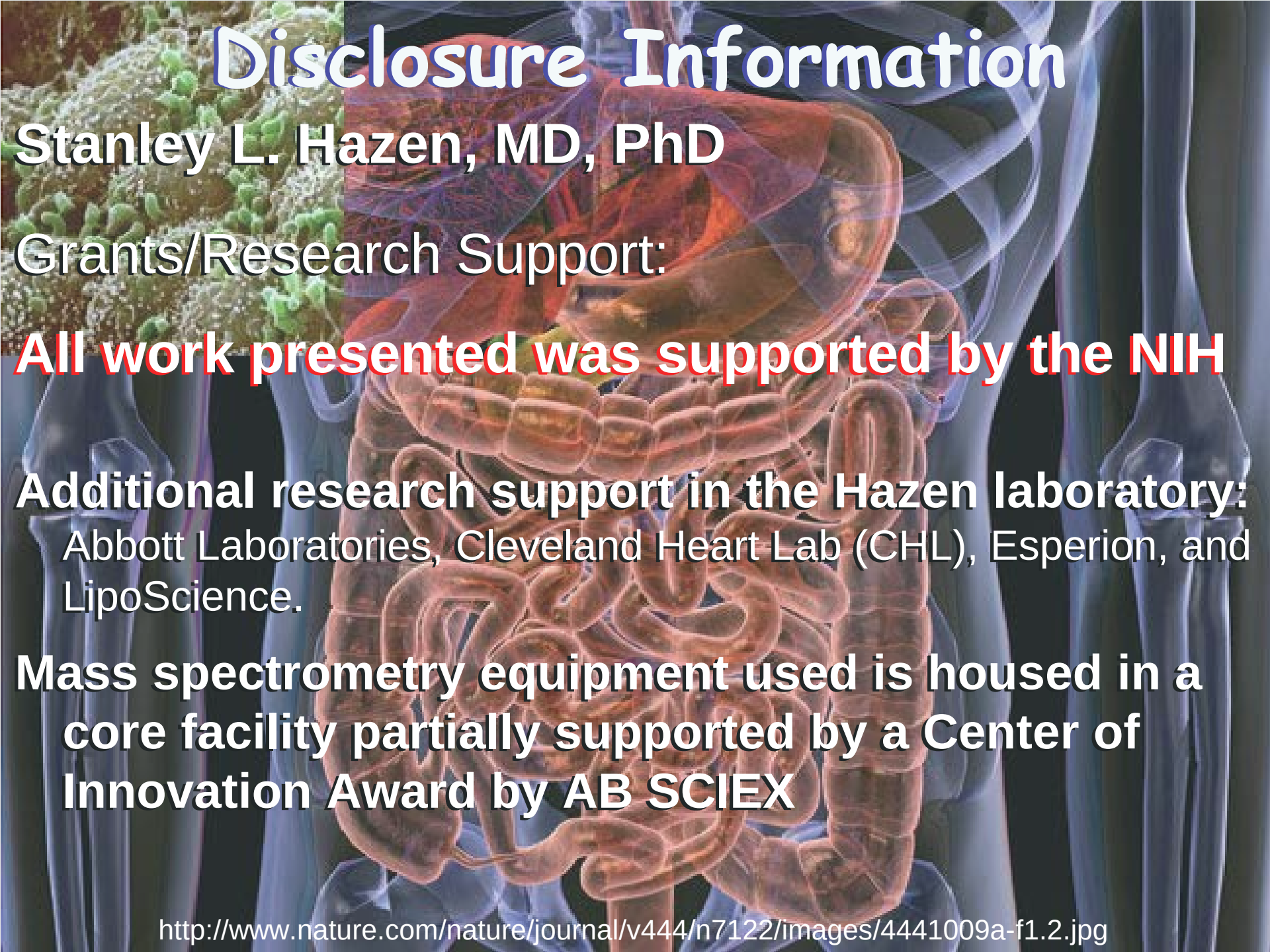


http://www.nature.com/nature/journal/v444/n7122/images/4441009a-f1.2.jpg

Phase 1: Discovery-based investigations

Metabolomics screening and structural identification

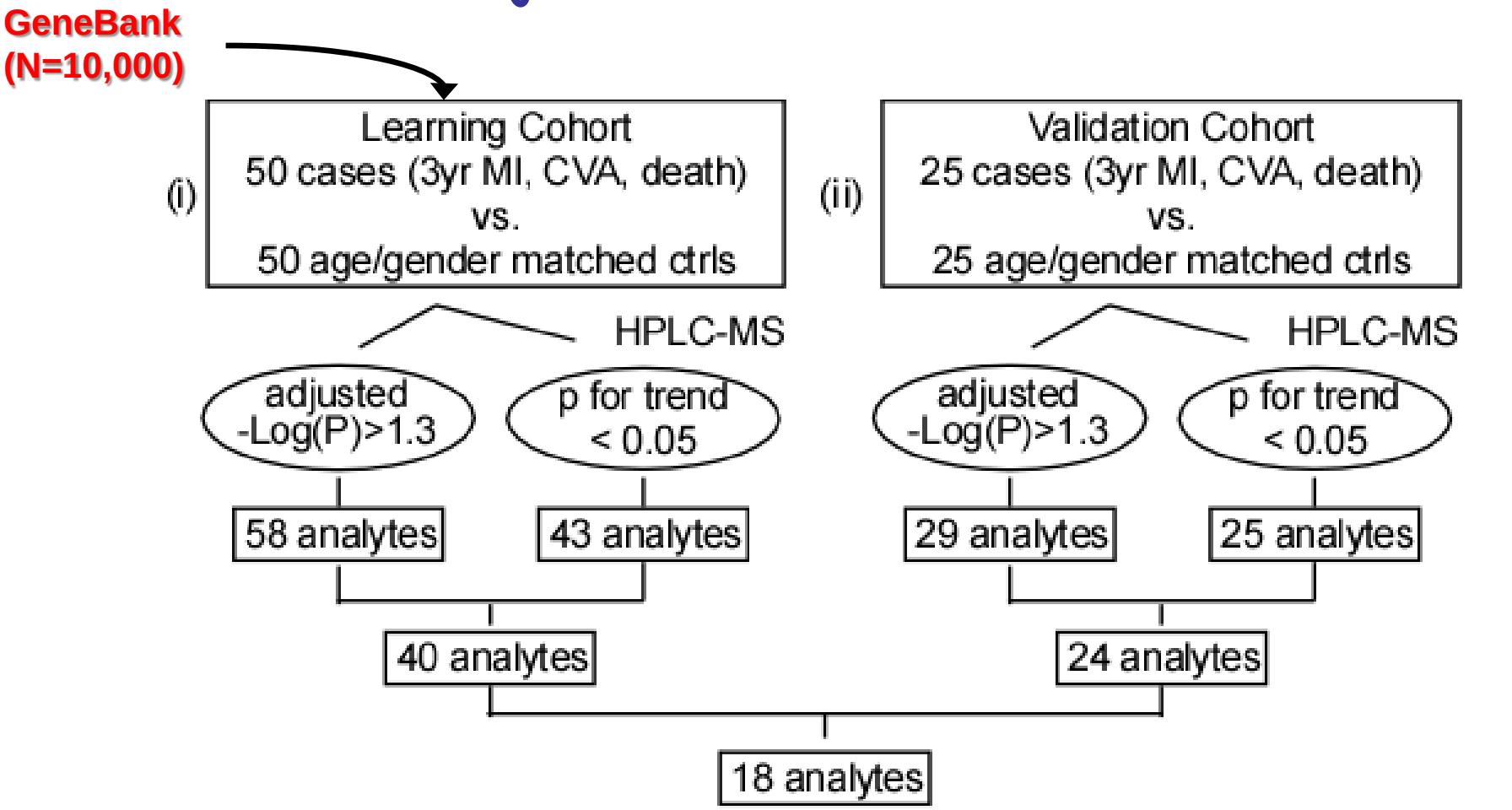
Phase 2: Clinical validation

Replication and demonstration of clinical utility

Phase 3: Mechanistic studies

Demonstration of causality for a novel pathway

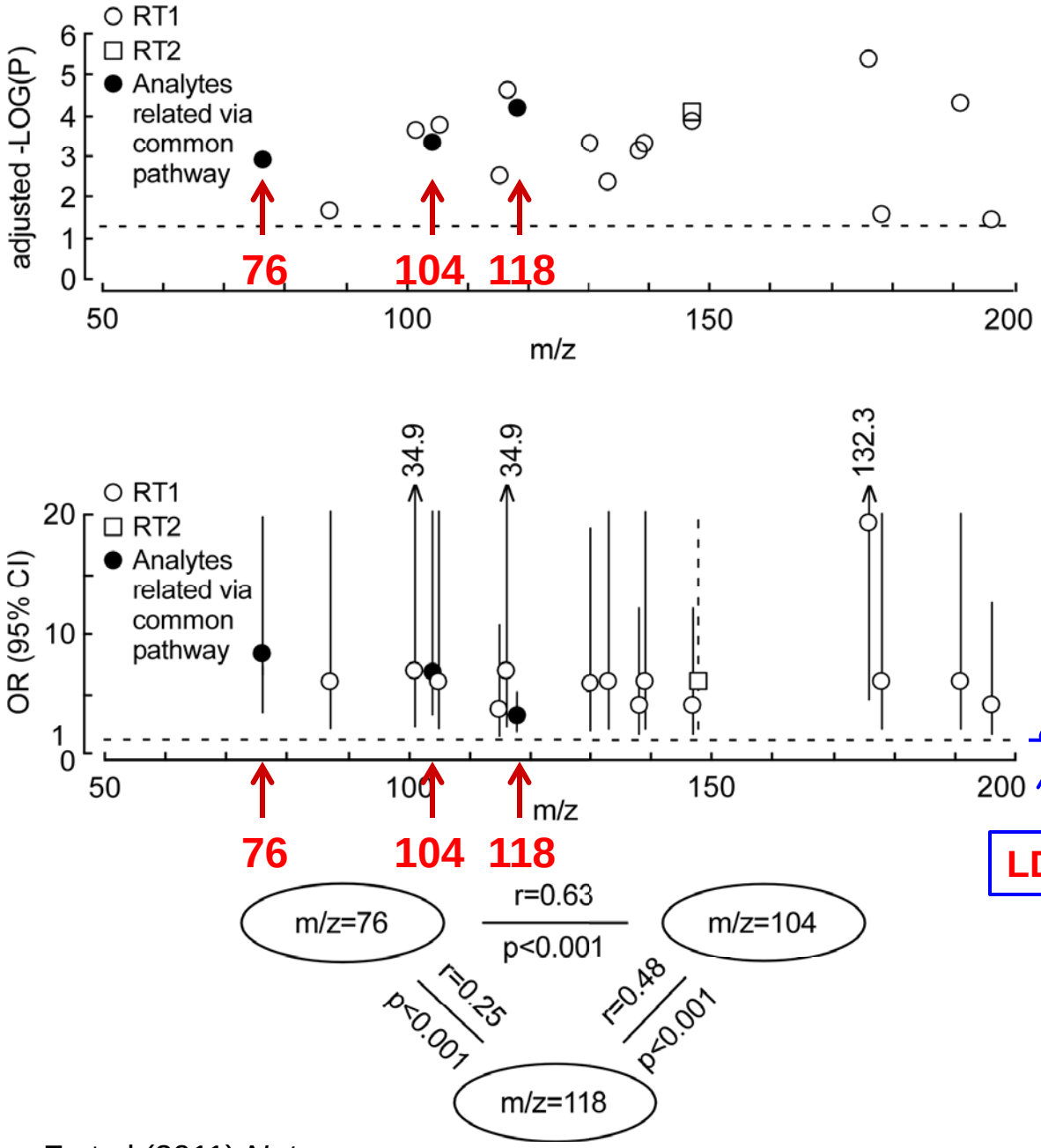
Strategy of metabolomics study design for identifying unbiased small molecule profiles predictive of incident risks for major adverse cardiovascular events



(iii) Structural identification of analytes

(iv) Confirm clinical prognostic utility in Independent Prospective Cohort (N>1000)

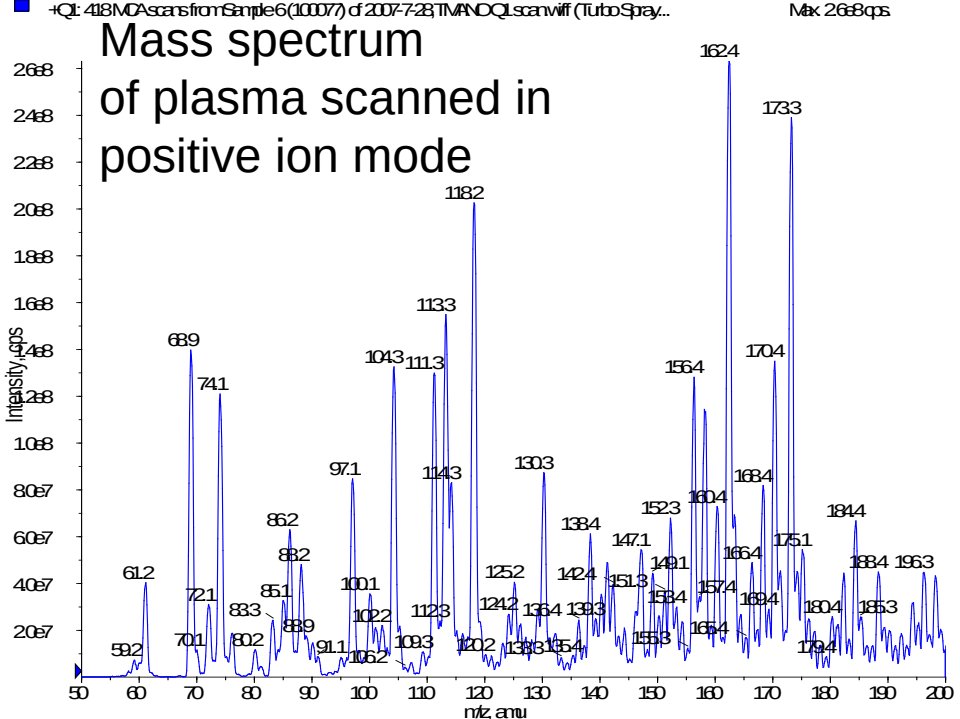
Wang Z et al (2011) *Nature*

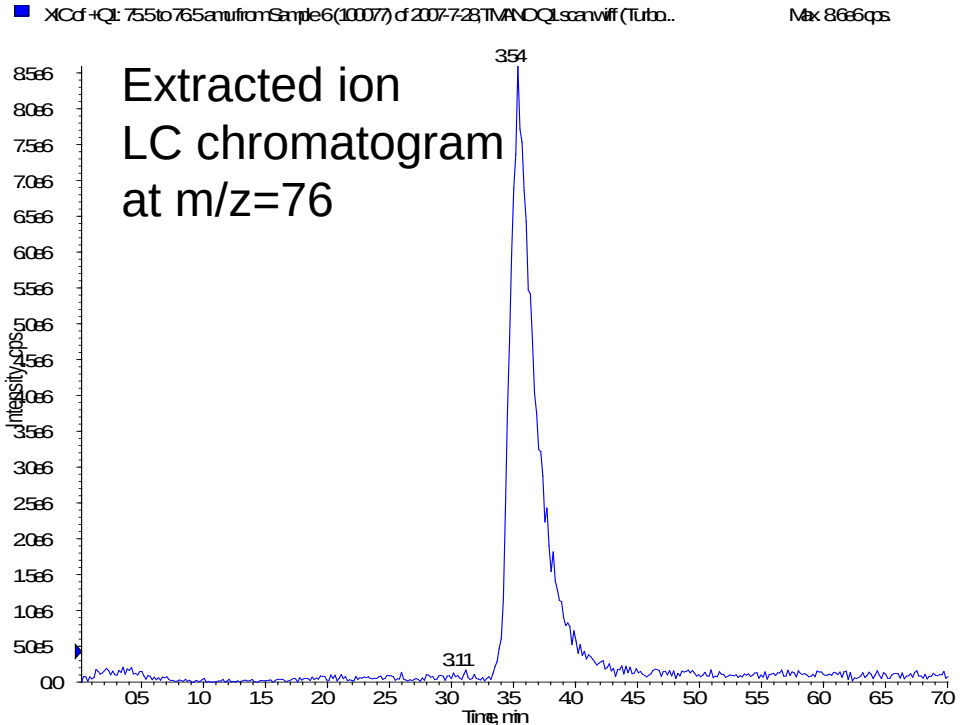


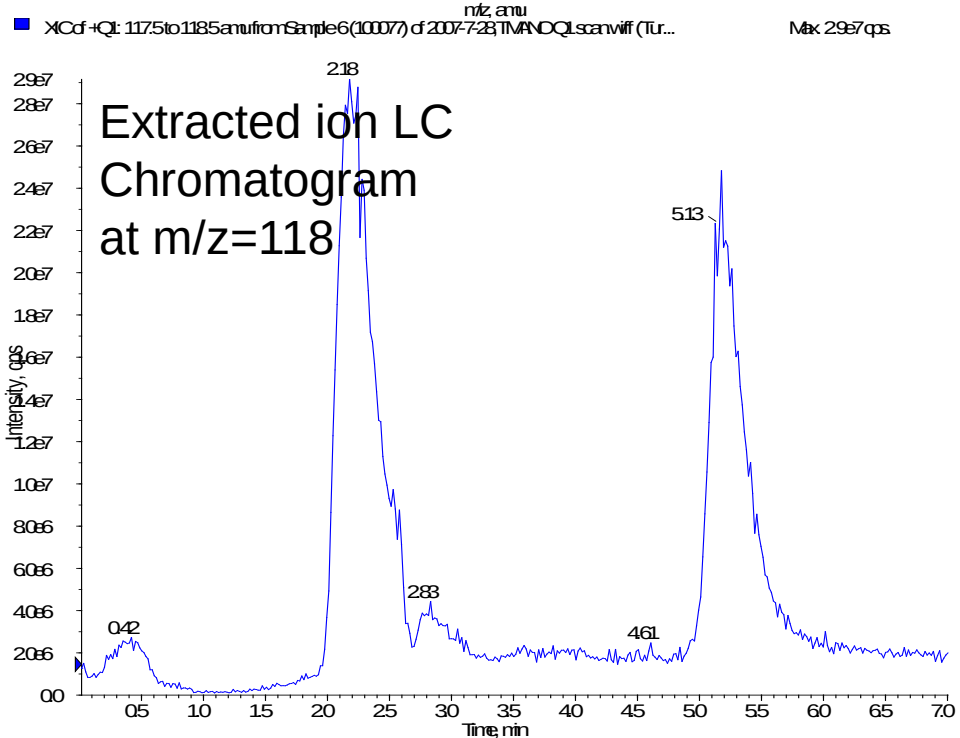
Plasma analytes with m/z 76, 104, and 118 are associated with CVD, show a dose-response relationship with MACE (3yr MI stroke or death) and are correlated, suggesting participation in a common pathway

Wang Z et al (2011) *Nature*

Example data from metabolomics study

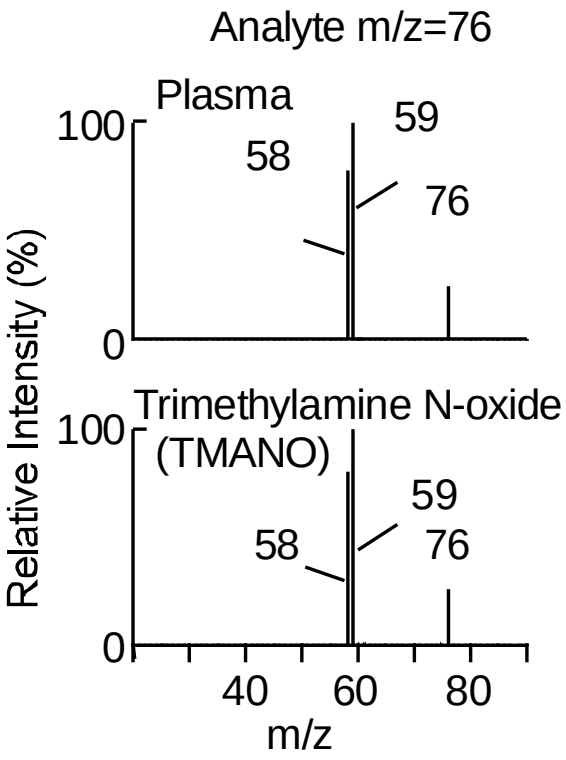




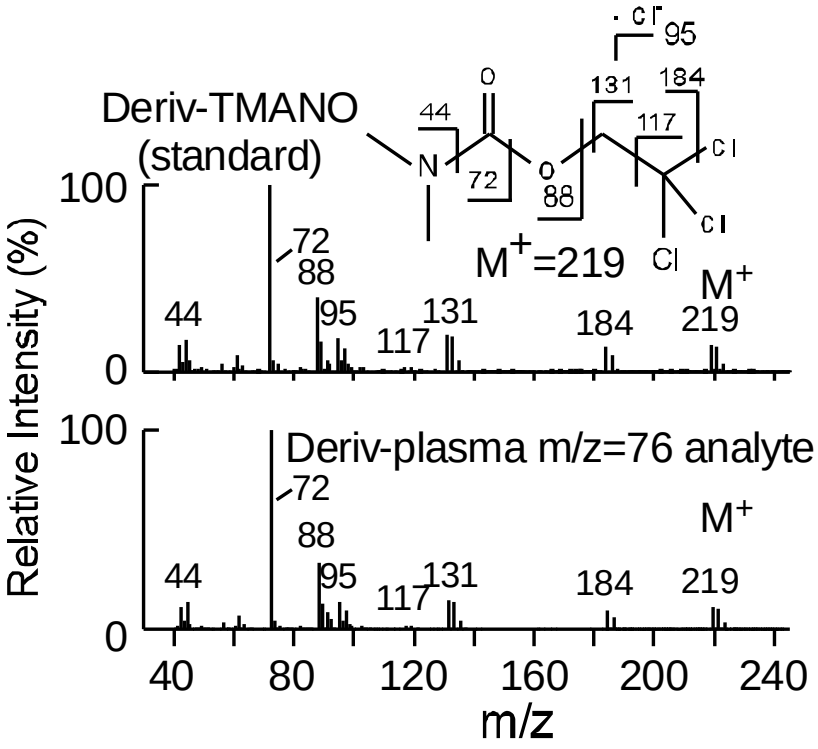


Structural identification/validation of plasma analyte at m/z=76 as TMANO (trimethylamine N-oxide)

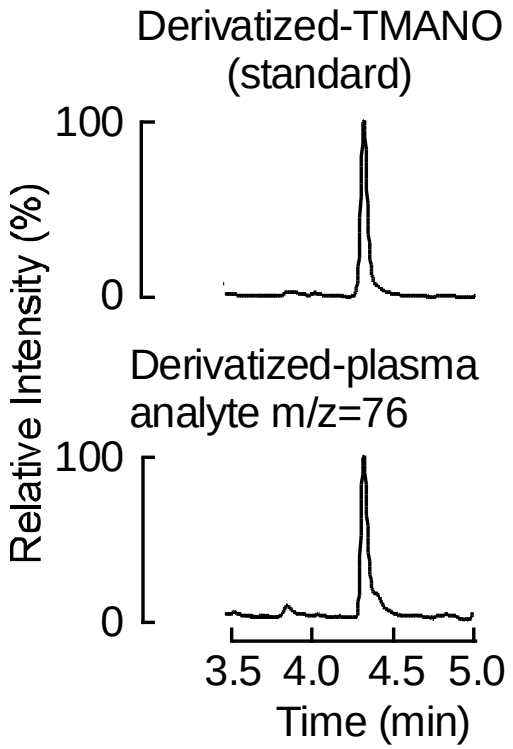
CID MS Spectrum



MS Deriv of TCEF

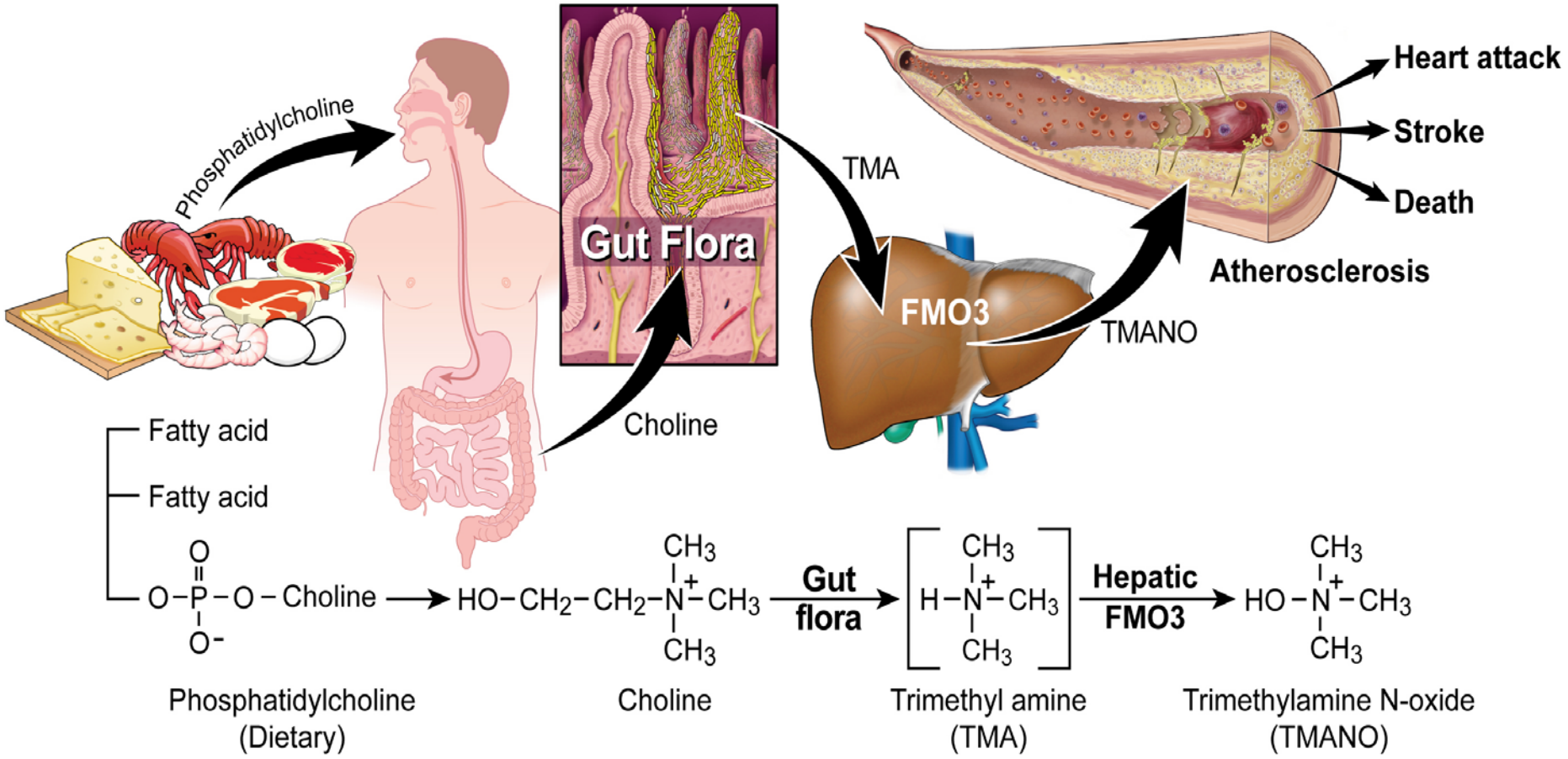


GC Chromatogram Of Deriv of TCEF



Wang Z et al, *Nature* (2011)

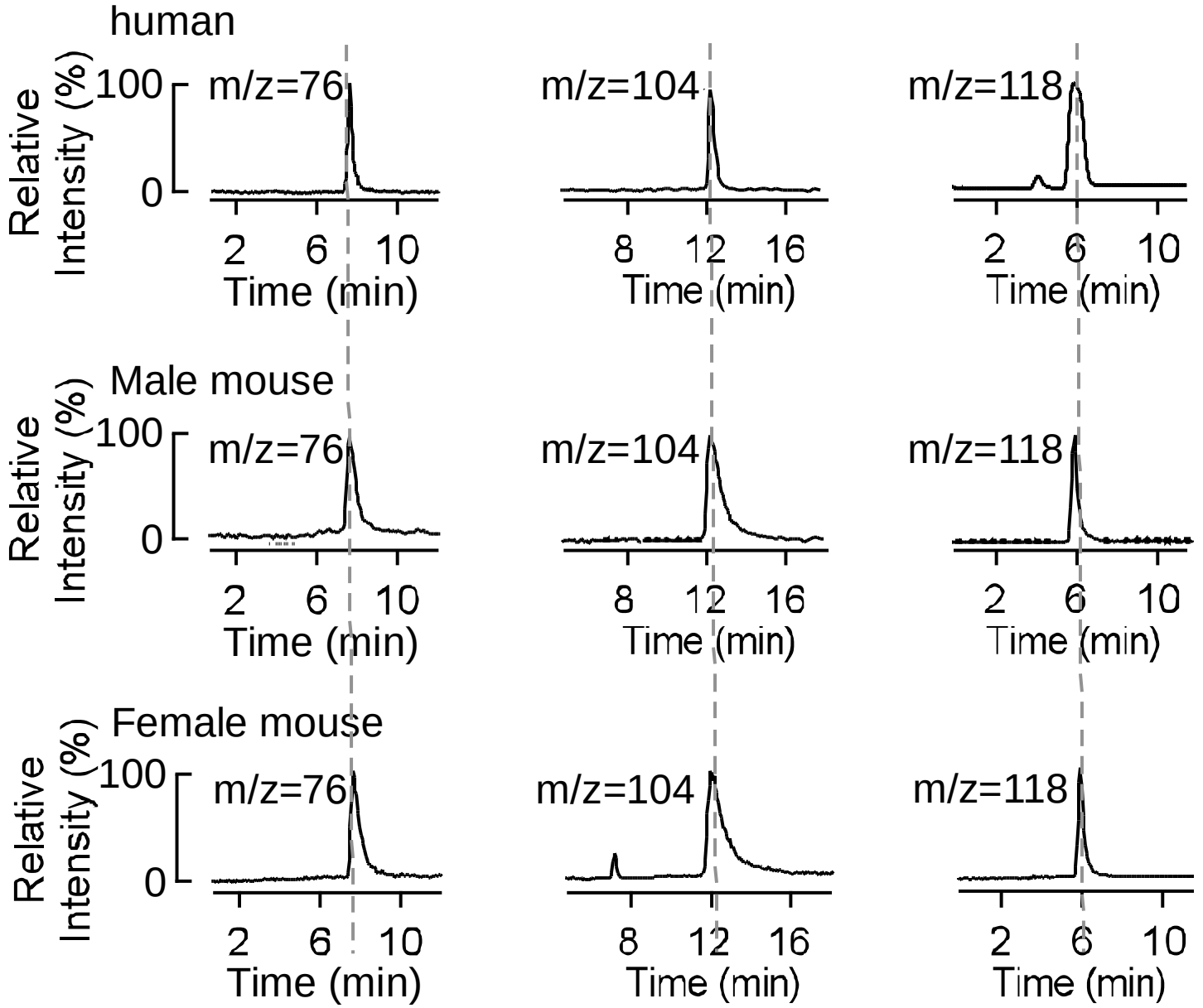
What is TMANO? - It is proposed to be a gut flora-dependent metabolite of dietary lecithin (phosphatidylcholine, PC)



Wang Z et al, *Nature* (2011)

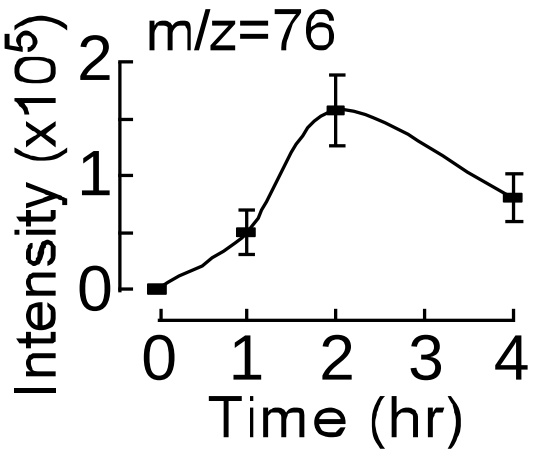
m/z 76

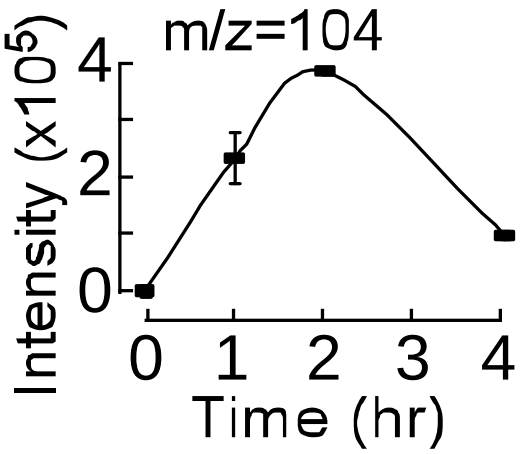
Dietary egg yolk PC produces increases in analytes with m/z 76, 104,and 118 in both human and mouse plasma

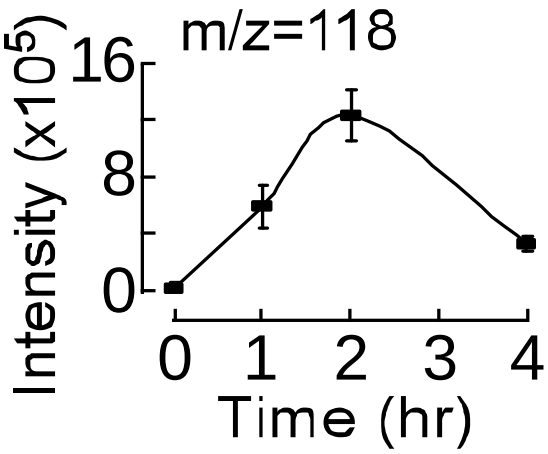


Dietary phosphatidylcholine enhances levels of the 3 analytes, indicating they are metabolites of PC

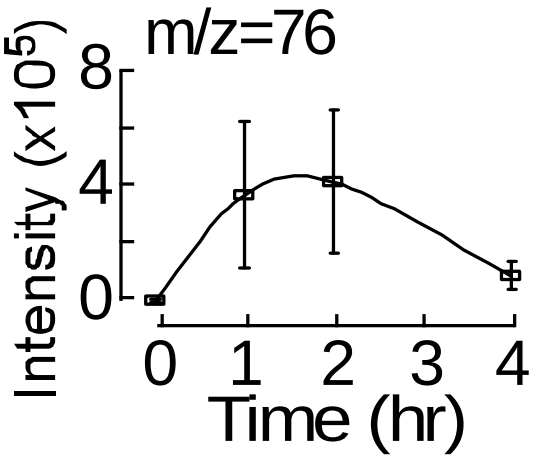
Male mouse, 1.5 mg PC gavage

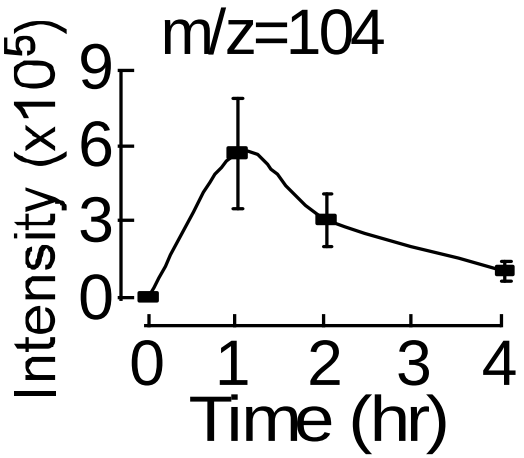


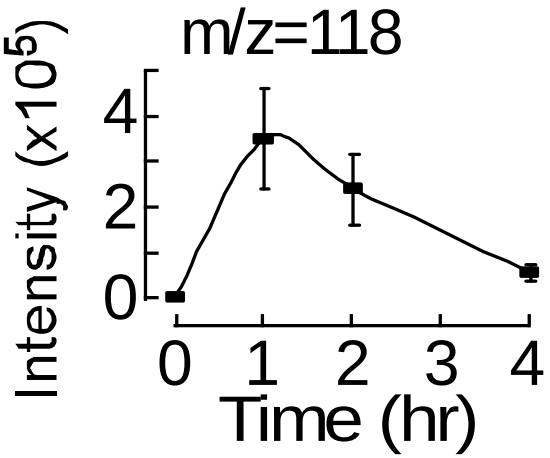




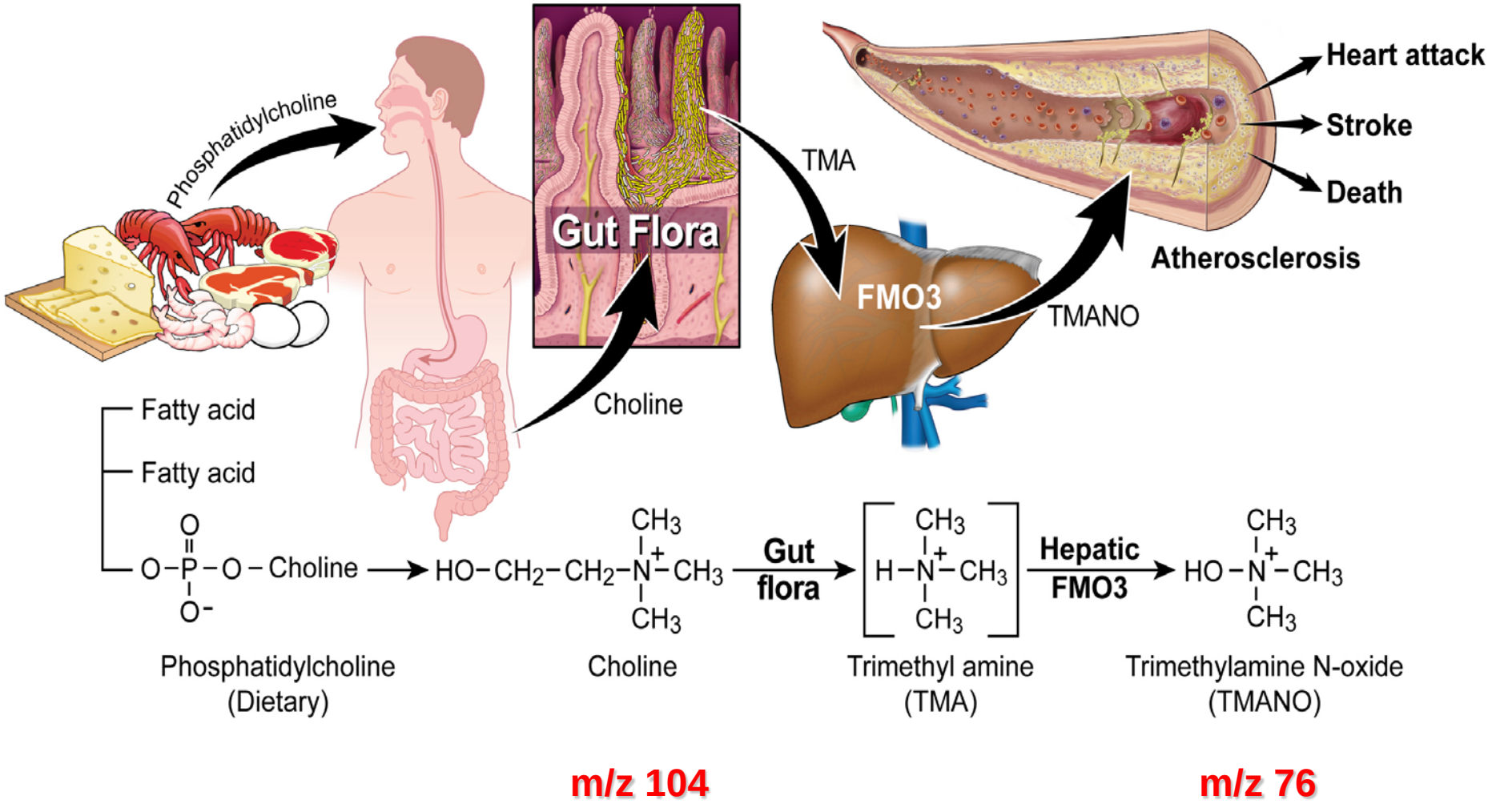
Female mouse, 1.5 mg PC gavage



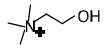
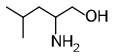
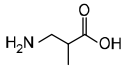
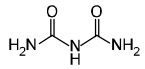
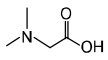
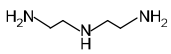
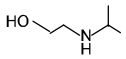
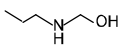


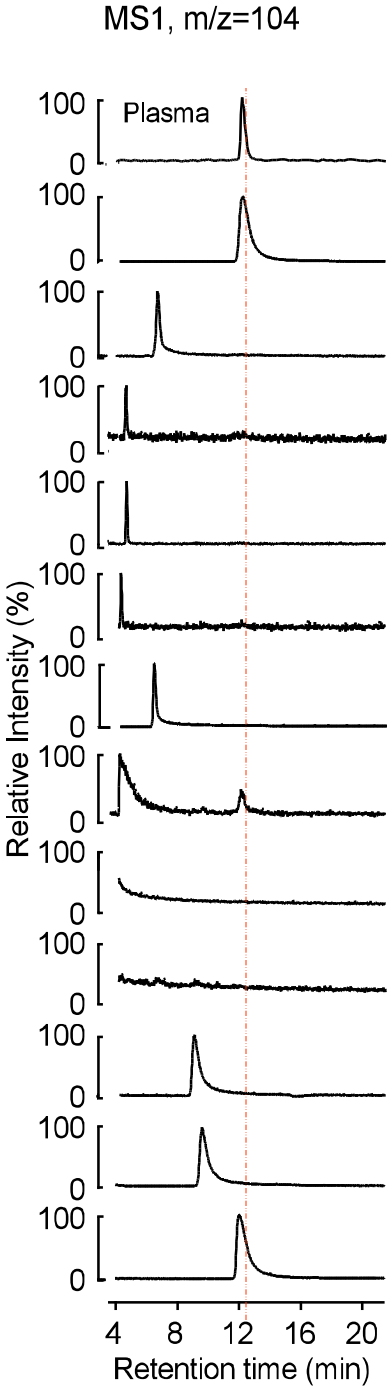


Maybe choline is the plasma analyte associated with CVD with m/z 104



Wang Z et al, *Nature* (2011)

Plasma analyte m/z=104		MH^+
choline		104
2-amino-3-methyl-1-butanol		104
3-aminoisobutyric acid		104
2-aminobutyric acid		104
biuret		104
N,N-dimethyl glycine		104
benzonitrile		104
diethylenetriamine		104
ethyl-N-hydroxyl acetimidate		104
2-isopropyl-aminoethanol		104
2-propyl-aminoethanol		104
1-dimethyl-amino-2-propanol		104

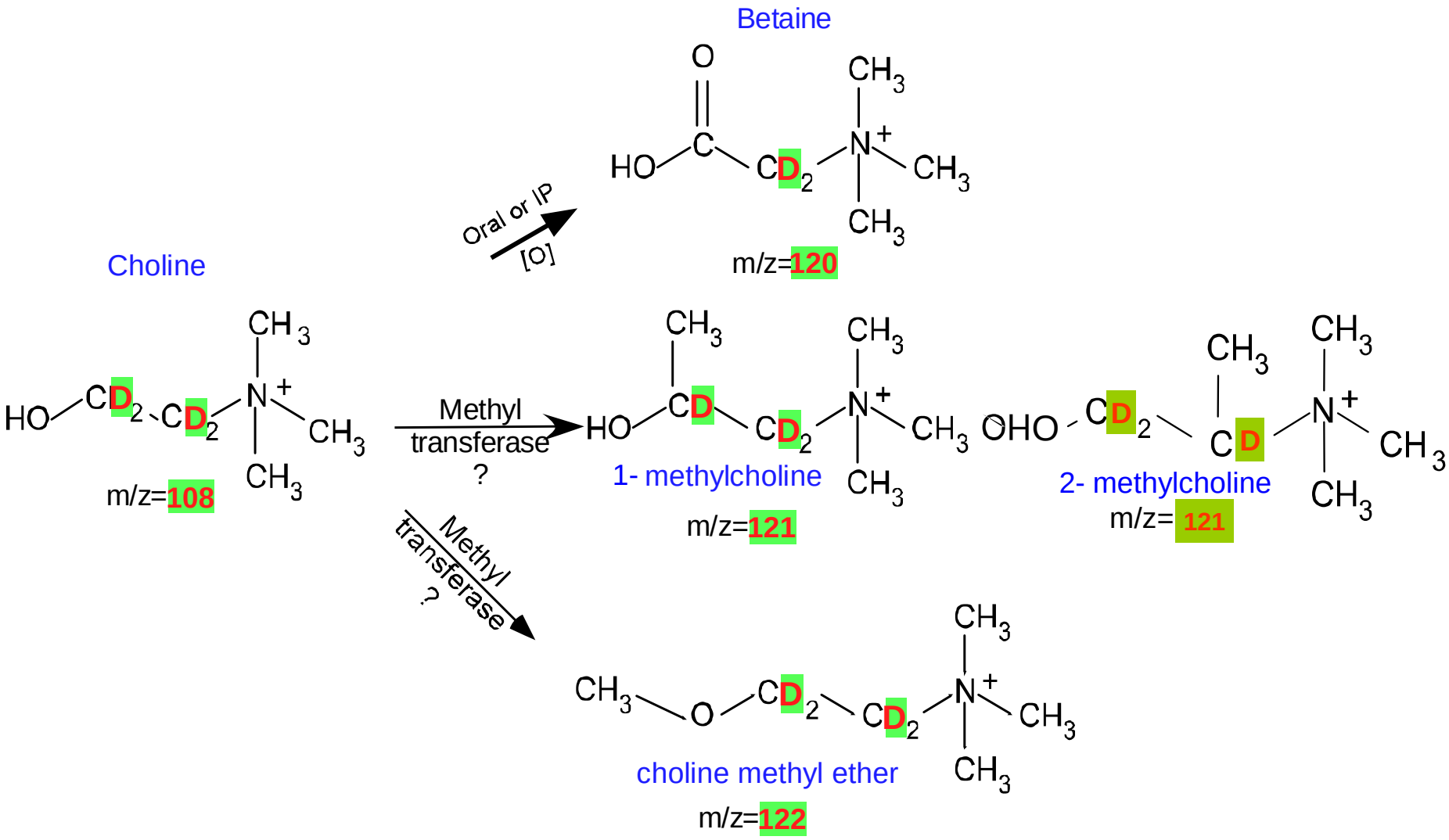


Candidate plasma analytes linked to CVD risks with m/z 104

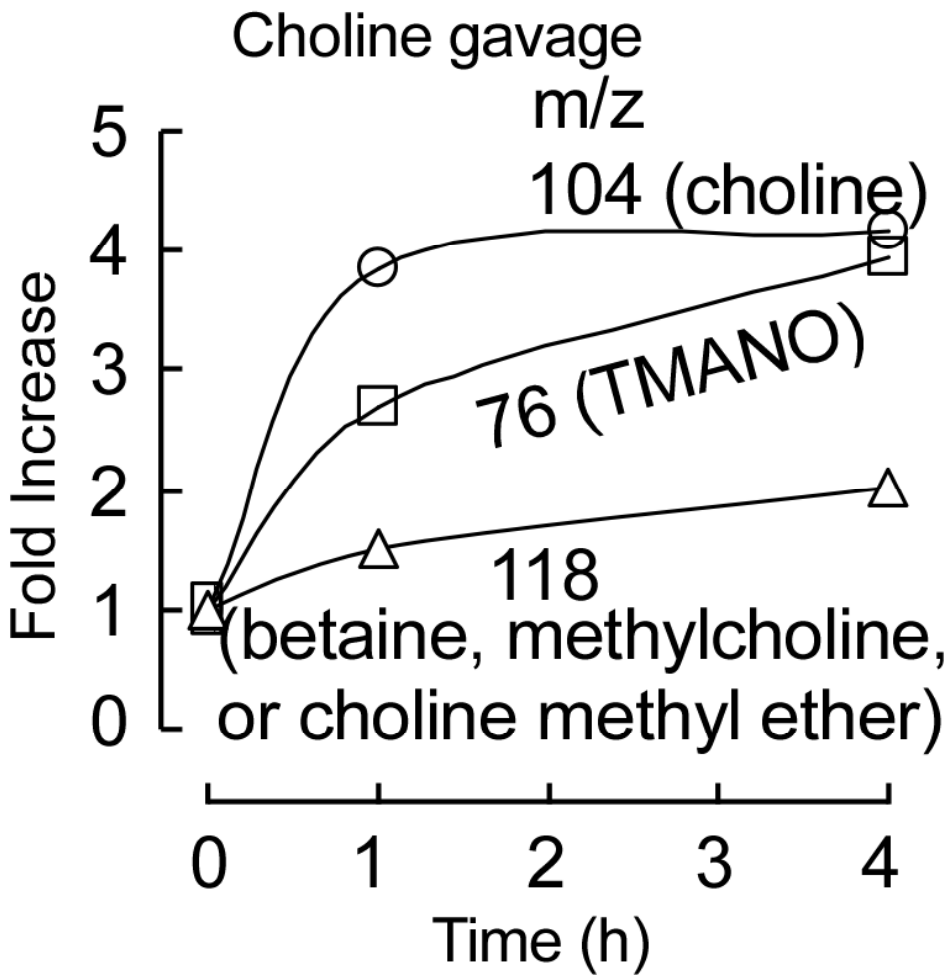
Identity as Choline was confirmed by:

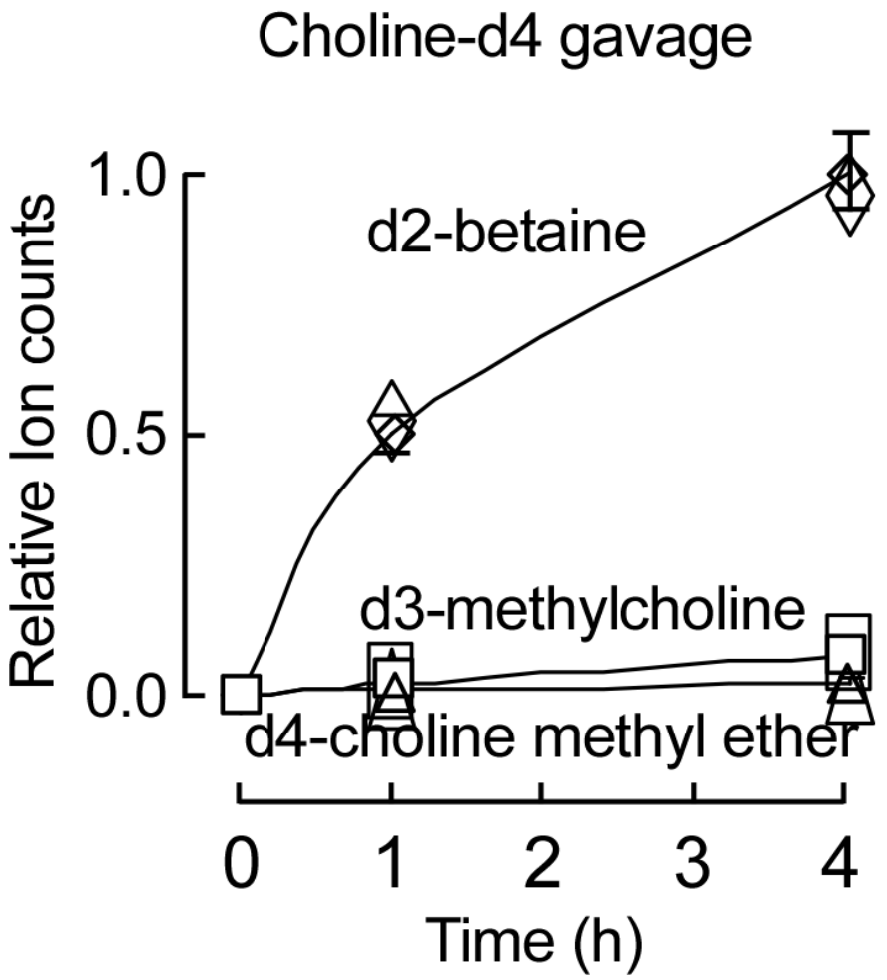
- LC-MS^n
- GC/MS/MS
- NMR
- Isotope tracer studies: d9-choline and d4-choline

Strategy to determine the analyte at m/z=118 by choline deuterated isotopologue feeding study



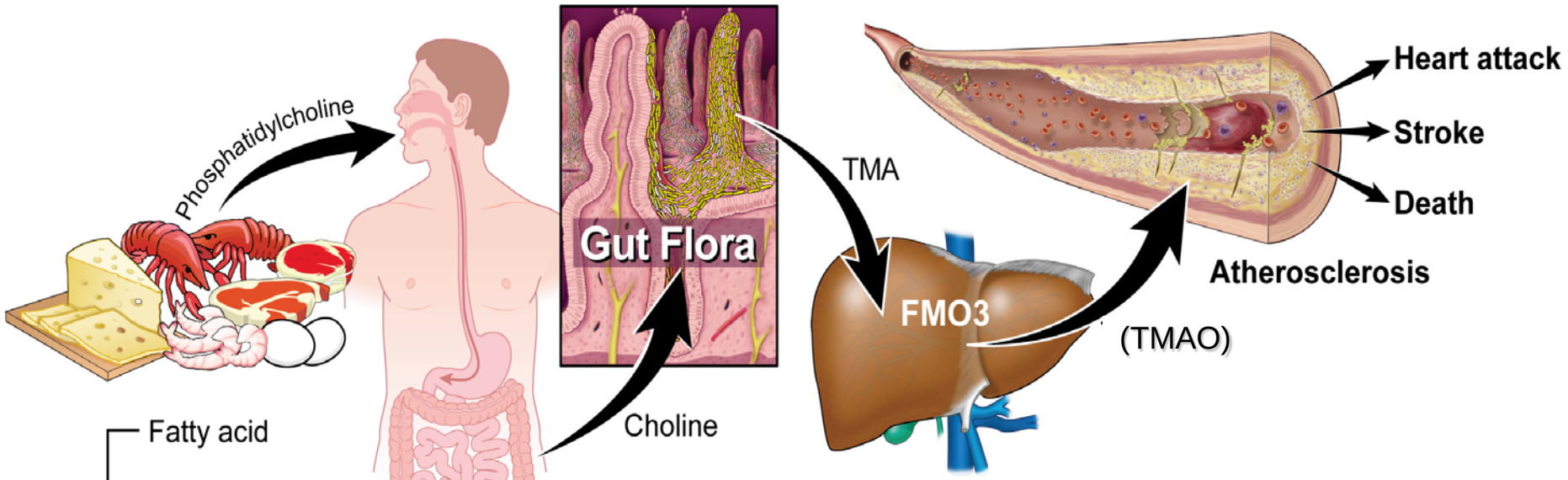
Isotope challenge studies confirm the identities of TMAO, choline and betaine as the plasma analytes predicting CVD risk





Wang Z et al, *Nature* (2011)

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

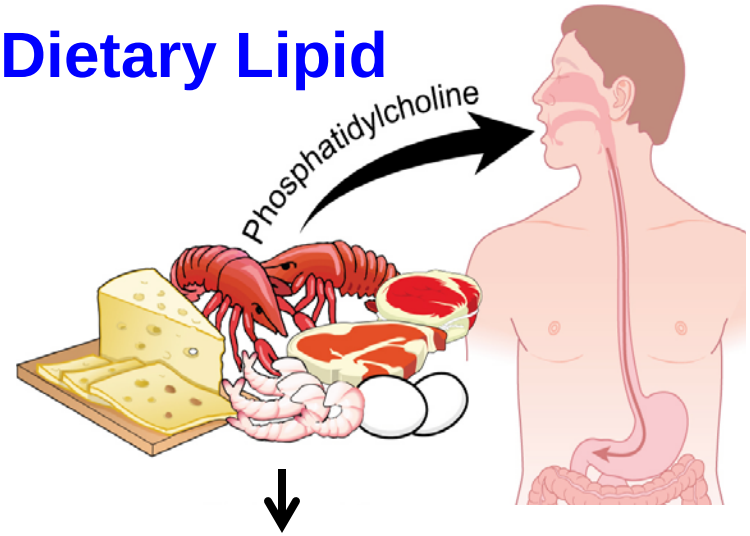


Identities confirmed by:
LC-MS^n, ^{1}H, ^{13}C, ^{15}N NMR
GC/MS/MS, Isotope tracer studies

Betaine
m/z 118

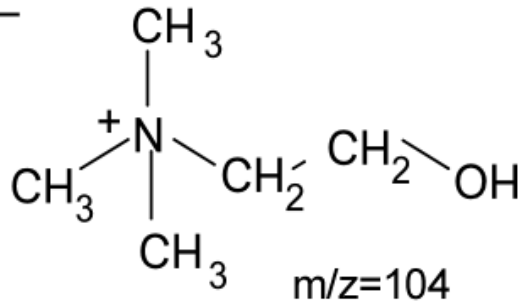
Wang Z et al (2011) *Nature*

Dietary Lipid



What is the role of gut flora?

Choline



m/z=104

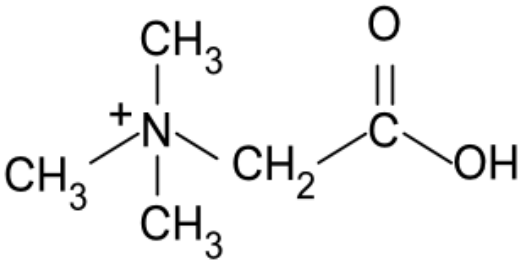
d4-choline: $(CH_3)_3{}^+NCD_2CD_2OH$

d9-choline: $(CD_3)_3{}^+NCH_2CH_2OH$

Oral or i.p.

[O]

Betaine



m/z=118

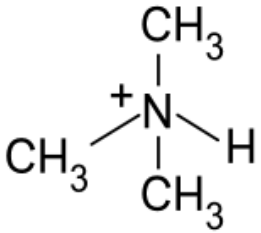
d4 metabolite m/z=120

d9 metabolite m/z=127

Oral only

Gut Flora

TMA



m/z=60

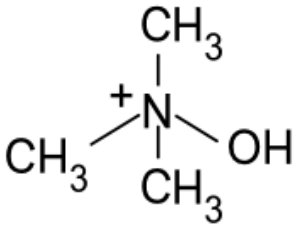
d4 metabolite m/z=60

d9 metabolite m/z=69

FMO3

[O]

TMANO



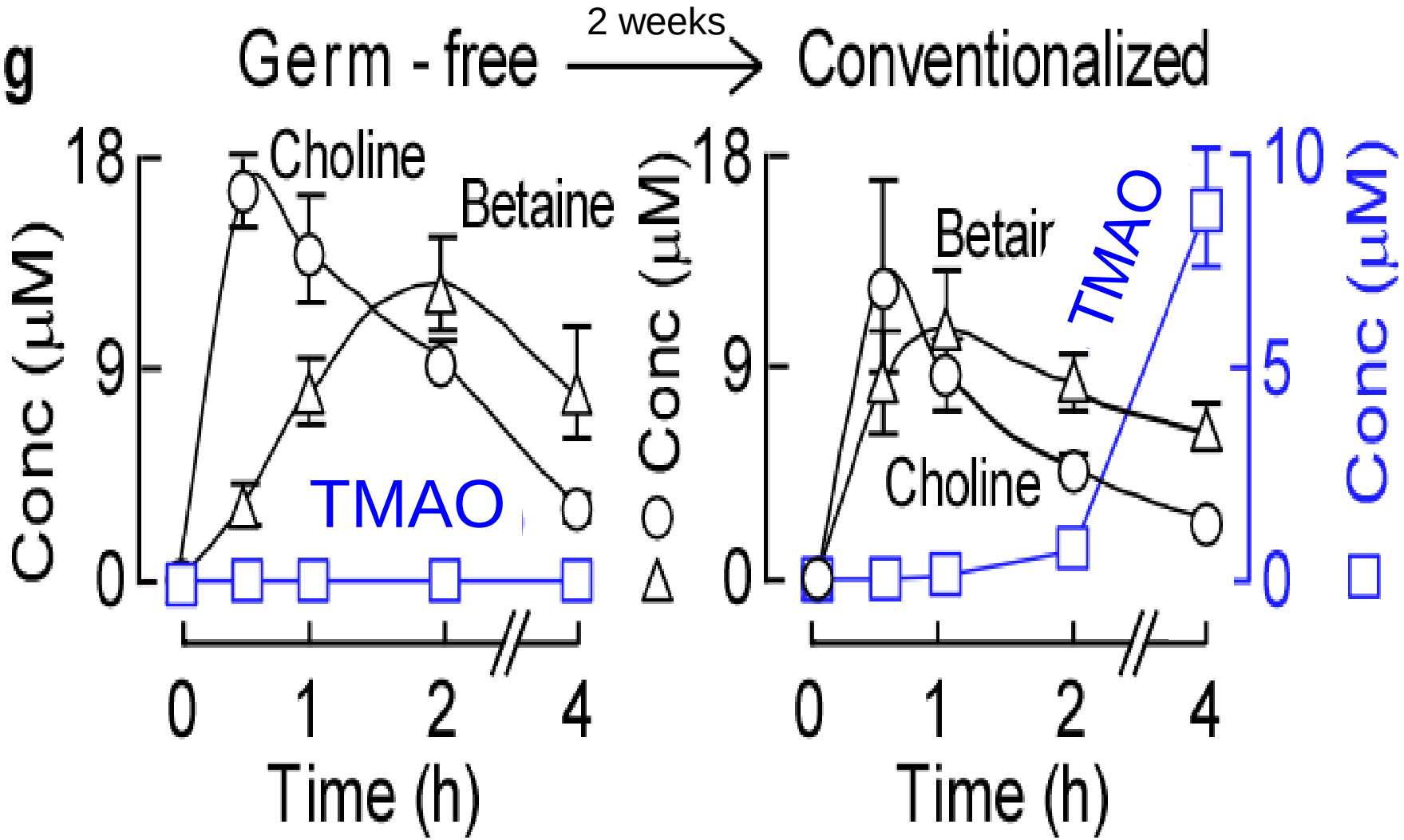
m/z=76

d4 metabolite m/z=76

d9 metabolite m/z=85

Wang Z et al, *Nature* (2011)

Intestinal Microbial Organisms Play an Obligatory Role in TMAO Generation from Dietary Egg Yolk PC in Mice



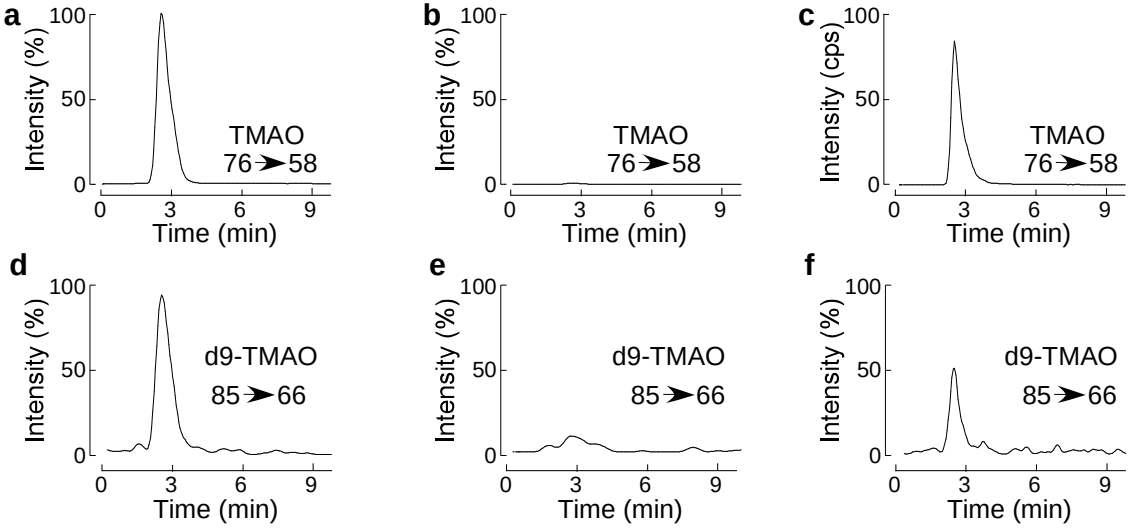
Wang Z et al, *Nature* (2011)

TMAO is a gut flora dependent metabolite in humans : PC challenge - Oral d9-PC and 2 hard boiled eggs at each visit

Pre-antibiotics (visit 1) → Antibiotics: Suppression of gut flora → Post-antibiotics (visit 2) → Acquisition of gut flora → visit 3

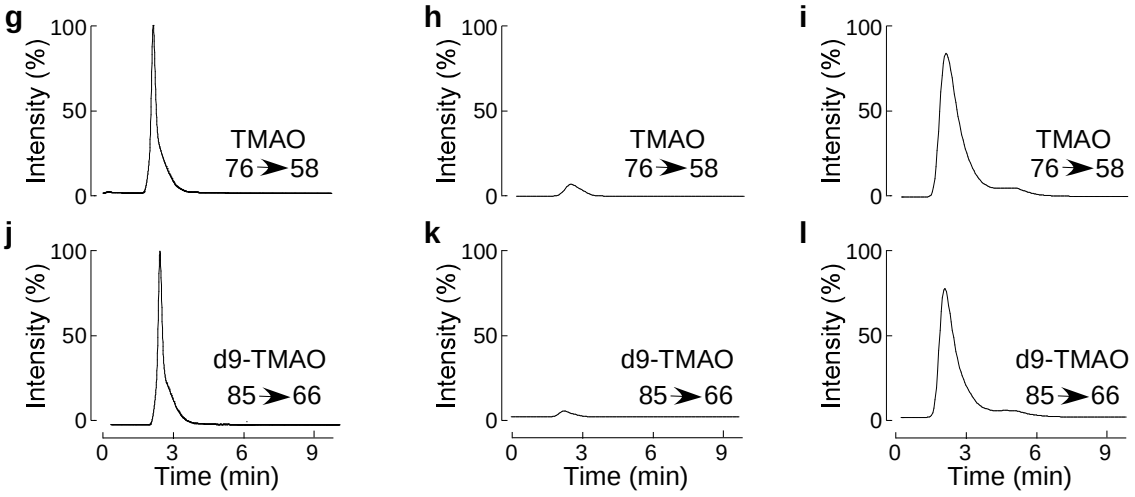
6 h post PC challenge

Plasma



24 h post PC challenge

Urine



Tang, Wang et al, NEJM (2013)

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Replication and demonstration of clinical prognostic utility

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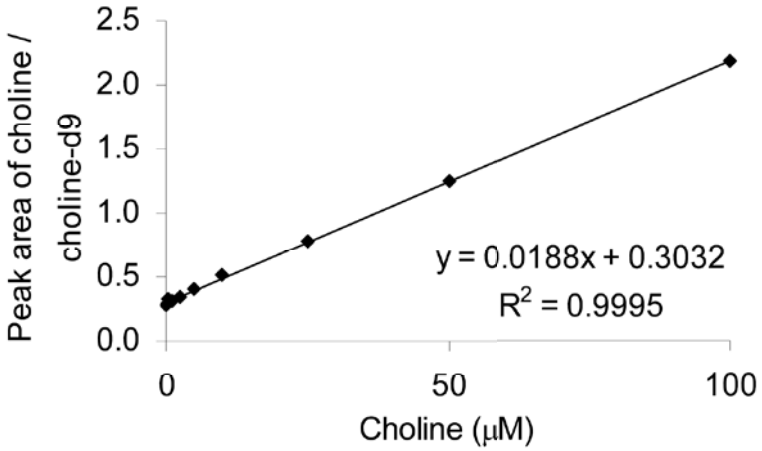
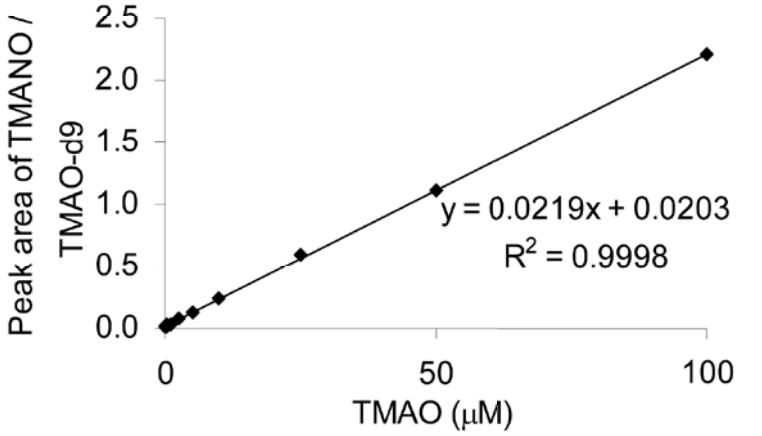
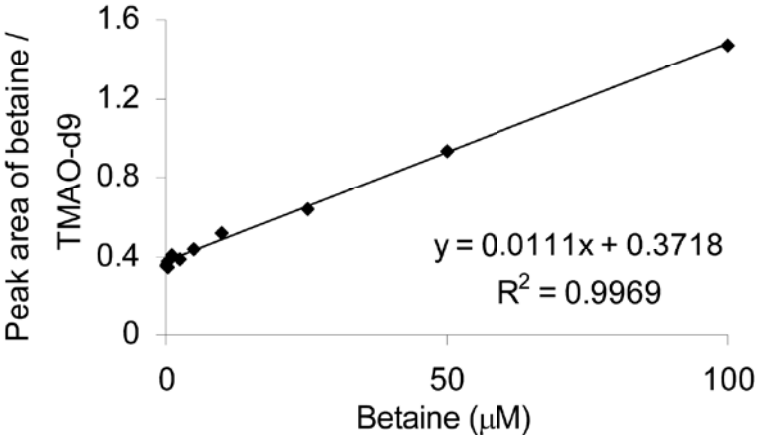




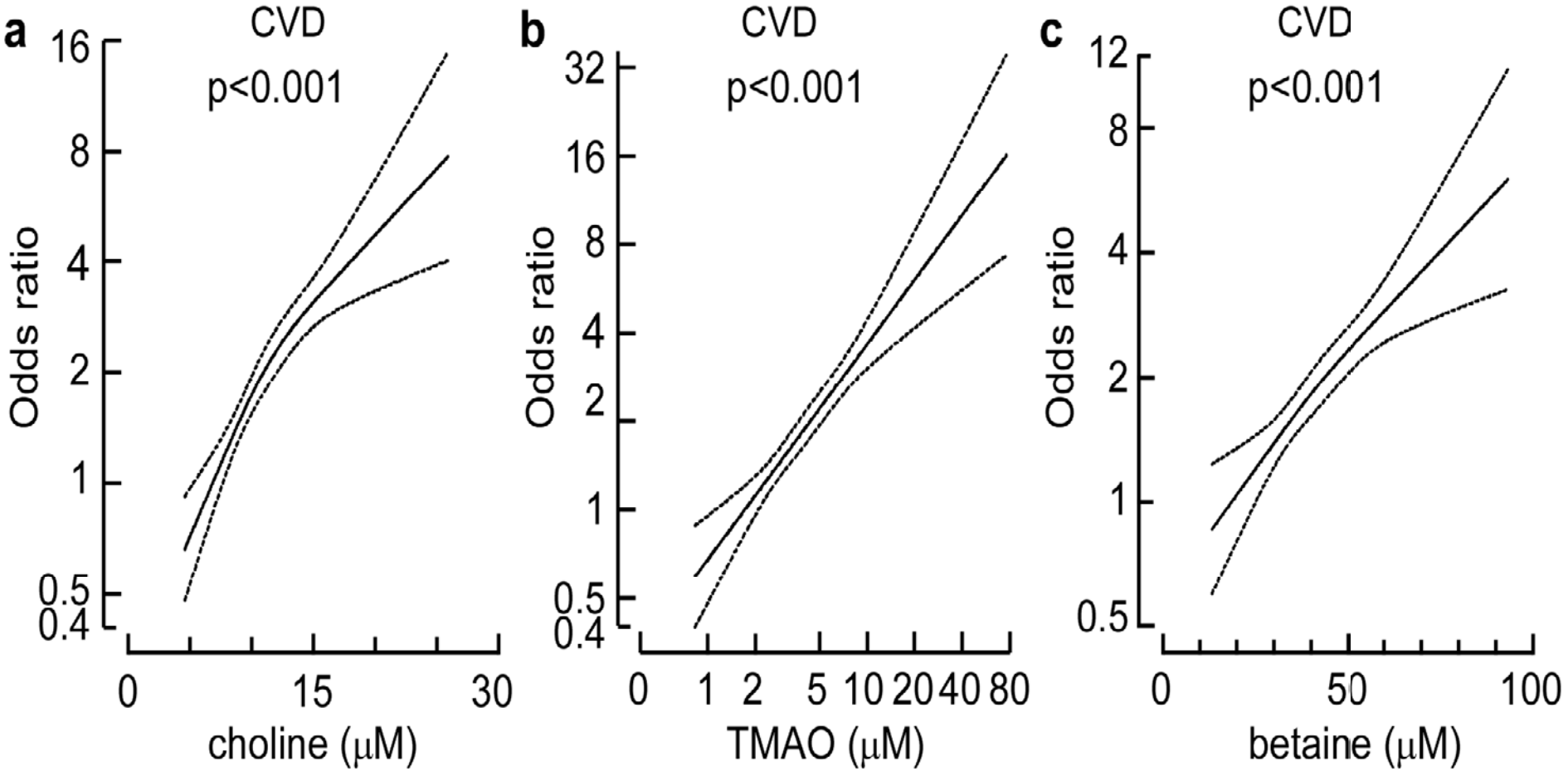


Development of stable isotope dilution LC/MS/MS assays for choline, TMAO and betaine using d9(trimethyl) isotopologues as internal standards

(controls)	Intraday CV	Interday CV
TMAO (low)	4.7	4.9
(high)	3.1	4.4
Betaine (low)	5.2	5.4
(high)	3.0	6.1
Choline (low)	4.8	6.9
(high)	3.4	3.9

Prospective Cohort: N=1865 Sequential Cardiology Patients

Plasma choline, TMAO and betaine levels predict CVD risks (N=1865)



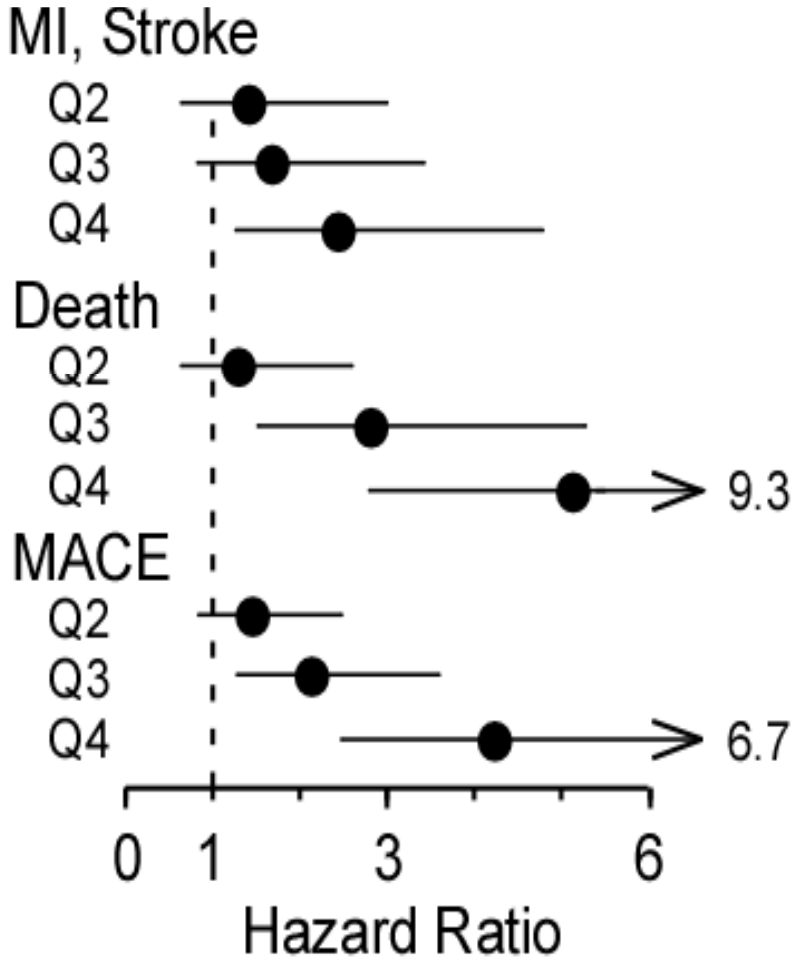
Odds ratio (95%CI) adjusted for age, sex, DM, HTN, smoking, LDL, HDL, TG, CRP, eGFR

Z Wang et al, *Nature* (2011)

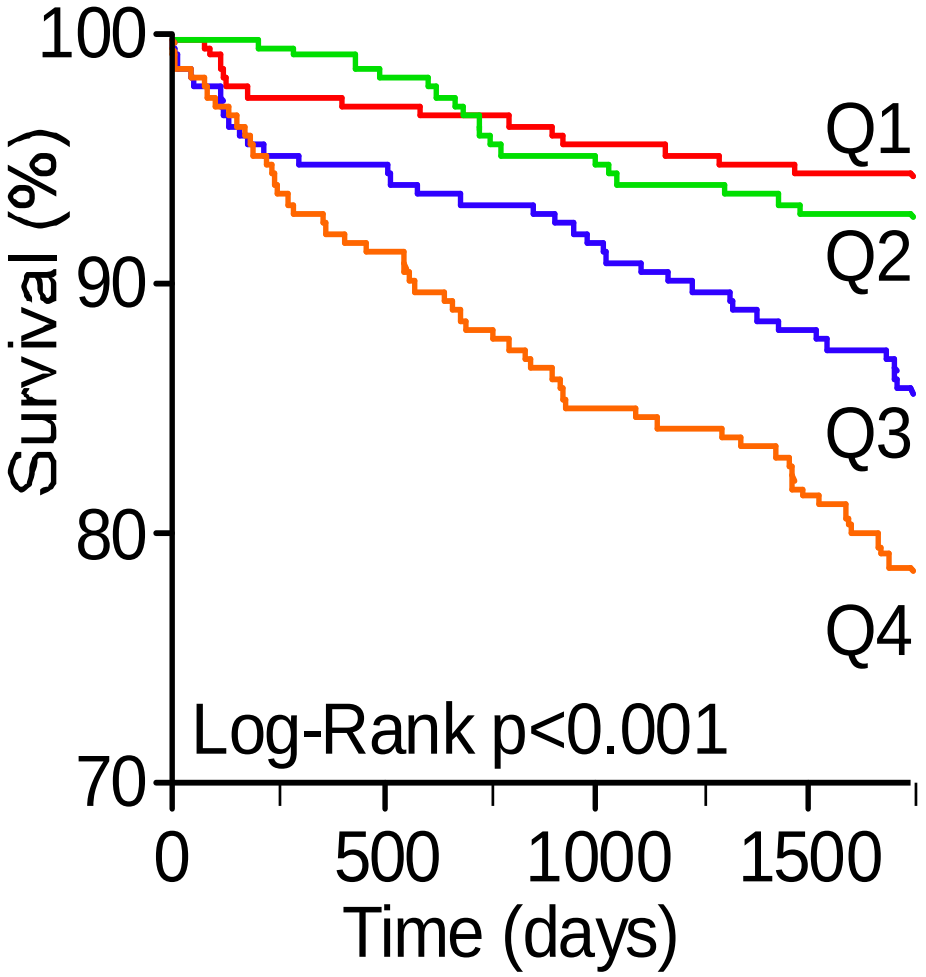
Plasma levels of the gut flora dependent metabolite TMAO predict incident (3 year) CVD risks

New Independent Cohort: N=4007 Sequential Subjects

TMAO



TMAO



Adjusted for age, sex, DM, HTN, smoking, LDL, HDL, TG, CRP, eGFR

Tang, Wang et al, NEJM (2013)

Phase 1: Discovery-based investigations

Metabolomics screening and structural identification

Phase 2: Clinical validation

Replication and demonstration of clinical utility

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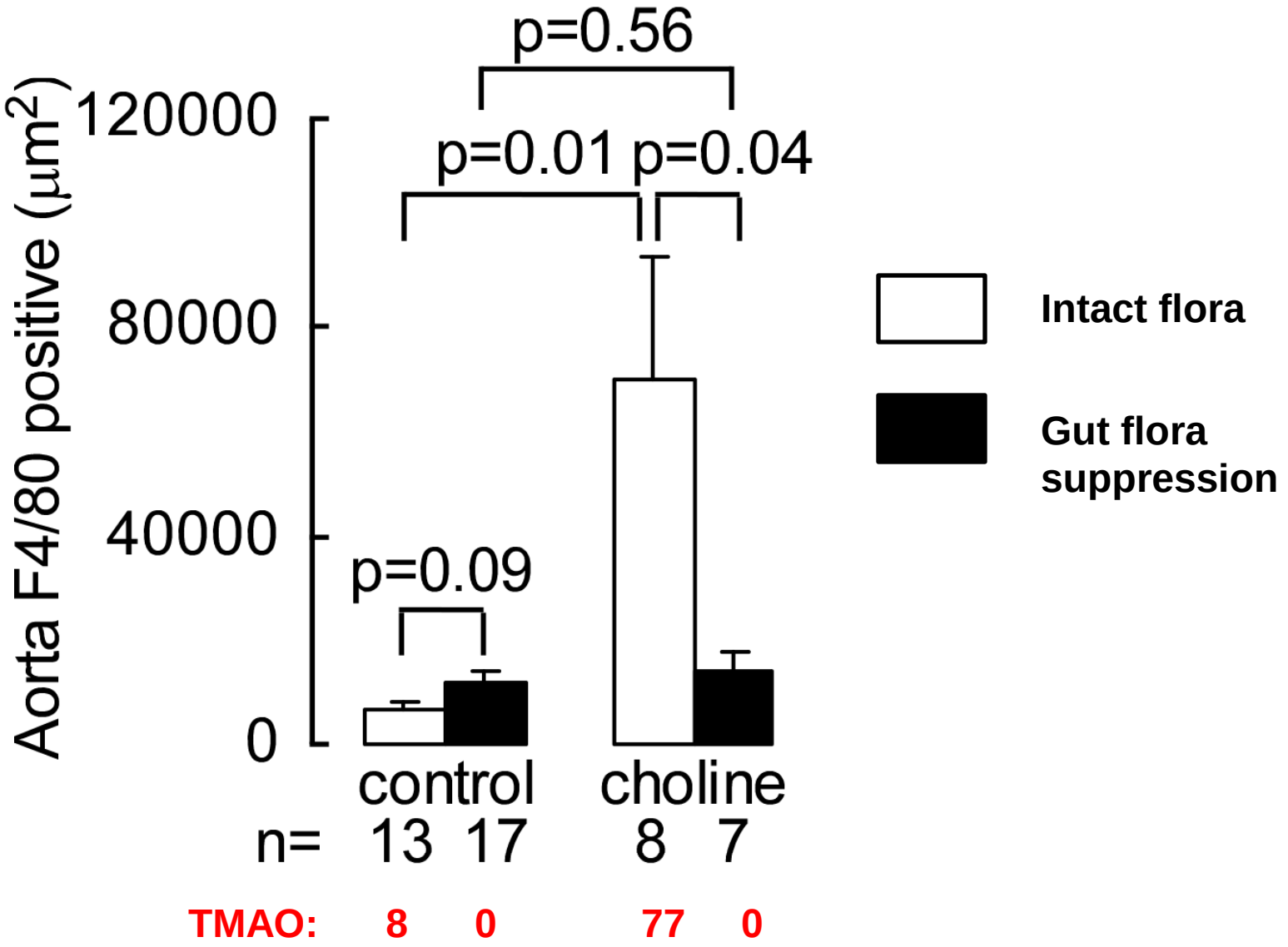
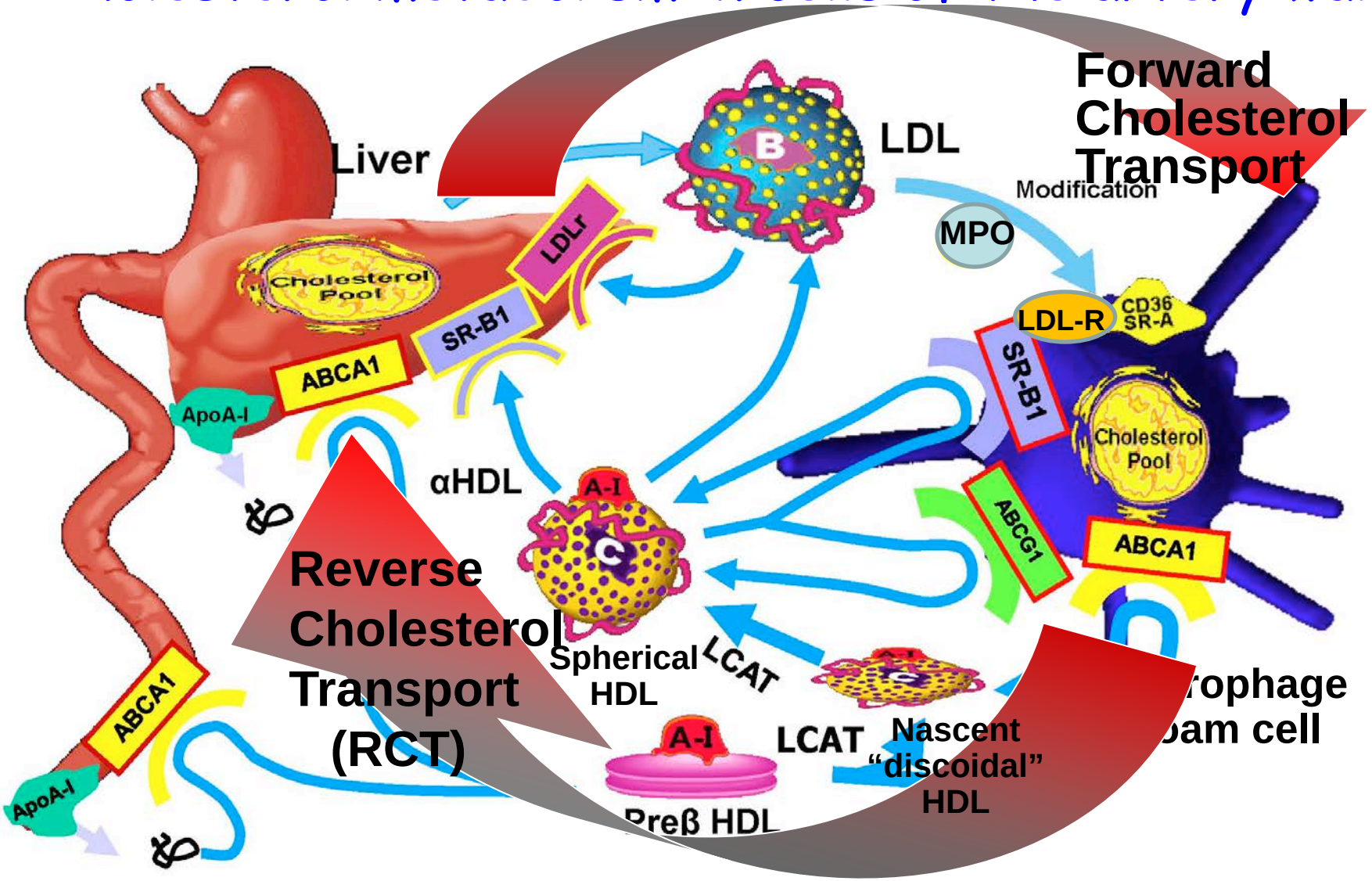
Demonstration of causality for a novel pathway

Suppression of gut flora inhibits TMAO formation and dietary choline induced atherosclerosis

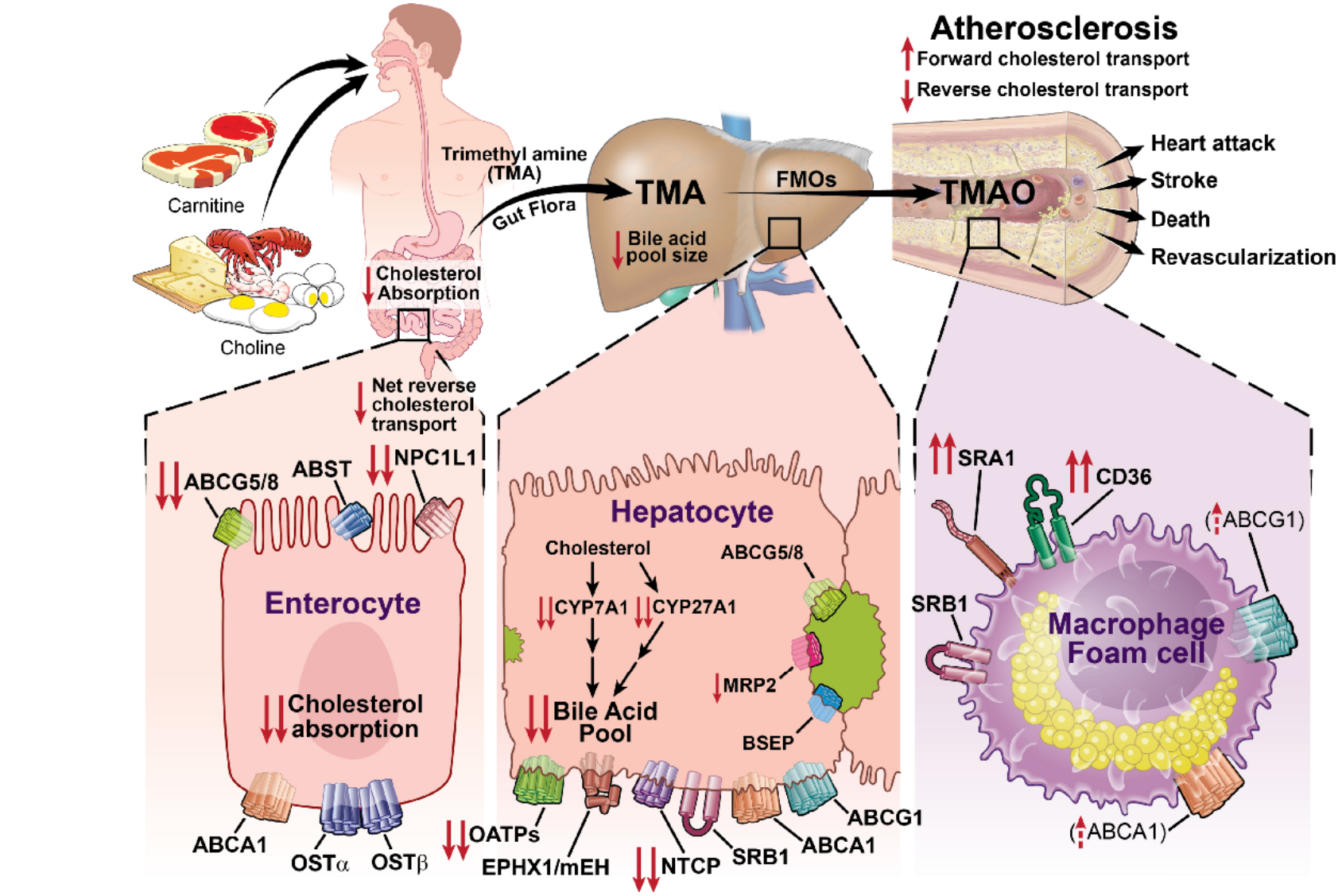


Wang Z et al, *Nature* (2011)

Cholesterol metabolism in cells of the artery wall:

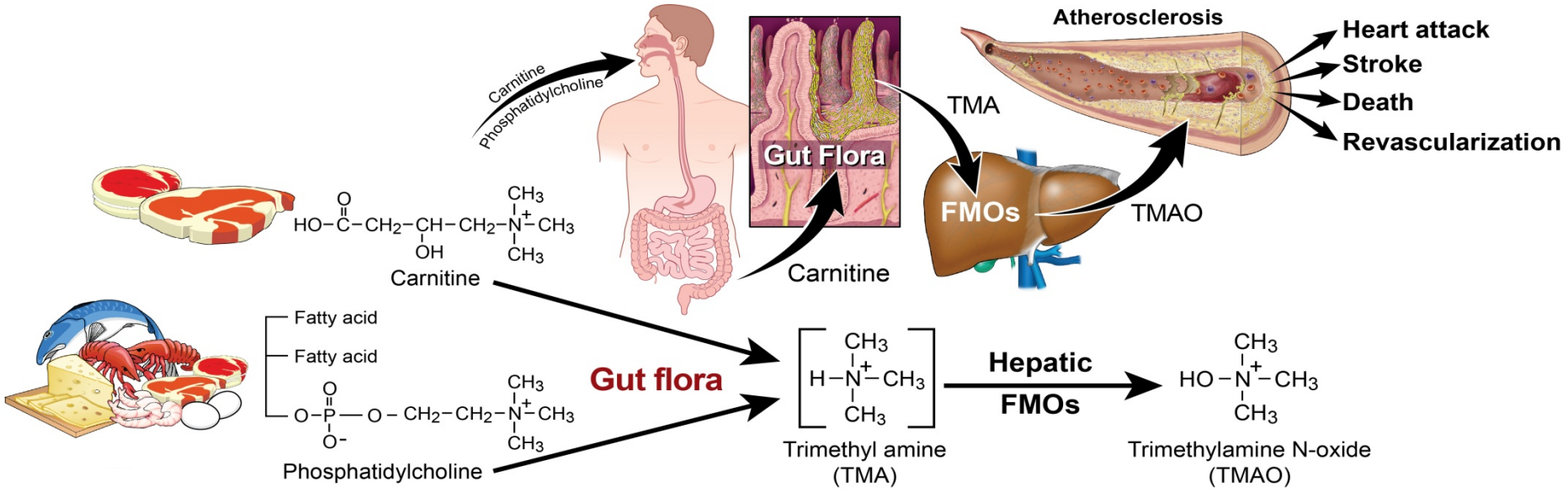


TMAO alters cholesterol and sterol metabolism in multiple compartments - net effect - increased atherosclerosis



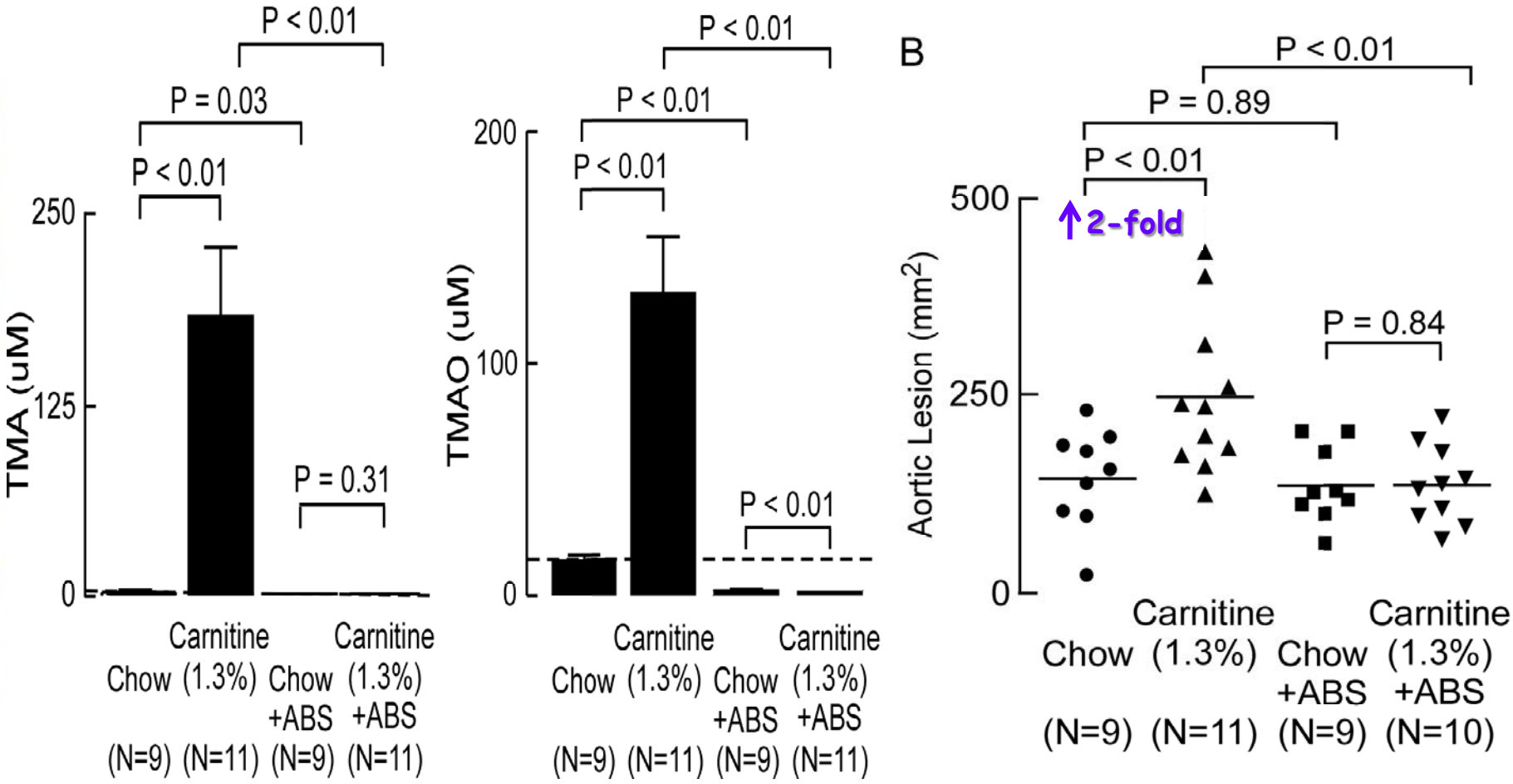
Koeth, et al, Nature Med., 2013

Carnitine, an abundant nutrient in red meat, is pro-atherogenic too



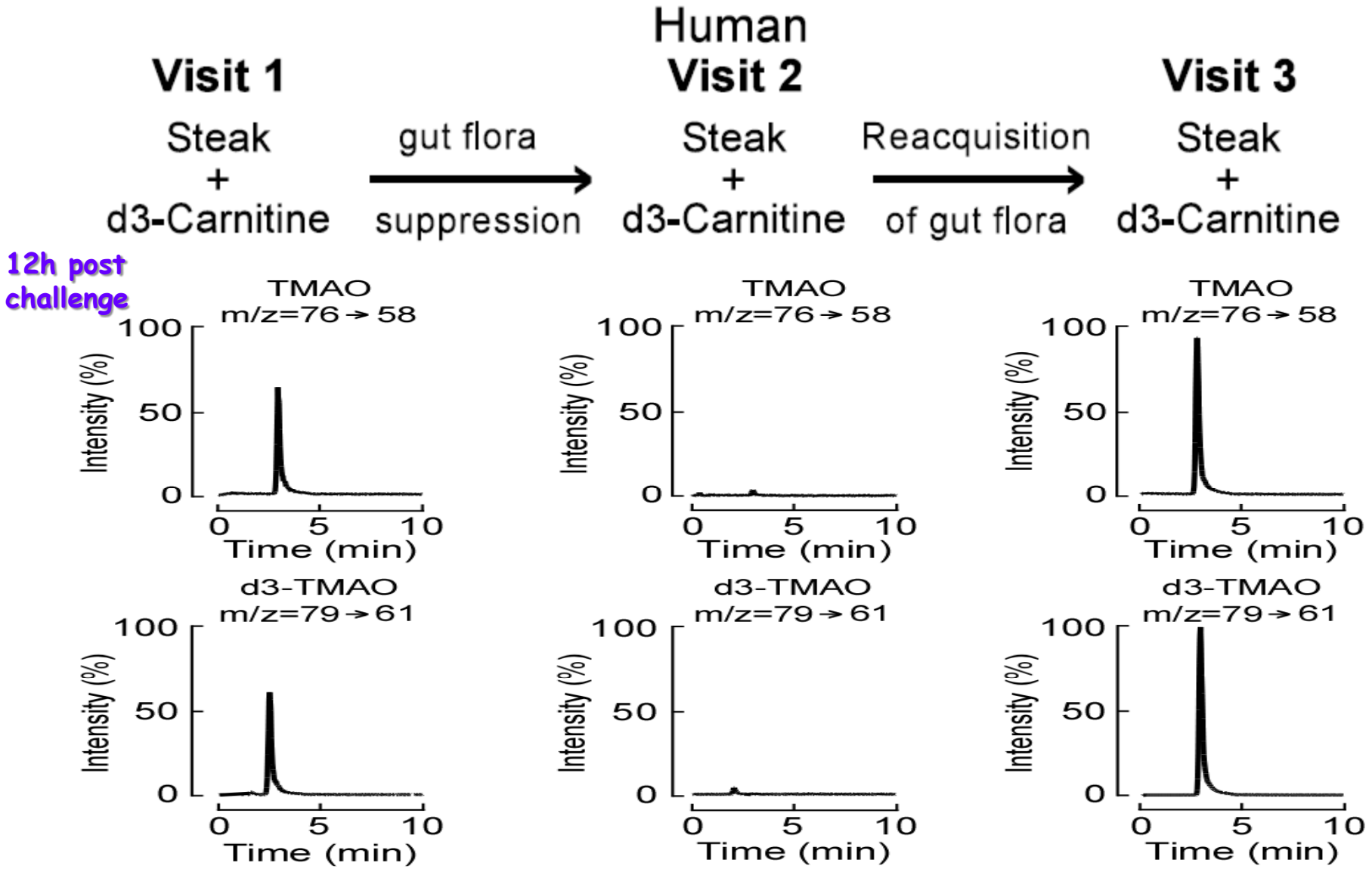
Robert Koeth et al, Nature Medicine (2013)

Carnitine supplementation accelerates atherosclerosis in apoE-/- mice, but not with suppression of intestinal flora (and suppression of TMA/TMAO formation)



Robert Koeth et al, Nature Medicine (2013)

Human carnitine tolerance study: There is an obligatory role for gut flora in TMAO production from oral carnitine



Robert Koeth et al, Nature Medicine (2013)

Hypothesis: Dietary patterns alter the composition of the gut microbial community

N=30

N=23

Omnivore and Vegans/Vegetarians

Stool Collected

Gut Microbiota Composition

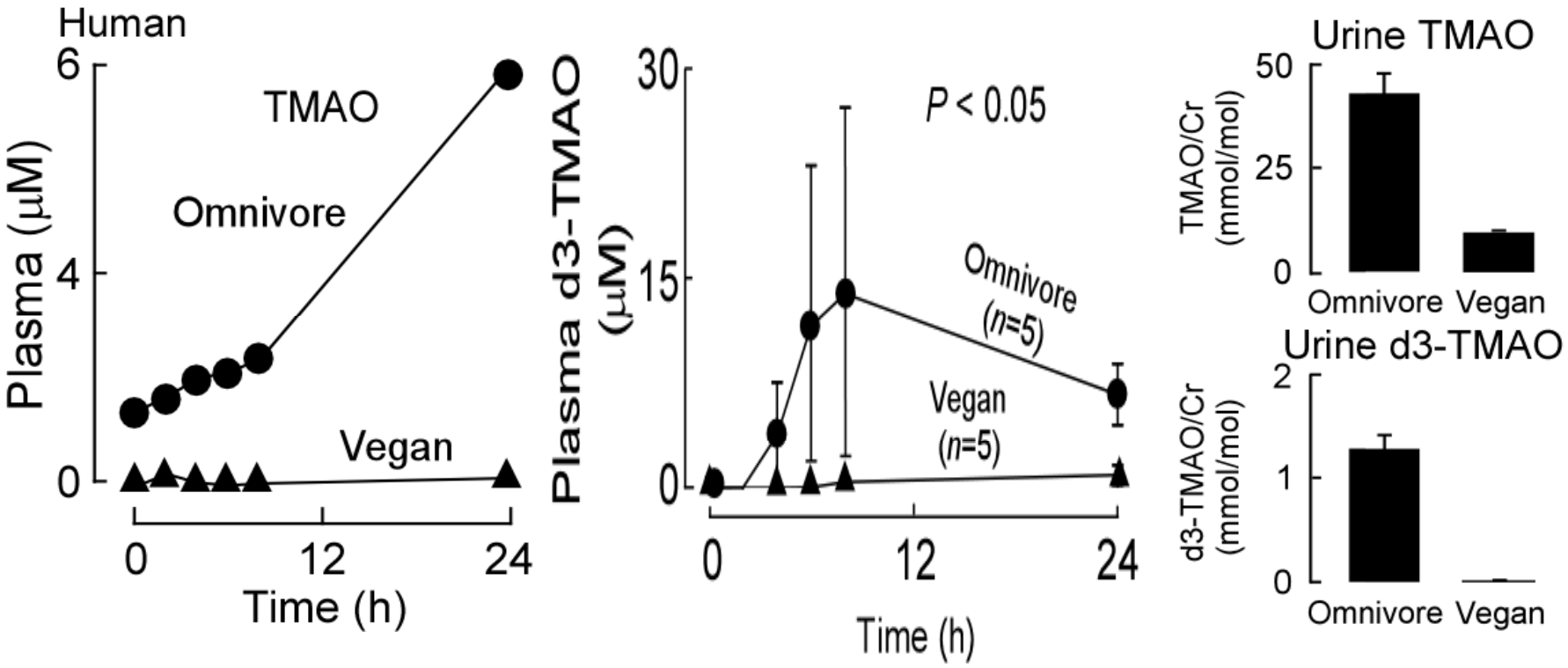
Blood Collected

TMAO measured by mass spectrometry

TMAO is formed from dietary carnitine in omnivors, but minimally in vegans

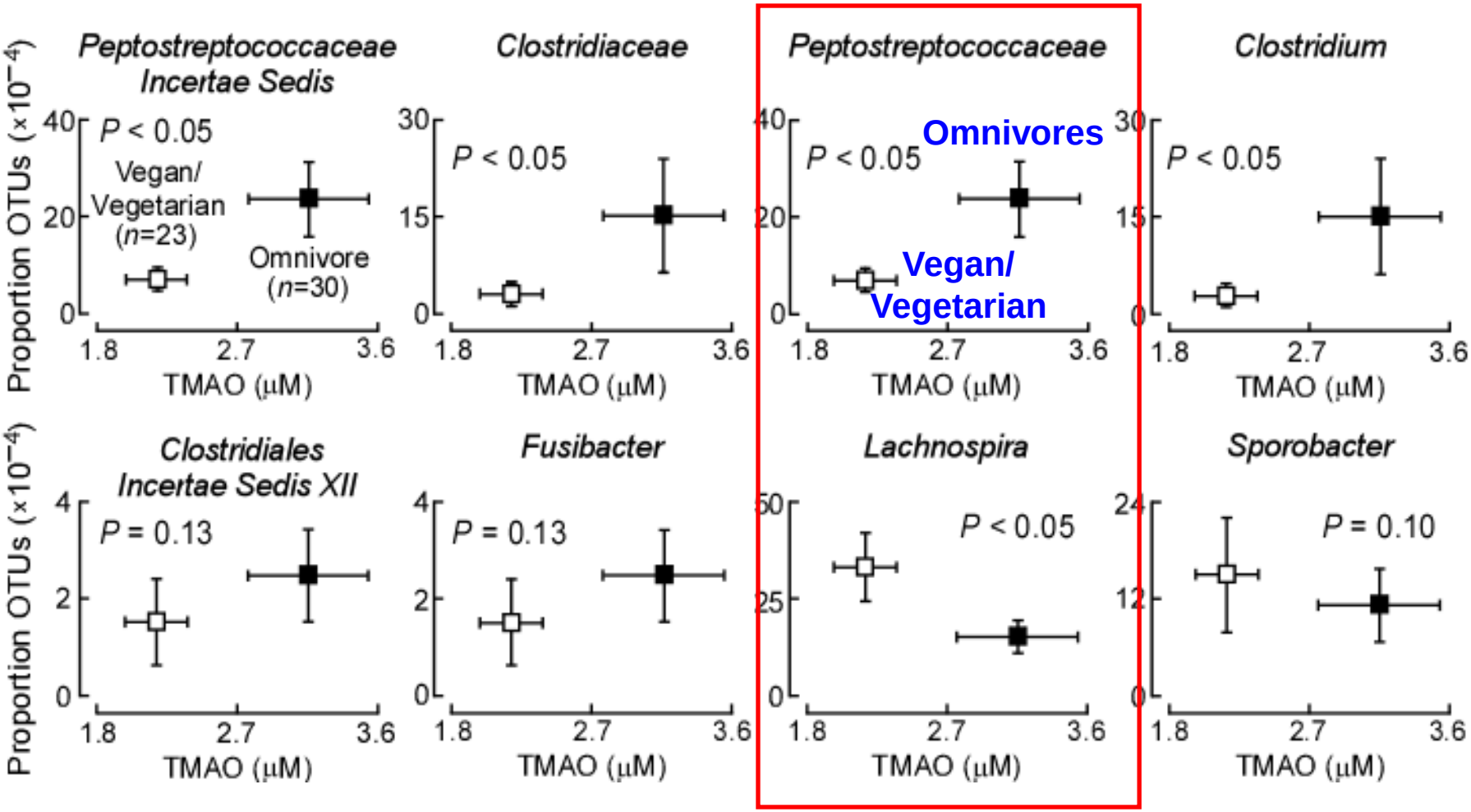
Carnitine challenge:
8oz tenderloin
+d3(methyl)-carnitine

Carnitine challenge:
d3(methyl)-carnitine



Robert Koeth et al, Nature Med (2013)

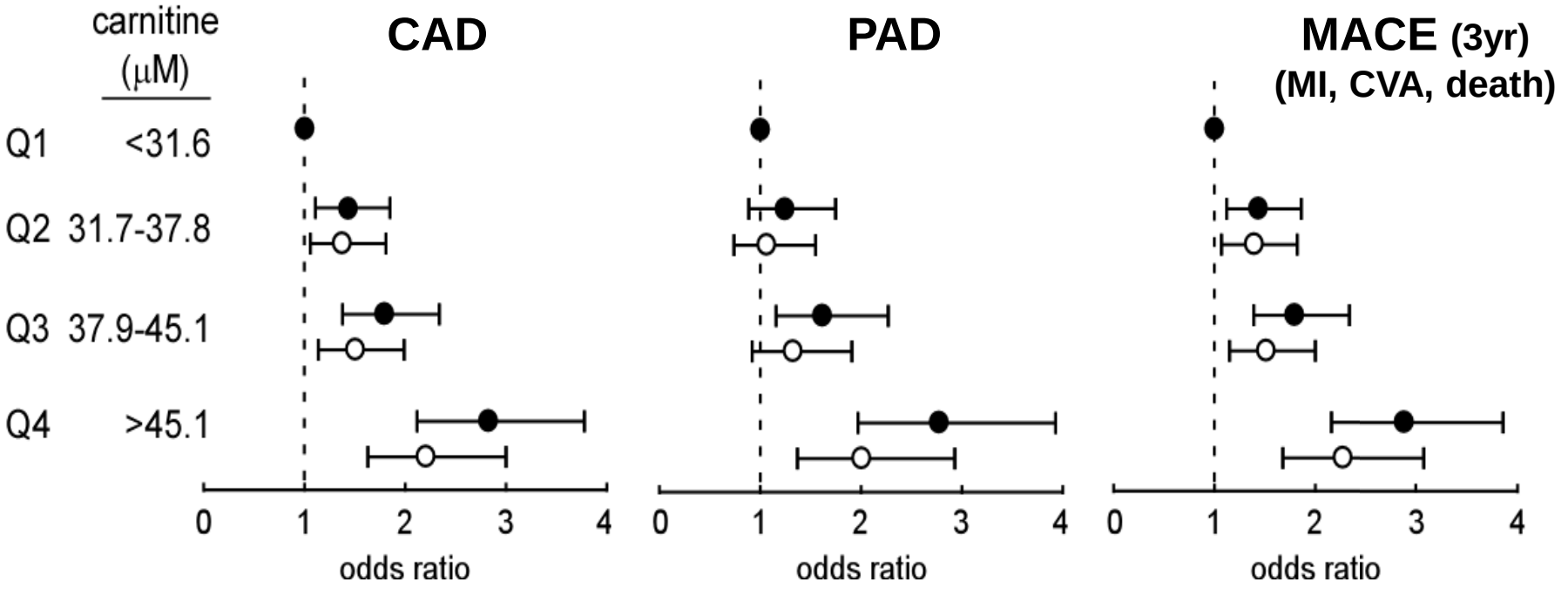
Specific microbiota taxa are associated with long-term dietary patterns and plasma TMAO levels



Robert Koeth et al, Nature Medicine (2013)

Plasma levels of carnitine in subjects predict cardiovascular risks

Sequential subjects (N=2595) undergoing cardiac evaluation at the Cleveland Clinic Preventive Cardiology Clinic



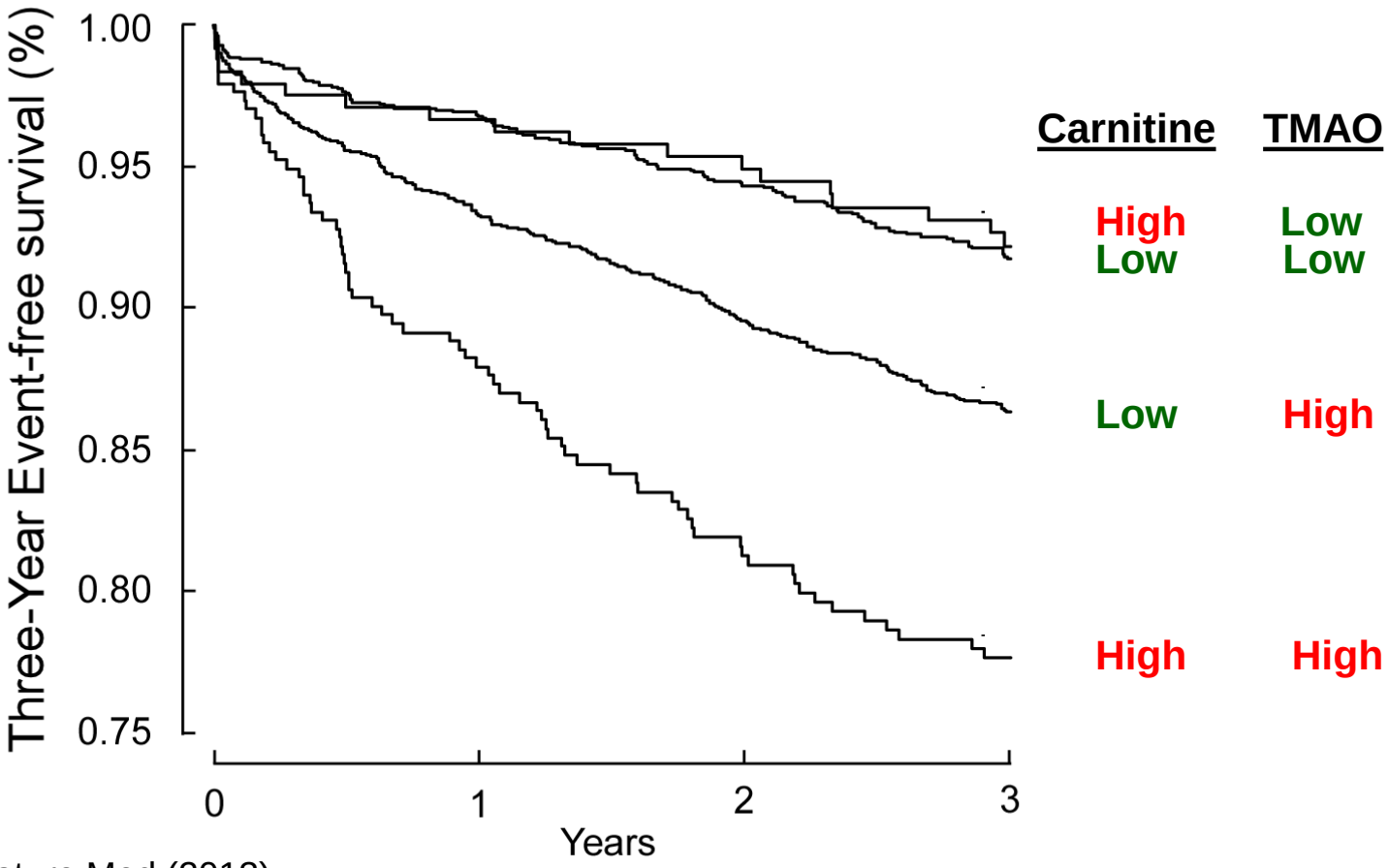
● Unadjusted

○ Adjusted for age, gender, DM, SBP, DBP, smoking, hsCRP, LDL, HDL, TG, eCrCl, hsCRP

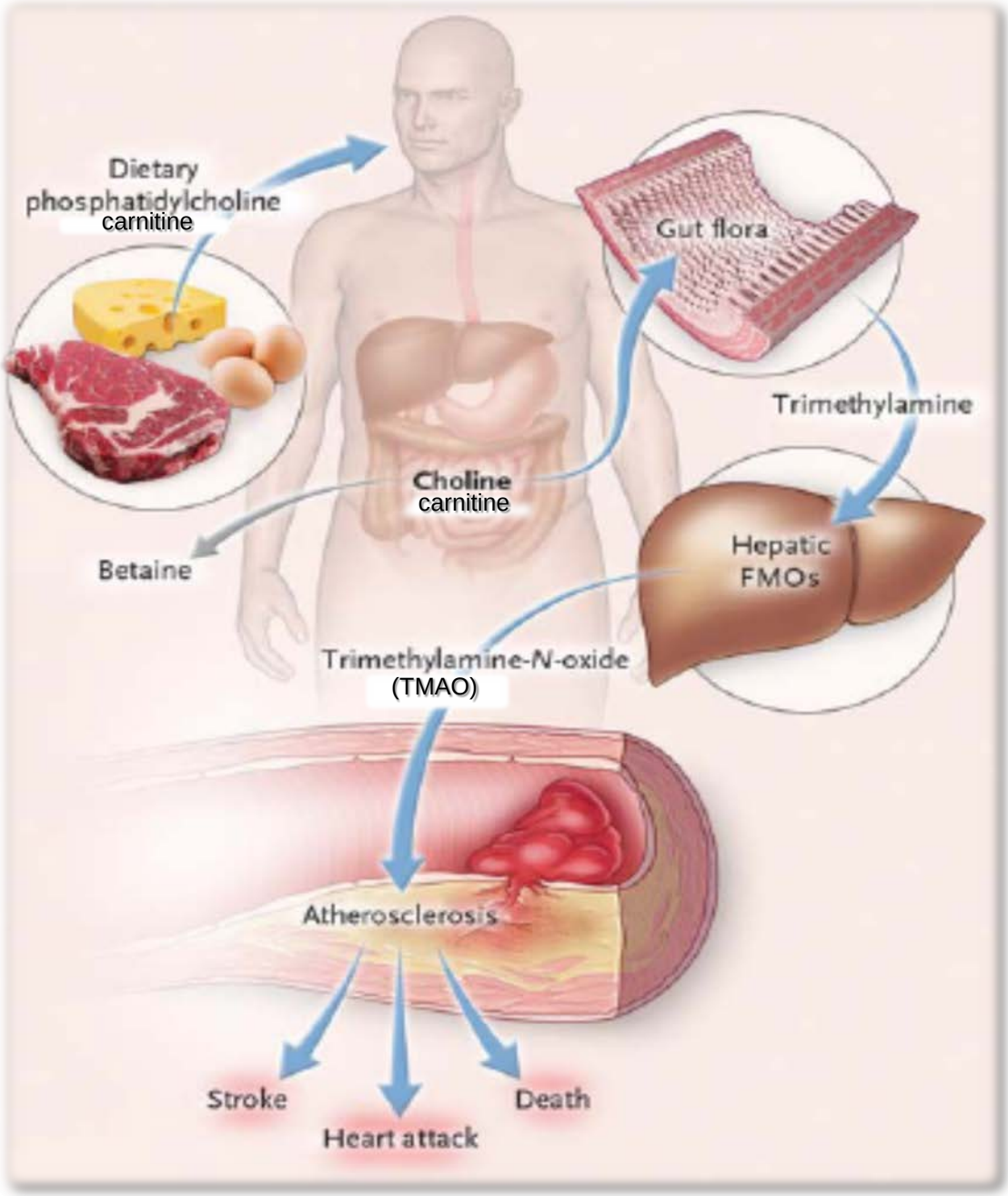
Robert Koeth et al, Nature Med (2013)

Plasma levels of carnitine in subjects predict cardiovascular risks - only if TMAO is high

Sequential subjects (N=2595) undergoing cardiac evaluation at the Cleveland Clinic Preventive Cardiology Clinic



Robert Koeth et al, Nature Med (2013)



Metabolomics studies are a powerful tool for discovery of new diagnostic and therapeutic targets

Gut flora contributes to atherosclerotic heart disease

Wang Z et al (2011) *Nature*

Bennett B et al (2013) *Cell Metab*

Koeth RA et al (2013) *Nature Medicine*

Tang WHW et al (2013) *NEJM*

Wang Z et al (2014) *Euro Heart J (in press)*

Tang WHW et al (2014) *JACC (in press)*

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Jun Chen

Gary Wu

James Lewis

Hongzhe Li

USC

Hooman Allayee