Gut Flora – a Newly Recognized Participant in Cardiac and Metabolic Diseases

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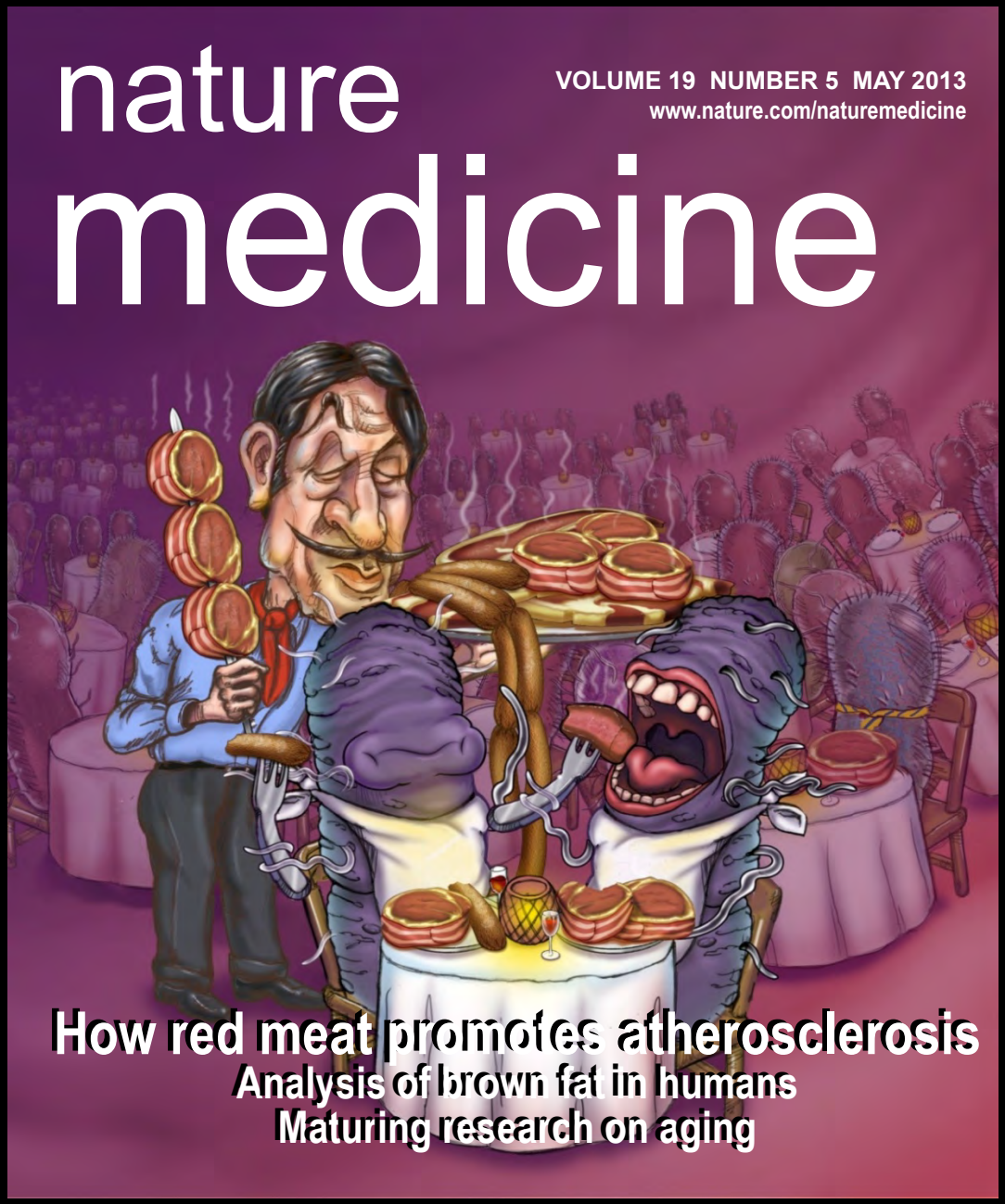
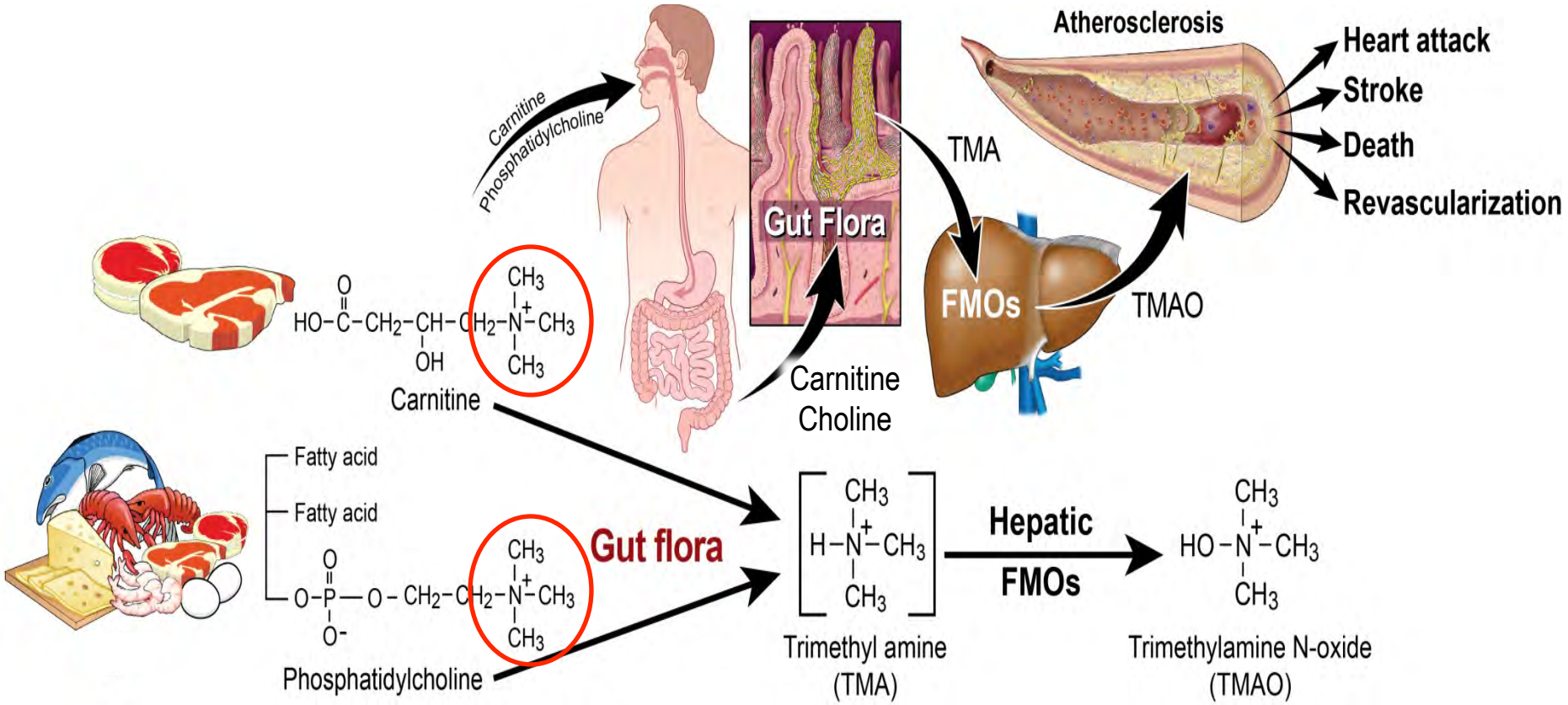


Take home summary:

Gut microbiota participates in atherosclerosis in the presence of specific dietary exposures



Wang Z et al, Nature (2011). Koeth RA et al, Nature Medicine (2013). Tang WHW et al, NEJM (2013)

Additional take home concepts:

- The microbiome is a filter of our largest environmental exposure – what we eat
- The microbiome can be considered as our largest endocrine organ
- The micrbiome is a "drugable" target

Phase 1: Discovery-based investigations

Metabolomics screening and structural identification

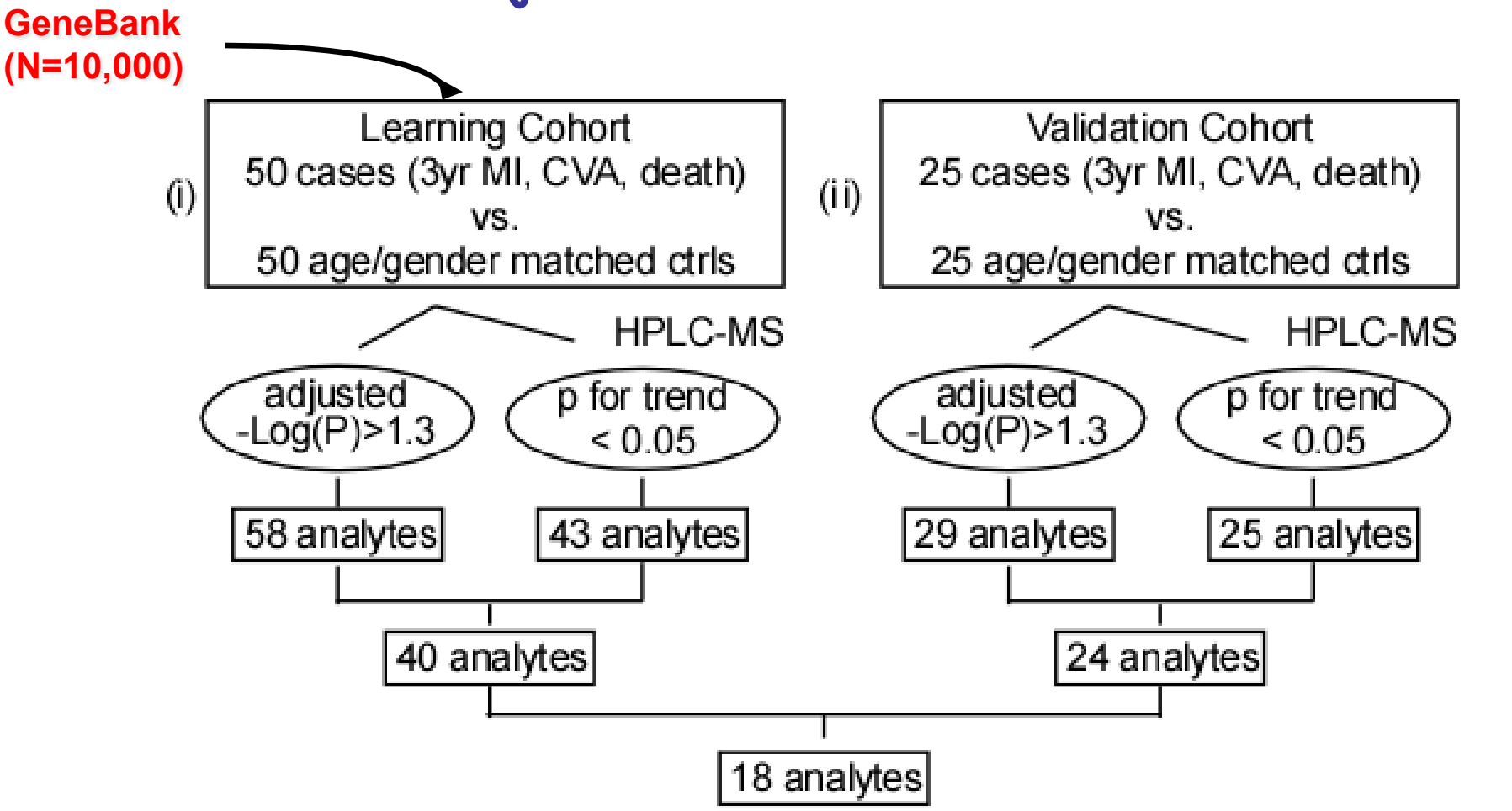
Phase 2: Clinical validation

Replication and demonstration of clinical utility

Phase 3: Mechanistic studies

Demonstration of causality for a novel pathway

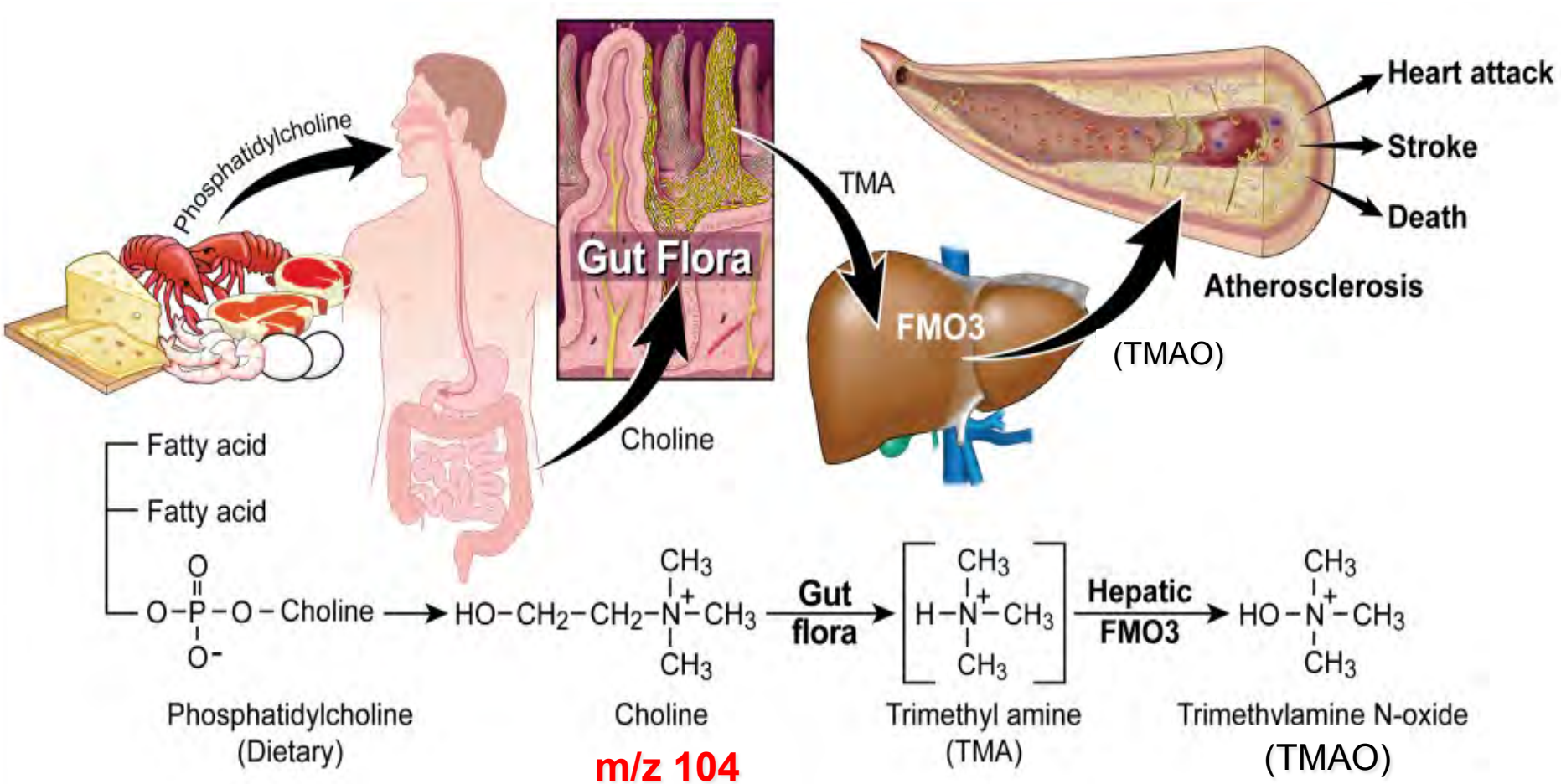
Strategy of metabolomics study design for identifying unbiased small molecule profiles predictive of incident risks for major adverse cardiovascular events



(iii) Structural identification of analytes

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

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

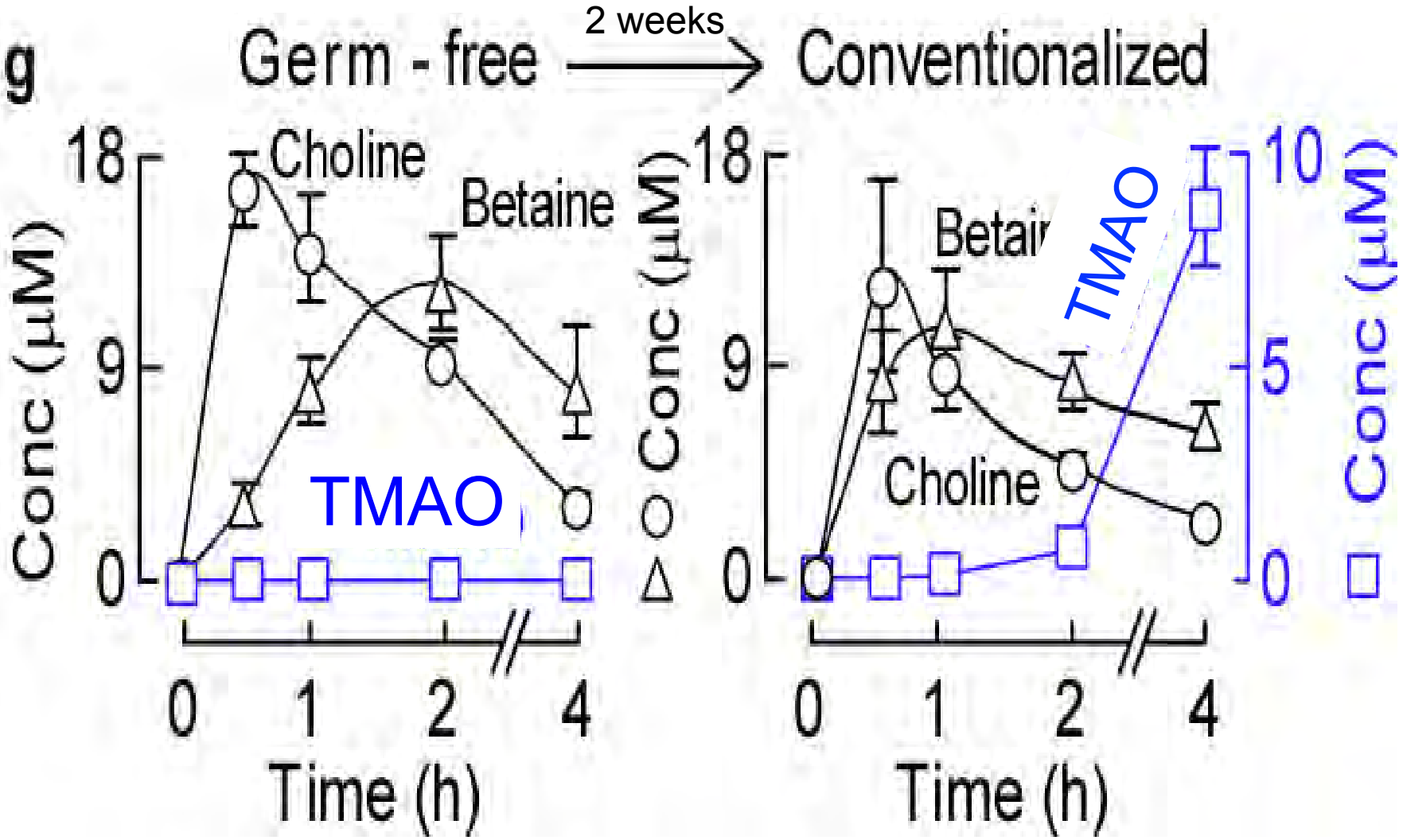


Identities confirmed by:

LC-MS^n, ^{1}H, ^{13}C, ^{15}N NMR

GC/MS/MS, Isotope tracer studies

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



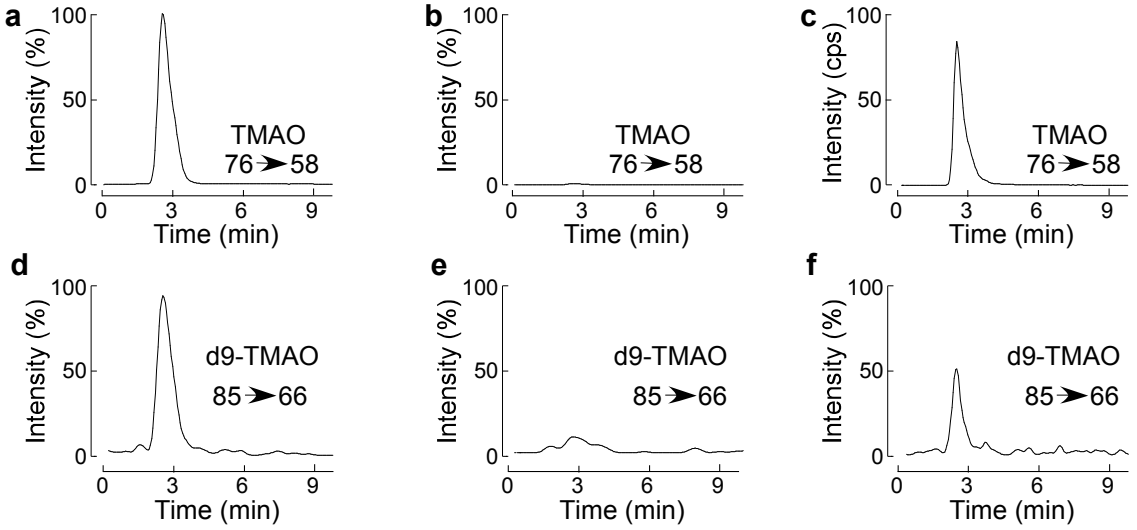
Wang Z et al, *Nature* (2011)

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

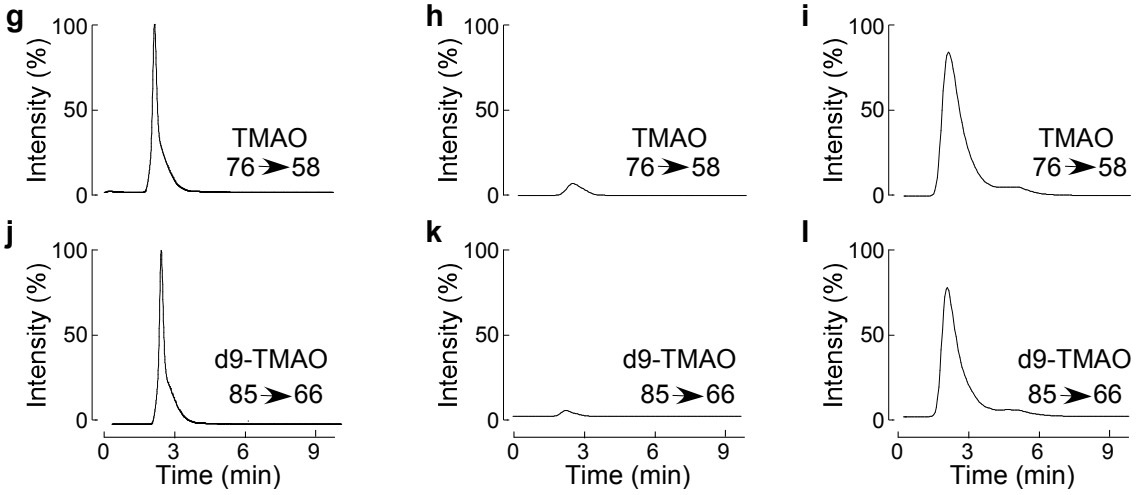
6 h post PC challenge

Plasma



24 h post PC challenge

Urine

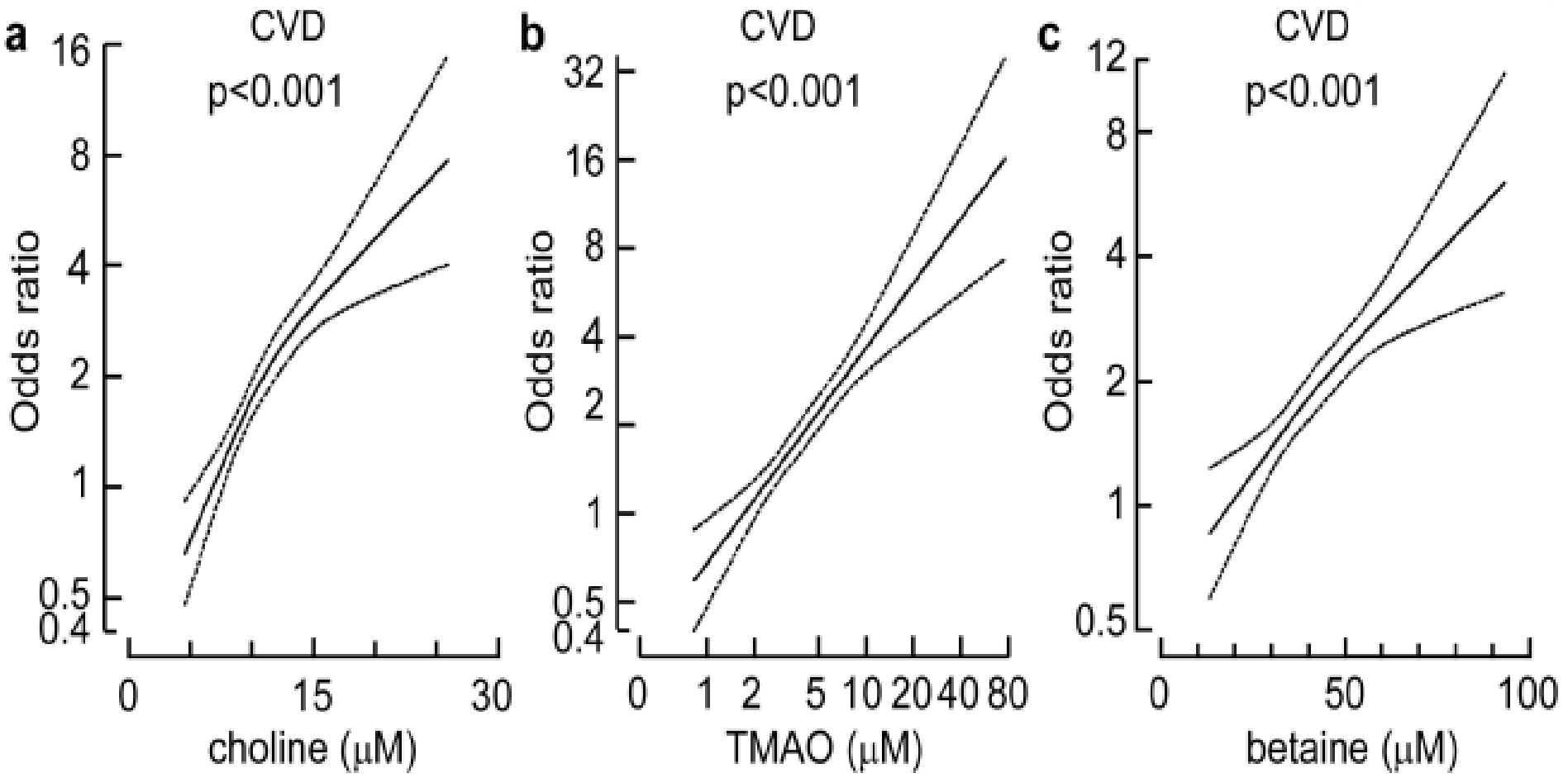


Tang, Wang et al, NEJM (2013)

Prospective Cohort: N=1865 Sequential Cardiology Patients

Plasma choline, TMAO and betaine levels predict CVD risks

(N=1865)



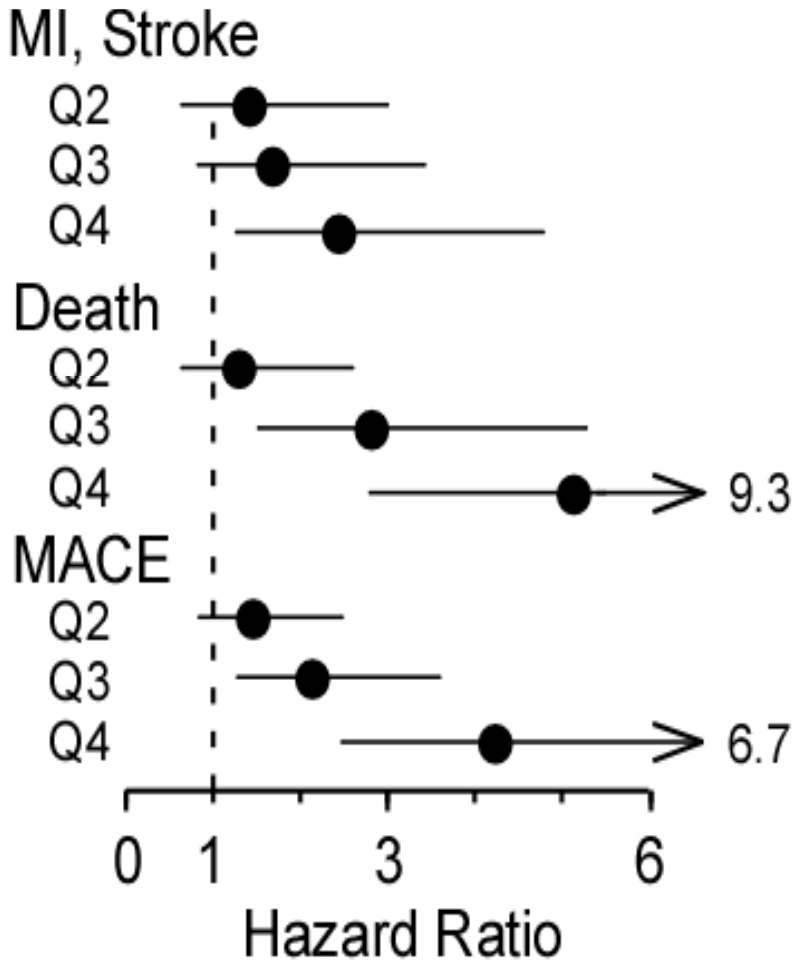
Odds ratio (95%CI) adjusted for age, sex, DM, HTN, smoking, LDL, HDL, TG, CRP, eGFR

Z Wang et al, *Nature* (2011)

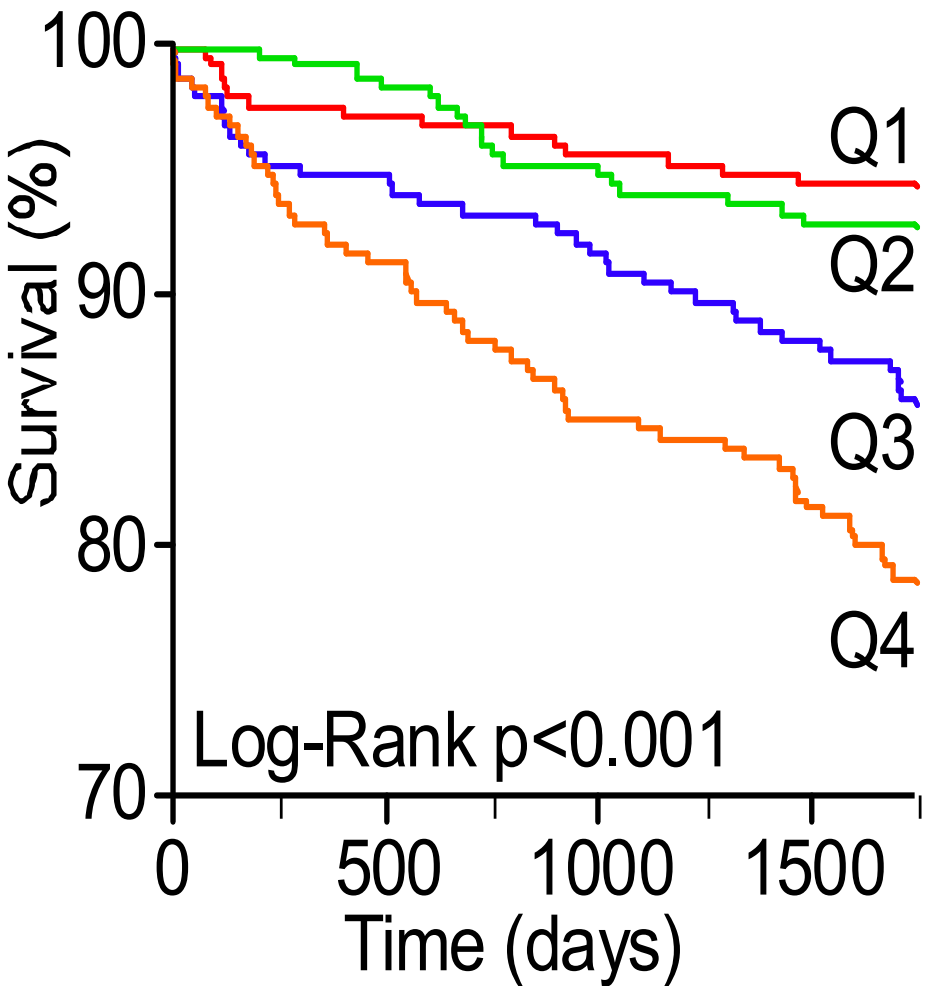
Plasma levels of the gut flora dependent metabolite TMAO predict incident (3 year) CVD risks

New Independent Cohort: N=4007 Sequential Subjects

TMAO



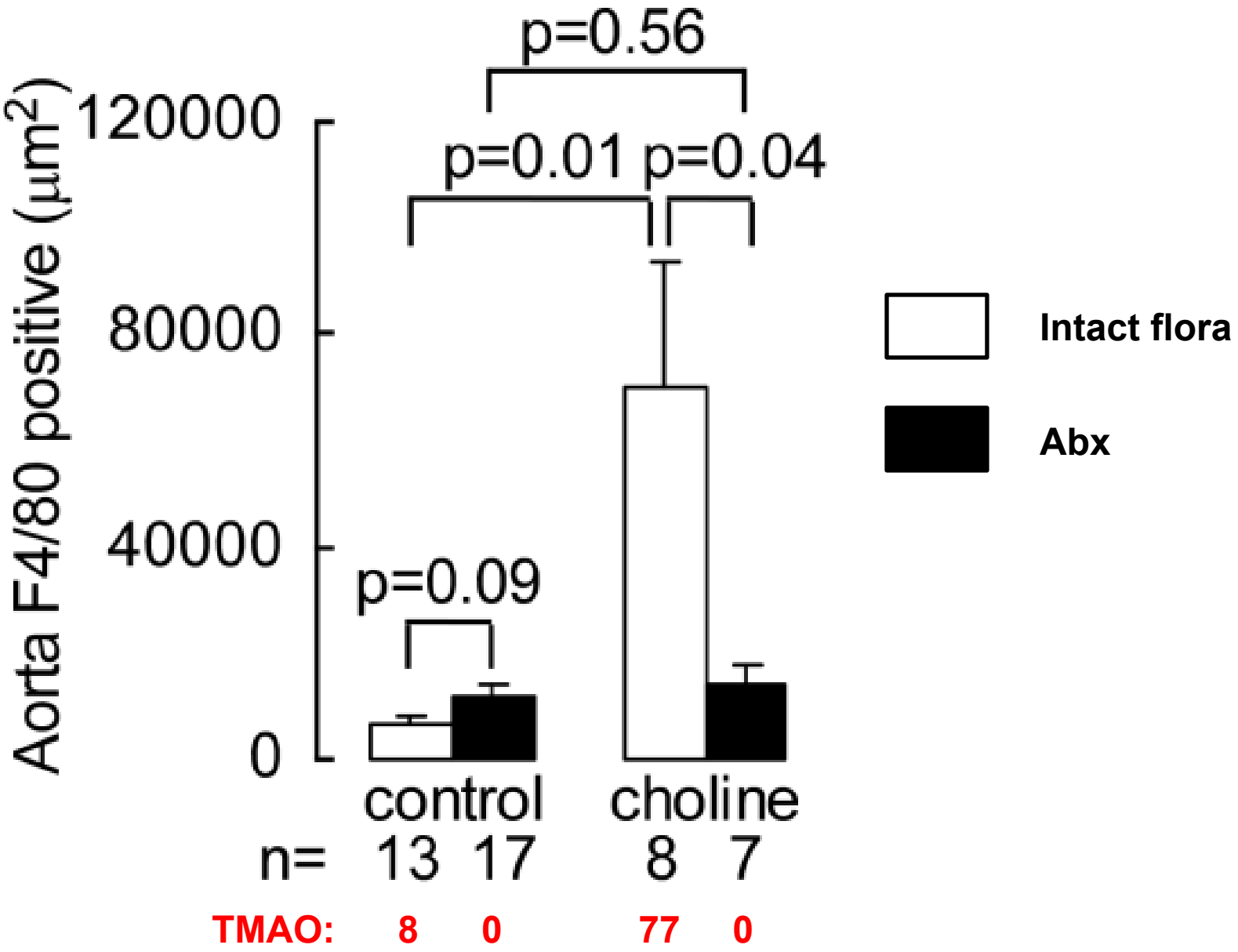
TMAO



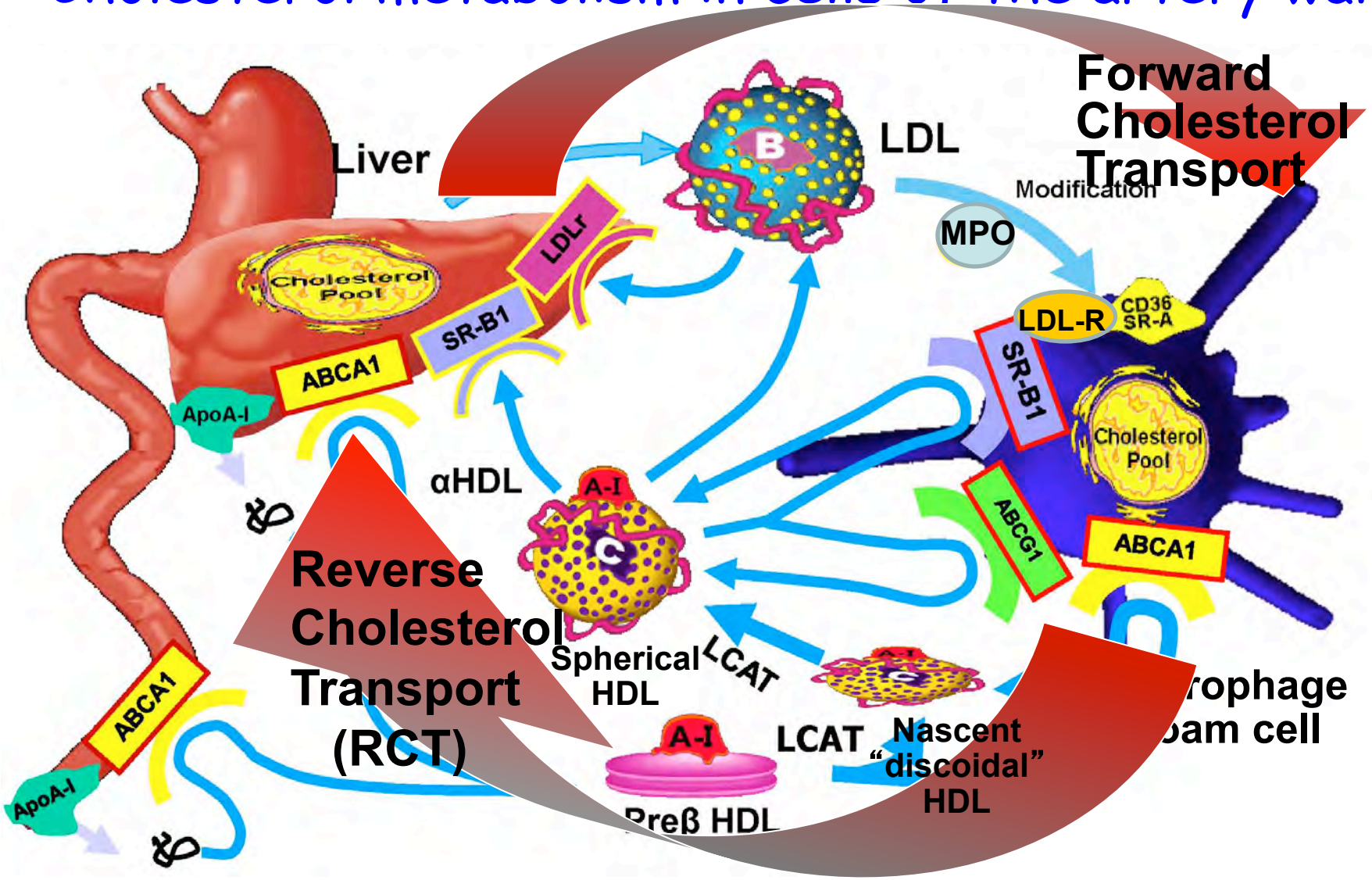
Adjusted for age, sex, DM, HTN, smoking, LDL, HDL, TG, CRP, eGFR

Tang, Wang et al, NEJM (2013)

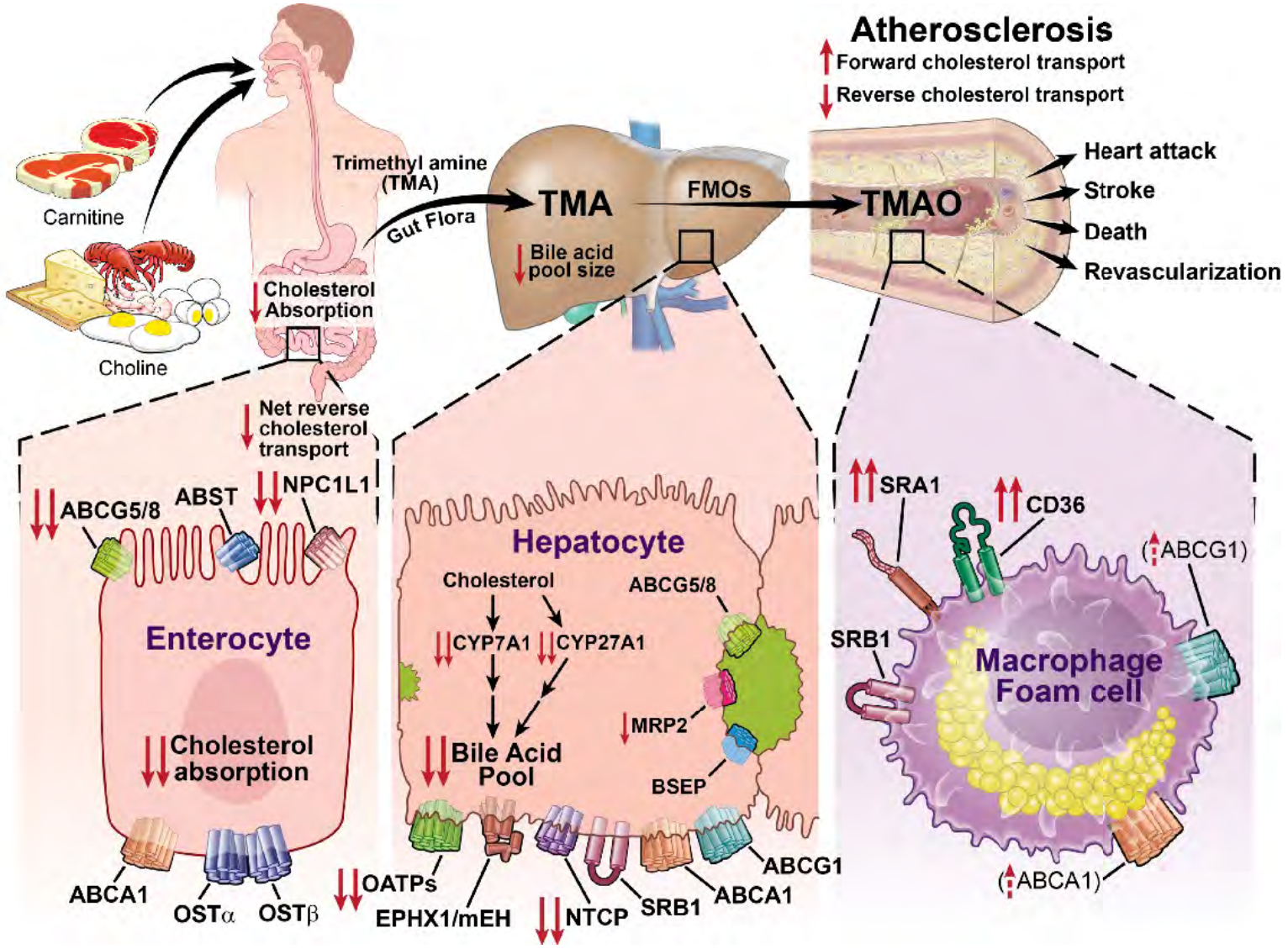
Suppression of gut flora inhibits TMAO formation and dietary choline induced atherosclerosis



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

Epidemiology studies show red meat ingestion is associated with increased mortality risk

An Pan, PhD et al, **Red Meat Consumption and Mortality: Results from 2 Prospective Cohort Studies,** *Archives of Internal Medicine*. 2012; 172(7):555-563.

Health Professionals Follow-up Study
(n=37,698)
men, 40-75 yo
1986 - 2008

Nurses Health Study
(n=83,644)
women, 35-55 yo
1980 - 2008

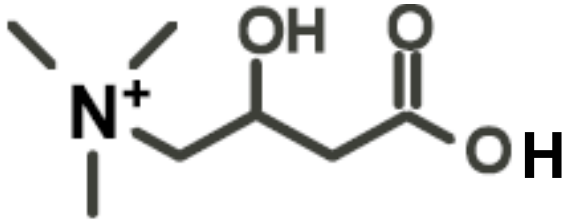
Combined -
2.96 million years of follow-up
23,926 deaths

1 serving per day increase in red meat corresponds to:

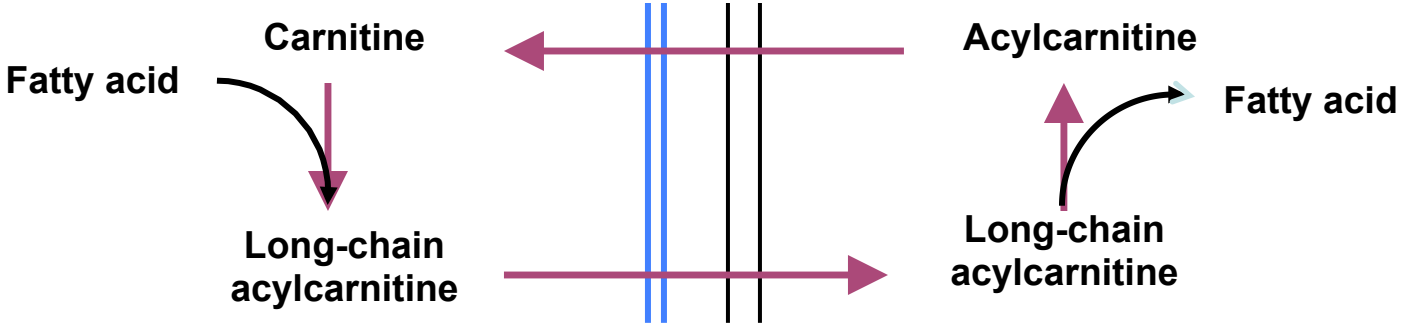
13% increase in total mortality (unprocessed red meat)

20% increase in total mortality (processed red meat)

Carnitine (*from carnis (carnivore), meaning flesh*) participates in fatty acid translocation into mitochondria for β-oxidation

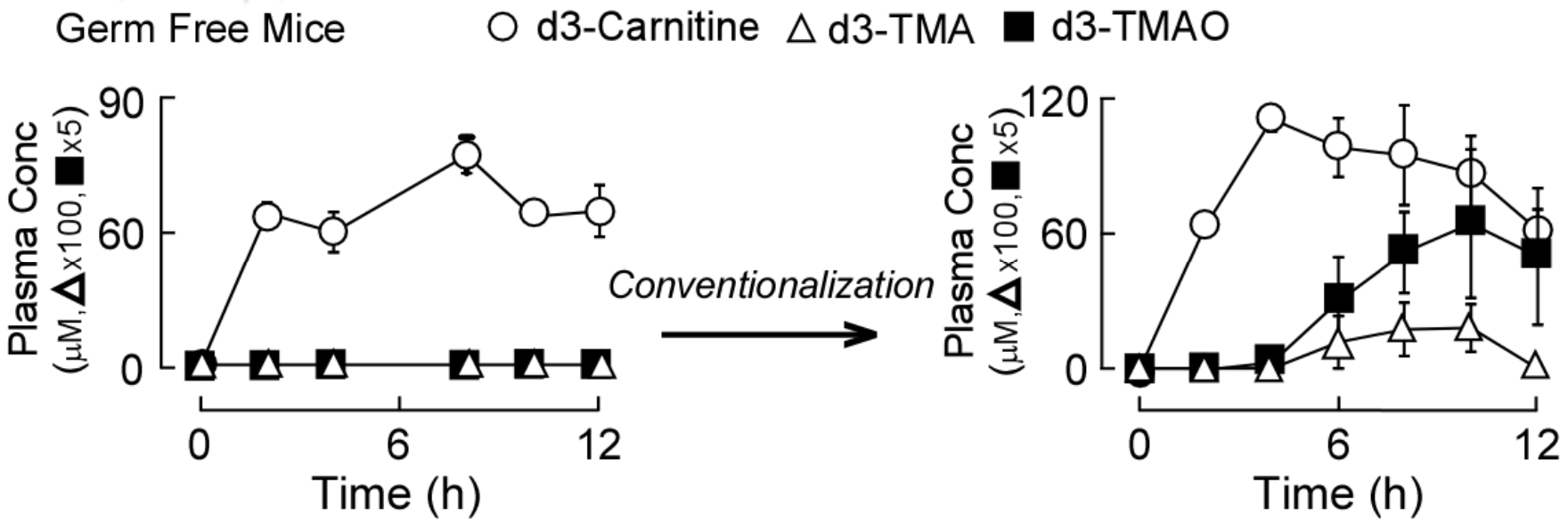


Carnitine



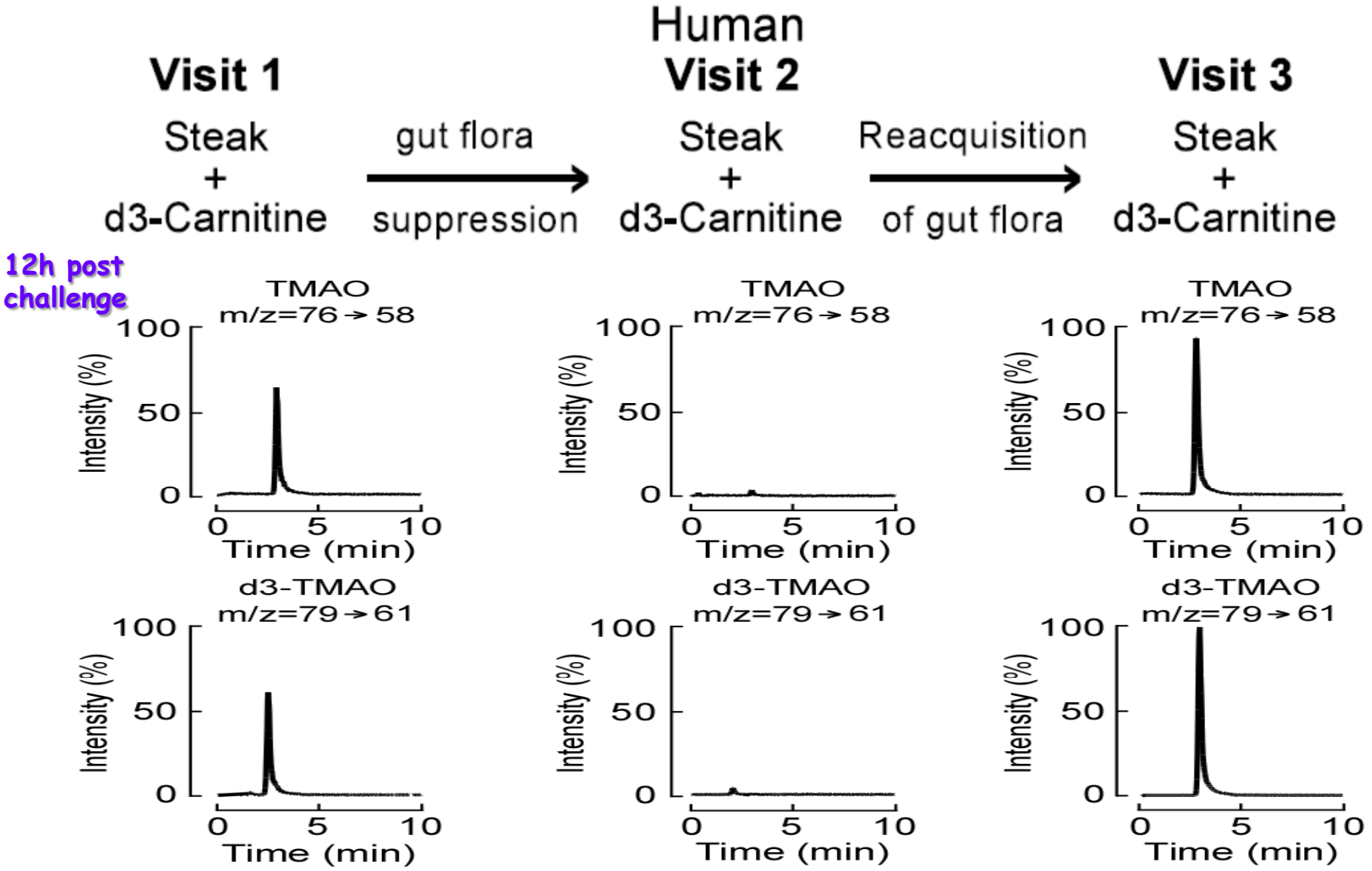
There is an obligatory role for gut flora in TMAO formation from dietary carnitine in mice

d3-(methyl)-carnitine oral dose

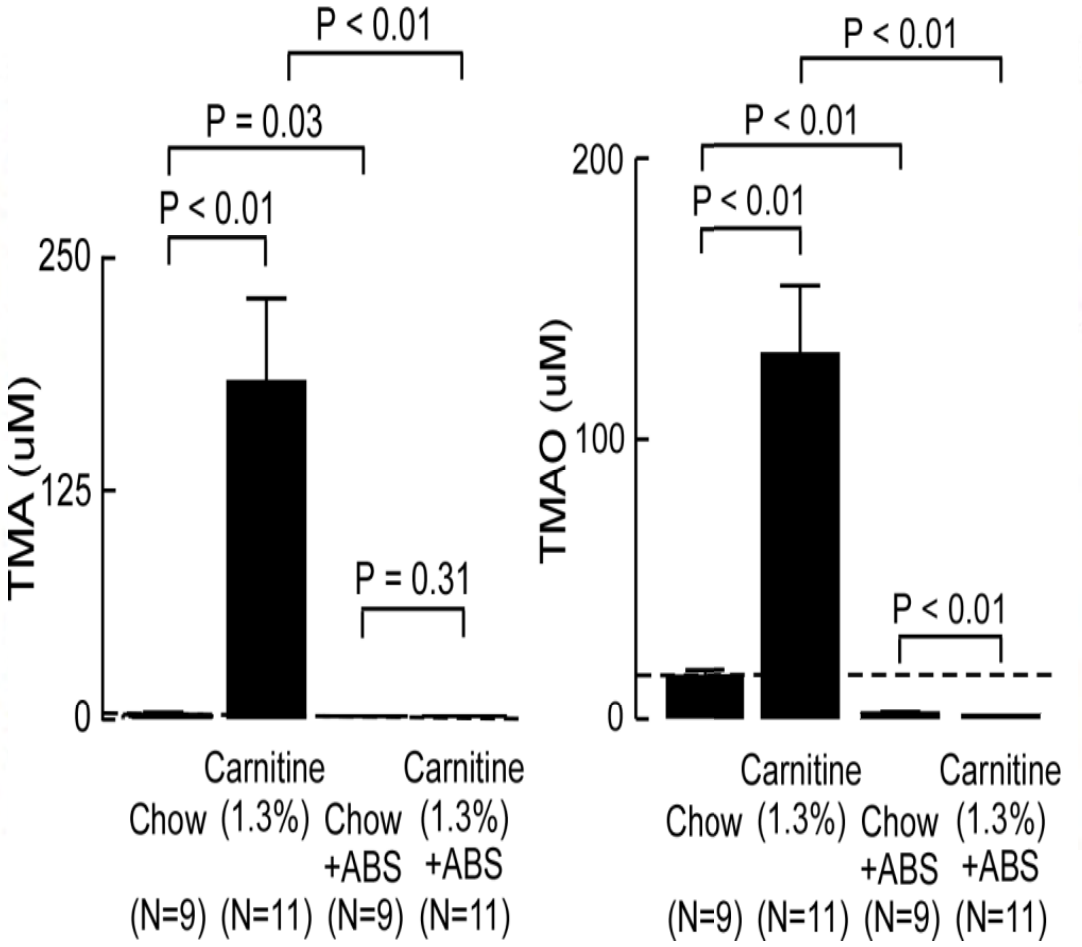


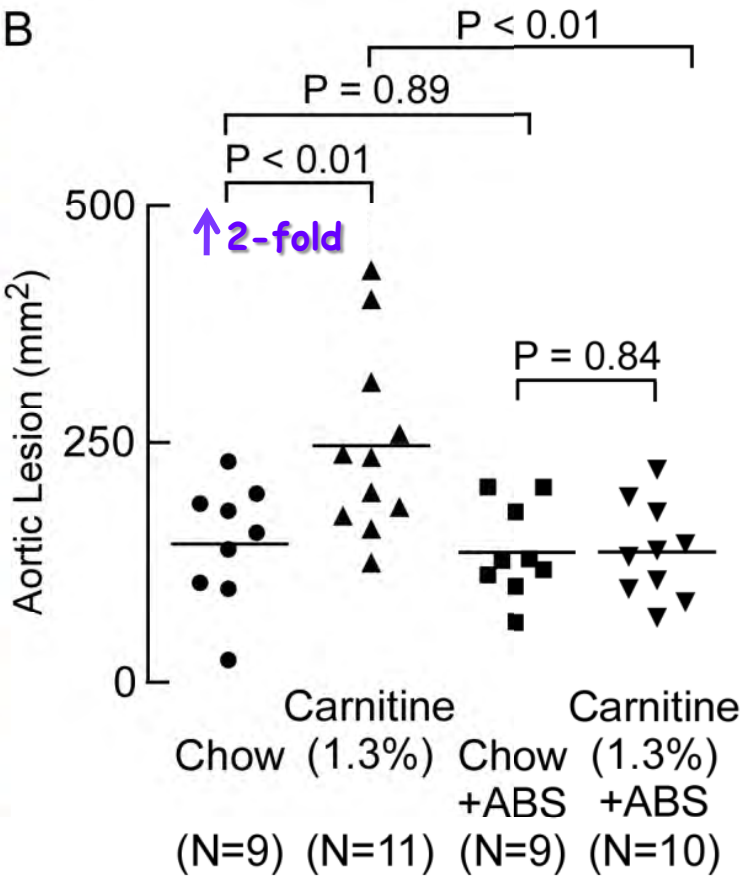
Robert Koeth et al, Nature Medicine (2013)

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



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

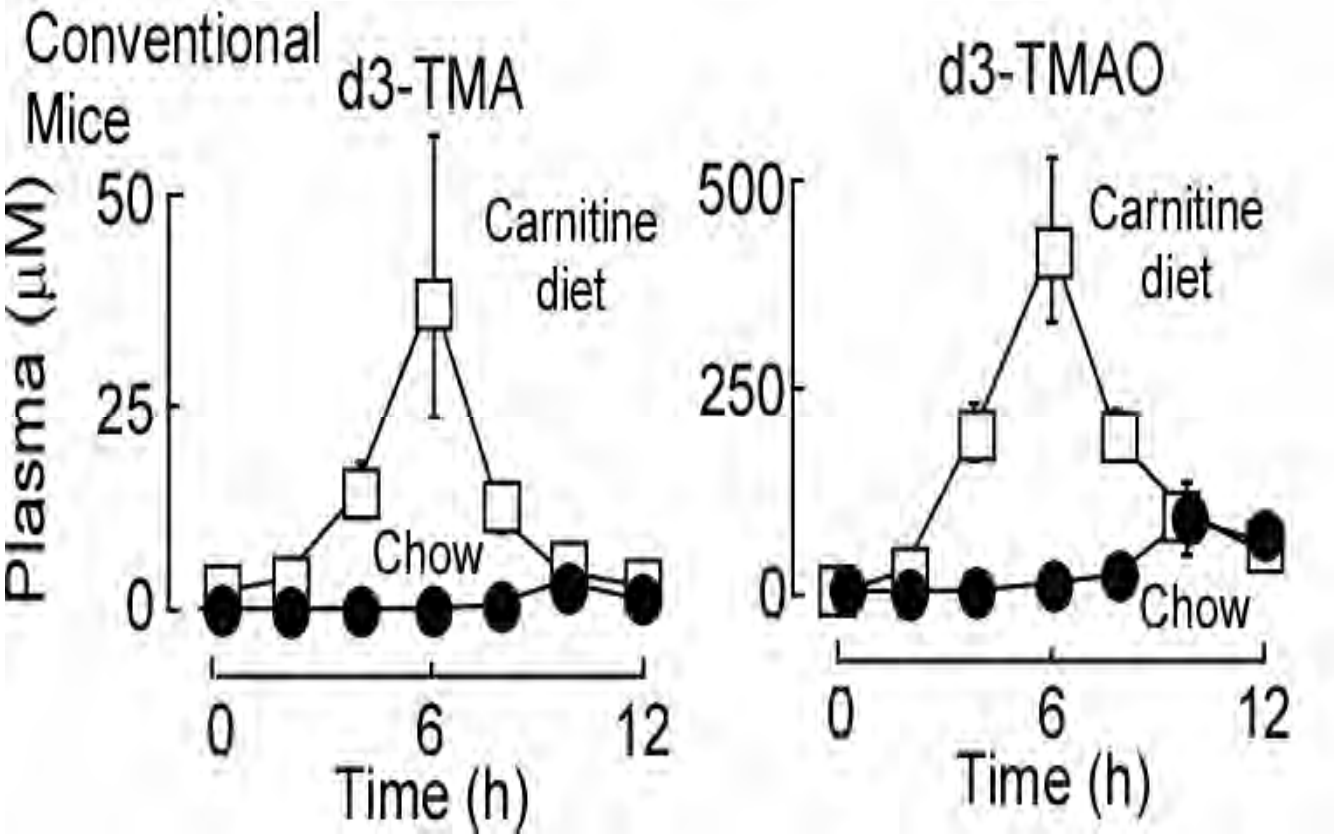




Robert Koeth et al, Nature Medicine (2013)

Production of the gut flora metabolite TMAO from carnitine is inducible

d3-(methyl)-carnitine oral dose



Chronic dietary exposure to carnitine alters gut microbial composition and thus, host metabolism of carnitine

Robert Koeth et al, Nature Medicine (2013)

Scheme of human gut microbiota analysis

N=30

N=23

Omnivore and Vegans/Vegetarians

Stool Collected

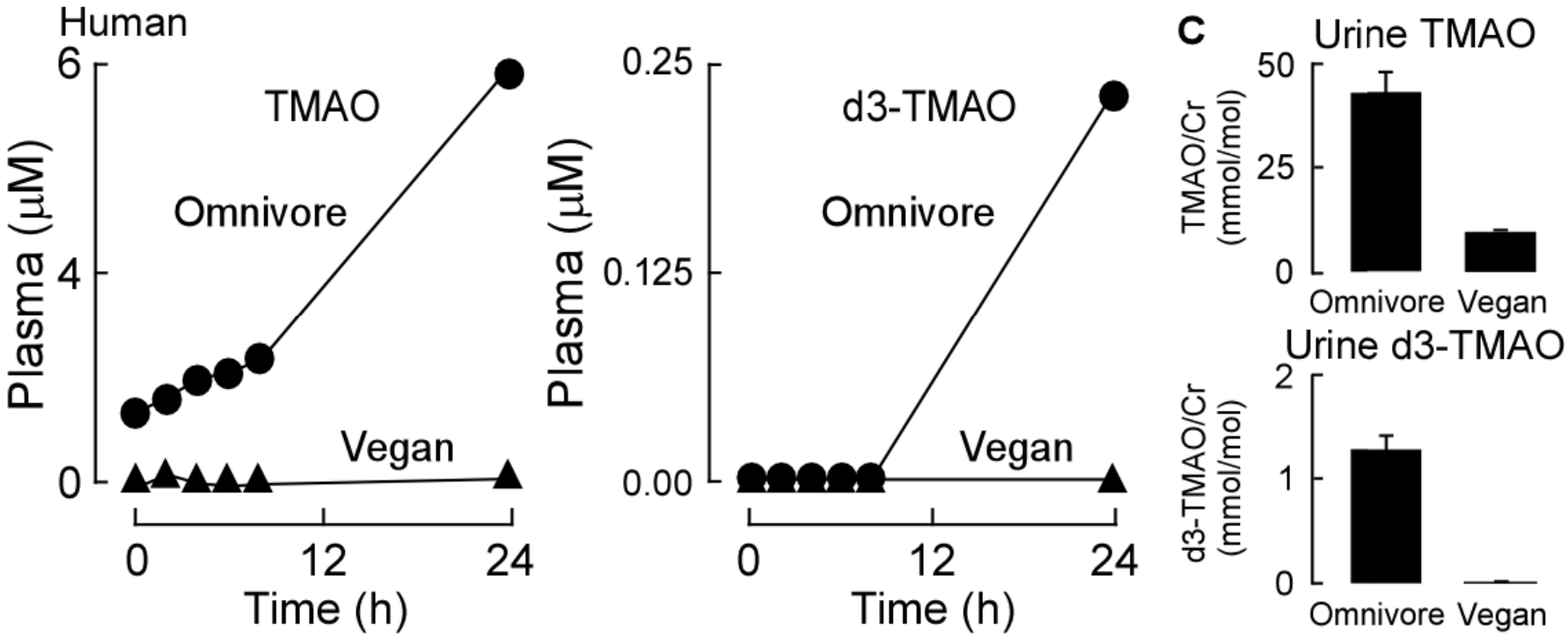
Blood Collected

Gut Microbiota Composition

TMAO measured by mass spectrometry

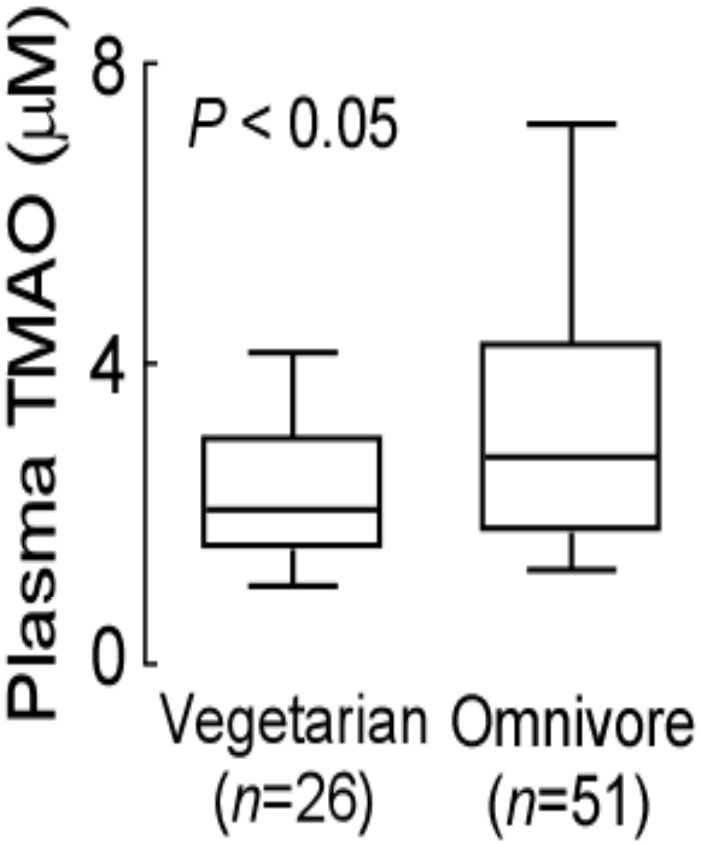
TMAO is formed from dietary carnitine in omnivors, but minimally in vegans

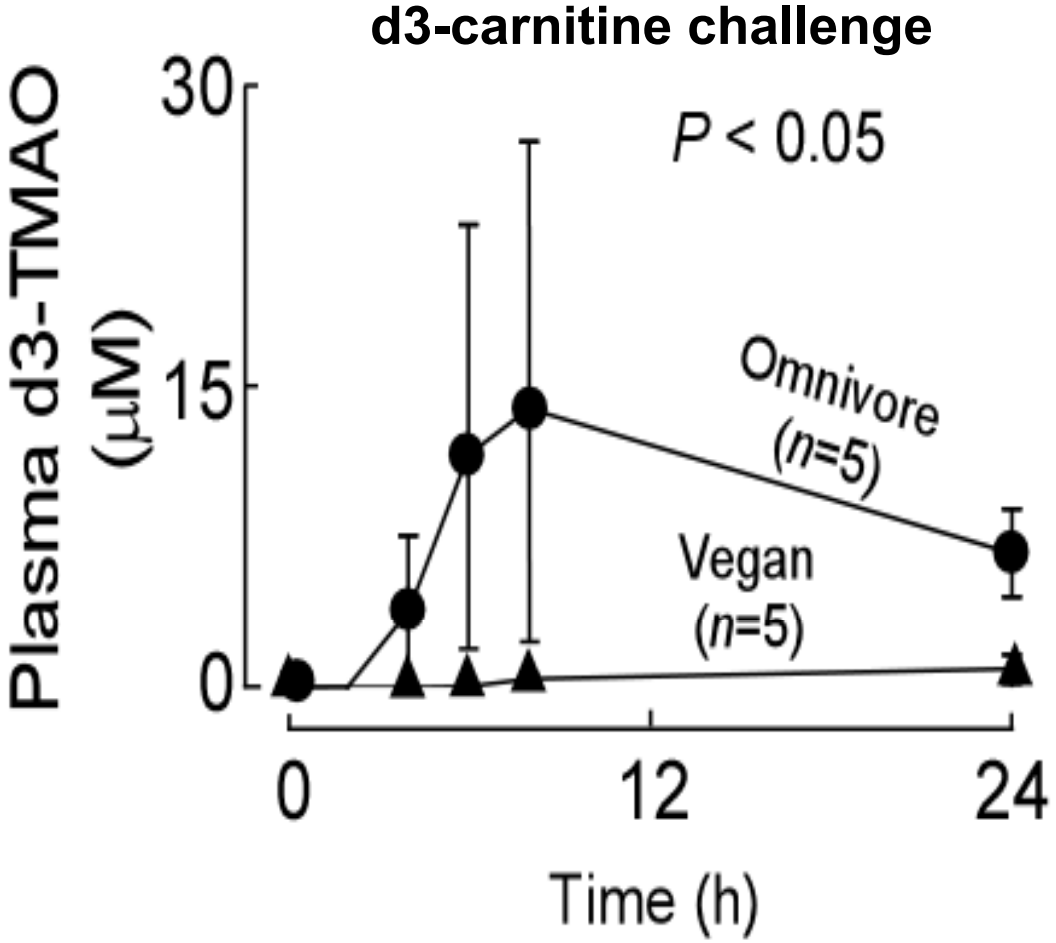
Carnitine challenge: 8oz tenderloin + d3(methyl)-carnitine



Robert Koeth et al, Nature Med (2013)

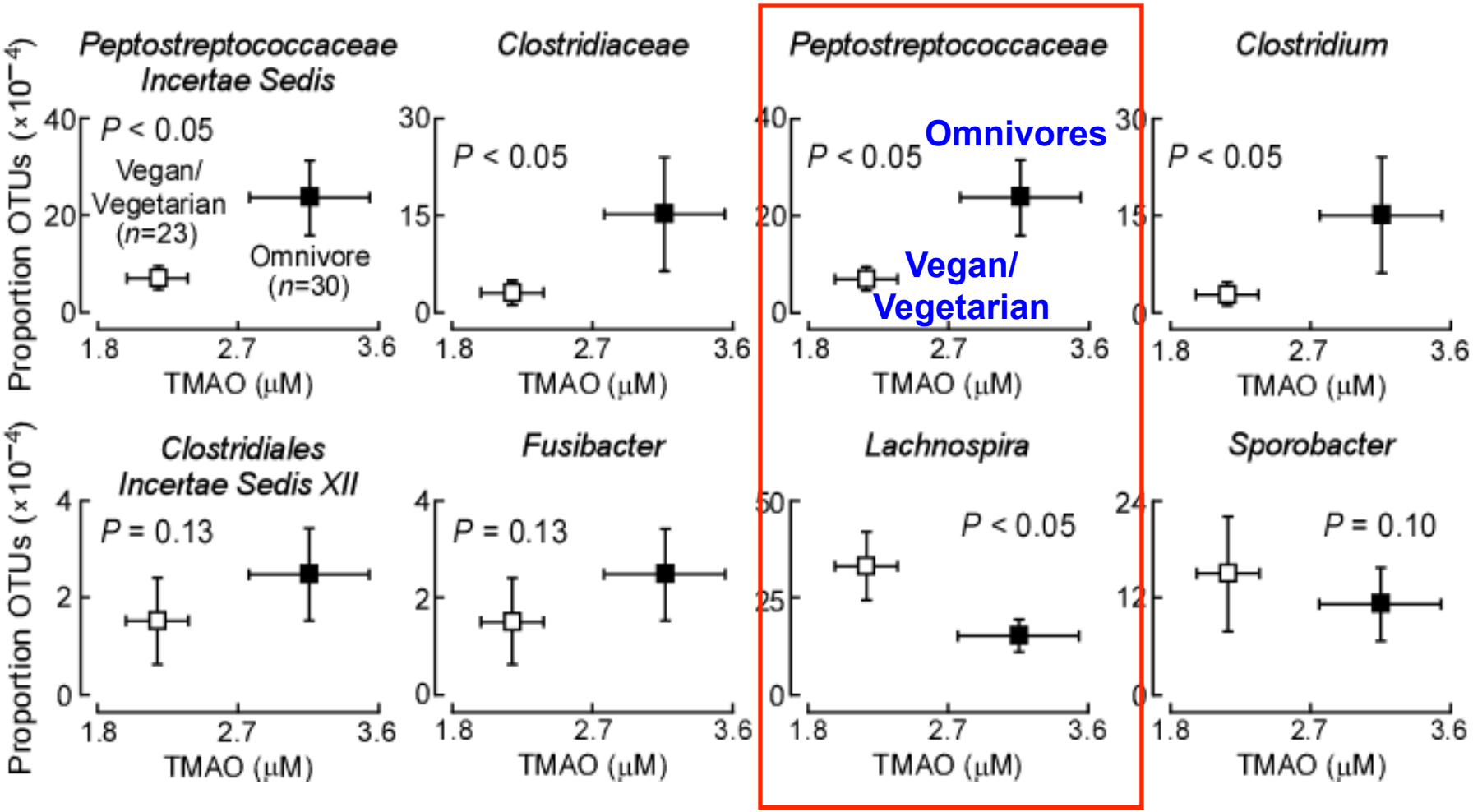
Chronic dietary exposure significantly influences carnitine metabolism





Robert Koeth et al, Nature Medicine (2013)

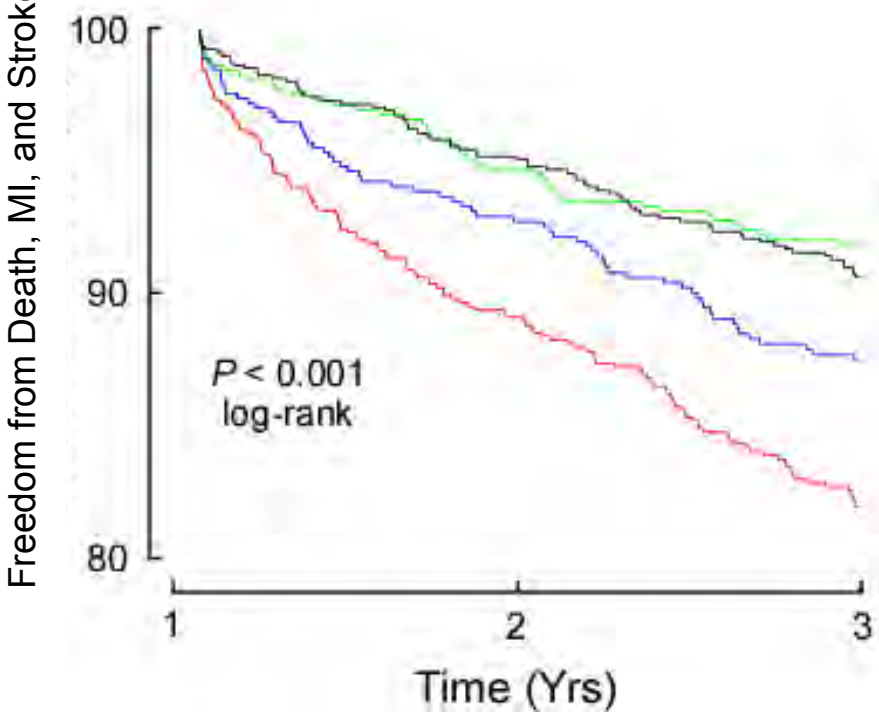
Specific microbiota taxa are associated with long-term dietary patterns and plasma TMAO levels



Robert Koeth et al, Nature Medicine (2013)

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

(N=2595)



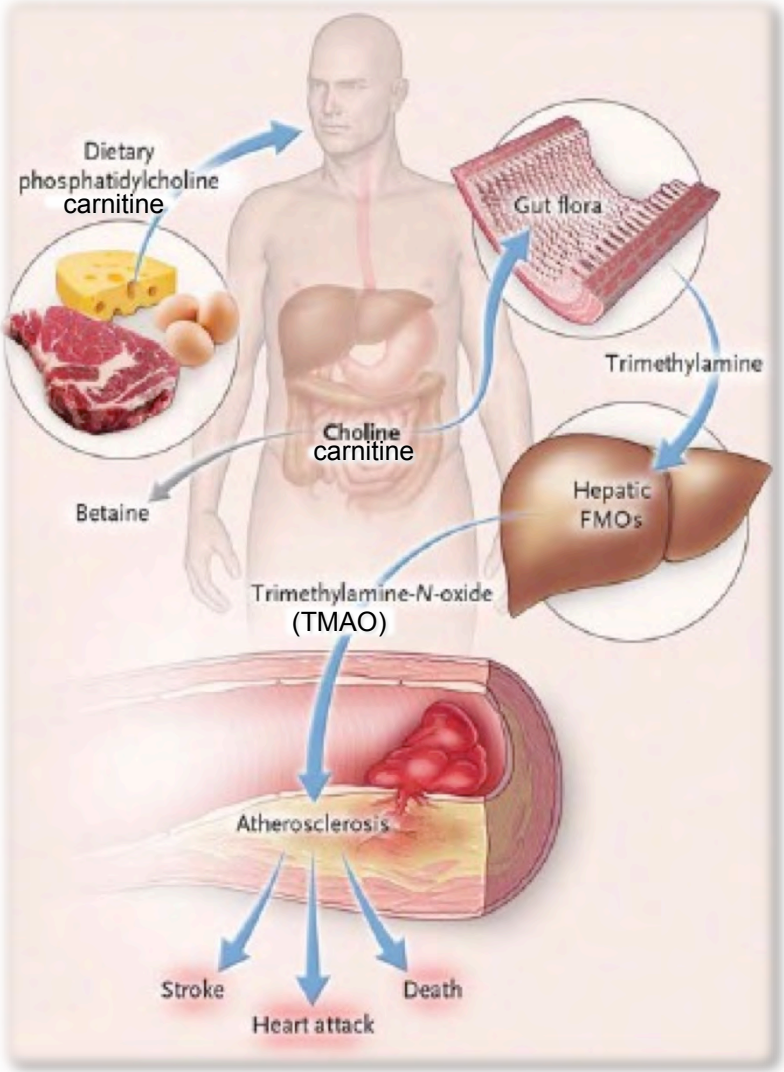
Hazard Ratios

Carn	TMAO	Unadjusted HR (95%)	Adjusted HR (95%)
High	Low	0.9 (0.6 - 1.4)	0.8 (0.5 - 1.3)
Low	Low	1.0 (Reference)	1.0 (Reference)
Low	High	1.6 (1.2 - 2.0)	1.3 (1.02 - 1.7)
High	High	2.5 (1.8 - 3.4)	2.1 (1.5 - 2.8)

Robert Koeth et al, Nature Medicine (2013)

Take Home Summary:

Diet and Intestinal Microbes are Mechanistically Linked to Atherosclerotic Heart Disease



Wang Z et al (2011)
Nature

Koeth RA et al (2013)
Nature Medicine

Tang WHW et al (2013)
New Engl J Med

Bennett B et al (2013)
Cell Metab

Other carnitine sources - are there long term adverse health effects ?

Energy Blend 2500mg
L-Carnitine, Glucose,
Caffeine, Guarana, Inositol,
Glucuronolactone, Maltodextrin

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ENERGY INGREDIENTS PER CAN: MALTODEXTRIN 2000mg, TAURINE 1800mg, L-CARNITINE 450mg, INOSITOL 180mg, PANAX GINSENG 325mg, GUARANA 90mg.

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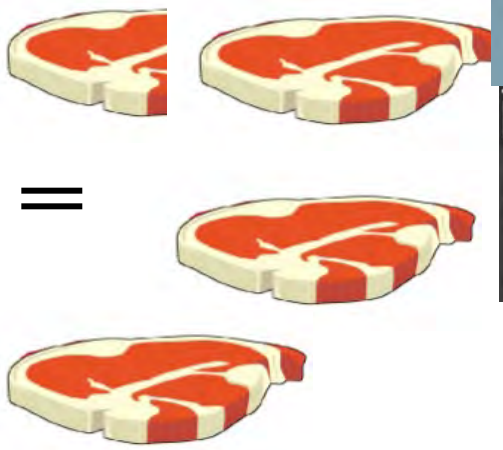

Actually, >600 mg carnitine/can

Acknowledgments

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