Our Gut Microbiome in Health and Disease

Edwin Cox, M.D.

OLLI

Gastrointestinal tract

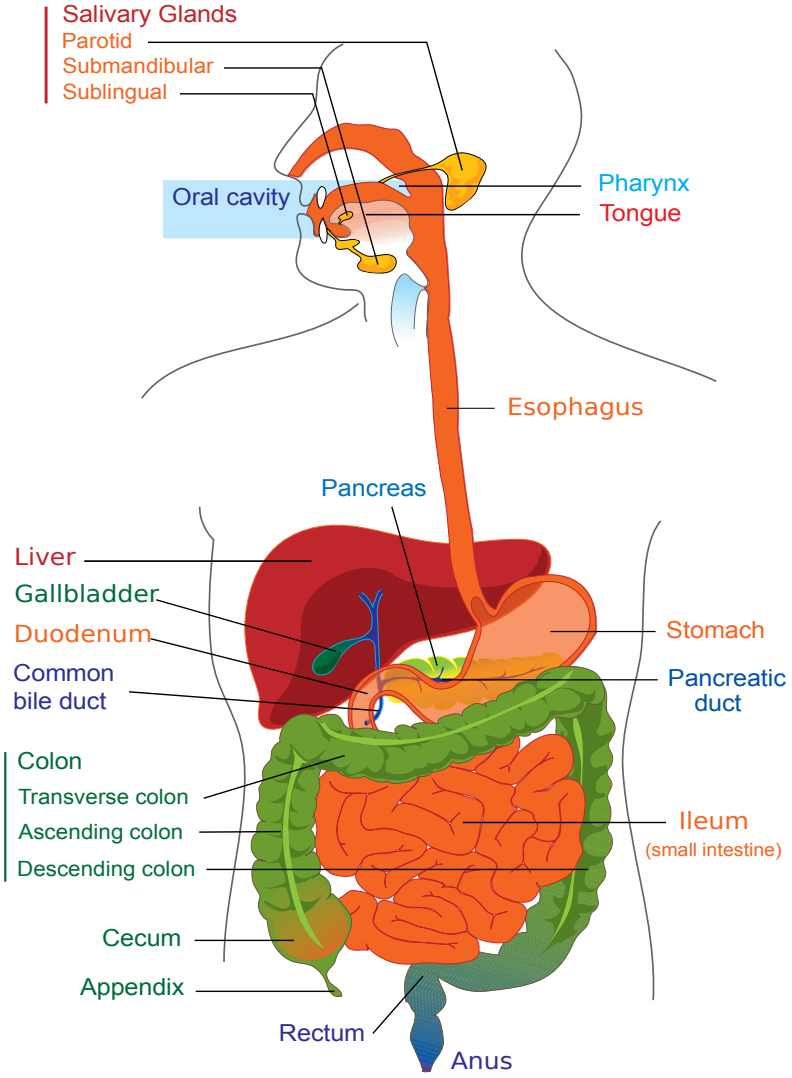
AKA digestive tract, gut

Takes in food, beverage, medication, and everything else

Sorts it out; keeps some of it; passes the rest along

Main parts

- Oral cavity, esophagus, stomach, small intestine, large intestine
- Liver, pancreas, gall bladder

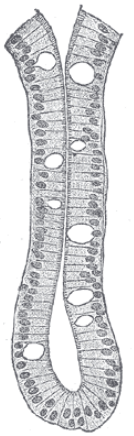


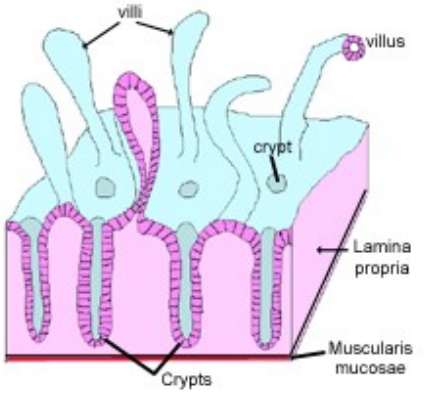
Crypt? Intestinal gland

The intestinal lining is studded with millions of tubular glands

The glands were first described in the 1700s by a German doctor, and in his honor came to be called Crypts of Lieberkühn

Also upward projections called villi that drastically increase surface area for efficient absorption





Humans as host

We play host to roughly 50 trillion microbes, mostly bacteria

- By comparison, we are comprised of roughly 37 trillion human cells
- The majority of bacteria are in the large intestine and terminal small bowel
- 500 to 1000 individual bacterial species are present

Greater diversity (larger number of species and balance among species) is associated with gut health

- Compete out pathogenic (disease-causing) organisms
- Produce substances that inhibit pathogens

Tending our inner garden

Our gut bacteria provide valuable benefits (discussed later)

We do little in exchange except provide nutrients

The quality of their benefits is dependent on what we feed them

They feed on the residue from our diet, mainly indigestible carbohydrates ("fiber")

- Modern name for fiber is *prebiotics*

Our best strategy is to provide good quantity and diversity of dietary fiber

- Prebiotic supplements ("nutraceuticals") may not suffice

How do we get our gut bacteria?

Babies acquire bacteria from the birth canal, breast feeding, person-to-person contact, crawling on the floor, etc.

Develop core population and species distribution over time

- Stable over extended periods
- Diet affects proportions of different species

Acquire new species as we come into contact

Balance altered by antibiotic therapy

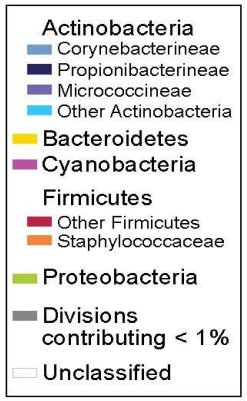
Microbiome

Biome / biota

- A community of living things – plants, animals, bacteria, etc. – occupying a specified region
- "Ecosystem" refers to a biome plus its environment (e.g., water, climate, minerals)

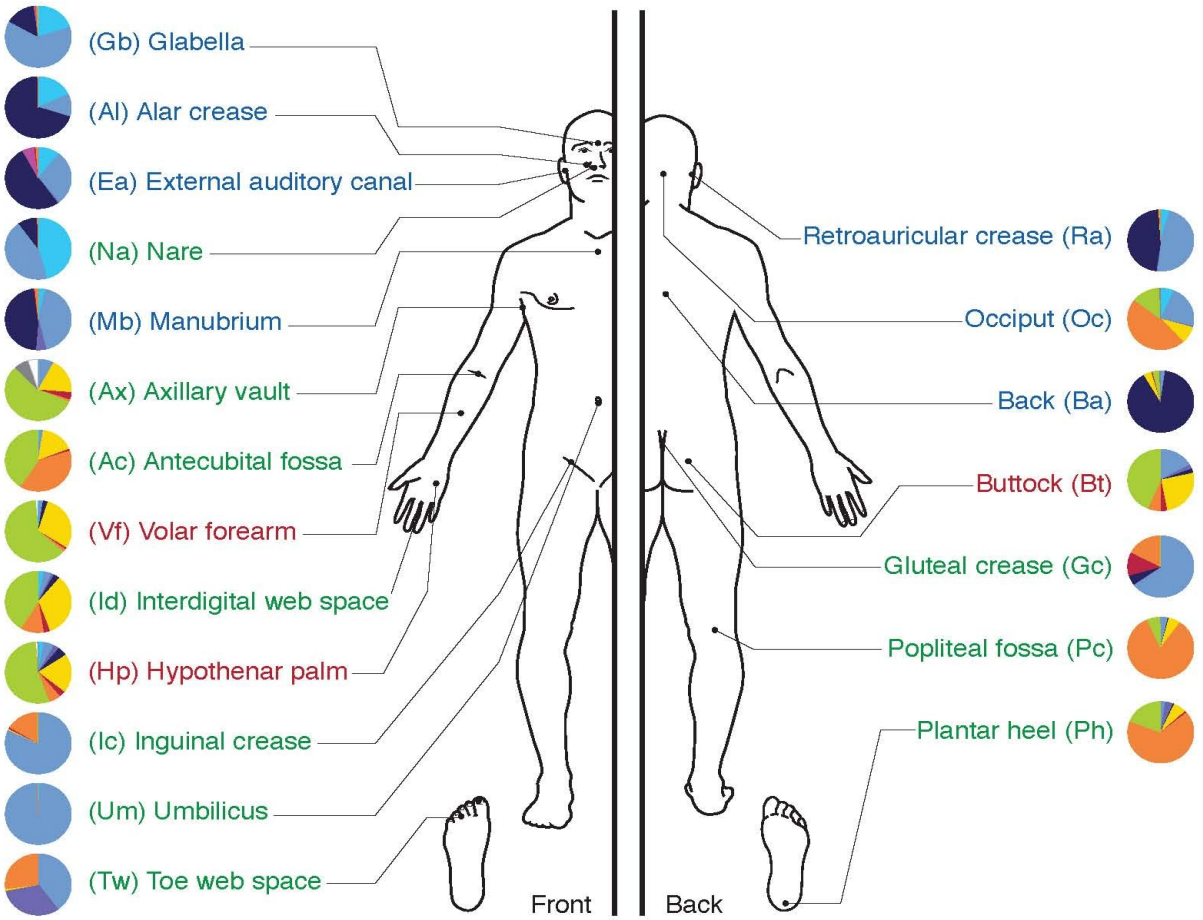
Microbiome

- The community of microscopic organisms that typically lives in commensal or mutualistic relationship with a macroorganism
- Bacteria, fungi, archaea, viruses, and others



Different locations are host to different species, because microenvironment determines where organisms thrive.

Factors such as pH, electrolyte and sebum concentration, temperature, mechanical forces, etc., determine favorability of a location to habitation.



Humans as a meta-organism

A modern interpretation of life forms

- Macro-organisms are not monoliths; we are hybrids of our base genome/cells and all the passengers that we carry with us; that hybrid has been given the term "meta-organism"
- The meta-organism has a core set of passengers, but the menagerie is dynamic, as species and proportions of micro-organisms change in response to diet, age, BMI, among other factors
- Most passengers, at best, contribute to the well-being of the host in return for being fed and at worst, live without harming the host

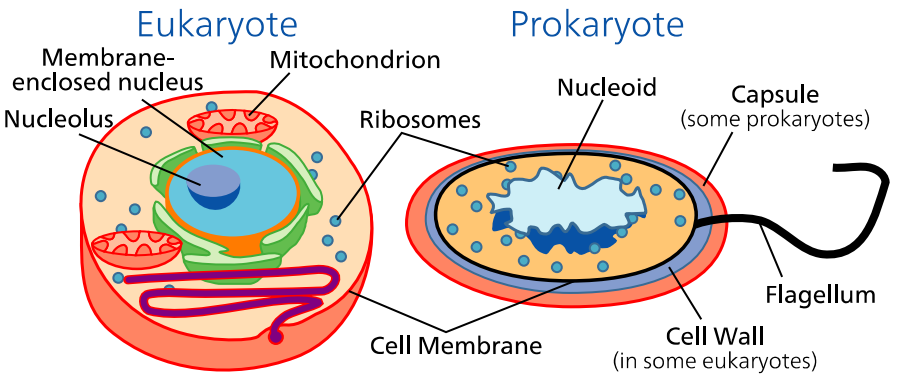
Pro- and eukaryotes

Cells that have no nucleus and have their DNA in the cytoplasm are called "prokaryotes"

- Bacteria, and similar but distinct single-cell organisms called archaea, are prokaryotes

The other main category, eukaryotes, have a nucleus, containing the DNA, and other membrane-bound organelles

Prokaryotes were the first organisms on Earth; higher forms evolved from them



Bacteria

Single-cell prokaryotic organisms that are a complete living thing on their own

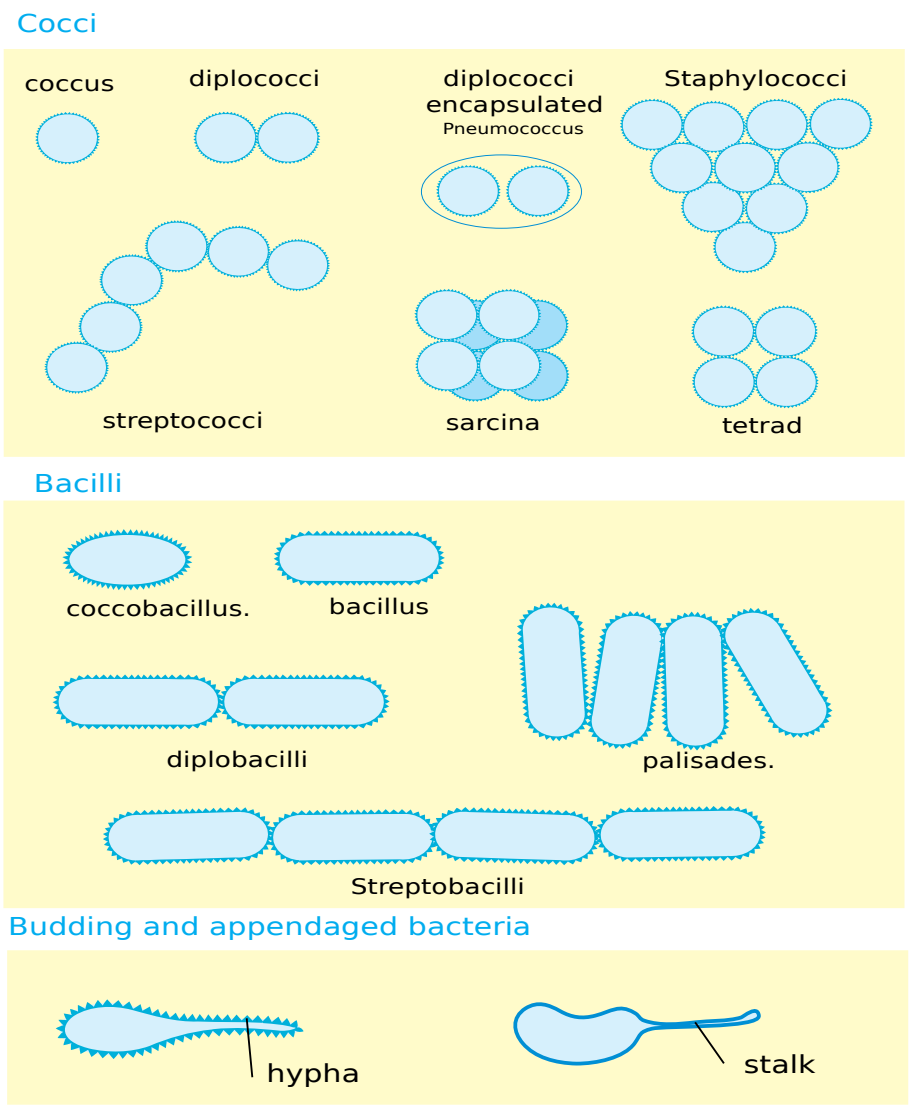
- Viruses, by contrast, cannot live on their own; must inhabit a free-living organism to replicate

Size generally 0.5-5 μm and visible only by microscope, but rarely as large as 700 μm (0.7 mm) or as small as a virus

- Red blood cell ~ 7 μm

Impressive variety of shapes, energy sources, environmental preference, and other characteristics

Bacteria


Cocci
coccus
diplococci
diplococci
encapsulated
Pneumococcus
Staphylococci
streptococci
sarcina
tetrad
Bacilli
coccobacillus.
bacillus
diplobacilli
palisades.
Streptobacilli
Budding and appendaged bacteria
hypha
stalk
Others
enlarged rod
Fusobacterium
Vibrio
Comma's form
Bdellovibrio
Club Rod
Corynebacteriaceae
Helical form
Helicobacter pylori
Corkscrew's form
Borrelia burgdorferi
Filamentous
spirochete

Bacteria: known and unknown

Almost everything we knew about bacteria until quite recently was based on cultures

- Spread a sample onto culture medium in a Petri dish (agar with other nutrients)
- Single bacteria proliferate to form a colony
- Sample from colony examined under microscope with Gram staining to identify (colony morphology, organism morphology, preferred media, etc)
- Subcultured to further determine characteristics and identify

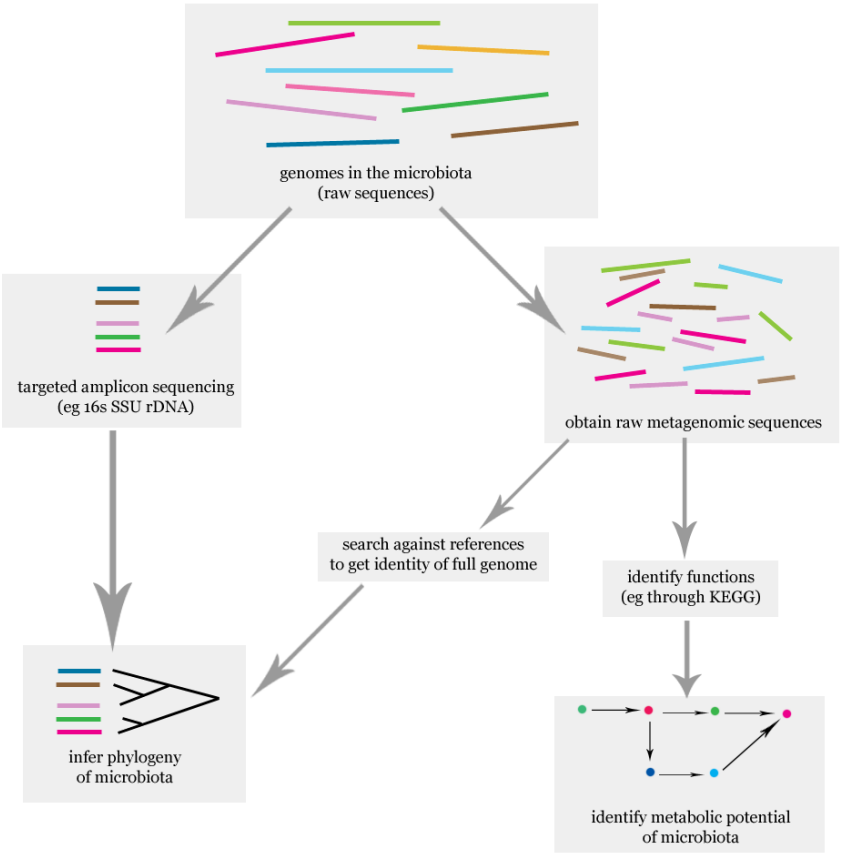
We knew how much we didn't know about bacteria, because many bacteria can be seen but not cultured

Bacteria in the 21th century

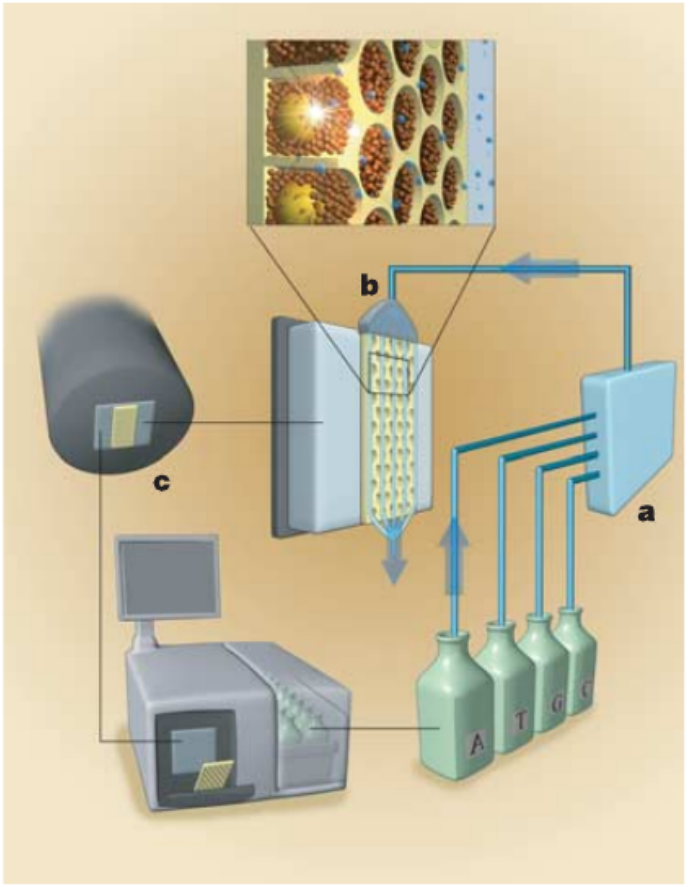
Revolution due to DNA sequencing extends to bacteria

Bacteria not culturable by current techniques can be identified

Genomic analysis allows identification of bacteria without culturing



DNA Sequencing Instrument



3" square slide holds 500,000 samples, each a different bacteria

Automated process reads all sequences in 2 hours

Sequencing output: Taxonomy tree

Operational taxonomic units (OTU)

Branching structure represents relationships between the various bacteria analysed

Reports percentages of each OTU

Some can be identified by reference to gene bank; others are as yet unidentified

Taxonomy of bacteria

Taxonomy

- Classification in the "tree of life", implying the evolution pathway

Species is the "leaf" of the "tree", a basic defining unit within which hybrids are possible; genus is the next level up on the tree

- Name of each organism is a two word epithet, "genus species"; e.g., Homo sapiens

Structure of taxon (classic)

- Domain, [kingdom], phylum, class, order, family, genus, species
- "Do kings play chess on fine glass sets"
- Often, a phylum is named for the first identified genus of the phylum; naming rules
- Not an absolute, clear-cut process; taxonomy is work in progress; re-organization as new information and concepts evolve (e.g., genomics)
- There are three domains – bacteria, archaea, eucaryota

Taxonomy of bacteria

Bacteria constitutes a domain

The biomass of bacteria exceeds that of plants and animals

- Estimated to be 10^{30} bacteria on Earth

Phyla of bacteria

- 12 phyla known in 1987
- Now, ~ 30 accepted phyla
- ~ 20 candidate phyla in process

Branching order vs. multiple speciation event

Horizontal gene transfer

Main phlya important to humans

- Actinobacteria, Bacteriodetes, Firmicutes, Spirochaetes, Proteobacteria, Tenericutes, Verrucomicrobia

Importance of our gut bacteria

Intestinal microbiome provides valuable services

- Synthesize vitamins - vitamin K, biotin, folate
- Ferment indigestible fiber to make short-chain fatty acids that supply energy to colon and body
- Make compounds that condition the immune system
- Promote integrity of intestinal lining (gut permeability)
- Inhibit growth of disease-causing bacteria

Diseases and disorders occur when bacterial community balance is upset

- Disordered microbiome may play important roles in obesity, diabetes
- May contribute to inflammatory bowel diseases (Crohn's, ulcerative colitis) and irritable bowel syndrome

Three Rights Make a Wrong

The story of unexpected danger that lurks in our diet - and gut

How a liver enzyme, that normally protects us from toxins we ingest, turns vital dietary nutrients into a killer molecule, with the unwitting help of normally beneficial intestinal bacteria

- A cruel, seemingly random joke of Nature, aided and abetted by our love of eating meat

Atherosclerosis (AS)

The disease process which affects the walls of arteries, eventually leading to blockage

Consequences of AS are effects on specific organs

- Myocardial infarction, or heart attack (heart) – ASHD, CHD
- Cerebrovascular accidents, or strokes (brain) - CVD
- Infarction, or tissue death (extremities, organs) - ASPVD
- Known collectively as atherosclerotic cardiovascular disease (ASCVD, ASVD)

ASCVD are the leading preventable causes of death and disability in the U.S. and other developed nations

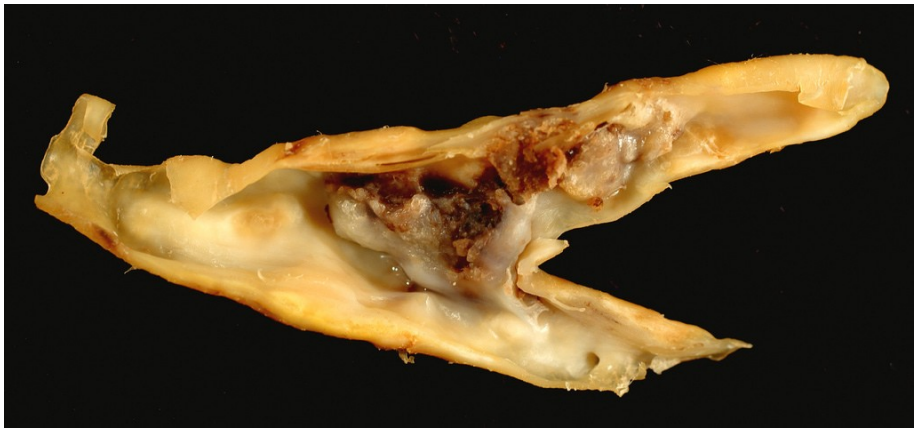
- Most cases could have been avoided by appropriate diet
- A small minority are genetically driven by errors in metabolic pathways (familial hyperlipidemias) or anatomic defects (aneurysms)

Atheroma

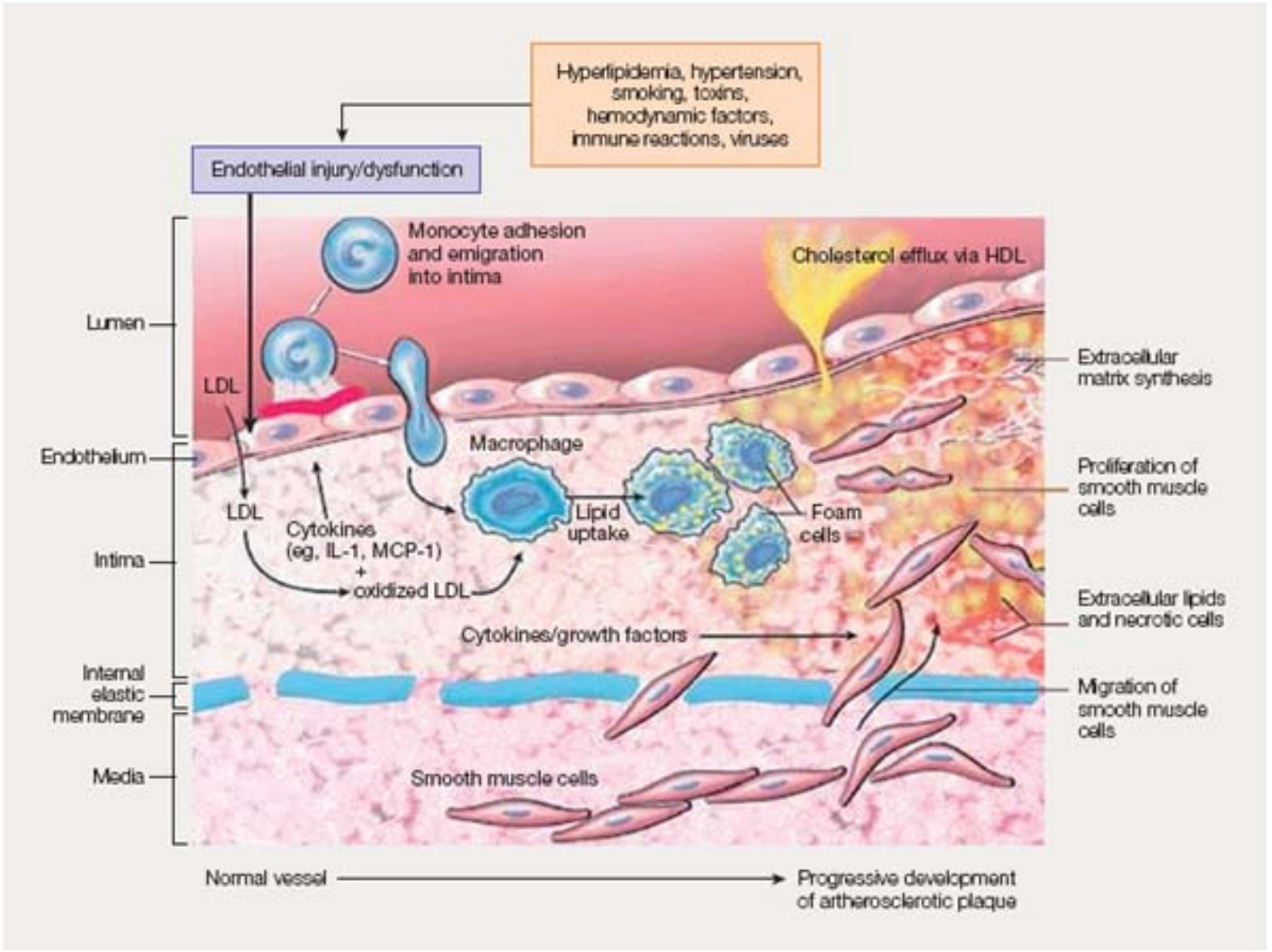
The basic abnormality is a long-term vicious cycle of fat deposition, injury, repair, scarring, rupture, again and again

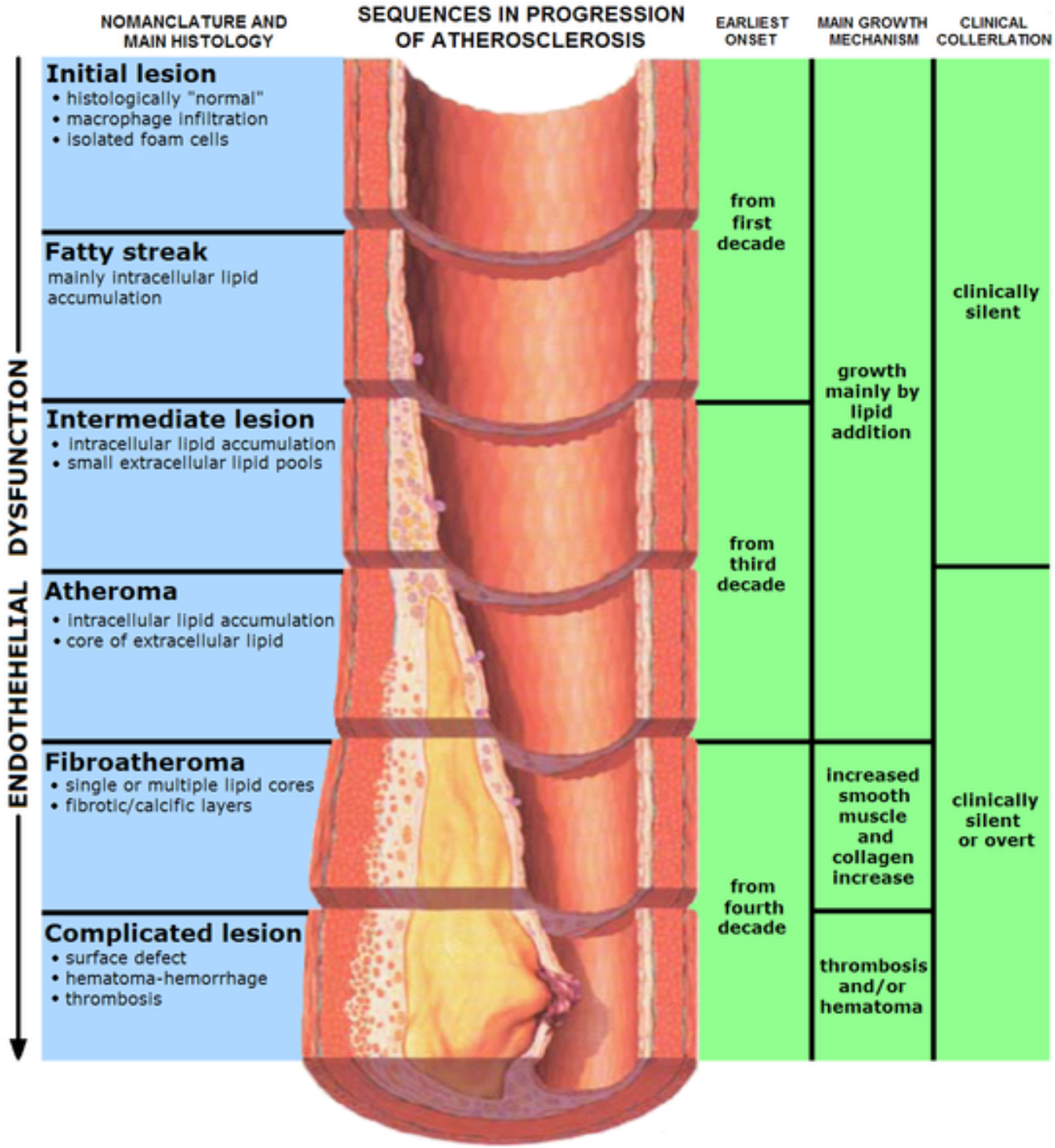
Etymology: Atheroma - "An abnormal mass of fatty or lipid material with a fibrous covering, existing as a discrete, raised plaque within the intima of an artery"

[G. athērē, gruel, + -ōma, tumor]



Atherosclerosis Formation




NOMANCLATURE AND MAIN HISTOLOGY
SEQUENCES IN PROGRESSION OF ATHEROSCLEROSIS
EARLIEST ONSET
MAIN GROWTH MECHANISM
CLINICAL COLLERLATION
ENDOTHEHELIAL DYSFUNCTION
Initial lesion
• histologically "normal"
• macrophage infiltration
• isolated foam cells
Fatty streak
mainly intracellular lipid accumulation
Intermediate lesion
• intracellular lipid accumulation
• small extracellular lipid pools
Atheroma
• intracellular lipid accumulation
• core of extracellular lipid
Fibroatheroma
• single or multiple lipid cores
• fibrotic/calcific layers
Complicated lesion
• surface defect
• hematoma-hemorrhage
• thrombosis
from first decade
from third decade
from fourth decade
growth mainly by lipid addition
increased smooth muscle and collagen increase
thrombosis and/or hematoma
clinically silent
clinically silent or overt

ASCVD conundrum

Risk factors identified

- Lipoprotein ("cholesterol") metabolism – higher LDL and triglycerides, lower HDL
- Hypertension
- Obesity
- Cigarette smoking
- Metabolic syndrome → diabetes

Yet, in statistical analysis of populations, much of the disease risk remained unaccounted for by these factors

- Non-smokers with normal blood pressure who are not diabetic or obese and have "favorable" cholesterol profiles can have MI, CVA

ASCVD & diet

- Cohort observational studies pointed to diet as an independent factor
  - ^ red meat consumption associated with higher ASCVD
  - ^ nuts, dietary fiber associated with lower ASCVD
- Randomized clinical trials demonstrate that Mediterranean diet is an independent favorable factor
- What's responsible?

Red meat & ASCVD

Association of red meat & ASCVD recognized from 1940s

Ancel Keys – Seven Countries Study: Blood cholesterol associated with ASHD

- Blood cholesterol highly correlated with saturated fat consumption

McGovern Committee (1970s) focused on saturated fat, leading to USDA emphasis

A generation of nutrition advice based on concept that dietary fat → ASCVD

Political aspect: Industry associations lobbied tirelessly against placing blame on beef, pork, diary; fat didn't have an industry association lobbying on its behalf!

Industry groups promoted low-fat cuts of meat, low-fat dairy as the answer

Food manufacturers promoted low-fat products

- Fat has no trade association or lobbyists

USDA promoted substitution of grains and other carbs for fat in the diet

- Food Pyramid

21st century findings

Saturated fat is not the pariah it was once thought to be

- Dietary SF not correlated with ASHD

Red meat – and especially processed meat – is highly associated with ASHD and mortality

If not the fat, what is the lethal nutrient?

- One candidate: dietary heme iron, from myoglobin in beef muscle
- But the actual perpetrator appears to be…

21st century findings

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Red meat – and especially processed meat – is highly associated with ASHD and mortality

If not the fat, what is the lethal nutrient?

- One candidate: dietary heme iron, from myoglobin in beef muscle
- But the actual perpetrator appears to be…
- CARNITINE (and its cousins, choline & phosphatidyl choline)

Carnitine

Necessary for movement of fatty acids into mitochondria in human cells

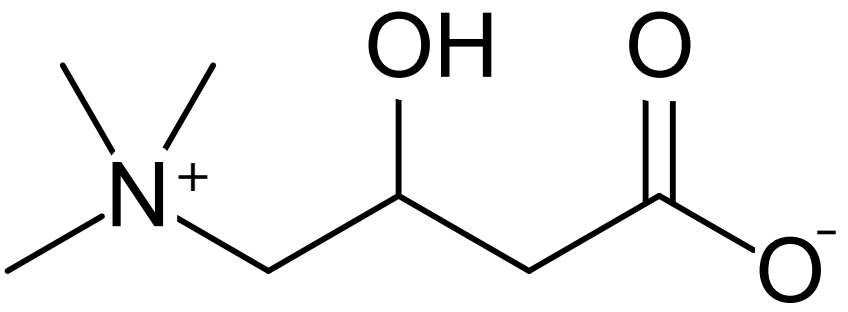
Synthesized in humans from other substrates

- Not required in diet

Abundant in animal-based foods, especially red meats

Taken by athletes to enhance muscle development

Some evidence of protective effect in unstable heart disease



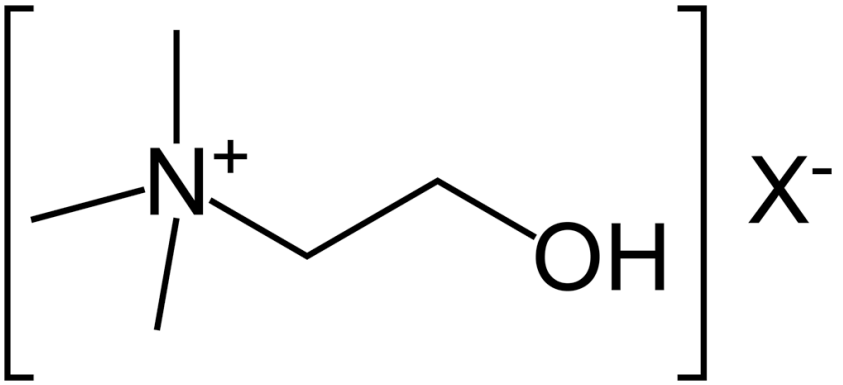
Choline and lecithin

Choline and lecithin (AKA phosphatidylcholine) are vital molecules in the assembly of cell membranes and many other metabolic pathways

Essential to human development and health, and must be obtained from the diet

Abundant in egg yolks, adequate amounts in many other foods (meat, fish, poultry, dairy)

Taken as a supplement in hopes of forestalling cognitive decline and dementia



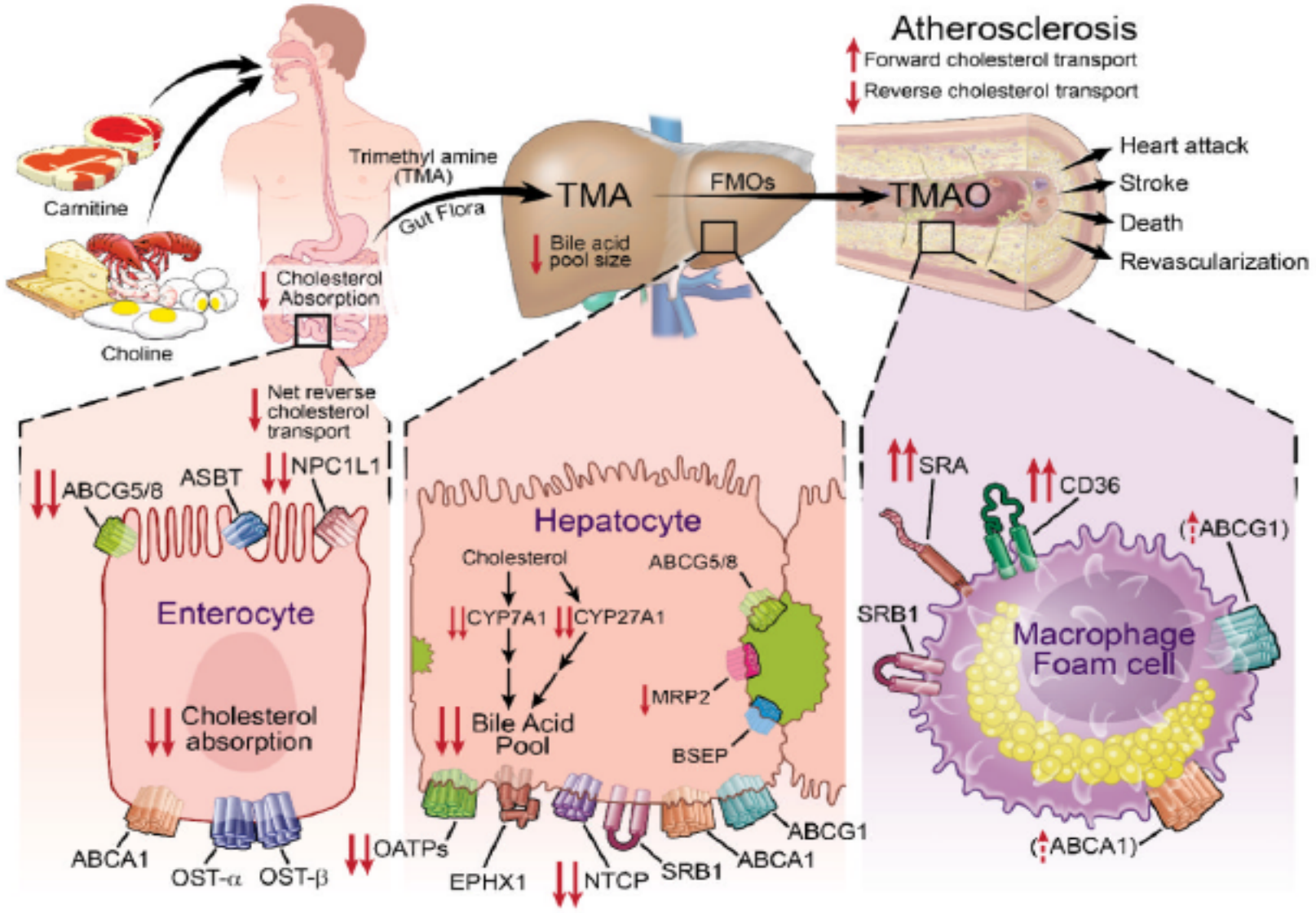
How come?

How could these compounds, so vital to our existence, be harming us?

This is the "three rights make a wrong" story

- Carnitine and choline are consumed by gut bacteria, with trimethylamine (TMA) as the by-product
- TMA is absorbed into the blood
- Liver FMO3, a detoxifying enzyme, converts TMA to TMA oxide (TMAO)
- TMAO interferes with cholesterol transport, activates macrophages to foam cells in blood vessel walls
- Regular consumption of carnitine and choline thus apparently lead to atherosclerosis

TMAO & ASCVD


Atherosclerosis
Forward cholesterol transport
Reverse cholesterol transport
Heart attack
Stroke
Death
Revascularization
Trimethyl amine (TMA)
Gut Flora
TMA
FMOs
TMAO
Bile acid pool size
Carnitine
Choline
Cholesterol Absorption
Net reverse cholesterol transport
ABCG5/8
ASBT
NPC1L1
Enterocyte
Cholesterol absorption
ABCA1
OST-α
OST-β
Hepatocyte
Cholesterol
CYP7A1
CYP27A1
Bile Acid Pool
ABCG5/8
MRP2
BSEP
OATPs
EPHX1
NTCP
SRB1
ABCA1
ABCG1
SRA
CD36
ABCG1
SRB1
Macrophage Foam cell
ABCA1

Discoverer of the TMAO-ASCVD link

Cleveland Clinic is a major heart disease referral center

- Project GeneBank started around 2000, aiming to advance knowledge of the causes, prevention and treatment of cardiovascular disease
- Goal of enrolling 10,000 subjects
- Blood samples obtained for studies

Stanley Hazen, M.D., Ph.D.

- Proposed looking for molecules in blood associated with ASCVD

LC/MS – High Tech Chemistry

Liquid chromatography

- Separation of molecules by flow through a column

Mass spectrometry

- Determines the charge to mass ratio of molecules in an emerging cohort

Marriage of the two in highly automated system allows high-throughput

- ~ 1000 different compounds in a blood sample can be separated and characterized / identified in one run
- Quantity of compounds can be determined in each sample

Fishing expedition: find molecules associated with ASCVD

Blood contains some 2,000 non-protein compounds (molecules)

Liquid chromatography / mass spectrometry

- Molecules separated in an LC column by speed with which they move
- Each band of identical molecules identified in MS by mass-to-charge ratio (m/z)

Compare amount of each compound in cases (patients with ASCVD) to that in normal controls

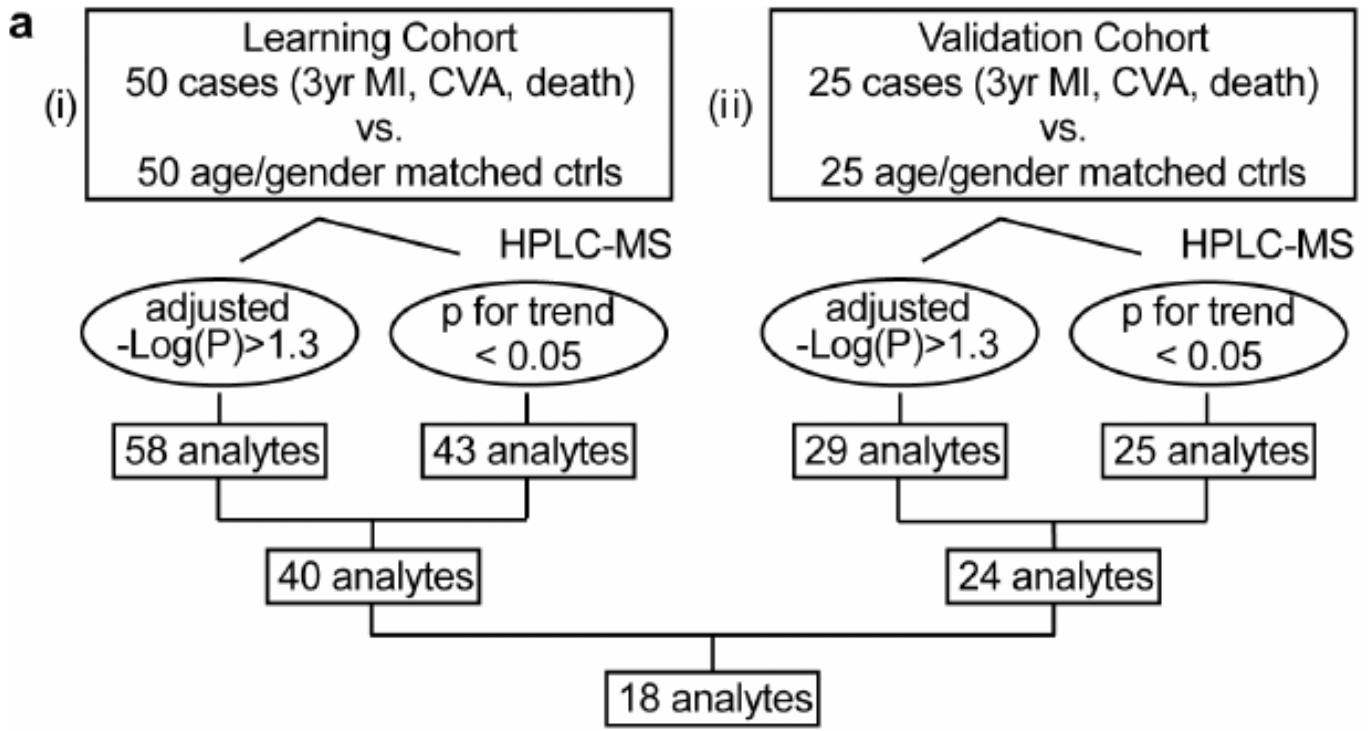
Focus in on those compounds with significantly different levels in cases vs. controls

Nature 2011

Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Zeneng Wang[1,2], **Elizabeth Klipfell**[1,2], **Brian J. Bennett**[3], **Robert Koeth**[1], **Bruce S. Levison**[1,2], **Brandon DuGar**[1], **Ariel E. Feldstein**[1,2], **Earl B. Britt**[1,2], **Xiaoming Fu**[1,2], **Yoon-Mi Chung**[1,2], **Yuping Wu**[4], **Phil Schauer**[5], **Jonathan D. Smith**[1,6], **Hooman Allayee**[7], **W. H. Wilson Tang**[1,2,6], **Joseph A. DiDonato**[1,2], **Aldons J. Lusis**[3], and **Stanley L. Hazen**[1,2,6,8]

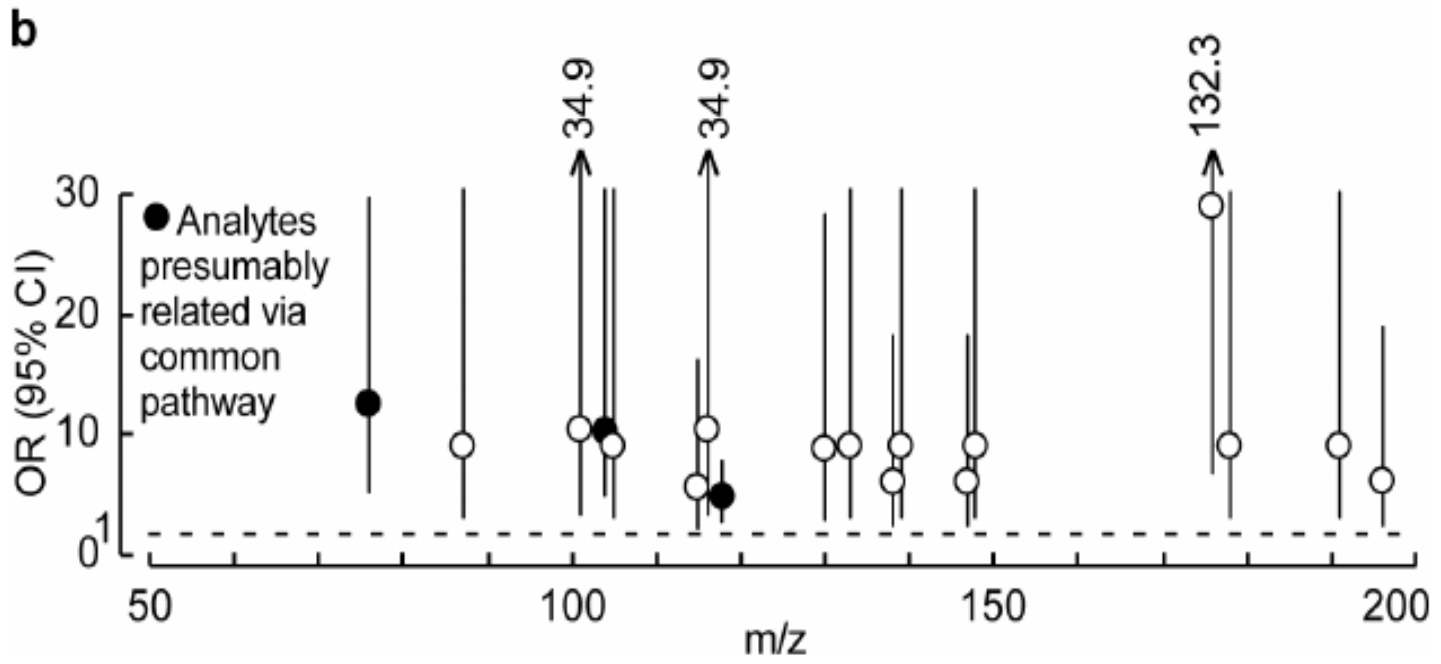
Identification strategy



(iii) Structural identification of analytes

(iv) Confirm clinical prognostic utility in independent prospective cohort (N=1876)

LC/MS analytes associated with higher ASCVD



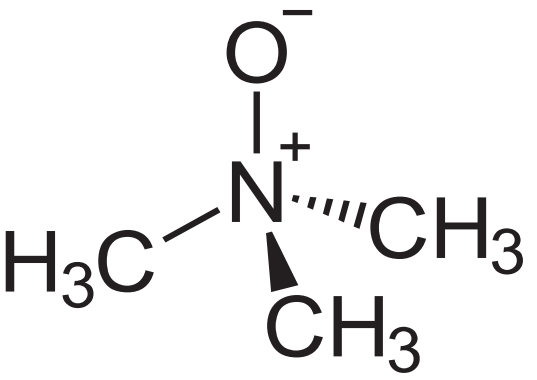
Three compounds that were associated with ASCVD were highly correlated among themselves; m/z 76, 104 and 118

Trimethylamine N-oxide (TMAO)

M/Z 76 compound unequivocally ID'd as TMAO

TMAO is not in the typical human diet and plays no normal role in human metabolism

- What the heck is it doing there ??



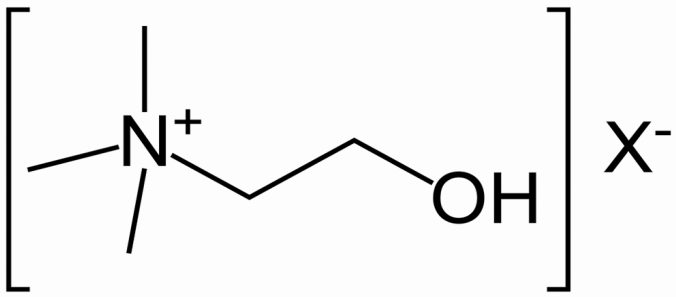
Trimethylamine N-oxide (TMAO)

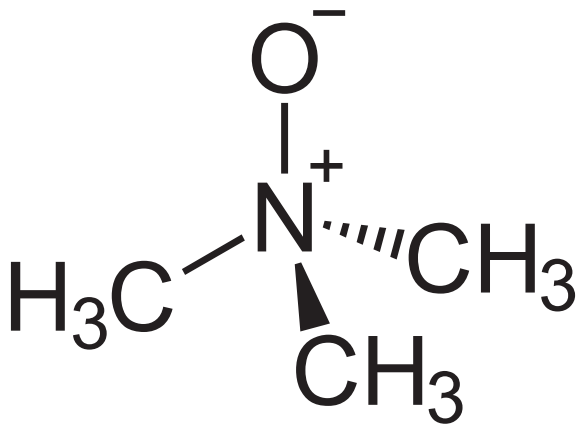
Important compound in deep-sea fish

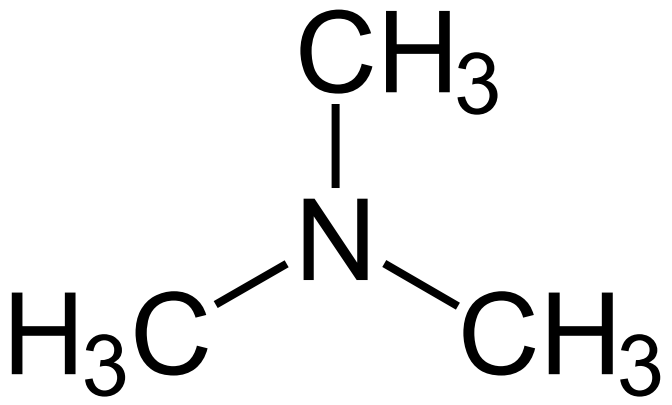
- Stabilizes protein molecules against effects of pressure and osmolarity

Found in other animals, and its metabolism is known

- It's a metabolic product of choline, by way of trimethylamine







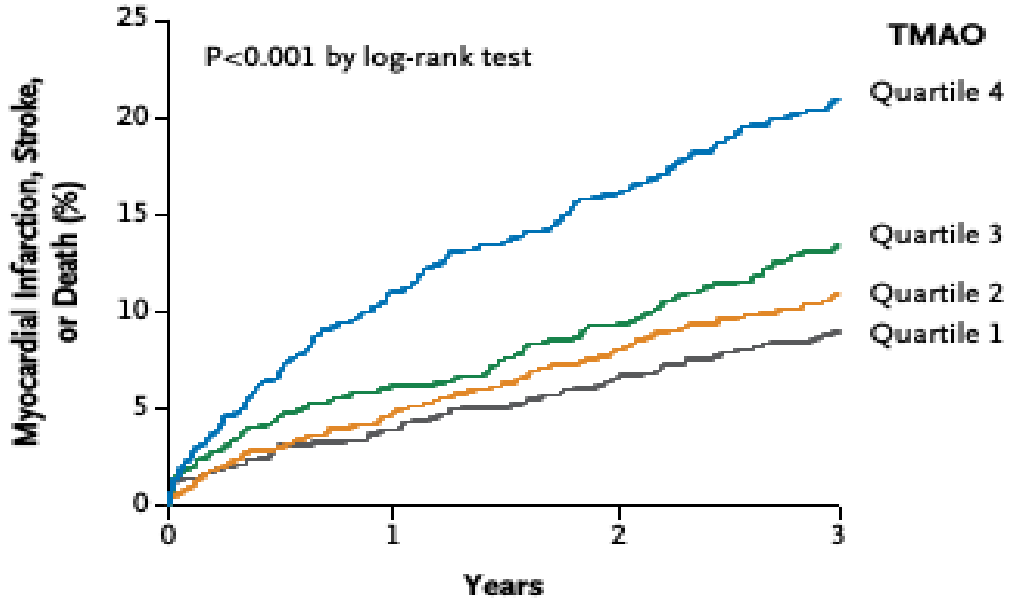
The other analytes

M/Z 104 was found to be choline

M/Z 118 was found to be betaine

Both are compounds in the metabolic pathway with TMAO, so their correlation was not surprising

ASCVD events and TMAO



No. at Risk				
Quartile 1	1001	933	869	827
Quartile 2	998	940	884	843
Quartile 3	1003	938	888	835
Quartile 4	1005	913	849	791

Figure 2. Kaplan–Meier Estimates of Major Adverse Cardiovascular Events, According to the Quartile of TMAO Level.

Data are shown for 4007 participants in the clinical-outcomes study. The P value is for all comparisons.

TMAO levels and ASCVD risk

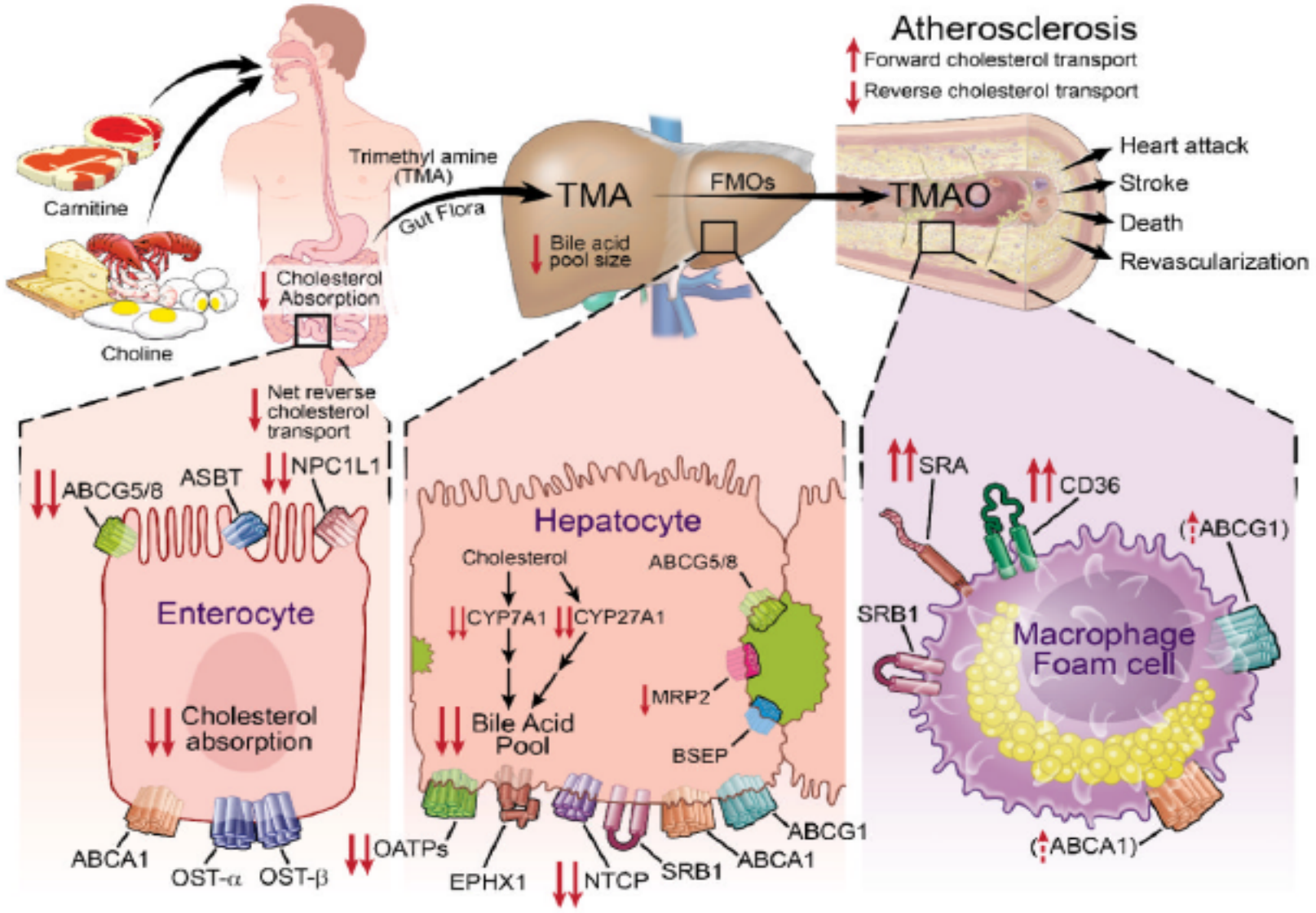
TMAO levels are strongly associated with subsequent ASCVD events

After adjustment for known risk factors, TMAO level remained a significant additional independent risk factor

These findings in a large population (~4000) validated the "fishing expedition" results

What biological mechanism accounts for this relationship, and what is the roles of choline?

TMAO & ASCVD


Atherosclerosis
Forward cholesterol transport
Reverse cholesterol transport
Heart attack
Stroke
Death
Revascularization
Trimethyl amine (TMA)
Gut Flora
TMA
FMOs
TMAO
Bile acid pool size
Carnitine
Choline
Cholesterol Absorption
Net reverse cholesterol transport
ABCG5/8
ASBT
NPC1L1
Enterocyte
Cholesterol absorption
ABCA1
OST-α
OST-β
Hepatocyte
Cholesterol
CYP7A1
CYP27A1
Bile Acid Pool
ABCG5/8
MRP2
BSEP
OATPs
EPHX1
NTCP
SRB1
ABCA1
ABCG1
SRA
CD36
ABCG1
SRB1
Macrophage Foam cell
ABCA1

Trimethylamine (TMA)

TMA is a simple volatile molecule that gives rotting fish their smell

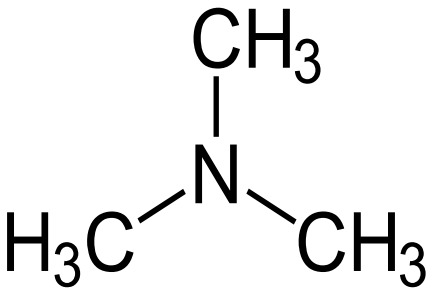
When we eat foods with choline and carnitine, certain gut bacteria turn it into TMA

TMA is absorbed into the blood

FMO3 turns TMA into TMAO

TMA normally only present in small quantities in humans

- Exception: those with abnormal FMO3
- TMA builds up in blood, urine
- Fish odor syndrome



FMO, the detoxifying enzyme

Flavin-containing monooxygenase (FMO) is an important enzyme in our liver

- Can seek out, bind foreign substances and bond oxygen to them to assist excretion
- Many of these substances are toxic, could harm the body if not eliminated
- FMO is important in the disposition of drugs

FMO efficiently converts most TMA into TMA oxide (TMAO)

Of mice and TMAO

Hazen & associates used a mouse model to investigate mechanisms – ASCVD-prone mice (APM)

APM fed TMAO show accelerated atherosclerosis (AAS)

APM fed choline have TMAO in blood -> AAS

Germ-free APM, or those given antibiotics, fed choline → No TMAO, and no AAS !

Choline in mouse diet is converted to TMAO by way of gut bacteria; if bacteria aren't present, no TMAO is produced

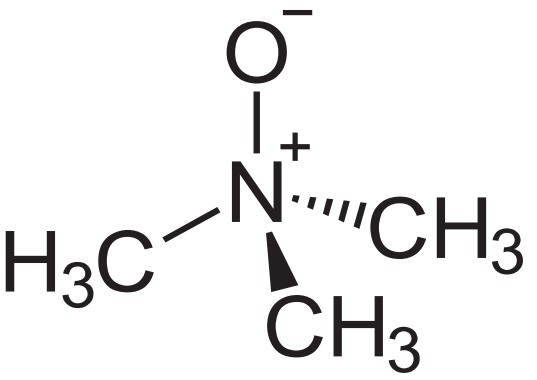
Choline is converted to TMA by bacteria, is absorbed, and converted to TMAO by liver enzyme FMO3

Trimethylamine N-oxide (TMAO)

Vigorously promotes ASCVD by several mechanisms

- Enhances forward cholesterol transport, inhibits reverse cholesterol transport
- Activates macrophages into foam cells

Oops!

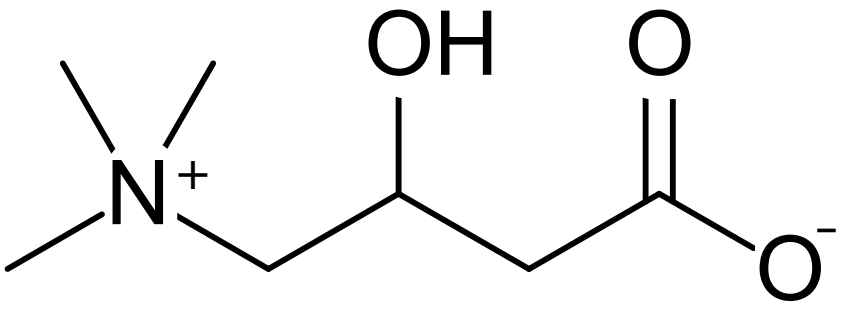


Carnitine

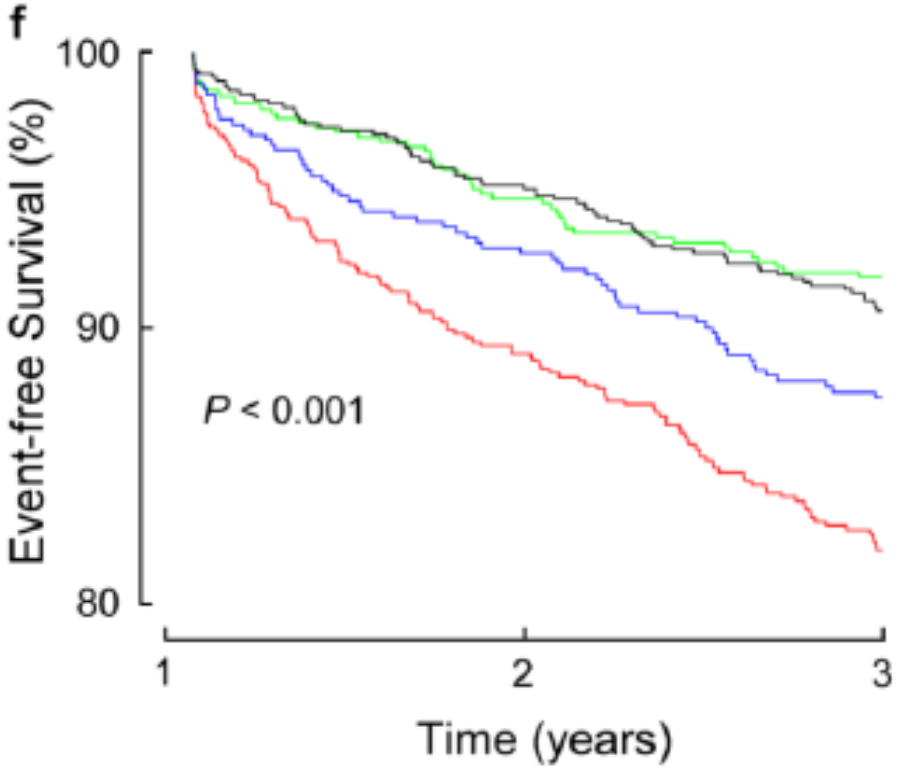
Abundant in animal-based foods, especially red meats

Taken by athletes to enhance muscle development

Like choline, it is converted by gut bacteria to TMA, which is absorbed and converted to TMAO by FMO3



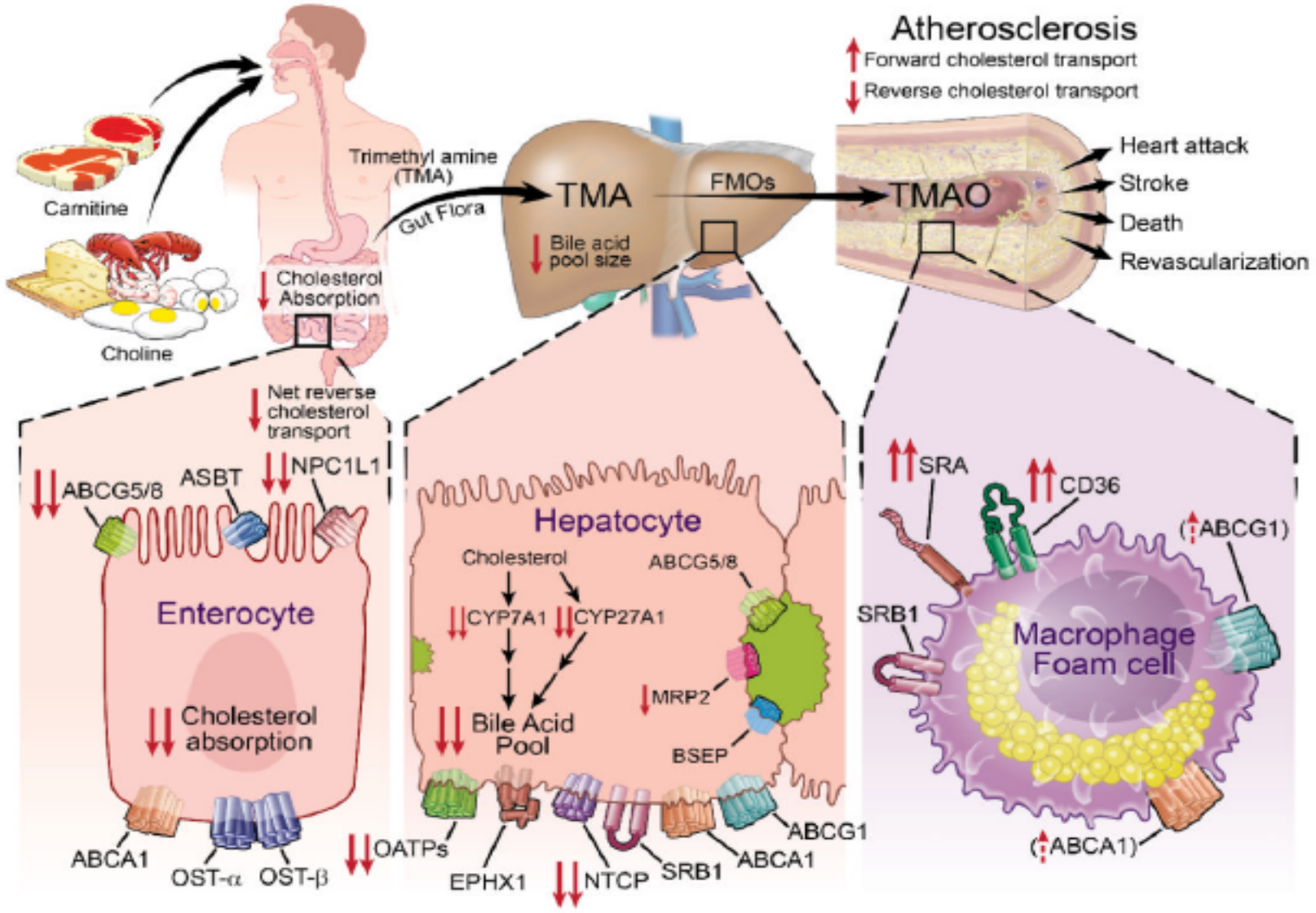
ASCVD events and TMAO



Carnitine	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)

Product	Quantity	Carnitine
Beef steak	100 g	95 mg
Ground beef	100 g	94 mg
Pork	100 g	27.7 mg
Bacon	100 g	23.3 mg
Tempeh	100 g	19.5 mg
Cod fish	100 g	5.6 mg
Chicken breast	100 g	3.9 mg
American cheese	100 g	3.7 mg
Ice cream	100 ml	3.7 mg
Whole milk	100 ml	3.3 mg
Avocado	one medium	2 mg[20]
Cottage cheese	100 g	1.1 mg
Whole-wheat bread	100 g	0.36 mg
Asparagus	100 g	0.195 mg
White bread	100 g	0.147 mg
Macaroni	100 g	0.126 mg
Peanut butter	100 g	0.083 mg
Rice (cooked)	100 g	0.0449 mg
Eggs	100 g	0.0121 mg
Orange juice	100 ml	0.0019 mg

TMAO & ASCVD


Atherosclerosis
Forward cholesterol transport
Reverse cholesterol transport
Heart attack
Stroke
Death
Revascularization
Trimethyl amine (TMA)
Gut Flora
TMA
FMOs
TMAO
Bile acid pool size
Carnitine
Choline
Cholesterol Absorption
Net reverse cholesterol transport
ABCG5/8
ASBT
NPC1L1
Enterocyte
Cholesterol absorption
ABCA1
OST-α
OST-β
Hepatocyte
Cholesterol
CYP7A1
CYP27A1
Bile Acid Pool
ABCG5/8
MRP2
BSEP
OATPs
EPHX1
NTCP
SRB1
ABCA1
ABCG1
SRA
CD36
ABCG1
SRB1
Macrophage Foam cell
ABCA1

The cast, in order of appearance

Choline / phosphatidyl choline / carnitine / betaine

- Nutrients in certain foods we eat

Gut bacteria

- Consume nutrients, convert them to TMA

TMA

- Trimethylamine
- The intermediate

FMO

- Flavin-containing monooxygenase
- Liver enzyme, converts TMA → TMAO

TMAO

- Trimethylamine N-oxide
- The toxin: inhibits favorable cholesterol metabolism, activates foam cells, and activates other mechanisms promoting AS

Conclusions

Choline is an essential nutrient, our gut bacteria perform many beneficial services and FMO3 is an important detoxifying enzyme – three "rights"!

TMAO produced by FMO3 from dietary choline, and from carnitine, is a strong candidate for the "missing link" in ASCVD genesis in humans – a huge "wrong"!

Further work needed to confirm and extend

In the meantime, it's reasonable to limit one's consumption of foods and supplements potentially producing TMAO to :

- Sufficient choline to avoid deficiency
- As little carnitine as possible, while meeting other nutritional requirements; mainly, limit red meat

Other means of interrupting the pathway may be discovered

Eat Sh*t and Live!: Say WHAT !?!

Remember when we were young and foolish, and we thought someone disrespected us?

- What did we yell at them? ***Eat sh*t and die!***

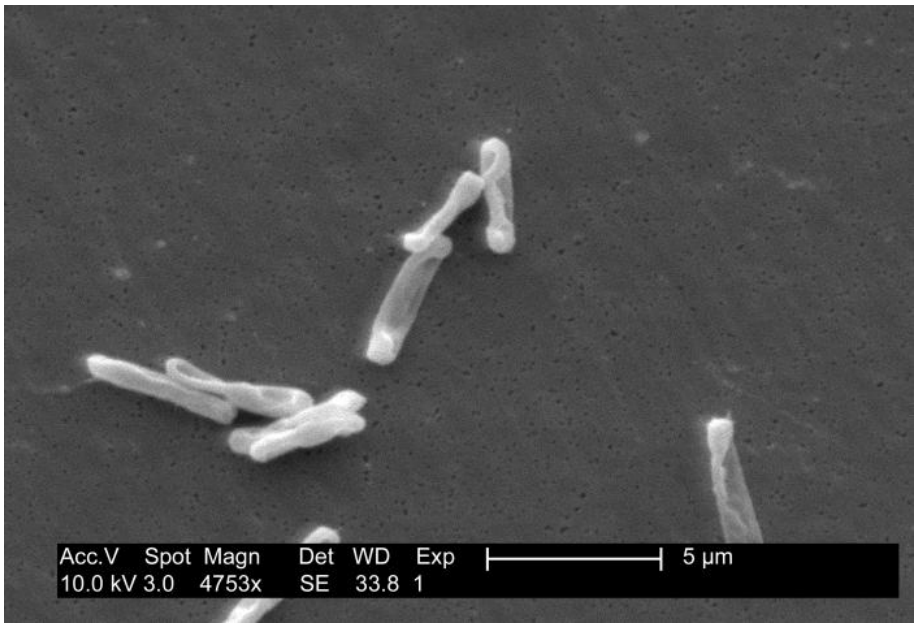
Consuming fecal matter was the most disgusting, degrading thing we could imagine, and death would surely be the result of doing it

Ironically, it turns out that a life-threatening, antibiotic-resistant infection can *only* be cured with just that!

C. diff: The difficult bacillus

Clostridium difficile (*C. diff*)

- Originally called *Bacillus difficile* when found in the stools of healthy neonates in 1935; so-named because it was **difficult** to culture and isolate; reclassified as *Clostridium difficile*
- Nowadays **difficult** because it causes a common diarrheal illness, most often found in hospitals and nursing homes, spread by staff, and can be difficult to eradicate and prevent



C. diff: The difficult bacillus

Clostridium difficile (*C. diff*)

- Forms spores which survive antibacterials, heat, acid, and promote spread
- *C. diff* not normally prominent in human gut microbiome, inhibited by competition from normal inhabitants
- Gets opportunity to thrive when normal gut bacteria are suppressed by antibiotic therapy, weak immunity, debilitation
- Virulent strains emerging after 2000; infection, resistance and death rates rising

Curing *C. diff* with Fecal Transplant

Medical breakthrough (2013) !

- Resistant cases of *C. diff* can almost always be cured with a transplant of feces from a normal, healthy donor
- Demonstrated superior to conventional therapy in a randomized controlled clinical trial

Pseudomembranous colitis

Diarrheal disease known since 1890s

Characteristic findings in the colon – patchy pseudomembranes

Most often occurred after being treated with broad-spectrum antibiotics, especially clindamycin

Pseudomembranous colitis and *Clostridium difficile*

Clostridium difficile / PMC connection

- Established, reported in 1978
- Illness was due not so much to the number of organisms but due to two toxins secreted by *C. diff*, A & B
- Diagnostic test based on finding toxins in the stool
- PMC is the more extreme form of *C. diff* infection

Treatment of *C. diff* infection

Unresponsive to usual antibiotics (e.g., penicillins, cephalosporins)

Routinely responds to oral metronidazole (Flagyl) or oral vancomycin

Vancomycin has a significantly higher response rate in moderate to severely ill patients

Treatment of *C. diff* infection

Relapses are not infrequent

- *C.* diff may recolonize faster than desirable bacteria → recurrent illness
- Retreatment with vancomycin or metronidazole may achieve long-term control after relapse, but some patients have multiple relapses
- Even as of 2008, a review in NEJM deemed relapsing, resistant *C. diff* to be a major health issue without a fully satisfactory answer
- CDC reported mortality rising to 14,000 deaths per year by 2007

Gut bacteria transfer (GBT) for *C. diff*

Desperate times call for desperate measures

- Fecal bacteria from normal healthy individual transferred to patient with *C. diff* infection
- Sporadic anecdotal use of GBTs for pseudo-membranous colitis – colon to colon – reported from 1950s on
- Based on knowledge that disease often followed antibiotic use, hypothesized role of normal flora in suppressing pathogenic bacteria; analogy – healthy lawn suppresses weed growth

Case series in Norway in 1990s

Gut bacteria transfer (GBT) for *C. diff*

Procedure most often referred to as "fecal transplant"

- Sample of feces from donor is processed to harvest bacteria
- Bacteria are introduced directly into colon, or via naso-duodenal tube into the small intesting
- Crucially, must avoid or bypass stomach to avoid destruction by acid and enzymes

I use a different term, GBT

- Sounds nicer

GBT for *C. diff*

Case series from Duluth, MN, hospital, published in 2003

- Homogenized, filtered fecal preparation given by naso-gastric tube
- 18 consecutive patients with 2+ relapses over 9 years
- 15 had durable remission with 1st transplant, 1 relapsed and successfully treated with vancomycin
- 2 severely ill patients died

GBT status - 2008

NEJM review article "*Clostridium difficile* – More difficult than ever"

- "Several case series describe efficacy [of fecal transplant] in preventing recurrent infection, but in the absence of controlled trials, fecal transplantation remains unpopular for practical and aesthetic reasons."
- Motivated a controlled trial in the Netherlands

Controlled trial of GBT

Van Nood & colleagues from the Netherlands

- Published 2013 in NEJM

Results of 42 patients with relapsed *C. diff* infection randomized into 3 groups (response/treated)

- Infusion of feces by duodenal tube (15/16)
- Vancomycin (4/13)
- Vancomycin + colon lavage (3/13)

Gut microbiota analysis confirmed restoration of normal bacterial diversity following transplant, by contrast to limited diversity pre-transplant

Further developments

Oral enteric-coated capsule developed to protect bacteria from acid-protease destruction in stomach; avoids tube

Stable preparations which can be standardized and stored for prolonged periods to avoid necessity of keeping donors on standby and doing involved preparation for each transplant

Potential uses for GBT

Obesity

- Obese individuals have a distinct and different core microbiome, even twins
- Studies in mice shows obese or lean trait transmissible by GBT

Inflammatory bowel disease

Neurologic and behavioral diseases

- Gut bacteria make psychoactive compounds
- Autism, depression
- Gut / microbiota / brain axis

Metabolic syndrome, type II diabetes

PROBIOTICS

Probiotics

Preparations of live bacteria consumed with the expectation that they convey health benefits

- Foods – Yogurt, fermented plants
- Pills – Single or multiple organisms

Evidence of benefit is mostly testimonial

- Scientific studies are few and equivocal

Tiny fraction of ingested bacteria arrive in colon

- Stool samples shows no lasting alteration of microbiome species proportions
- Beneficial effects are not due to direct replacement of despicables with desirables

Probiotics

I can provide no guidance on what preparations to take

- Data lacking to support rational choice

Best way to a healthy microbiome is through dietary prebiotics (fiber)

- Whole-grain cereals and grain products
- Fruits and vegetables
- Nuts and seeds

Thank you!

Find out more about diet and health at:

Olli-what-to-eat-and-why.weebly.com

Contact me at: ebcox at yahoo dot com

Edwin B. Cox, M.D.