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

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How red meat promotes atherosclerosis
Analysis of brown fat in humans
Maturing research on aging
Take home summary:
Gut microbiota participates in atherosclerosis in the presence of specific dietary exposures

The microbiome can be considered as our largest endocrine organ.

- The microbiome is a "drugable" target.
- The microbiome is a filter of our largest environmental exposure - what we eat.

Additional take home concepts:
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

GeneBank (N=10,000)

(i) Learning Cohort
50 cases (3yr MI, CVA, death) vs.
50 age/gender matched ctrls

HPLC-MS
- adjusted $-\log(P) > 1.3$
- $p$ for trend $< 0.05$

58 analytes
43 analytes

40 analytes

(ii) Validation Cohort
25 cases (3yr MI, CVA, death) vs.
25 age/gender matched ctrls

HPLC-MS
- adjusted $-\log(P) > 1.3$
- $p$ for trend $< 0.05$

29 analytes
25 analytes

24 analytes

(iii) Structural identification of analytes
(iv) Confirm clinical prognostic utility in Independent Prospective Cohort (N>1000)
Choline, betaine and trimethylamine-\textit{N}-oxide are plasma analytes associated with CVD.

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

**Key Mass-to-Charge Ratios:**
- m/z 104
- m/z 76
- m/z 118

**Diagram Highlights:**
- Phosphatidylcholine (Dietary)
- Fatty acid
- Gut Flora
- Choline
- Trimethylamine (TMA)
- Trimethylamine N-oxide (TMAO)
- Heart attack
- Stroke
- Death
- Atherosclerosis

**Chemical Structures:**

- Phosphatidylcholine
- Choline
- Trimethylamine
- Trimethylamine N-oxide
Intestinal Microbial Organisms Play an Obligatory Role in TMAO Generation from Dietary Egg Yolk PC in Mice

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

6 h post PC challenge

24 h post PC challenge

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

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

New Independent Cohort: N=4007 Sequential Subjects

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

Suppression of gut flora inhibits TMAO formation and dietary choline induced atherosclerosis.
Cholesterol metabolism in cells of the artery wall:

Forward Cholesterol Transport

Reverse Cholesterol Transport (RCT)

Liver

Macrophage

Foam cell

MPO

Nascent “discoidal” HDL

HDL

LDL-R

LDL

Modification

αHDL

A-I

LCAT

Spherical HDL

ABCA1

SR-B1

ABCA1

ApoA-1

CD36

Preβ HDL

ApoA-1
TMAO alters cholesterol and sterol metabolism in multiple compartments - net effect - increased atherosclerosis

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)

One serving = 3 oz. steak
Carnitine (from carnis (carnivore), meaning flesh) participates in fatty acid translocation into mitochondria for $\beta$-oxidation.
There is an obligatory role for gut flora in TMAO formation from dietary carnitine in mice.

**d3-(methyl)-carnitine oral dose**

Germ Free Mice

- ○ d3-Carnitine
- △ d3-TMA
- ■ d3-TMAO

**Conventionalization**

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

Visit 1:
Steak + d3-Carnitine → gut flora suppression

Visit 2:
Steak + d3-Carnitine

Visit 3:
Steak + d3-Carnitine

12h post challenge

![Graphs showing TMAO production over time]
Carnitine supplementation accelerates atherosclerosis in apoE-/- mice, but not with suppression of intestinal flora (and suppression of TMA/TMAO formation)

Production of the gut flora metabolite TMAO from carnitine is inducible by d3-(methyl)-carnitine oral dose.

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

Scheme of human gut microbiota analysis

Omnivore and Vegans/Vegetarians

N=30

Stool Collected

Gut Microbiota Composition

N=23

Blood Collected

TMAO measured by mass spectrometry
TMAO is formed from dietary carnitine in omnivores, but minimally in vegans.
Chronic dietary exposure significantly influences carnitine metabolism

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

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

(N=2595)

Take Home Summary:

Diet and Intestinal Microbes are Mechanistically Linked to Atherosclerotic Heart Disease


Bennett B et al (2013) *Cell Metab*
Actually, >600 mg carnitine/can

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