The Gut Microbiome Metabolism of Choline and L-Carnitine and Cardiovascular Risk: Implications for HIV

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Cleveland Clinic
Presenter Disclosure

W. H. Wilson Tang, MD

Disclosed no conflict of interest.
“Food Metabolome”

The biggest environment exposure is represented by what we ingest as food and filtered by gut microbiota.

Crosstalks between Microbiota and Host and Dietary Influence

Tilg & Moschen, *Gut* 2014

Untargeted Metabolomic Survey of Small Molecule Profiles Predictive of Incidence MACE Risk

Selection Criteria (out of 2000+ analytes):
- Case-control Bonferoni adjusted two sided $t$-test $P<0.05$
- Dose-response (analyte vs phenotype) Cochran-Armitage $P<0.05$
- Minimal signal-to-noise ratio of 5:1
- LC/MS in positive MS1 mode

Wang et al., *Nature* 2011
Untargeted Metabolomics Predictive of Future Risks for Major Adverse Cardiovascular Events

Wang et al., Nature 2011
What is Choline/Phosphatidylcholine (Lecithin)?

Lekithos (Greek) = Egg yolk
Chole (Greek) = Bile

Sources of Choline
- Diet
- PEMT/Phospholipase
- PC Catabolism

Acetylcholine Catabolism

Choline

Choline Utilization
- Betaine
- Excretion
- Phosphocholine/PC
- Acetylcholine

Li & Vance, J Lipid Res 2008
What is Trimethylamine N-oxide (TMAO)?

Table 1. Accumulation of TMA or DMA in amended sewage

<table>
<thead>
<tr>
<th>Substrate (µg/ml)</th>
<th>Maximum rate of accumulation (ng/ml per h)</th>
<th>Maximum product yield (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (200)</td>
<td>40</td>
<td>259 DMA.</td>
</tr>
<tr>
<td>Choline (200)</td>
<td>11</td>
<td>192 TMA.</td>
</tr>
<tr>
<td>Phosphatidylcholine (1000)</td>
<td>2,450</td>
<td>67,000 TMA.</td>
</tr>
</tbody>
</table>

Lidstrom, *The Prokaryotes* 2006

Prokarotic Aerobic Metabolism

Bennett et al., *Cell Metabolism* 2013

TMAO Production by Flavin Mono-Oxygenase (FMO) family members

Gut Microbiota Play Obligatory Role in TMAO Generation from Dietary Egg Yolk PC in Mice

Wang et al., *Nature* 2011

**Involvement of Gut Microbiota**

**Involvement of Foam Cells**

![Graphs showing concentration changes of Choline, Betaine, and TMAO over time in germ-free and conventionalized mice.](image1)

![Graphs showing comparison of arterial damage between normal chow and high-fat diet, with and without antibiotics.](image2)

Wang et al., *Nature* 2011
Transmission of Atherosclerotic Susceptibility

C57BL/6J

NZW/LacJ

Gregory et al, J Biol Chem 2015
Limited number of TMA-producing strains in Mice leading to TMAO production

Romano et al, MBio 2015
Working Hypothesis: Gut Microbiota-dependent Phosphatidylcholine Metabolism in CV Risk

Wang et al., *Nature* 2011
Gut Flora-dependent Phosphatidylcholine (PC) Metabolism in Humans by PC Challenge Test

Human: PC Challenge Testing
Increased Plasma TMAO Levels Portends Higher Risk of CVD and Adverse Events in Humans

Human: CVD

Wang et al., *Nature* 2011


Human: Major Adverse Cardiac Events
Prognostic Value of Plasma Choline and Betaine Largely Confined to High TMAO Levels in Humans

Wang et al., Eur Heart J 2014
Prognostic Value of Plasma TMAO Levels in Chronic Heart Failure Independent of Natriuretic Peptides

Human

Tang et al, J Am Coll Cardiol 2014
Dietary Choline/TMAO Exposure Contributes to Progressive Renal Fibrosis and Dysfunction

Tang et al, Circ Res 2015
TMAO infusion prolongs Ang-II hypertensive hemodynamic effects in rats

Changes in systolic blood pressure

Ufnal et al, *Can J Cardiol* 2014
Lack of Significant Genetic Determinants of TMAO Levels in Humans

rs2075988: significantly associated with FMO3 mRNA in liver eQTL (p=4.5x10^{-4})
Plaque burden in HIV-infected patients is associated with serum intestinal microbiota-generated trimethylamine

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<table>
<thead>
<tr>
<th>Dietary intake</th>
<th>Controls ((n = 67))</th>
<th>HIV-infected patients ((n = 155))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaine (mg)</td>
<td>155.9 (90.3–208.9)</td>
<td>128.9 (94.2–182.3)</td>
<td>0.43</td>
</tr>
<tr>
<td>Choline (mg)</td>
<td>349.2 (251.5–464.8)</td>
<td>330.7 (218.9–429.4)</td>
<td>0.60</td>
</tr>
<tr>
<td>Serum metabolites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betaine ((\mu)mol/l)</td>
<td>6.1 (4.7–7.7)</td>
<td>5.8 (5.1–7.0)</td>
<td>0.32</td>
</tr>
<tr>
<td>L-Carnitine ((\mu)mol/l)</td>
<td>5.9 (4.9–6.7)</td>
<td>5.8 (4.5–6.9)</td>
<td>0.58</td>
</tr>
<tr>
<td>Choline ((\mu)mol/l)</td>
<td>2.0 (1.8–2.2)</td>
<td>1.9 (1.7–2.3)</td>
<td>0.88</td>
</tr>
<tr>
<td>TMA ((\mu)mol/l)</td>
<td>0.3 (0.3–0.4)</td>
<td>0.3 (0.3–1.5)</td>
<td>0.50</td>
</tr>
<tr>
<td>TMAO ((\mu)mol/l)</td>
<td>0.7 (0.5–1.0)</td>
<td>0.7 (0.5–1.1)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Srinivasa et al, AIDS 2015
Correlation between Coronary Plaque Characteristics and Serum TMA Levels in HIV-Infected Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Log serum TMA</th>
<th>Log serum TMAO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
</tr>
<tr>
<td>Coronary plaque characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log calcium score</td>
<td>0.22</td>
<td>0.006</td>
</tr>
<tr>
<td>Total plaque segments (#)</td>
<td>0.20</td>
<td>0.02</td>
</tr>
<tr>
<td>Noncalcified plaque segments (#)</td>
<td>0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>Calcified plaque segments (#)</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Total calcium volume of plaque (mm(^3))</td>
<td>0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Total calcium mass of plaque (mg)</td>
<td>0.22</td>
<td>0.009</td>
</tr>
<tr>
<td>Inflammatory indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log CRP (mg/dl)</td>
<td>0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>Log hsIL-6 (pg/ml)</td>
<td>–0.10</td>
<td>0.25</td>
</tr>
<tr>
<td>Log LPS (ng/ml)</td>
<td>0.19</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Srinivasa et al, AIDS 2015
Gut Flora-dependent Phosphatidylcholine Metabolism: Trimethylamine N-oxide (TMAO)

L-Carnitine as Alternative Dietary Source of Gut Microbiota-Dependent TMAO Production in Mice

Dietary Influence of Gut Microbiota Metabolism: Differences Between Omnivores and Vegans

Prognostic Value of Plasma Carnitine is Largely Confined to High TMAO Levels

Koeth et al, Nat Med 2013
Gut microbes convert L-carnitine into \( \gamma \)BB in different locations and quantities

Koeth et al, Cell Metab 2014
Gut microbe-dependent pathways for conversion of dietary $\gamma$BB into TMAO

Koeth et al, *Cell Metab* 2014

C57BL/6J *ApoE*-/- mice
Gut microbiome-dependent pathways for conversion of dietary L-carnitine into TMAO

Koeth et al, Cell Metab 2014
Effect of Gut Microbiota-Dependent TMAO Production on Cholesterol & Sterol Metabolism

Dietary Intake and Microbial TMA Lyases as Therapeutic Targets

Strategies to Target the Gut Microbial Endocrine Organ for Improving Cardiovascular Health

Implications for HIV-Infected Patients

• Food metabolome comprises beyond single microbial pathway.

• Choline and L-carnitine conversion to TMA and TMAO depends beyond dietary exposures - microbial and host metabolism that can be affected by HIV infection.

• Multiple adverse consequences have been associated with TMA/TMAO accumulation, yet precise pathogenetic mechanisms remain unclear.

• Strategies to minimize adverse consequences associated with TMAO generation are emerging.
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