ARTICLE

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Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

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Study rational

- The purpose of the study was to determine if there was a link between gut-flora phospholipid metabolism and risk of atherosclerosis
- Currently there is known relationships to CVD for blood cholesterol and triglycerides, but little is known about how lipids and phospholipids affect the parthenogenesis of CVD
- Prior studies have claimed that there is a possible link to CVD parthenogenesis from infectious agents, but studies have failed to make a positive link
- Based on previous studies using both a learning and validation cohorts of human plasma samples they were able to identify 18 analyses that would be used for the remainder of the study

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Sample preparation/analytical platform

- Lipids were extracted using a chloroform:methanol method
- Metabolites were analysed after running through a phenyl column using a cohesive HPLC with a PE Sciex API triple quadrupole mass spectrometer
- Targeted analysis was used
  - Metabolites isolated from HPLC were vacum dried and dissolved in water
  - Redisolved metabolites were put back through the phenyl column with a HLPC gradient
    - 0.2% formic acid over 2 min
    - 18% acetonitrile containing 0.2% formic acid over 18 min and further
    - 100% acetonitrile containing 0.2% formic acid over 3 min
- GC/MS and TMAO
  - m/z 76 also included initial reduction by titanium (III) chloride47 and further reaction with 2,2,2-trichloroethylchloroformate
  - J&W scientific DB-1 column for separations
  - LC/MS/MS and NMR
- LC/M/MS
  - Used for TMAO, choline, and betaine

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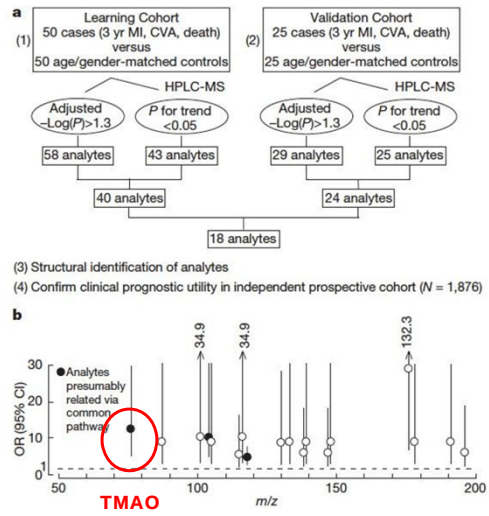
Method critiques

- No good explanation on how the samples were normalized
- Not clear on how metabolites were analyzed for the initial studies identifying the metabolites studied
- Would have been nice to see untargeted approach to problem to see potential other targets
- Based on previous papers, would have been nice to see additional data analysis softwares used to analyze the data
- Not clear on the parameters used for the analysis

Identifying Metabolites of Interest in CVD: TMAO



Selection Criteria

1. Statistically significant difference between cases and controls (Bonferroni adjusted two-sided *t*-test with $p < 0.05$)
2. Significant dose-response relationship between analyte level and clinical phenotype (Armitage trend test with $p < 0.05$)
3. Minimal signal-to-noise ratio of 5:1 for the given analyte

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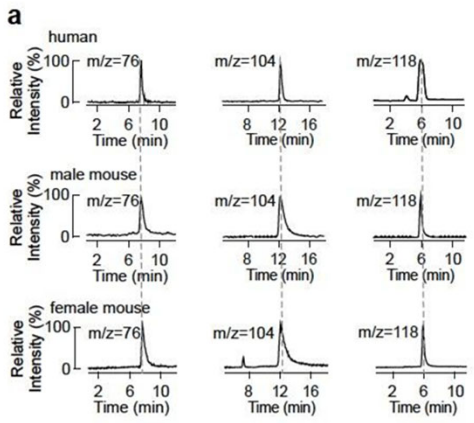
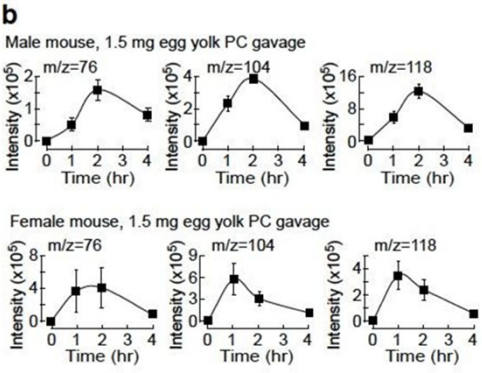
Identifying Metabolites of Interest in CVD: Choline





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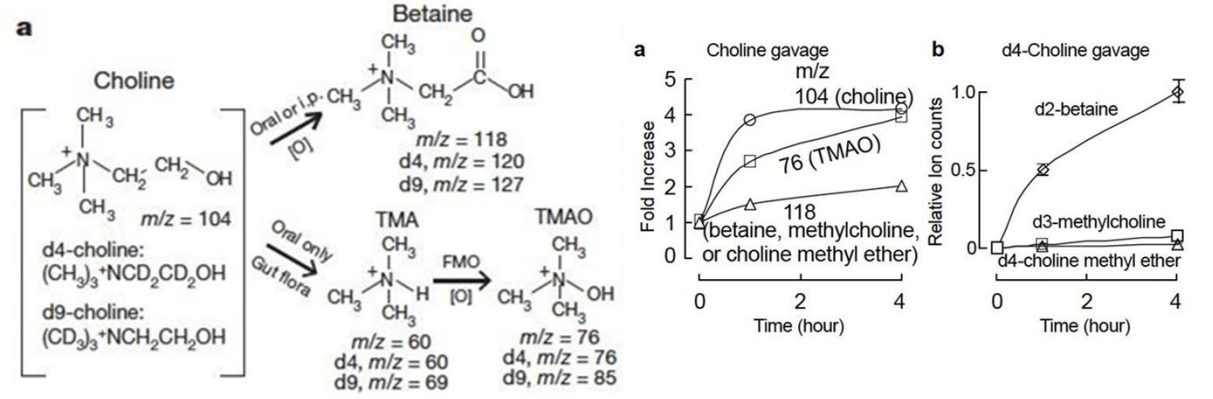
Identifying Metabolites of Interest in CVD: Betaine



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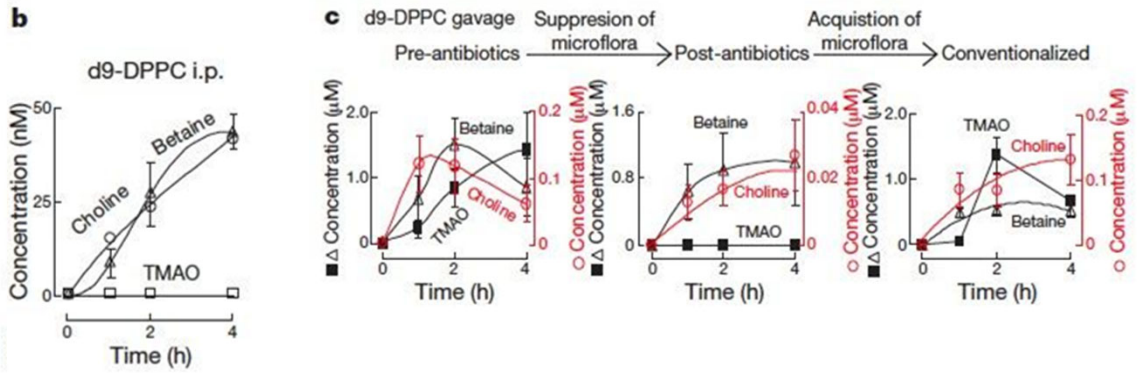
The Influence of Gut Flora on the Production of Plasma Analytes



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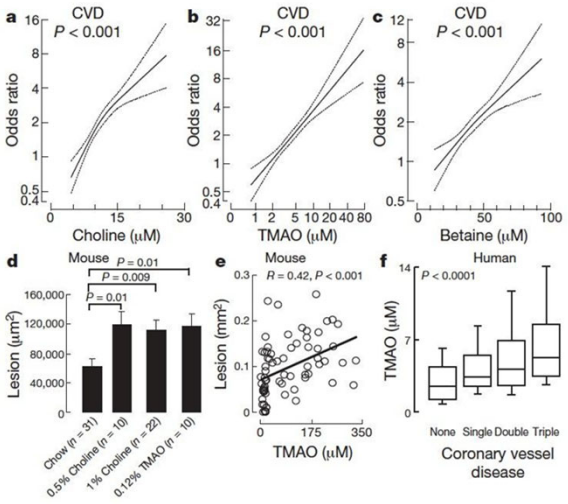
Dietary PC metabolites predict CVD Risk and Promote Atherosclerosis



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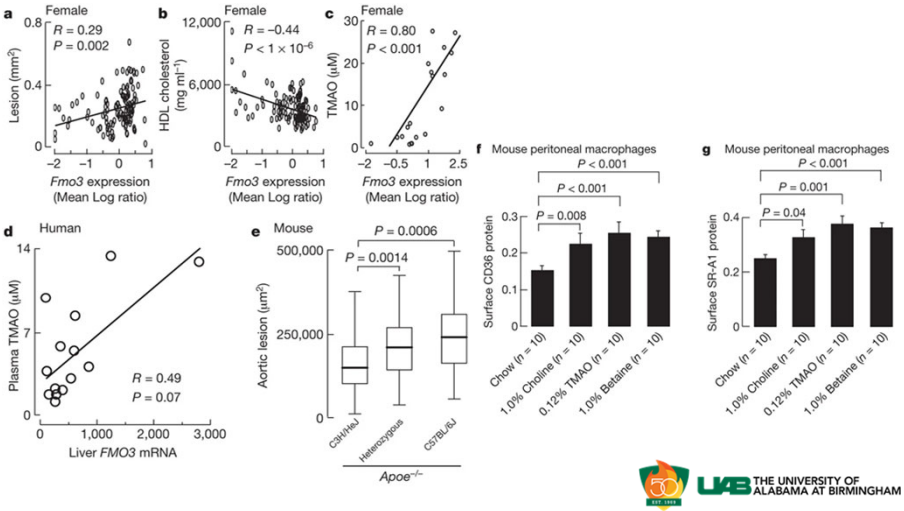
Hepatic *Fmo* genes are linked to atherosclerosis and dietary PC metabolites enhance macrophage scavenger receptor expression.

- F2 intercross used from atherosclerosis-prone C57BL/6J Apoe -/- and atherosclerosis-resistant C3H/ HeJ Apoe -/- mice



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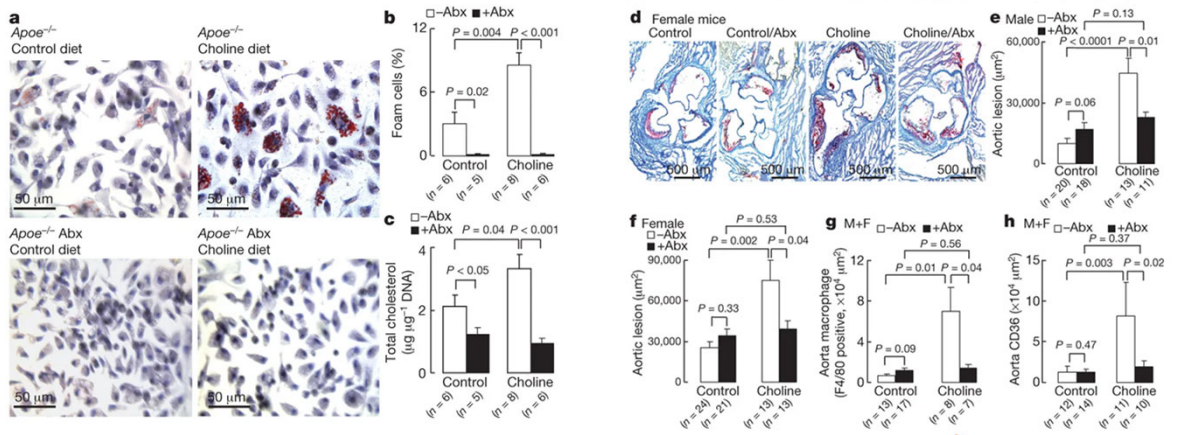
Obligatory role of gut flora in dietary choline enhanced atherosclerosis.



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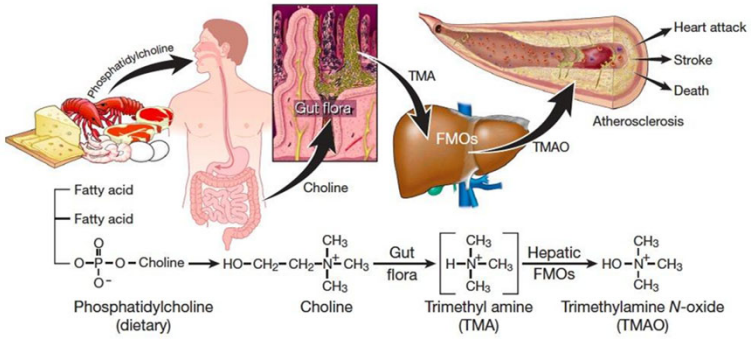
CONCLUSION



Probiotic supplements, if designed properly, may be a valuable therapeutic method for CVD.

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