Dietary phosphatidylcholine supplementation decreased atherosclerosis development in Ldlr\(^{-}\) mice

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Choline an essential nutrient for important biological functions

- Cell-membrane signaling (phospholipids)
- Lipid transport (lipoproteins)
- Neurotransmitter synthesis (acetylcholine)
- Betaine methyl-group metabolism

**CHOLINE**
Dietary choline has been linked to heart diseases


Relationship between plasma TMAO levels and cardiovascular events


- **Quartile 4**: $6.18 \mu M$
- **Quartile 3**: $3.67 \text{ to } 6.18 \mu M$
- **Quartile 2**: $2.43 \text{ to } 3.66 \mu M$
- **Quartile 1**: $< 2.43 \mu M$

P $< 0.001$ by log-rank test
Atherosclerotic plaque area is increased with choline and TMAO supplementation


**Panel a**

- Mouse
- Lesion (μm²)
- Chow
- 0.5% choline
- 1% choline
- 0.12% TMAO
- (n=8) (n=10) (n=10) (n=13)

Significance levels:
- p=0.050
- p=0.045
- p=0.20

**Panel c**

- Male
- TMAO (μM)
- Chow
- 0.5% choline
- 1% choline
- 0.12% TMAO
- (n=9) (n=10) (n=10) (n=13)

Significance levels:
- p=0.0001
- p=0.02
- p=0.005

_Apoe<sup>−/−</sup> male mice_
Dietary choline does not influence atherosclerosis development in \textit{Ldlr}^{-/-} male mice


**A:**

- LC, \textit{Ldlr}^{-/-} control (0.1% choline)
- LCS, \textit{Ldlr}^{-/-} choline-supplemented (1% choline)
- LBS, \textit{Ldlr}^{-/-} betaine-supplemented (0.1% choline and 0.9% betaine)

**F:**

- Plasma TMAO (\textmu M)
  - LC
  - LCS
  - LBS

HFD (40% calories from fat) with 0.5% of cholesterol 8 or 16 weeks
Dietary TMAO does not influence atherosclerosis development in \textit{Ldlr}⁻/⁻ male mice

8 weeks

HFD (40\% calories from fat) with 0.5\% of cholesterol
8 or 16 weeks

LC, \textit{Ldlr}⁻/⁻ control (0.1\% choline)
LTS, \textit{Ldlr}⁻/⁻ TMAO-supplemented (0.1\% choline and 0.2\% TMAO)

Dietary choline or TMAO supplementation does not influence atherosclerosis development in Apoe\(^{-/-}\) male mice


**28 weeks**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Lesion area (% of total area)</th>
<th>Plasma cholesterol (mM)</th>
<th>Plasma TG (mM)</th>
<th>Plasma choline (μM)</th>
<th>Plasma betaine (μM)</th>
<th>Plasma TMAO (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow diet 12 or 28 weeks</td>
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<tr>
<td>EC, Apoe(^{-/-}) control (0.1% choline)</td>
<td><img src="image" alt="Lesion area" /></td>
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<tr>
<td>ECS, Apoe(^{-/-}) choline-supplemented (1% choline)</td>
<td><img src="image" alt="Lesion area" /></td>
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<tr>
<td>EBS, Apoe(^{-/-}) betaine-supplemented (0.1% choline and 0.9% betaine)</td>
<td><img src="image" alt="Lesion area" /></td>
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<tr>
<td>ETS, Apoe(^{-/-}) TMAO-supplemented (0.1% choline and 0.2% TMAO)</td>
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</table>
Conclusion

• Increasing plasma TMAO levels with choline or TMAO supplementation did not increase atherosclerosis in either \( Ldlr^{-/-} \) or \( Apoe^{-/-} \) mice\(^1,2\)

• The effects of dietary choline on atherosclerosis development may depend on housing facilities, age, and the source or form of dietary choline supplementation\(^1\)

“The present studies suggest that the reduced ingestion of L-carnitine and total choline by vegans and vegetarians, with attendant reductions in TMAO levels, may contribute to their observed cardiovascular health benefits.”

Research question: Does the form of dietary choline influence atherosclerosis development?

- Aortic atherosclerotic plaque area --- Oil Red O
- Choline metabolites --- LC-MS/MS
- Lipids --- colorimetric assays
- Lipoprotein fractions --- FPLC

N=66 male mice (8-10 weeks)
HFD (40% calories from fat) with 0.5% of cholesterol 12 weeks

CON, n=21 0.1% choline
CS, n=21 0.4% choline
PCS, n=24 0.1% choline 0.3 % choline from PC

IP Injection POLOXAMER 407
Blood collection 0, 1, 2, 3, and 4 h
Hypothesis

Dietary choline supplementation (either as free choline nor PC) would not enhance atherosclerotic plaque formation in male *Ldlr*^{-/-} mice.
Dietary PC supplemented *Ldlr*⁻/⁻ male mice have lower atherosclerotic size lesion

Values are reported as means ± SEMs, n = 21-24. Groups without a common letter differ, p<0.05. Multiple comparison One-Way ANOVA
Plasma TMAO and TMA concentrations are elevated in PCS group

Values are reported as median [95% CI] in Whisker’s plots for normalized data, n = 21-24.
Groups without a common letter differ, p<0.05.
Multiple comparison One-Way ANOVA
PCS group has lower and VLDL-C and higher HDL-C fraction in plasma

n = 9
VLDL secretion did not change among dietary groups

Values are reported as means ± SEMs, n = 9, p<0.05
Multiple comparison One-Way ANOVA
Take home messages

• PC supplemented diet:
  ↓ Atherosclerotic lesion size
  ↑ Plasma TMA and TMAO levels
  = Plasma cholesterol and TG levels
  ↑ Plasma HDL-C and ↓ VLDL-C fractions

• Dietary PC supplementation might improve atherosclerosis through increased lipoprotein clearance

• Our data do not support the suggestion to reduce the consumption of meat and eggs due to be rich in PC to reduce the risk of CVD.
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Questions, comments?