Eicosapentaenoic Acid (EPA) Reduces Hyperglycemia-Induced Small Dense Low-Density Lipoprotein Oxidation \textit{In Vitro} in a Manner Distinct from Docosahexaenoic Acid (DHA)

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Background

• Low-density lipoprotein (LDL) oxidation contributes to inflammation and endothelial dysfunction in atherosclerosis.\textsuperscript{1-3}

• This process may be accelerated in patients with hyperglycemia and small dense LDL (sdLDL), an atherogenic particle susceptible to oxidative damage.

• Eicosapentaenoic acid (EPA), an omega-3 fatty acid (O3FA), has been shown to significantly reduce plasma levels of oxidized LDL in patients with elevated TGs but the mechanism is not well understood.\textsuperscript{4,5}

CV Risk Factors Promote Oxidative Stress and Membrane Cholesterol Domain Formation

EPA Inhibits Glucose-Enhanced Model Membrane Lipid Peroxidation in a Dose-Dependent Fashion

**EPA Concentration (µM)**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2.5</th>
<th>5.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid Hydroperoxides (µM)</strong></td>
<td>2000</td>
<td>1600</td>
<td>1200</td>
<td>800</td>
<td>400</td>
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**p<0.001 versus vehicle-treated control; †p<0.001 versus 1.0 µM EPA; §p<0.001 versus 2.5 µM EPA; ¶p<0.001 versus 5.0 µM EPA (Student-Newman-Keuls multiple comparisons test; overall ANOVA: p<0.0001, F=561.62). Values are mean ± SD (N=6). Mason RP, Jacob RF. Biochim Biophys Acta. 2015;1848:502-509.**
EPA Inhibits Glucose-Enhanced Model Membrane Lipid Peroxidation as Compared to other TG-Lowering Agents

**p < 0.001 versus vehicle-treated control; *p < 0.001 versus all other TG-lowering agents (Student-Newman-Keuls multiple comparisons test; overall ANOVA: p < 0.0001, F = 9.940). Values are mean ± SD (N=6).

Mason RP, Jacob RF. *Biochim Biophys Acta*. 2015;1848:502-509.

Fenofib = fenofibrate; Gemfib = gemfibrozil
EPA, But Not Other TG-lowering Agents, Inhibit Lipid Oxidation & Cholesterol Domain Formation

Adapted from Mason RP, Jacob RF. Biochim Biophys Acta. 2015;1848:502-509.
LDL Modification Leads to Plaque Development and Reduced Clearance

High Glucose

Oxidative Stress

↑ LOOH

Oxidized LDL

Modified LDL (apoB)

Foam-cell formation

Monocyte motility

Endothelial adhesion

Chemoattraction

Free-radical production

Reduced clearance and plaque instability

Normal LDL

LOOH = lipoprotein lipid hydroperoxides

Small Dense LDL-C Predicts CVD


SD LDL-C = small, dense LDL-C; LB LDL-C = large, buoyant LDL-C.
Hypothesis

We hypothesized that EPA would reduce sdLDL oxidation in a manner that may be distinct from other long chain omega-3 fatty acids, such as DHA.
Methods

• Small dense (sdLDL) was isolated from human plasma by isopycnic centrifugation and incubated with EPA, DHA, or EPA/DHA at a final omega-3 fatty acid concentration of 10.0 µM and elevated glucose (200 mg/dL).

• Vitamin E was tested at the same concentration as an additional control.

• Lipid oxidation was initiated with CuSO$_4$ (1.0 µM) and followed over 6 hours with hourly measurements of malondialdehyde (MDA), a biomarker of lipid oxidation.
Results
Effects of EPA, DHA, EPA/DHA, and Vitamin E on sdLDL Oxidation under Hyperglycemic Conditions

The effect of EPA reached statistical significance from DHA at 2 hours and significance was maintained through 6 hours.
Effects of EPA, DHA, EPA/DHA, and Vitamin E on sdLDL Oxidation under Hyperglycemic Conditions (5 Hr)

![Graph showing the effects of different treatments on sdLDL oxidation](image)

- **Vehicle**: 13.5 ± 2.5 MDA Equivalents (µM)
- **Vit E**: 15.0 ± 3.0 MDA Equivalents (µM)
- **EPA**: 7.5 ± 1.0 MDA Equivalents (µM)
- **EPA + DHA**: 17.0 ± 4.0 MDA Equivalents (µM)
- **DHA**: 18.0 ± 5.0 MDA Equivalents (µM)

*p<0.01 and **p<0.001 vs. vehicle-treated control; †p<0.01 and ‡p<0.001 vs. vitamin E (Vit E); §p<0.001 vs. DHA alone; ¶p<0.001 vs. EPA + DHA (Student-Newman-Keuls multiple comparisons test; overall ANOVA: p<0.0001, F=149.85). Values are mean ± SD (N=3).
Dose-Dependent Effects of EPA and ATM on sdLDL Oxidation under Hyperglycemic Conditions
Additive Effects of EPA and ATM on sdLDL Oxidation under Hyperglycemic Conditions

- Vehicle
- EPA 0.25 µM
- ATM 0.25 µM
- ATM 0.5 µM
- EPA 0.25 µM + ATM 0.25 µM
- EPA 0.25 µM + ATM 0.5 µM

Time Point (hr) vs sdLDL Oxidation (MDA Equivalents (µM))
Schematic Illustration of the Proposed Protective Effects of EPA on Small Dense LDL Lipid Oxidation

Adapted from Mason RP, Jacob RF. Diabetes. 2015;64(Suppl 1):A178-A179.
Summary

• Incubation with EPA at therapeutically-relevant concentrations had potent antioxidant activity in sdLDL under conditions of high glucose.

• The *in vitro* activity of EPA was superior to DHA and EPA/DHA combination over time.

• Vitamin E had no antioxidant activity under these conditions.

• The antioxidant activity of a widely used statin was enhanced with the addition of EPA at low concentrations.
EPA and ATM, Alone or in Combination, Inhibited Glucose- and Oxidized LDL-Induced Endothelial Dysfunction Ex Vivo in Rats

Conclusions

• EPA inhibited sdLDL oxidation under conditions of hyperglycemia, potentially due to free radical scavenging activity.

• The combination of a statin with EPA had enhanced antioxidant benefit even at low concentrations.

• The benefit with EPA and statin was extended ex vivo into rat endothelial cells exposed to glucose and oxLDL.

• These findings may have important clinical implications, especially for patients with diabetes mellitus or impaired glucose metabolism.
Research Team

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