New Insights into Omega-3 Fatty Acids and Lipids Using Nanotechnology Approaches

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Disclosure Information

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Integrated Perspective on CV Risk Factors and Atherosclerosis

- Endothelial Dysfunction

- Dyslipidemia

- Oxidative Stress & Inflammation

Mason RP, Jacob RF
Effects of Omega-3 Fatty Acids on Inflammation and Oxidation
Omega-3 PUFAs are Metabolized into Anti-inflammatory Mediators

Omega-6 PUFAs
- Arachidonic acid (AA)
  - Proinflammatory mediators: Prostaglandin E3, Leukotriene B5
  - Less potent mediators: Prostaglandin E2, Leukotriene B4

Omega-3 PUFAs
- Eicosapentaenoic acid
  - Anti-inflammatory mediators: Resolvin E1, Protectin D1, Maresin
- Docosahexaenoic acid

Omega-3 Fatty Acids Incorporate into Lipoprotein Particles

https://commons.wikimedia.org/wiki/File:Structure_of_a_Lipoprotein.png
Lipoprotein Particles Vary in Size and Atherosclerotic Potential

Adapted from Ballantyne CM. Clinical Lipidology. 2009. Saunders, Philadelphia, PA. Apo = apolipoprotein; HDL = high-density lipoprotein (Lp); IDL = intermediate density Lp; LDL = low-density Lp; VLDL = very-low-density Lp.
Lipid Oxidation Markers Predict CV Events in 634 Patients with CAD


- oxLipid levels: highest quartile
- oxLipid levels: lowest quartile

*P < 0.0001*

*P = 0.0140*

*P = 0.0003*
Comparative Effects of TG-Lowering Agents on Human LDL Oxidation In Vitro

Vehicle EPA Fenofib Niacin Gemfib Vit E

0 2 4 6 8 10 12 14 16
MDA Equivalents (µM)

*P<0.001 versus vehicle-treated control; †P<0.001 versus Fenofib, Niacin, or Gemfib; ‡P<0.001 vs Vit E (Student-Newman-Keuls multiple comparisons test; overall ANOVA: P<0.0001, F=132.38). Values reported are mean ± SD (N=3). Each agent was tested at 10.0 µM. Mason RP, et al. J Cardiovasc Pharm. 2016 (in press).
Small Dense LDL-C Alone Predicts CVD

Risk of CHD Over Time by SD LDL-C Quartiles

Risk of CHD Over Time by LB LDL-C Quartiles

SD LDL-C = small, dense LDL-C; LB LDL-C = large, buoyant LDL-C.
Comparative Effects of TG-Lowering Agents on Human sdLDL Oxidation \textit{In Vitro}

*\(P<0.001\) versus vehicle-treated control; †\(P<0.001\) versus Fenofib, Niacin, or Gemfib; ‡\(P<0.001\) vs Vit E (Student-Newman-Keuls multiple comparisons test; overall ANOVA: \(P<0.0001\), \(F=1268.1\)). Values reported are mean ± SD (N=3). Each agent was tested at 10.0 µM. Mason RP, \textit{et al.} \textit{J Cardiovasc Pharm.} 2016 (in press).
Schematic of Proposed Protective Effects of Omega-3 Fatty Acids on sdLDL Lipid Oxidation

Adapted from: Mason RP, Jacob RF. Diabetes. 2015; 64, Suppl 1:A178-A179
Omega-3 Fatty Acids Differentially Inhibit Human sdLDL and VLDL Oxidation *In Vitro*

\[ *P<0.001 \text{ versus vehicle-treated control; } \dagger P<0.05 \text{ and } \ddagger P<0.001 \text{ versus DHA (Student-Newman-Keuls multiple comparisons test; overall ANOVA—sdLDL data: } P<0.0001, F=391.88; \text{ VLDL data: } P<0.0001, F=1074.8). \text{ Values reported are mean } \pm \text{ SD (N=3). Each agent was tested at 10.0 } \mu\text{M (sdLDL) and 2.5 } \mu\text{M (VLDL). Mason RP, et al. } J \text{ Cardiovasc Pharm. } 2016 \text{ (in press).} \]
Effects of TG-lowering Agents on Atherosclerosis and Cholesterol Crystal Formation
Small Angle X-ray Diffraction of Cell Membranes

Membrane suspension

Lucite Sedimentation Cell

Interbilayer water space

Centrifugation 35,000 \( \times \) g

Curved glass support

Membrane multibilayer sample is mounted on curved glass support

Water space

\( d \cdot \sin \theta \)

\( 2 \theta \)

X-ray Source

\( \lambda \)
SEM Photomicrograph of Cholesterol Crystal and Macrophage Foam Cells

Cholesterol Crystals Associated with Apoptotic Cell Death

Characterizing Model Membrane Cholesterol Crystalline Domains by X-ray Diffraction

Effects of Omega-3 Fatty Acid EPA and Vitamin E on Cholesterol Domain Formation

Mason RP, Jacob RF. *Biochim Biophys Acta*. 2015;1848:502-509.
Effects of TG-Lowering Agents on Cholesterol Domain Formation

Mason RP, Jacob RF. *Biochim Biophys Acta*. 2015;1848:502-509.
Comparative Effects of TG-lowering Agents on Cholesterol Domain Formation

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

EPA blocks free radical propagation through the lipid bilayer, preventing lipid oxidation and cholesterol domain formation.
Effects of Omega-3 Fatty Acids with Statins on Endothelial Function with Dyslipidemia
Nitric Oxide Is a Key Mediator of Vascular Protection

Behrendt D, Ganz P. Am J Cardiol. 2002;90(10C):40L-48L.
Nanotechnology Approaches Used to Measure Endothelial Function


Normal Endothelial Function Is a Balance Between NO and ONOO⁻ Release Levels

- ONOO⁻
  - Vasoconstriction
  - Inflammation
  - High Angiotensin II
  - Pro-thrombotic
  - Trophic
  - Fibrotic

- NO
  - Vasodilation
  - Anti-inflammatory
  - Natriuresis
  - Anti-fibrotic
  - Anti-thrombotic

Mason RP, Jacob RF.
CV Risk Factors Lead to Loss of NO Release from Rat Aortic and Glomerular Endothelium

*P*<0.001 versus control; †*P*<0.05 versus NO release measured from hypertensive animals (Student-Newman-Keuls multiple comparisons test; overall ANOVA—aortic EC data: *p*<0.0001, *F*=89.991; glomerular EC data: *p*<0.0001, *F*=74.629). Values are mean ± SD (N=5-6). Spontaneously hypertensive (SH) rats: BP = 167 ± 5; STZ-induced blood glucose = 354 ± 83. ECs = endothelial cells. Mason RP, *et al*. *Am J Hypertens*. 2009;22:1160-1166.
Omege-3 Fatty Acid and Atorvastatin Pretreatment Inhibits the Effects of Oxidized LDL on Human Endothelial Cell Function

Atorvastatin active metabolite (ATM) was used in this study. Values are mean ± SD (N=3-6). *P<0.05 and ***P<0.001 vs. oxidized LDL (oxLDL); †P<0.01 vs. oxLDL + EPA; §P<0.001 vs. oxLDL + Atorv (Student-Newman-Keuls multiple comparison test; overall ANOVA: P<0.0001, F=25.827). Mason RP, et al. J Clin Lipidol. 2014;8:342-343.
Illustration of Postulated Combined Effects of EPA and Statin on Reversal of Endothelial Dysfunction

Mason RP et al. *J Am College Cardiol.* (abstract presented April, 2016).
Antioxidant effects
Cholesterol crystalline domains
Ox-LDL
RLP-C
Improved endothelial function
Adhesion of monocytes
Macrophages
Foam cells
Fish Oil Supplements as Source of Omega-3 Fatty Acids

- Fish oil is the most commonly used dietary supplement among US adults.¹
- Based on the 2012 National Health Interview Survey, about 7.8% of adults (19 million) had taken a fish oil supplement in the previous 30 days.²
- Although numerous dietary supplements containing OM3FA are widely available, their integrity and efficacy remain unverified.³

Can Fish Oil Supplements be Used to Treat Patients?

- Most common dietary supplements report 30% of their contents as omega-3 fatty acids (OM3FAs)
- Each 1 gram capsules may contain 300 mg of EPA/DHA
- In order to reach 4 g/day of OM3FAs, a patient would need a large number of capsules

Fish Oil Supplements Contain Various Levels of OM3FA and Saturated Fat

Saturated Fat Content in Fish Oil Supplement Leads to Solid Mass following Isolation

Rx of Pure Omega-3 Fatty Acids is a Clear Fluid

Fish Oil Dietary Supplement forms Solid Mass

Sherratt, CR, Mason RP
International Fish Oil Supplements Exceed Recommended Levels of Oxidation Markers

- 83% of fish oil products tested exceeded recommended PV threshold
- 25% exceeded recommended AV
- 50% exceeded recommended TOTOX
- Only 3 of 36 (8%) met the international recommendations, not exceeding any of these indices
- Best-before date, cost, country of origin, and exclusivity were all poor markers of supplement quality

Recommended international thresholds are indicated by dotted lines in each panel.
U.S. Leading Fish Oil Supplements Exceed Recommended Levels of Oxidation Markers

*Global Organization for EPA and DHA Omega-3s (GOED). Available at: http://www.goedomega3.com/index.php
Mason RP et al. Poster presented at the AMCP 2015 Nexus, Orlando, FL.
Effects of a DS Fatty Acid Extract vs Non-Oxidized and Partially Oxidized Preparations of EPA and DHA on Human sdLDL Oxidation In Vitro

Each agent was tested separately at 10.0 µM or in combination at 5.0 µM against vehicle-treated controls. *p<0.001 versus vehicle alone; †p<0.001 versus DS; §p<0.001 versus oxEPA + oxDHA (Student-Newman-Keuls multiple comparisons test; overall ANOVA: p<0.0001, F=993.26). Values are mean ± S.D. (N = 3). Mason RP et al. Poster presented at the AMCP 2015 Nexus. Orlando, FL.
Conclusions

• Lipids modify cell function through association with specific domains that regulate signal transduction. At high levels, cholesterol forms domains that lead to cytotoxic crystals.

• Omega-3 FA such as EPA inhibit oxidative damage to ApoB-containing particles and cellular membranes leading to reduced inflammation, endothelial dysfunction and cholesterol crystals, potentially improving LDL clearance.

• Supplements contain variable amounts of omega-3 fatty acids, saturated FAs and elevated peroxidation products that limit their benefit. Given the lack of oversight, supplements should not be used as a replacement for Rx.
Using nanotechnology approaches, we have characterized novel effects of omega-3 fatty acids like EPA on pathogenic mechanisms associated with CV disease, including oxidative stress, inflammation, and endothelial dysfunction.
Research Team

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