Cholesterol and Cholesterol Oxides on Coronary Heart Diseases
Trends in egg consumption in U.S. and the reason

A. Egg Consumption:
- 403 in 1945
- 234 in 1993 (176 Table eggs, 58 Processed eggs)
- 256 in 2004

B. Major concerns: Cholesterol and Fat in eggs
- Cholesterol in egg: 213 mg/large egg (1989)
  30% of Yolk
- Cholesterol in meat: 70-80 mg/100 g
How Cholesterol is Used in the Animal Body

Cholesterol

- Vitamin D
- Cell Membrane
- Hormones
- Bile
- Skin
Cholesterol and CHD: Theories

- Plasma lipid theory
- Response-to-Injury hypothesis: Lipid and cholesterol oxidation products cause injury
- Combined “Lipid” and “Injury” Hypothesis
Arteriosclerosis and CHD

**Atherosclerosis**: general term for a condition characterized by thickening, hardening, and loss of elasticity of the walls of the blood vessels.

**CHD**: condition that results when the coronary arteries are narrowed or occluded, most commonly by atherosclerotic deposits of fibrous and fatty tissue. Coronary artery disease is the most common underlying cause of cardiovascular disability and death.
The infiltration of LDL cholesterol into the wall of an artery is the major factor in the infiltration and progression of atherosclerotic plaques.

Cholesterol plays a pivotal role.
Plasma lipid theory (Infiltration theory)

- Anitschew (1913): High frequency atherosclerosis in rabbits when fed cholesterol
- Feeding 2% cholesterol diet increased serum cholesterol level by 90 folds and increased atherosclerotic lesion (Langner and Bement, 1985).
- Epidemiological Studies: High serum Cholesterol is related to atherosclerosis
- Intervention reduced atherosclerosis: Cholestryamine (increase the rate of bile excretion)
- High cholesterol induced atherosclerosis but the response varied among rabbits (Duff et al., 1957)
- LDL receptor theory of Goldstein and Brown (1976): familial hypercholesterolemia
Does dietary cholesterol raise serum cholesterol?

- At very high level of intake (> 1000 mg per day): yes. Because physiological compensation mechanisms (decreased synthesis and absorption of Cholesterol increased excretion via bile salts) are not good enough to maintain SC level.

- At 500 - 800 mg level: 70% can maintain homeostasis by the compensation mechanisms. Approximately 30% increased Sc level temporarily and went back to normal.

- Only U.S. has dietary cholesterol level in a dietary guideline.
Response-to-Injury Hypothesis

- An injury to the endothelium is the initiating event in atherogenesis
- Reinterpretation of cholesterol study
  Questions on the purity of cholesterol (Taylor et al., 1979)
- USP grade cholesterol was contaminated with cholesterol oxidation products (COPS)
- Epidemiological study: Indian immigrants to London
Biological effects of cholesterol oxides

- Atherogenicity
- Angiotoxicity
- Cytotoxicity
- Mutagenecity
- Inhibition of enzyme activities: HMGCoAR
Cholesterol Oxides

Cholesterol (cholest-5-en-3β-ol)

7α-OH or 7β-OH cholesterol

5,6α- or 5,6β-epoxides

Cholestanetriol

7-keto cholesterol

5 α-hydroperoxycholesterol
Endothelial cell injury: Mechanically and chemically

- Hypertension
- Plasma LDL: familial and diet related
- Toxins: COPS, virus, and drugs
- Pure cholesterol was not atherogenic nor angiototoxic in spite of being able to induce hypercholesterolemia
- Each COPS were angiotoxic and atherogenic: Cholestan triol, 25-hydroxycholesterol are the most potent atherogenic agents
Combined “Lipid” and “Injury” Hypothesis

- Endothelial injury by Lipid Oxidation Products and then plaque accumulation by LDL-cholesterol
- COPS are initiators of atherosclerosis
- Cholesterol is involved in plaque deposition
- Cholesterol deposition is merely a secondary process preceded by a complex series of pathological changes involving the interplay of endothelial cells, macrophages, platelets, growth factors, chemotactic factors, lipoproteins, and foam cells.
Angiotoxicity and cytotoxicity

- Explain their role as initiators of atherosclerotic lesions

- Endothelial cell damage initiates the complex series of pathological changes leading ultimately to plaque, wall thickening, and lumen narrowing
Atherosclerotic cascade

- Wound by toxin or hypertension (right brachiocephalic artery is more susceptible)
- Release of chemokines, accumulation of platelets for healing
  - Monocytes cross the endothelial cell layer and scavenge oxidized LDL
  - Foam cell formation and then fatty streak
  - Foam cells secrete chemokines, and platelets and foam cells secrete growth factors
Atherosclerotic cascade

- More monocytes/macrophages and T-lymphocytes accumulate
- Swelling and damage of endothelial cells by the free radicals and smooth cell growth
  - Cell rupture and platelets aggregation as a process of wound healing
  - LDL, cholesterol esters, lipid, collagen, calcium deposition
- Plaque formation
- Obstruct arteries and thrombosis
Cholesterol Oxidation

- In solid phase (crystalline cholesterol): side chain oxidation
- In aqueous solution: autoxidation similar to that of other lipids
- Exposure to air, heat, light and radiation accelerate cholesterol oxidation
- Variety of COPS are found in foods: milk products, egg products, fish products
- COPS are absorbed in intestine and transferred by LDL and VLDL
### Types of heat treatment on oxysterols formation in spray-dried egg mix

<table>
<thead>
<tr>
<th>Oxysterol</th>
<th>Indirect heat (mg/g)</th>
<th>Direct heat (mg/g)</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7α-hydroxy cholesterol</td>
<td>1.8</td>
<td>7.0</td>
<td>289</td>
</tr>
<tr>
<td>7β-hydroxy cholesterol</td>
<td>1.5</td>
<td>18.5</td>
<td>1133</td>
</tr>
<tr>
<td>α-epoxide</td>
<td>21.5</td>
<td>50.0</td>
<td>132</td>
</tr>
<tr>
<td>β-epoxide</td>
<td>1.9</td>
<td>37.4</td>
<td>1868</td>
</tr>
<tr>
<td>25-hydroxy cholesterol</td>
<td>1.4</td>
<td>5.1</td>
<td>264</td>
</tr>
<tr>
<td>7-ketocholesterol</td>
<td>2.0</td>
<td>37.0</td>
<td>1750</td>
</tr>
<tr>
<td>Cholestantriol</td>
<td>11.6</td>
<td>13.0</td>
<td>12</td>
</tr>
</tbody>
</table>

Missler et al. (1985, J. Food Sci. 50: 229)
### Oxysterols (ppm/food sample) in processed meats

<table>
<thead>
<tr>
<th>Processed Meat</th>
<th>25-OH</th>
<th>7 α-OH</th>
<th>7 β-OH</th>
<th>triol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked Bratwurst</td>
<td>Tr</td>
<td>820.0</td>
<td>Tr</td>
<td>1335.0</td>
</tr>
<tr>
<td>Beef Franks</td>
<td>Tr</td>
<td>98.5</td>
<td>34.0</td>
<td>Tr</td>
</tr>
<tr>
<td>Chicken rolls</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>Cooked Hamburgers</td>
<td>Tr</td>
<td>72.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cooked lean bacon</td>
<td>Tr</td>
<td>Tr</td>
<td>NA</td>
<td>Tr</td>
</tr>
<tr>
<td>Raw hamburger</td>
<td>Tr</td>
<td>Tr</td>
<td>36.0</td>
<td>1298.0</td>
</tr>
<tr>
<td>Turkey bologna</td>
<td>Tr</td>
<td>NA</td>
<td>NA</td>
<td>86.0</td>
</tr>
</tbody>
</table>

Higley et al. (1986, Meat Sci. 16:175)
Cholesterol oxides (ppm, dry wt. basis) in the edible portions of commercial fish products

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lipid</th>
<th>7 β-OH</th>
<th>7-keto epoxides</th>
<th>Triol</th>
<th>25-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salt-dried</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anchovy</td>
<td>11.5</td>
<td>37.1</td>
<td>46.4</td>
<td>45.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Northern cod</td>
<td>4.8</td>
<td>6.8</td>
<td>9.7</td>
<td>8.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Jap. Whiting</td>
<td>6.8</td>
<td>24.5</td>
<td>24.9</td>
<td>25.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Pacific herring</td>
<td>27.0</td>
<td>8.4</td>
<td>7.5</td>
<td>8.9</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Boiled-dried</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anchovy</td>
<td>11.2</td>
<td>55.8</td>
<td>60.6</td>
<td>61.3</td>
<td>8.5</td>
</tr>
<tr>
<td>Shrimp</td>
<td>6.2</td>
<td>3.7</td>
<td>4.0</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>Smoked salmon</td>
<td>8.7</td>
<td>7.3</td>
<td>6.3</td>
<td>5.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Ohshima et al. (1993, JAOCs 70:595)
Efforts to solve cholesterol problems

- Use of drug: bile salt sequestrants (Cholestyramine), Nicotinic acid (decrease VLDL production), HMGCoAR inhibitor

- Diet: increase n3 fatty acids and decrease saturated fat consumption

- Removal of cholesterol from foods by physical and chemical methods
Proposed Benefit of N-3 Fatty Acids

Omega-3 fatty acids reduce:
- Platelet aggregation
- Serum triglycerides
- Arterial spasm
- Blood pressure
- Serum cholesterol
Pathways for Eicosanoids Synthesis

**Diet**
- LA → LA → AA
- AA → AA → EPA → DHA
- LNA → LNA → EPA → DHA
- EPA → EPA → DHA

**Body**
- E,D → AA
- LTB4 → LTB5
- LTC4 → LTC5
- AA + EPA, DHA
- C → PG12 → TXA2
- C → PG13 → TXA3
Mechanisms of action of EPA and DHA

LTB4, LTC4 ←------------ AA --------------→ PGI2, TXA2

LTB5, LTC5 ←------------ EPA, DHA --------------→ PGI3, TXA3

* LTB4, LTC4: increase adhesion of Leucocytes to endothelium and inflammatory effects
* LTB5, LTC5: less effective than LTB4, LTC4
* PGI2: vasodilator, inhibit platelet aggregation
* PGI3: help effect of PGI2
* TXA2: increase platelets aggregation, vasoconstriction
* TXA3: less potent than TXA2

N3 fatty acids compete with N6 for elongase, desaturase, cyclooxygenase and lipoxigenase
Possible adverse effects of high level consumption of omega-3 fatty acids

- Depletion of tissue vitamin E
- Increased propensity to bleed
- Increased consumption of lipid oxidation products
- Increased *in vivo* production of lipid oxidation products
Effect of dietary fats on blood cholesterol and the development of atherosclerosis in rabbits
### Fatty acid composition (%) and peroxide values of oils used

<table>
<thead>
<tr>
<th>Item</th>
<th>CO</th>
<th>FO</th>
<th>OO</th>
<th>HCO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
<td>-</td>
<td>7.56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>13.88</td>
<td>20.19</td>
<td>10.63</td>
<td>13.82</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>-</td>
<td>10.11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>28.39</td>
<td>10.74</td>
<td>37.15</td>
<td>33.55</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>-</td>
<td>0.68</td>
<td>-</td>
<td>1.11</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>54.93</td>
<td>1.70</td>
<td>50.15</td>
<td>49.17</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>0.36</td>
<td>3.31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>2.34</td>
<td>3.45</td>
<td>2.06</td>
<td>2.34</td>
</tr>
<tr>
<td>Eicosatetrenoic acid</td>
<td>-</td>
<td>0.65</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eicosapentanoic acid</td>
<td>-</td>
<td>12.23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Docosahexanoic acid</td>
<td>-</td>
<td>11.72</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other fatty acids</td>
<td>-</td>
<td>25.90</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

------------- meq peroxide/kg oil ---------------

<table>
<thead>
<tr>
<th>Peroxide value (PV)</th>
<th>CO</th>
<th>FO</th>
<th>OO</th>
<th>HCO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30.28</td>
<td>31.57</td>
<td>144.87</td>
<td>28.43</td>
</tr>
</tbody>
</table>

Abbreviations: CO, 2% fresh corn oil; FO, 2% fresh fish oil; OO, 2% oxidized oil; HCO, 2% heated corn oil. Cholesterol (CHO, 1.5 g/kg diet)
Effect of various dietary oils on cholesterol content in plasma

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>69.6</td>
<td>407.7</td>
<td>447.1</td>
<td>453.6</td>
<td>430.6</td>
</tr>
<tr>
<td>CO</td>
<td>69.6</td>
<td>333.8</td>
<td>379.0</td>
<td>321.0</td>
<td>281.8</td>
</tr>
<tr>
<td>FO</td>
<td>69.6</td>
<td>341.9</td>
<td>429.1</td>
<td>459.9</td>
<td>449.8</td>
</tr>
<tr>
<td>OO</td>
<td>69.6</td>
<td>396.2</td>
<td>444.9</td>
<td>474.4</td>
<td>435.8</td>
</tr>
<tr>
<td>HCO</td>
<td>69.6</td>
<td>281.4</td>
<td>246.0</td>
<td>192.7</td>
<td>184.4</td>
</tr>
</tbody>
</table>

Abbreviations: NO, 0% oil; CO, 2% fresh corn oil; FO, 2% fresh fish oil; OO, 2% oxidized oil; HCO, 2% heated corn oil. Cholesterol (CHO, 1.5 g/kg diet) was added to all the experimental diets.
Atherosclerotic lesions in aorta of rabbits fed diets containing cholesterol and different oils

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>9 week</th>
<th>12 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>2.25</td>
<td>3.75</td>
</tr>
<tr>
<td>CO</td>
<td>1.00</td>
<td>1.50</td>
</tr>
<tr>
<td>FO</td>
<td>2.25</td>
<td>2.75</td>
</tr>
<tr>
<td>OO</td>
<td>1.75</td>
<td>3.50</td>
</tr>
<tr>
<td>HCO</td>
<td>1.00</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Abbreviations: NO, 0% oil; CO, 2% fresh corn oil; FO, 2% fresh fish oil; OO, 2% oxidized oil; HCO, 2% heated corn oil. Cholesterol (CHO, 1.5 g/kg diet) was added to all the experimental diets.
Atherosclerotic lesions in aorta of rabbits fed with different levels of dietary cholesterol
Results

- No added oil and oxidized oil groups developed the highest degree of atherosclerotic lesions followed by fresh fish oil, and fresh corn oil and heated corn oil group had the lowest lesions.

- Plasma total cholesterol level was the most critical factors involved in the development atherogenic lesions in rabbit.

- Dietary cholesterol and oxidation status of dietary lipids had significant impact on lipid metabolism and the development of atherosclerosis in rabbits.

- Oxidized oils in diet accelerated the progress of atherosclerotic lesions, but polymerization of oils by heating lowered plasma cholesterol level and the development of atherogenic lesions in rabbits by inhibiting the absorption of cholesterol in guts.