Polyphenol Bioactivity: Antioxidants?

Prof Kevin D Croft
University of Western Australia

Other dietary polyphenols with biological activity

- **Phenolic acids**, eg caffeic acid (fruits, coffee etc)
- **Lignans**, eg. Sesamin (sesame seed), isolariciresinol etc (flax seed, bran)
- **Stilbenes**, eg. resveratrol (grapes)
- **Phenylpropanoids**, eg Curcumin (turmeric), zingerone (ginger)
- **Terpenoids**, eg. Oleuropein (olive oil)

Dietary polyphenols and cardiovascular disease: *more than antioxidants*

- low concentrations in vasculature
- metabolic transformation
- specific targets, key enzymes
Flavonoid Metabolism

Human neutrophils stimulated with Ca ionophore

Antioxidant activity

Atherosclerosis (animal models)

Apo E deficient mouse

Faulty uptake of lipoproteins in the liver

Very high circulating cholesterol
Effect of pure polyphenols incorporated into the diet, apoE-/- , ~1 mg/day, 20 weeks

Table 1. Effects of specific polyphenols on tested pathways at week 26 (expressed as % change compared to the ApoE-/- mice fed on control diet).

<table>
<thead>
<tr>
<th>Polyphenol</th>
<th>Aortic sinus lesion formation</th>
<th>Thoracic aorta lesion formation</th>
<th>Plasma cholesterol</th>
<th>Aortic F2-isoprostanes</th>
<th>HO-1 protein</th>
<th>Aortic LTB4</th>
<th>Plasma soluble P-selectin</th>
<th>Urinary nitrate</th>
<th>Vascular eNOS activity</th>
<th>Urinary ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>-79*</td>
<td>-57*</td>
<td>+0.1</td>
<td>-60*</td>
<td>+190*</td>
<td>-63*</td>
<td>-29*</td>
<td>+90*</td>
<td>+1446*</td>
<td>-52*</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>-14</td>
<td>-15</td>
<td>-2</td>
<td>-77*</td>
<td>+4</td>
<td>-34*</td>
<td>-33*</td>
<td>+44</td>
<td>+631</td>
<td>-51*</td>
</tr>
<tr>
<td>Theaflavin</td>
<td>-56*</td>
<td>-24</td>
<td>-</td>
<td>-39</td>
<td>ND</td>
<td>-10</td>
<td>ND</td>
<td>+44</td>
<td>+923*</td>
<td>-1</td>
</tr>
<tr>
<td>Sesamin</td>
<td>-41</td>
<td>-24</td>
<td>-</td>
<td>-27</td>
<td>ND</td>
<td>-12</td>
<td>ND</td>
<td>+631</td>
<td>+305</td>
<td>-4</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>-24</td>
<td>-43</td>
<td>-</td>
<td>-9</td>
<td>ND</td>
<td>-24</td>
<td>ND</td>
<td>+4</td>
<td>+466</td>
<td>-40</td>
</tr>
</tbody>
</table>

*p < 0.05 vs ApoE-/- control mice. ND = not determined.

Evidence for effects of dietary flavonoids on major CVD risk factors in humans

Endothelial Function
Blood Pressure

Heme oxygenase-1 is the inducible form of the enzyme involved in heme degradation and the release of iron, carbon monoxide and the bile pigment biliverdin that is then converted to bilirubin. HO-1 exerts an anti-inflammatory and antioxidant action within the vasculature and can protect against oxidative damage.
Comparison of quercetin, epicatechin, epigallocatechin gallate on plasma NO

- Cross-over study, 12 healthy subjects
- 200mg of each flavonoid or water control
- Blood collected at baseline and after 2 hours, urine at baseline and 5 hours.
- Plasma nitrosothiols measured by gas phase chemiluminescence. Nitrate/nitrite measured by GCMS
- Plasma and urinary flavonoids measured by GCMS

### Bioavailability of flavonoids - plasma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma Flavonoids (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>1</td>
</tr>
<tr>
<td>3'-MQ</td>
<td>2</td>
</tr>
<tr>
<td>Total Q</td>
<td>3</td>
</tr>
</tbody>
</table>

#### How do flavonoids effect NO ?

- Increase eNOS enzyme activity, produce more NO
- Prevent the breakdown of NO
- Recent in vitro evidence that methylated metabolites of epicatechin can inhibit NADPH oxidase (*Steffan, Sies, FRBM 2007*)
- Reduction of $\text{NO}_3^-$ to $\text{NO}_2^-$ and NO

### PARTICIPANTS AND PROCEDURE

- 30 healthy men and women (18 to 65 years of age) recruited from the general population
- 4 visits in random order
- 1 week washout between testing days
- Same dinner night before each visit
- Same low flavonoid / low nitrate breakfast on morning of each visit

*Bondonno et.al. Free Rad Biol Med. 2012*
Study design

<table>
<thead>
<tr>
<th>Visit</th>
<th>Pre and during visit</th>
<th>During visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>FLAVONOID CONTROL</td>
<td>NITRATE CONTROL</td>
</tr>
<tr>
<td>B</td>
<td>FLAVONOID ACTIVE</td>
<td>NITRATE CONTROL</td>
</tr>
<tr>
<td>C</td>
<td>FLAVONOID CONTROL</td>
<td>NITRATE ACTIVE</td>
</tr>
<tr>
<td>D</td>
<td>FLAVONOID ACTIVE</td>
<td>NITRATE ACTIVE</td>
</tr>
</tbody>
</table>

Breakfast and flavonoid treatment 4 hr  
Lunch and flavonoid / nitrate treatment 2 hr  
FMD Blood sample

% FMD BY TIME AFTER CUFF INFLATION

NOx BY TREATMENT:

(-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans  
H Schroeter et.al  PNAS 2006

Mechanisms of action

• Activation of key signalling pathways or enzymes
  – AMPK activation, enzymes involved in lipid and glucose metabolism, eNOS. Resveratrol, quercetin etc
• Estrogenic effects, isoflavones, some lignans
• Redox sensitive transcription factors
  – NF-kB → inflammation
  – Nrf2 → binds antioxidant response elements of genes encoding for antioxidant enzymes eg HO-1

• Cardiovascular health effects
  – vascular function, BP
  – Thrombosis
  – Lipids
  – Inflammation
  – Glucose metabolism

Bioactivity overview

• Polyphenols are a diverse range of compounds.
• Global measures such as Total Antioxidant Capacity (TAC) may be of little value.
• Bioactive polyphenols may be beneficial either as food components or isolated compounds. Food matrix effects on bioavailability may influence absorption and metabolism.
• Different polyphenols may have very different and specific activity, eg grapefruit flavonoids and drug metabolising enzymes.

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