The Importance of Flavonoids (Plant phenolic compounds) for Gut Health

Speaker: Dr Matt Frevel
What are Flavonoids?

- Secondary plant metabolites
- Often play a role in plant defence against viruses, bacteria, fungi, herbivores
- Growth regulators, environment communicators
- Synthesised from L-phenylalanine

- Plant compounds that contribute to the blue, red, orange, and brown colours in many different plant parts, i.e. leaves, flowers, fruits, bark, nuts and seeds
- Main dietary sources include: fruits and vegetables, seeds, nuts, grains, legumes, tea, coffee, wine, beer
Metabolism of Dietary Polyphenols

Dietary polyphenols
- Hydrolysis of most glycosides (LPH, CBG)
- Conjugation reactions (methylation, glucuronidation, sulfation)
  - Small intestine
  - Not absorbed
  - Colon
    - Action of bacterial enzymes (α-rhamnosidases)
  - Aglycones
    - Portal vein
  - Liver
    - Conjugation reactions
      - Methylation
      - Glucuronidation
      - Sulfation
    - Bile
    - Tissues
  - Aglycones
    - Portal vein
    - Urine
    - Feces
What happens to Flavonoids in the Gut?

- Most flavonoids present as Glycosides (have sugars attached)
- Aglycones (without sugars) are easily absorbed in small intestine
- Monoglucosides transported with sodium glucose transporter-1
- Absorption rates in small intestine are typically low
- Significant portion of flavonoids reach the large intestine
- Gut bacteria quickly hydrolyze glycosylated and conjugated flavonoids to aglycones or methylated forms
Effects & Activities of Flavonoids Include

- Anti-oxidant activity
- Metal-chelating activity
- Anti-inflammatory activities
- Immune-modulatory activities
- Anti-carcinogenic activities
- Anti-bacterial & Anti-viral activities
- Anti-platelet aggregation activity
- Anti-hypertensive, Pro-endothelial function
- Enzyme-inhibitory activities: COX, phospholipase A2, lipooxygenase, xanthinoxidase, collagenase, elastase, hyaluronidase, beta-glucoronidase, several kinases

- Effects on and in the Gut
Effects on and in the Gut

• Microflora
  – modulating intestinal bacterial population

• Gut Inflammation
  – modulating inflammatory cascades

• Barrier Function
  – modulating tight junction proteins and mucus
Modulating Intestinal Microflora
(pre-biotic & anti-bacterial)

A Survey of Modulation of Gut Microbiota by Dietary Polyphenols

Montserrat Dueñas

BioMed Research International
Volume 2015, Article ID 850902, 15 pages

Population Increases
Lactobacilli spp.
Bifidobacteria spp.
Enterococci spp.
C. coccoides grp.
Eubacterium rectale grp.
E. Coli
Klebsiella spp.
Akkermansia spp.

Population Decreases
C. histolyticum grp.
Staphylococcus aureus
Salmonella typhimurium

Modulating Bacterial Populations (pre-biotic & anti-bacterial)

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<table>
<thead>
<tr>
<th>Phenolic compound/food</th>
<th>Dose</th>
<th>Time of incubation</th>
<th>Microbial technique</th>
<th>Growth enhancement</th>
<th>Growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-catechin</td>
<td>150 mg/L, 1000 mg/L</td>
<td>&lt;48 h</td>
<td>FISH</td>
<td><em>Lactobacillus-Enterococcus</em> spp. <em>Bifidobacterium</em> spp.</td>
<td><em>C. histolyticum</em> group</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. coccoides-E. rectale</em> group <em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td>Blueberry extracts</td>
<td>5, 10 and 25%</td>
<td>48 h</td>
<td>FISH</td>
<td><em>Lactobacilli Bifidobacteria</em></td>
<td></td>
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<tr>
<td>Pomegranate extract and punicalagin</td>
<td>10%</td>
<td>48 h</td>
<td>FISH</td>
<td><em>Total bacteria</em></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td><em>Bifidobacterium</em> spp. <em>Lactobacillus-Enterococcus</em> spp.</td>
<td></td>
</tr>
<tr>
<td>Almond skins</td>
<td>1%, w/v predigested almond skins</td>
<td>&lt;24 h</td>
<td>FISH</td>
<td><em>Bifidobacteria C. coccoides-E. rectale</em> group</td>
<td><em>C. histolyticum</em> group</td>
</tr>
</tbody>
</table>
# Effects of Tea Polyphenols on Microflora

<table>
<thead>
<tr>
<th>Polyphenol</th>
<th>Inhibitory Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea polyphenols including: epigallocatechin gallate (EGCG), epicatechin gallate, epigallocatechin, gallocatechin, epicatechin, catechin</td>
<td>Helicobacter pylori, Staphylococcus aureus, E. coli O157:H7, Salmonella typhimurium, Listeria monocytogenes, methicillin-resistant S. aureus, Pseudomonas aeruginosa, Hepatitis C virus, Influenza virus, HIV, Epstein–Barr virus, Fungi of the Candida genus</td>
</tr>
</tbody>
</table>

Examine the effects of polyphenols on:

- colonic environment
- intestinal barrier function
- gut microbiota
Study Design

Rats on five different diets:

1. Low fat
2. High fat
3. High fat + Aronia
4. High Fat + Haskap
5. High Fat + Bilberry

Faecal samples analysed.
Effect of Dietary Polyphenols on Microbiota

Means/Ratios which differ significantly using the Tukey’s test (p < 0.05) do not share a common letter. LF low fat. HF High fat.

Conclusion
Polyphenols obviated change in Firmicutes and Bacteroidetes populations seen with a high fat versus low-fat diet
Effect of Dietary Polyphenols on Mucin & IgA

Conclusion
Polyphenols improve barrier function by increasing mucin and IgA (first line of defence in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms)
Effects on Microflora - Conclusion

- Dietary polyphenols may help maintain intestinal health by preserving gut microbial balance
- Growth of potential pathogens eg. C. perfringens and C. histolyticum, and certain Gram-negative Bacteroides spp. and other pathogens are inhibited
- Growth of beneficial clostridia, bifidobacteria and lactobacilli is stimulated
Modulating Gut Inflammation


Anti-inflammatory effects of dietary phenolic compounds in an in vitro model of inflamed human intestinal epithelium

Thérèse Sergent\textsuperscript{a}, Neil Piront\textsuperscript{b}, Julie Meurice\textsuperscript{a}, Olivier Toussaint\textsuperscript{b}, Yves-Jacques Schneider\textsuperscript{a,}\textsuperscript{*}

Experiment:

1. Differentiated Caco-2 - small intestine epithelial cells including tight junctions and microvilli
2. Add pro-inflammatory cocktail: IL-1, TNF-α, IFN-γ and LPS
3. Add phenolic compounds: Resveratrol, Ellagic acid, Ferulic acid, Curcumin, Quercetin, Chrysin, EGCG, Genistein
4. Measure production of pro-inflammatory cytokines: IL-6, IL-8, MCP-1

MCP-1 = monocyte chemoattractant protein-1
Conclusion:
Significant inhibition of IL-6 and MCP-1 by Genistein (soy-isoflavone), and of IL-6 and IL-8 by EGCG (green tea flavanol)
SHORT COMMUNICATION

Therapeutic Efficacy of Pycnogenol in Experimental Inflammatory Bowel Diseases

Miyako Mochizuki and Noboru Hasegawa*

Experiment:
Pre-treat rats with a high antioxidant pine bark extract before inducing IBD to determine the effect on the severity of damage in the colon mucosa.

Figure 1. Effect on severity of colitis.
Pycnogenol (P-L: 0.5 mg/kg; P-H: 10 mg/kg) was given for 10 days prior to the administration of TNBS. Results are presented as the mean +/- SE of six experiments: * p < 0.05; ** p < 0.01, compared with the TNBS-control.

⇒ Significant reduction in macroscopic damage score
Experiment:
Pre-treat rats with a high antioxidant pine bark extract before inducing IBD to determine the effect on the severity of damage in the colon mucosa.

Figure 2. Effect on neutrophil granulocyte infiltration into the distal colon mucosa. Experimental procedure is as described in Fig. 1. Results are presented as the mean +/- SE of six experiments: ** p < 0.01; *** p < 0.001, compared with the TNBS-control.

⇒ Significant inhibition of myeloperoxidase activity = neutrophil infiltration
Intestinal anti-inflammatory activity of ellagic acid in the acute and chronic dextrane sulfate sodium models of mice colitis

Marta Marín, Rosa María Giner, José-Luis Ríos, María Carmen Recio*

Pomegranate traditionally used for treatment to inflammatory diseases including ulcerative colitis. Ellagitannins are one of the bioactive compounds in pomegranate, hydrolysed to ellagic acid in the intestine thought to be at least partly responsible for the activity of pomegranate extracts.

**Experiment:**

Model of chronic IBD: One week on, one week off, treat mice with Dextran Sulphate Sodium on a background of +/- 0.5% Ellagic acid.
Ellagic acid reduced Diarrhea Bleeding Disease Activity Index COX-2 amounts iNOS amounts p38-MAPK NFkB
Dietary polyphenols can modulate the intestinal inflammatory response

<table>
<thead>
<tr>
<th>Anti-inflammatory Effect</th>
<th>Flavonoids</th>
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<tbody>
<tr>
<td>Reduced IL-6</td>
<td>Kaempferol, Biochanin-A, Genistein, EGCG</td>
</tr>
<tr>
<td></td>
<td>Quercetin, EGCG, Ellagic acid</td>
</tr>
<tr>
<td>Reduced MCP-1</td>
<td>Genistein</td>
</tr>
<tr>
<td>Reduced NFkB activation</td>
<td>Genistein, Quercetin, EGCG, Apigenin, Chrysin, Luteolin, Ellagic acid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Polyphenol dose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-stim.</td>
<td>30 μM</td>
<td>Parada et al.</td>
</tr>
<tr>
<td>TNF-α</td>
<td>40–44 μM</td>
<td>Ruiz et al.</td>
</tr>
<tr>
<td>TNF-α</td>
<td>50–100 μM</td>
<td>Kim et al.</td>
</tr>
<tr>
<td>non-stim. or IL-1β</td>
<td>0.1–10 μM</td>
<td>O’Leary et al.</td>
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<tr>
<td>IL-1β, TNF-α, or LPS</td>
<td>100 μM</td>
<td>Romieu et al.</td>
</tr>
<tr>
<td>LPS</td>
<td>10–50 μM</td>
<td>Kim et al.</td>
</tr>
<tr>
<td>non-stim.</td>
<td>30 μM</td>
<td>Parada et al.</td>
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<td>IL-1β, TNF-α, or LPS</td>
<td>100 μM</td>
<td>Romieu et al.</td>
</tr>
<tr>
<td>IL-1β + TNF-α + INF-γ</td>
<td>4 μM</td>
<td>Potokar et al.</td>
</tr>
<tr>
<td>non-stim.</td>
<td>100 μg/mL</td>
<td>Liu et al.</td>
</tr>
<tr>
<td>IL-1β, TNF-α, or LPS</td>
<td>50 μM</td>
<td>Romieu et al.</td>
</tr>
<tr>
<td>IL-1β, TNF-α, or LPS</td>
<td>25–50 μM</td>
<td>Parada et al.</td>
</tr>
</tbody>
</table>
Lower colitis severity with these Flavonoids

Flavonols: Balchalein, Quercetin, Quercitrin, Rutin, Sylimarin

Flavones: Diosmin, Dosmalfate, Morin

Isoflavones: Genistein, Diadzin, Galbridin, Coumarin

Flavanone: Hesperidin, Wogonin

Flavanols: Catechin, EGCG, Theaflavin

Proanthocyanidins: Pine bark extract

Stilbenes: Resveratrol, Piceatannol

Phenolic acids: Curcumin, 4-Coumaric acid, Ellagic acid, Paepalantine, Paenol
Modulating Gut Barrier Function

Tight Junctions Function:
• Building physical barrier function that keeps pathogens, micro-debris and toxins out, and lets solutes, nutrients and water in

Things that impair Tight Junction Function:
• Inflammatory cytokines
• Reactive oxygen species
• Pathogenic bacteria

Tight Junctions Defects associated with:
• IBD, Food allergies, Alcoholic liver disease, TBI, …
**Normal barrier**
- Intestinal lumen
- Epithelial cells
- ZO-1
- Claudin
- F-actin
- Occludin

**Impaired barrier**
- Bacterial products and Dietary antigens
- Inflammatory cytokines
  - Reactive oxygens
  - Pathogenic bacteria etc.

Lamina propria
Tight Junctions

• Main determinant of the intestinal barrier
• Regulate paracellular movement of ions, solutes, and water through the intestinal epithelium
• Multi-protein complexes of 4 trans-membrane proteins: Occludin, Claudin, Junctional adhesion molecule, Tricellulin
• Transmembrane proteins interact with intracellular plaque proteins zonula, occludens (ZO) and cingluin
• These anchor the TJ proteins to the actin cytoskeleton
• The interaction of TJ’s with the cytoskeleton determines permeability
Protective Effect of Quercetin on Intestinal Tight Junction Barrier Function in Caco-2 Cell Model

Fig. 3. Promotive effect of quercetin on intestinal TJ barrier function. Human intestinal Caco-2 cells were incubated with or without 10–100 μM quercetin for 48 h in a Transwell culture system. Lucifer yellow flux (A) across the cell monolayers was evaluated for the last 3 h of incubation, and TER (B) was measured before and at 0.5, 1, 3, 6, 12, 24 and 48 h. Values are means±S.E.M., n=4. Values not sharing a common letter differ significantly, P<.05 (A). Asterisks indicate a significant difference from the values before quercetin administration, P<.05 (B).
Protective Effect of Genistein on Tight Barrier Function Under Oxidative Stress in Cellular Model

Table 1  Effect of genistein on $XO + X$-induced paracellular permeability

The basal TER of monolayers varied from 400 to 500 $\Omega \cdot cm^2$. Values are means ± S.E.M. ($n = 4$). Results that are significantly ($P < 0.05$) different from values for control (*) or 3 h $XO + X$ monolayers (†) are indicated.

<table>
<thead>
<tr>
<th>Property</th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>3 h + genistein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal TER (%)</td>
<td>103 ± 5</td>
<td>72 ± 4*</td>
<td>30 ± 6*</td>
<td>89 ± 7†</td>
</tr>
<tr>
<td>Mannitol flux (%/h/cm²)</td>
<td>0.21 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td>0.85 ± 0.06*</td>
<td>0.23 ± 0.02†</td>
</tr>
</tbody>
</table>

$XO + X$ xanthine oxidase + xanthine (used to induce oxidative stress)
TER transepithelial electrical resistance
Fig. 1. (−)-Epigallocatechin gallate (EGCG) inhibits IFN-γ increased epithelial permeability. Filter-grown T84 cells were treated with IFN-γ (20 ng/ml) ± EGCG (100 μM) for 48 h. Monolayer permeability was assessed by transepithelial resistance (TER) (n = 6–9) (A) and horseradish peroxidase (HRP) flux (n = 3) (B). *P ≤ 0.05 compared with control monolayers; starting TER range: 2,780–3,880 Ω·cm²

Conclusion  Genistein, Quercetin, EGCG have shown the potential to improve intestinal barrier function. Several other flavonoids have been shown to do the same!
Modulating Gut Barrier Function

Dietary Grape-Seed Procyanidins Decreased Postweaning Diarrhea by Modulating Intestinal Permeability and Suppressing Oxidative Stress in Rats

Peixia Song,†,** Ruoji Zhang,‡,** Xiaoxiao Wang,† Pingli He,† Lulin Tan,† and Xi Ma*‡

Experiment:
Weaned rats fed:
1. basal diet
2. basal diet supplemented with 250 mg/kg procyanidins (GSP)
3. basal diet supplemented with 2000 mg/kg ZnO.

Measured:
1. Intestinal permeability
2. Expression of mucosal tight junctions
3. Antioxidant status of small intestine tissues
Conclusion: Procyanidins reduced intestinal permeability
Effect of Procyanidins on Expression of TJ Proteins without and with Oxidative Stress

Conclusion
Procyanidins increase expression of tight junction proteins, and rescue reductions in tight junction proteins caused by oxidative stress.
Summary & Conclusion

Flavonoids / polyphenols have beneficial effects on and in our gut

a) by improving the growth of beneficial gut bacteria and inhibiting the growth of pathogenic strains

b) by reducing inflammation

c) by improving intestinal barrier function

Eat the Rainbow

Thank You!