The role of microbiota metabolism in the bioavailability and efficacy of polyphenols

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• 3 Interindividual variability in the production of polyphenols GM metabolites
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• 6 Research needs and future prospects
Monomeric and oligomeric phenolics present in many healthy food products
1) Background: Polyphenols and Health

Problems to prove their efficacy on human health

**Large inter-individual variability** (intervention studies lack significance)

Bioavailability of polyphenols is very low

Polyphenol concentration in the gut can be high

Interactions with gut microbiota lead to relevant effects?

Gut microbiota metabolites are more bioavailable than polyphenol precursors?

Are colon and systemic effects related to metabolites?
Gut microbes are surrounded by a large number of polyphenols and often at high concentrations (> 100 µM)
Interactions with gut microbiota
Interactions with gut microbiota
‘Prebiotic-like’ effects of polyphenols

Positive modulation of probiotics: *Lactobacillus* & *Bifidobacterium*

Negative modulation of other gut bacteria considered not so healthy

The three ‘Ps’ for gut health (Marchesi et al., 2015, Gut)

**Probiotics**

**Prebiotics**

**Polyphenols**

New definition of ‘Prebiotics’ needed (Cani & Everard, 2016, MNFR)

Many studies show prebiotic-like effects of polyphenols (tannins)
Mainly *in vitro* or in animal models
Mainly Polyphenol-rich extracts. **Not only polyphenols?**
Polyphenols / gut microbiota interaction
‘Prebiotic-like’ effects

Proanthocyanidins and flavonols. Green tea extract

Promotes the growth of *Bifidobacterium* in humans

Proanthocyanidins. Water-insoluble polyphenols Cocoa

Promotes the growth of *Lactobacillus* and *Bifidobacterium* *in vitro* human fecal microbiota fermentation
Fogliano et al., *MNFR*, 2011, 55, S44-S55.

Anthocyanins and proanthocyanidins. Water soluble blueberry extract

Increase growth *Lactobacillus* and *Bifidobacterium* species *in vitro* study with human fecal microbiota

Proanthocyanidins and anthocyanins. Grape phenolics extract

Promotes growth *Lactobacillus acidophilus*
Polyphenols / gut microbiota interaction

‘Prebiotic-like’ effects

Hydrolyzable tannins. Ellagitannins. Pomegranate extract

Promotes the growth of *Lactobacillus* and *Bifidobacterium*

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Li et al., 2015, *Food & Funct.* 6, 2487. *Humans* (pomegranate peel extract). Decrease *Clostridium*, *Bacteroides* and Enterobacteriaceae. No effect.

Mosele et al., *MNFR*, 2015. *Humans*, No effect (juice from arils)

Bacchus project. ClinicalTrials.gov Identifier: NCT02061098

Overweight *humans*. Pomegranate extract. 40 volunteers. No effect.
Polyphenols health effects through gut microbiota modulation

- **Akkermansia muciniphila** mucin-degrading, anti-inflammation and anti-obesity (Derrien et al., 2016).
  
  Increased by cranberry, grape and pomegranate polyphenol-rich extracts.
  
  Decreased (30 fold) by lignan-rich flaxseed.

- **Faecalibacterium prausnitzii**, butyrate-producing, depleted in IBD
  
  - Increased by wine polyphenols
  
  - Increased by cocoa extract rich in polyphenols

- **Firmicutes/Bacteroidetes** increased in T2 diabetes and obesity
  
  - Decreased by grape, pomegranate and cocoa extract rich in polyphenols and by quercetin

Interaction with gut microbiota
Polyphenols catabolism by human gut microbes

De-Conjugation

Flavonoid ring fission
EA ring fission (lactonase)
Glycoside de-conjugation
De-acylation
De-hydroxylation
De-carboxylation
De-methylation
Methylation

Selma et al., J. Agric. Food Chem., 2009, 57, 6485

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Gut bacteria transforming phenolics

- **Beer (hops) flavanones (8-prenyl naringenin)**
  - *Eubacterium limosum.*

- **Soy isoflavones (equol)**
  - *Slakia isoflavoneconvertens*
  - *Adlerkreutzia equolifaciens*

- **Lignans (enterolactone)**
  - *Eggerthella lenta*

- **Resveratrol (dihydroresveratrol)**
  - *Slakia isoflavoneconvertens*
  - *Adlerkreutzia equolifaciens*

- **Ellagitannins (urolithins)**
  - *Gordonibacter urolithinfaciens*
2) Are microbiota metabolites are more bioavailable than native dietary polyphenols?

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Ellagic acid</td>
<td>0.07 μM</td>
<td>Urolithins</td>
<td>20 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gonzalez-Sarrias et al 2015</td>
<td>Cerda et al., 2004</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>-</td>
<td>Hesperetin</td>
<td>1.5 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vallejo et al., 2010</td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>0.5 μM</td>
<td>Equol</td>
<td>0.04-15 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tamura et al 2009</td>
<td>Atkinson et al., 2016</td>
</tr>
<tr>
<td>Lignans</td>
<td>-</td>
<td>Enterolactone</td>
<td>0.004-0.04 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milder et al., 2007</td>
<td></td>
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</tbody>
</table>
Plasma concentrations of daizdein and equol after soya intake

Fig. 1. Plasma isoflavonoids (aglycones+metabolites) of mice in the DA group and the CDA group. Values are means ± SE (n = 7). *Significantly different (P < .05) from the DA group. The data were analyzed using t-test analysis.

Tamura et al., 2009, Nut. Res.

LeeCole et al. 2014, JAFC
3) Inter-individual variability
COST ACTION FA 1403 - POSITIVe

Inter-individual variation in response to consumption of plant food bioactives and determinants involved

http://www6.inra.fr/cost-positive
POSITIVe Web Page

http://www.cebas.csic.es/
POSITIVe Newsletter
Human gut microbiome and enterotypes

- Fecal metagenomes
- Individuals from 4 countries
- 3 robust clusters identified (not nation or continent specific)
  - Bacteroides, Prevotella and Ruminococcus.

ENTEROTYPES

- They could explain large variability in intervention studies
- They could explain responders/non-responders


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Polyphenols and enterotypes

- Equol production
- Urolithin production
- Rhamnosidases
- 8-Prenyl-naringenin production
- Dihydroresveratrol production
- Valerolactone production
- Enterolactone production

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Polyphenols and enterotypes

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### Inter-individual variability (gut microbiota)

<table>
<thead>
<tr>
<th>Food polyphenol</th>
<th>Microbial metabolite</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Flavanone rutinosides</td>
<td>Hesperetin</td>
<td>Vallejo et al., 2010, JAFC</td>
</tr>
<tr>
<td>Lignans</td>
<td>EL, ED</td>
<td>Clavel et al., 2005, Appl. Env. Micr.</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Equol</td>
<td>Rowland et al., 2000, Nutr. Cancer</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>Phenyl acetic</td>
<td>Gross et al., 2010, JAFC</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>Urolithins</td>
<td>Tomas-Barberan et al., 2014, JAFC</td>
</tr>
</tbody>
</table>

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Flavonoid deconjugation

Intestinal lumen

Rhamnosidases

colon microbiota

Bacteroides
Lactobacillus
Bifidobacterium

Pereira-Caro et al., 2014, AJCN

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Citrus flavanone urinary excretion levels

Large interindividual variability
Interindividual variability/ Flavanone Pharmacokinetics

Flavanone-enriched juice

Plasma Concentration (nM)

Time (h)

Vallejo et al., JAFC 2010, 58, 6516.
Equol producer phenotype

Equol non-producer phenotype
Equol production inter-individual variability

- Equol producer (30-50%)
- Non Equol-producer (50%-70%)
- ODMA(80-90 %)
- Non ODMA-Producer (10-20%)

Rowland et al., 2000 Nut. Cancer
Equol producer phenotype

Equol non-producer phenotype

Phenotype A

Phenotype B

Phenotype 0
Phenotypes of ellagitannin microbial metabolism

Phenotype 0

Phenotype B

Phenotype A

Cerdá et al., 2005, JAFC, 53, 227
González-Barrio et al., 2011, JAFC, 59, 1152
Inter-individual variability

No Gut dysbiosis N=137

Gut dysbiosis N=67
Colorectal cancer, Metabolic syndrome

Phenotypes

Tomas-Barberan et al., JAFC 2014, 62, 6535
4) Health effects of metabolites

Mainly *in vitro* assays (human cell lines)
- Anti-inflammatory in vascular cells
- Anti-inflammatory in intestinal cells
- Anti-thrombotic activity
- Anti-proliferative activity

Some *in vivo* studies with animal models
- Colon inflammation model

**A few human studies (pharmaceutical approach)**

**Mechanisms**
- Anti-inflammatory
- Interaction with oxidative stress
- Interaction with estrogen receptors
4) Health effects of metabolites
Urolithin A induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents

Dongryeol Ryu¹,⁵, Laurent Mouchiroud¹,⁵, Pénélope A Andreux¹,²,⁵, Elena Katsyuba¹, Norman Moullan¹, Amandine A Nicolet-dit-Félix¹, Evan G Williams¹, Pooja Jha¹, Giuseppe Lo Sasso¹, Damien Hizard³, Patrick Aebischer⁴, Carmen Sandi³, Chris Rinsch² & Johan Auwerx¹

The biological effects of urolithins remain poorly characterized, despite wide-spread human exposure via the dietary consumption of their metabolic precursors, the ellagitannins, which are found in the pomegranate fruit, as well as in nuts and berries. We identified urolithin A (UA) as a first-in-class natural compound that induces mitophagy both *in vitro* and *in vivo* following oral consumption. In *C. elegans*, UA prevented the accumulation of dysfunctional mitochondria with age and extended lifespan. Likewise, UA prolonged normal activity during aging in *C. elegans*, including mobility and pharyngeal pumping, while maintaining mitochondrial respiratory capacity. These effects translated to rodents, where UA improved exercise capacity in two different mouse models of age-related decline of muscle function, as well as in young rats. Our findings highlight the health benefits of urolithin A and its potential application in strategies to improve mitochondrial and muscle function.
Stratification can help understanding health effects

Non significant for the whole population

Significant for specific groups
• Equol producer (30-50%)
• Non Equol-producer (50%-70%)

• Whole population non-significant results

• Equol producers show health effects

• Equol given to equol non-producers does not show health effects (Hazim et al., 2016 AJCN)
Selma et al., 2016, Food & Funct
The PomeCardio trial

**Design:** A double-blind, 2-arm, placebo-controlled randomised, **crossover**, dose-response trial. Six months follow-up with 4 intervention phases and 4 wash-out periods.

### Total cholesterol: Baseline levels

- **All**: 209±40 mg/dL (N=49)
- **Metabotype A**: 202±25 mg/dL (N=32)
- **Metabotype B**: 243±20 mg/dL (N=14)
- **Metabotype 0**: 170±30 mg/dL (N=3)

**Cardiovascular risk**

**Normal values**

Normal values for total cholesterol:
- **Metabotype A**: 202±25 mg/dL (N=32)
- **Metabotype B**: 243±20 mg/dL (N=14)
- **Metabotype 0**: 170±30 mg/dL (N=3)
Effect of pomegranate extract on total cholesterol

All individuals
Total effect: -12.9%

Baseline
6 weeks

Total Cholesterol (mg/dL)

Metabotype A
Total effect: -10.5%

Metabotype B
Total effect: -18%

Cardiovascular risk
Normal values
5) Similarities between different polyphenols

<table>
<thead>
<tr>
<th>Comparison</th>
<th>+</th>
<th>+</th>
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</thead>
<tbody>
<tr>
<td>Different metabotypes</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Different prevalence of metabotypes depending on health status</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Health effects non-significant in the whole population</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Health effects significant in specific metabotypes</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>?</td>
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<tr>
<td>Are the health effects due to the activity of the metabolites?</td>
<td>+?</td>
<td>+?</td>
<td>+?</td>
<td>+?</td>
<td>+?</td>
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<tr>
<td>Is a metabotype better prepared to respond to polyphenol intake?</td>
<td>+</td>
<td>+</td>
<td>?</td>
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6) Research needs and perspective

- Correlate Polyphenol metabolites stratification with gut microbiota composition: **metabotypes** with **enterotypes**
- Establish health effects of GM metabolites through intervention studies. (problems!!)
- Modulate GM to improve health through interaction with polyphenols: (Healthy GM composition; Ensure bioactive metabolites).
- Evaluate the role of GM in the interindividual variation after polyphenol intake.

- Understanding the health effects of polyphenols.
- Development of new functional foods, nutraceuticals and drugs: personalized nutrition.
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E-mail: fatomas@cebas.csic.es
2) Methods: Metabolome & Metagenome analysis
Fig. 1 – Frequency distribution of plasma equol concentration among control women (n = 1027).