Modulation of gut microbiota by plant polyphenols: A Paradigm Shift in Understanding their Effects on Diseases

Québec City, 22 October 2014

Yves Desjardins
@prof_yves

Institute of Nutrition and Functional Food
Laval University
Québec City, Québec, Canada
Why is bear poop blue???
Health effects have been attributed to phenolic compounds.
Blueberry Polyphenols

- Anthocyanins: 796 mg/100 g FW
- Procyanidins: 76 mg/100 g FW
- Chlorogenic acid: 905 mg/100 g FW
- Caffeic acid: 400 mg/100 g FW
- P-coumaric acid: 0 mg/100 g FW

Modulation of cellular signaling cascades (Cancer, CVD, Diabetes, NDD)

Stimulation of endogenous antioxidant network (SOD, Catalases)

Stilbenes
Antocyanins
Ellagic Acid
Ellagitannins
PAC
OPC

↓ CVD  
↓ LDL  
↑ Endothelial funct.

Reduction of inflammation in many tissues and organs

Interaction with cell cycle and induction of apoptosis

↓ cognitive decline

↓ Metabolic syndrome biomarkers
↓ Insulin resistance
↓ Glucose tolerance glucose
Bioavailability of polyphenols is very low

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conversion</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>100 mg quercetin</td>
<td></td>
<td>0.3 - 0.7 µmol/l</td>
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<tr>
<td>150 mg catechin</td>
<td></td>
<td>0.1 µmol/l</td>
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<tr>
<td>200 mg hesperin</td>
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<td>1.1 µmol/l</td>
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<tr>
<td>200 mg naringenin</td>
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<td>6 µmol/l</td>
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<tr>
<td>200 mg anthocyanin</td>
<td></td>
<td>~ 10 nmol/l</td>
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<tr>
<td>25 mg secoislariceresinol</td>
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<td>~ 30 nmol/l</td>
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</tbody>
</table>
Residence time in the body is relatively short
Polyphenols are recognized by the body as xenobiotics and are rapidly eliminated.
The Biological Relevance of Direct Antioxidant Effects of Polyphenols for Cardiovascular Health in Humans Is Not Established\textsuperscript{1–4}

Peter C. H. Hollman,\textsuperscript{5} Aedin Cassidy,\textsuperscript{6} Blandine Comte,\textsuperscript{3} Marina Heinonen,\textsuperscript{8} Myriam Richelle,\textsuperscript{9} Elke Richling,\textsuperscript{10} Mauro Serafini,\textsuperscript{11} Augustin Scalbert,\textsuperscript{7} Helmut Sies,\textsuperscript{12} and Stéphane Vidry\textsuperscript{13*}

First published online March 30, 2011; doi:10.3945/jn.110.131490.
Isolation of vitamin P 1936

1940

1944

1950

1984

1988

1995

1999

2007

2008

2013

Antioxidants

Direct antioxidant activity

In vitro

Protection against Capillary fragility

In vivo

Inhibition of endothelial NADPH oxidase

Preservation of Paraoxoanase activity

Modulation of lipd metabolism

Indirect antioxidants

Modulation of redox signals

Inhibition of Xanthine ox.

Decrease hypertension

Inhibition of hypertension

Inhibition of NADPH oxidase in neutrophils

Modulation of neutrophil function

Interference with Arachidonic acid metabolism

Modulation of platelet function

Modulation of GUT Microbiota

Inhibition of PPAR-g

Inhibition of adhesion molecules expression

Anti-inflammatory effects

Inhibition de Nf-κB

Inhibition of PARP

Modulation of platelet function

Epigenetic Modulators

Anti-atherosclerotic Compounds

Modulation of GUT Microbiota
Our genome: the microbiome

Metagenomic analysis of the colon microbiome
- 10X more bacterial cells than our body
- 100 more genes than our own genome.
- 1.5-2.0 kg microorganisms
- 100 trillion bacteria in our gut...

Microbial degradation of polyphenols

- Flavonols → Hydroxyphenyl acetic acid
- Flavanones → Hydroxyphenyl propionic acid
- Flavanols → Phenyl valerolactone
- Catechins → Hyppuric acid, Catecuic acid
- Lignans → Enterodiol
- Isoflavones → Equol
Procyanidins

Catechin

Gut microflora

3,4-Dihydroxyphenylvaleric acid

Gut microflora

Cyanidin

Protocatechuic acid

Gut microflora

Vanillyl acid

Liver

Peonidin

4-Hydroxybenzoic acid

Gut microflora

3-Hydroxybenzoic acid

Liver

3-Hydroxypropionic acid

Liver

3-Hydroxyphenylvaleric acid

Gut microflora

m-Coumaric acid

Liver

p-Coumaric acid ??

3,4-Dihydroxyphenylpropionic acid

Gut microflora

Gothier et al. 2003, Free Radical Biology & Medicine
Link between the microbiome and diabetes

Polyphenols

Prebiotics

Probiotics

↑ Bifidobacterium Lactobacillus

Gut microbiome

- Reduction in body weight
- Improvement in insulin resistance
- Increase tolerance to glucose
- Reduction of inflammatory biomarkers
Evolution of inflammation associated chronic diseases

Lean & Healthy

Obese & Diabetic

Sensitivity to insulin

Inflammation

Adiponectin

MCP-1

Leptin

ER Stress?

Necrosis

apoptosis?

Chemokines Cytokines Adipokines

APP'S

IL-6

TNFα

iNOS

Leptin

IL-1β

IFN-γ

NO

APP'S

Chemokines Cytokines Adipokines
Lipopolysaccharide is the most inflammatory molecule recognized by the body.
Polyphenols?

Gut microbiota and metabolic disorders: how prebiotic can work?

Nathalie M. Delzenne*, Audrey M. Neyrinck and Patrice D. Cani

*British Journal of Nutrition (2013), 109, S81–S85
Effect of polyphenols on gut microbes

Antimicrobial properties of phenolic compounds from berries

R. Puupponen-Pimiä¹, L. Nohynek¹, C. Meier¹, M. Kähkönen², M. Heinonen², A. Hopia² and K.-M. Oksman-Caldentey¹
¹VTT Biotechnology, and ²University of Helsinki, Department of Applied Chemistry and Microbiology, Food Chemistry Division, University of Helsinki, Finland

Table 6 Antimicrobial activity of selected pure phenolic compounds and berry extracts in liquid culture. (□) No inhibition: plate counts differ by < 5 × 10¹; (□) clear inhibition: plate counts differ by 5 × 10¹–5 × 10²; (□) strong inhibition: plate counts differ by 5 × 10²–5 × 10⁴; (□) very strong inhibition: plate counts differ by > 5 × 10⁴; (□) not tested

<table>
<thead>
<tr>
<th>Berry extracts 1 mg ml⁻¹</th>
<th>Lactobacillus rhamnosus E-800</th>
<th>Lact. rhamnosus E-666</th>
<th>Lact. reuteri E-849</th>
<th>Lact. paracasei E-510</th>
<th>Lact. johnsonii E-797</th>
<th>Lact. crispatus E-725</th>
<th>Lact. plantarum E-076</th>
<th>E. coli 50</th>
<th>E. coli CM871</th>
<th>Salmonella enterica SH-5014</th>
<th>Enterococcus faecalis E-203</th>
<th>Bifidobacterium lactis E-508</th>
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</table>
Effect of polyphenols on gut microbes

Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome

Rober A. Kemperman a,⁎,1, Gabriele Gross a,b,1, Stanislas Mondot c, Sam Possemiers b, Massimo Marzorati b, Tom Van de Wiele b, Joël Doré c, Elaine E. Vaughan a

a Unilever R&D, Olivier van Noortlaan 120, 3133 AT, Vlaardingen, The Netherlands
b Laboratory of Microbial Ecology and Technology (LabMET), Conagre Lines 653, Ghent University, B-9000 Gent, Belgium
c Institut National de la Recherche Agronomique, Unité Ecologie Physiologie Système Digestif, Batiment 440 – CNR – INRA, 78352 Jouy-en-Josas, Cedex, France

Food Research International, 2013. DOI: 10.1016/j.foodres.2013.01.034
The gut microbiota plays an essential role in the low-grade inflammation.

changes in gut microbiota

bacteroidetes

firmicutes

actinobacteria

proteobacteria

obesity

metabolic endotoxemia

inflammation

insulin sensitivity

increased gut permeability

zo-1

occludin

anandamide

cb1 r mrna

involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity
# Experimental design

**Mice C57Bl6** N=12 per group
8 weeks of chow diet or HFHS

<table>
<thead>
<tr>
<th>Chow</th>
<th>HFHS</th>
<th>HFHS</th>
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<tbody>
<tr>
<td>Gavage: <strong>vehicle</strong></td>
<td>Gavage: <strong>vehicle</strong></td>
<td>Gavage: <strong>300 mg Cranberry</strong></td>
</tr>
<tr>
<td>Drink: <strong>water</strong></td>
<td>Drink: <strong>water</strong></td>
<td>Drink: <strong>Water</strong></td>
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</table>

**Weight gain**
**Food intake**
**Gtt / Itt**

**Modulation of microbiota**
**Inflammation**

**Bioavailability**
ORIGINAL ARTICLE

A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of mice

Fernando F Anhê,1,2 Denis Roy,2 Geneviève Pilon,1,2 Stéphanie Dudonné,2 Sébastien Matamoros,2 Thibault V Varin,2 Carole Garofalo,3 Quentin Moine,3 Yves Desjardins,2 Emile Levy,3,4 André Marette1,2
Effect of cranberry extract on Weight Gain

![Graph showing the effect of cranberry extract on weight gain over time. The graph illustrates the body weight gain in grams (g) over days for different groups: Chow, HFHS, and Cranberry. The left graph shows the weight gain over time, while the right graph shows the total weight gain.](image-url)
Effect of cranberry extract on Total Energy Intake

Energy Intake

- Chow
- HFHS
- Cran

Total Energy Intake

Kcal
Effect of cranberry extract on visceral adiposity

**Visceral Adiposity**

- Chow
- HFHS
- Cran

**Subcutaneous Adiposity**

- Chow
- HFHS
- Cran

**Energy Efficiency**

- Chow
- HFHS
- Cran
Effect of cranberry extract on glucose and insulin tolerance

**ipITT**

![Graph showing glycemia over time for ipITT]

**OGTT**

![Graph showing glycemia over time for OGTT]

**AUC ipITT**

![Bar graph showing AUC for ipITT]

**AUC OGTT**

![Bar graph showing AUC for OGTT]
Effect of cranberry extract on glucose and insulin tolerance during OGTT

![Graph showing plasma insulin levels and AUC for insulinemia during OGTT, comparing Chow, HFHS, and Cran diets.](image)

![Bar graph showing HOMA-IR index values for Chow, HFHS, and Cran diets.](image)
Effect of cranberry extract on liver weight, triglycerides, lipid peroxidation, plasma triglycerides and cholesterol
Effect of a cranberry extract on hepatic steatosis

Chow  

HFHS  

HFHS + Cranberry
Effect of cranberry extract on Intestinal inflammatory reaction

- **G**: COX2 and β-actin expression levels compared to Chow.
- **H**: TNF-α and β-actin expression levels compared to Chow.
- **I**: NF-kB/I-κB ratio compared to Chow.

Graphs show: Chow, HFHS, and CE groups with significant differences indicated by ***, **, ###, and #.
454 Pyrosequencing of the gut metagenome

- Analysis of the V6-V8 region of the 16S rRNA bacterial genes by high-throughput pyrosequencing
- (~ 100,000 reads on 1/8 plate)
- 20 samples sequenced
- (Mice DNA was pooled for each diet and each week)
- A bar-code per sample allowed to assign the obtained sequences
- 2566 sequences were obtained per sample (after clean-up)
Evolution of the gut microbiota under a HFHS diet or a diet supplemented with cranberry extract.
Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity

Amandine Everarda, Clara Belzerb, Lucie Geurta, Janneke P. Ouwercerkb, Céline Druartc, Laure B. Bindelsa, Yves Guiotc, Muriel Derrienb, Giulio G. Mucciolid, Nathalie M. Delzennea, Willem M. de Vosb,e, and Patrice D. Cania,b,1

Metabolism and Nutrition Research Group, Walloon Excellence in Life sciences and BIOtechnology (WELBIO), Louvain Drug Research Institute, Université catholique de Louvain, B-1200 Brussels, Belgium; Laboratory of Microbiology, Wageningen University, 6703 HB, Wageningen, The Netherlands. Department of Pathology, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, B-1200 Brussels, Belgium; Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, B-1200 Brussels, Belgium; and Departments of Bacteriology and Immunology and Veterinary Biosciences, University of Helsinki, 00014 Helsingin yliopisto, Helsinki, Finland

Edited* by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 28, 2013 (received for review November 8, 2012)

NATURE | NEWS

Gut microbe may fight obesity and diabetes

Bacterium helps to regulate metabolism in mice.

Brian Owens

13 May 2013

The Buzz About Akkermansia muciniphila: It’s More Than Just Weight Loss
May 17, 2013 by Terri Sundquist

The bacterium Akkermansia muciniphila is creating quite a stir in science news, with people calling it the “weight loss bacterium”. While it’s exciting to think about a bacterium that has the ability to reduce body weight with no change in food intake, there’s another reason to get excited: The potential to treat obesity-related metabolic disorders such as type-2
WINOGRADSKY REVIEW

Microbes inside—from diversity to function: the case of Akkermansia

Clara Belzer\textsuperscript{1} and Willem M de Vos\textsuperscript{1,2,3}
\textsuperscript{1}Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands; \textsuperscript{2}Department of Veterinary Biosciences, Helsinki University, Helsinki, Finland; \textsuperscript{3}Department of Bacteriology and Immunology, Helsinki University, Helsinki, Finland

- True symbiont of humans
  - Represent 1-4% of intestinal bacterial population
  - Mucus degrading bacteria
  - Produces SCFA – immunological signals
  - Linked to obesity and low-grade inflammation
Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity

Amandine Everard, Clara Belzer, Lucie Geurts, Janneke P. Ouwerkerk, Céline Druart, Laure B. Bindels, Yves Guiot, Muriel Derrien, Giulio G. Mucchioli, Nathalie M. Delzenne, Willem M. de Vos, and Patrice C. Cani

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Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 28, 2013 (received for review November 8, 2012)
Effect of cranberry extract on metabolic endotoxemia induced by the HFHS diet

N= 6 for the Chow, HFHS et HFHS + Cranberry
*p < 0.05 vs. Chow; **p < 0.05 vs. HFHS
Figure S3

JEJUNUM

- **Muc2**
- **Klf4**
- **Reg3g**

COLON

- **Muc2**
- **Klf4**
- **Reg3g**
Mucus Production?

Akkermansia ??

T-Lymphoid Cells

PAC

Intestinal lumen

Gut microbes

Bile acids

Endotoxemia

Short-chain fatty acids

Chylomicron

Mucus Production?

LPS

Endocannabinoid system

SAA3

GPR43

PPARγ differentiation

LPL

Adipocytes

Effects linked to colonization

Effects linked to high-fat-diet/obesity

Effects counteracted by prebiotics

Why is bear poop blue??
Polyphenols

High nM/low µM

Chemical cues about the quality of the diet

(5%) Resveratrol/Quercetin/Ferulic acid

Phase I & II Metabolites

Microbial Metabolites

Esterol/Valerolactones/Coumaric acid
Urolithins/HBA

SCFA

Prebiotic effects

Gut Microbiota

Modified ecology
Defensins
Nutrient processing
Mucus production

Gut Microbiota effects

DC → T_{cell} → Cytokines

Akkermansia

↑ Nutrient processing
↑ Mucus production

Low-grade Inflammation

Low-grade Inflammation

Gut-Brain axis

Gut-Liver axis
## Acknowledgements

<table>
<thead>
<tr>
<th>Y. Desjardins</th>
<th>A. Marette</th>
<th>D. Roy</th>
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</table>
| Stéphanie Dudonné  
Pascale Dubé  
Véronique Richard | Geneviève Pilon  
Philippe St-Pierre  
Fernando Arhe  
Bruno Marcotte | Sébastien Matamoros  
Thibault Varin |

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<th>E. Levy</th>
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| M.-C. Denis  
Carole Garofalo  
Quentin Lemoyme |