Understanding specificities of fibre-microbiome interactions: how can this help?

MyNewGut final conference

Brussels, 18th October, 2018

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Université catholique de Louvain

www.mynewgut.eu
Objectives and key discoveries in preclinical studies

- Wheat bran as source of nutrients interacting with the gut microbiota to improve obesity
- "Common" enzymatic activities in host and gut microbiota as interesting targets (drug development and innovation in nutrition)
- New metabolites produced by the gut microbiota to be considered as anti-inflammatory
Dietary fibre in Europe: debates on properties and on definition

Consensus from about 2008: *Codex Alimentarius Alinorm*

Dietary fibre (DF) is made up of carbohydrate polymers with three or more monomeric units (MU), which are neither digested nor absorbed in the human intestine and includes:

1. **non-starch-polysaccharides (NSP)** from fruits, vegetables, cereals and tubers whether intrinsic or extracted, chemically, physically and/or enzymatically modified or synthetic (MU≥10)
2. **resistant (non-digestible) oligosaccharides (RO)** (MU 3–9)
3. **resistant starch (RS)** (MU≥10)

**For EFSA**: DF = NSP + RS + RO + lignin (when associated to DF polysaccharides)

Scientific evidence of benefits for health must be demonstrated!

- **Colonic function**: ↑stool production, stimulation of colonic fermentation, improved laxation, ↓intestinal transit time, ↑stool bulk, …
- **Blood cholesterol**: ↓fasting cholesterol/olaemia, ↓ blood cholesterol, ↓blood total and/or LDL-cholesterol levels, …
- **Blood glucose**: ↓post-prandial blood glucose/glycaemia and/or insulin, modulation of blood glucose, …

Others?
The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement (2017)

Prebiotic = substrate that is selectively utilized by host microorganisms conferring a health benefit

**Figure 1** Distinguishing what is considered a prebiotic with the proposed definition. Prebiotics must be selectively utilized and have adequate evidence of health benefit for the target host. Dietary prebiotics must not be degraded by the target host enzymes. *The figure shows candidate as well as accepted prebiotics in that levels of evidence currently vary, with FOS and GOS being the most researched prebiotics. CLA, conjugated linoleic acid; PUFA, polyunsaturated fatty acid; FOS, fructooligosaccharides; GOS, galactooligosaccharides; MOS, mannooligosaccharide; XOS, xyloooligosaccharide.*

Gibson et al. Nat Rev Gastroenterol Hepatol. 2017
Classification of fibre according to chemical components & properties:

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>class</th>
<th>solubility</th>
<th>viscosity</th>
<th>fermentability</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSP (MU≥10)</td>
<td>cellulose hemicellulose (heteroxylan, AX)</td>
<td>++ or -</td>
<td>++ or -</td>
<td>++</td>
</tr>
<tr>
<td>Mannans heteromannans</td>
<td>+++ or -</td>
<td>+++ or -</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>pectin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Other hydrocolloids (gums, mucilages)</td>
<td>+++ or ++</td>
<td>+++ or +</td>
<td>+++ or +</td>
<td></td>
</tr>
<tr>
<td>Inulin and fructan</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>RO (MU≤10)</td>
<td>Arabinobioxylo-oligosaccharides (wheat bran extract)</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>β-Fructo-oligosaccharides (FOS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α-galactooligosaccharides (GOS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant dextrins</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>polydextrose</td>
<td>+++</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS (MU≥10)</td>
<td>Type I, II, III, IV</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Associated substances (non carbohydrates)</td>
<td>Lignin Waxes Chitins</td>
<td>-</td>
<td>++ or -</td>
<td>-</td>
</tr>
</tbody>
</table>

Adapted from Stephen et al 2017
Newly tested prebiotic wheat bran extract (WBE)

27 mice (9 weeks old male C57Bl6J, Janvier Labs) divided into 3 groups, 3 mice/cage and will be fed with different diets:
- CT: D12450K diet (10% fat, 70% carbohydrates)
- WD: western diet (45% fat, 35% carbohydrates)
- WD+WBE: WD+5% Wheat bran extract (Cargill)

Fasting at 7h, sacrifice at 13h

Wheat bran extract (WBE) from

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit</th>
<th>Specification</th>
<th>Analysis Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinoyxlan Oligosaccharides (AXOS)</td>
<td>%dm</td>
<td>min. 70</td>
<td>72</td>
</tr>
<tr>
<td>Arabinose to Xylose Ratio of AXOS</td>
<td></td>
<td>0.17-0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>Average Degree of Polymerisation of AXOS</td>
<td></td>
<td>4-8</td>
<td>5</td>
</tr>
</tbody>
</table>
WBE \rightarrow body weight gain and fat mass expansion induced by the WD

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Suriano et al. Fat binding capacity and modulation of the gut microbiota both determine the effect of wheat bran fractions on adiposity. Sci, Rep 2017 Jul 17;7(1):5621
Fermentable carbohydrates with prebiotic properties: expected systemic effects in obesity

Complex carbohydrates

*Saccharolytic bacteria*

- Acetate
- Propionate
- Butyrate

+ *Akkermansia*

- Butyrate

Goblet cell
- Mucus production

L cell
- GLP-2
- PYY
- Appetite
- Insulin secretion/response

GLP-1

Gut barrier
- Endotoxaemia

Intestinal transit

Glucose homeostasis

Indicates Gut microbial activity
- GNG: gluconeogenesis

Saccharolytic bacteria

- GPR43/41
- TGR5

Bile acids

- Propionate
- succinate

Portal glucose

Portal propionate

Systemic glucose?

Substrate for GNG

Inhibitor of GNG from lactate

Portal glucose

Delzenne et al modified from Diabetologia 2015

www.mynewgut.eu
WBE deeply influenced the gut microbiota composition without changing intestinal SCFA level.

SCFA in caecal content (collaboration Kristin Verbeke KULeuven)

Effects independent on the increase in caecal SCFA concentration

An effect on endocrine function?
WBE modulates DPPIV activity

GLP-1(7-36) → GLP-1(9-36)-amide

act on GLP-1 R to modulate food intake (brain, portal neurones), glycemia (incretin effect on pancreatic beta-cells) and indirect effect on insulin response.

functional activity in the vasculature (improvement of vascular function), on the liver (Life Sci. 2014 May 2;102(2):134-8)
The Potential Role of the Dipeptidyl Peptidase-4-Like Activity From the Gut Microbiota on the Host Health

Marta Olivares¹, Valentina Schüppel²,³, Ahmed M. Hassan⁴, Martin Beaumont¹, Audrey M. Neyrinck¹, Laure B. Bindels¹, Alfonso Benitez-Páez⁵, Yolanda Sanz⁵, Dirk Haller²,³, Peter Holzer⁴ and Nathalie M. Delzenne¹*
The DPP-4 inhibitor vildagliptin impacts the gut microbiota and prevents disruption of intestinal homeostasis induced by a Western diet in mice

Marta Olivares¹ - Audrey M. Neyrinck¹ - Sarah A. Pötgens¹ - Martin Beaumont¹ - Nuria Salazar² - Patrice D. Cani¹,³ - Laure B. Bindels¹ - Nathalie M. Delzenne¹

3 groups of C57BL/6J mice, 9 weeks old (n=9)
Vildagliptin inhibits the DPP-4 activity in the caecal content and faeces.

As expected, vildagliptin reduces the DPP-4 activity and increases active GLP-1.

Vildagliptin also reduces the DPP-4 activity in the microbial ecosystem.

Olivares et al. The DPP-4 inhibitor vildagliptin impacts the gut microbiota and prevents disruption of intestinal homeostasis induced by a Western diet in mice. Diabetologia, 2018; 61(8): 1838–1848.
Vildagliptin improves the gastrointestinal function

**Expression of antimicrobial peptides in the ileum**

Vildagliptin restores the expression of antimicrobial peptides and the crypt depth

Olivares et al. The DPP-4 inhibitor vildagliptin impacts the gut microbiota and prevents disruption of intestinal homeostasis induced by a Western diet in mice. Diabetologia, 2018; 61(8): 1838–1848.
Vildagliptin influences the gut microbiota composition

**Gut microbiota composition**

(Pyrosequence the 16S rRNA gene, Illumina tech.)

Vildagliptin \(\downarrow\) the abundance of *Oscillibacter* spp. *in vivo* and inhibits its growth *in vitro*

Olivares et al. The DPP-4 inhibitor vildagliptin impacts the gut microbiota and prevents disruption of intestinal homeostasis induced by a Western diet in mice. Diabetologia, 2018; 61(8): 1838–1848.
Vildagliptin modulates bacterial components or metabolites related to metabolism and inflammation.

Proinflammatory Bacterial components

Fermentation and SCFA
DPPIV is also known as proteolytic against gluten
An effect on obesity?

Experimental design

C57BL/6J mice, 9 weeks old (n=9)

<table>
<thead>
<tr>
<th>Control</th>
<th>Western diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD + 5% gluten</td>
<td>WD + 5% gluten + AXOS</td>
</tr>
<tr>
<td>WD + 5% gluten + FOS</td>
<td></td>
</tr>
</tbody>
</table>

8 weeks

Olivares et al. *Arabinofuranosyl-oligosaccharides prevent obesity induced in mice by the addition of gluten in the Western diet* (to be submitted in 2018)
Gluten worsens the metabolic impairment induced by the Western diet while WBE prevents it

Body weight

Fat mass

Insulin

Adipocyte size

0 20 40 60 days

20 30 40 g

0 5 10 15 20 g

0 500 1000 1500 2000 Insulin (µg/l)

0 1000 2000 3000 4000 Adipocytes size(um²)

Control
Western diet (WD)
WD+gluten
WD+gluten+WBE
WD+gluten+FOS

Olivares et al. *Arabinoxylan-oligosaccharides prevent obesity induced in mice by the addition of gluten in the Western diet* (to be submitted in 2018)
Prebiotics reduce gluten immunogenic peptides in the cecal content: a role for the gut microbiota?

The reduction of toxic gluten peptides is negatively associated with *Bifidobacterium* and *Prevotella*

Olivares et al. *Arabinoyxylan-oligosaccharides prevent obesity induced in mice by the addition of gluten in the Western diet* (to be submitted in 2018)
Objectives and key discoveries in preclinical studies

- Wheat bran as source of nutrients interacting with the gut microbiota to improve obesity. WBE as prebiotic fiber: decreases gluten and lessens adiposity.
- "Common" enzymatic activities in host and gut microbiota as interesting targets for DPPIV in drug development and nutrition.
- New metabolites produced by the gut microbiota to be considered as anti-inflammatory.

Diet → Gut microbiota → specific bacteria metabolites → Preclinical models
Innovation in the context of MyNewGut
New anti-inflammatory bacterial metabolites gut-liver axis

What are the effects of amino-acid cometabolites on liver inflammation?

Beaumont et al
FASEB J 2018
Advantages:
✓ tissue preservation (hepatocytes-Kupffer cells)
✓ reproducibility
✓ economic
✓ histological & histochemical studies
✓ no hydrolytic enzymes (collagenase)
✓ possible to investigate human tissue

Precision cut-liver slices (PCLS): an original *in vitro* model which maintains hepatic architecture.

**Gut-liver axis:**
Interest of Precision-Cut Liver Slices
Indole reduces the LPS-induced overexpression of pro-inflammatory genes in the liver *in vivo*

**qPCR (Liver)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>LPS</th>
<th>Indole + LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Il1B</strong></td>
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<td><strong>Nos2</strong></td>
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<tr>
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</tr>
</tbody>
</table>

**NFκB pathway**

(Gerondakis, Nat Immunol, 2014)
**Gut microbiota**
- Tyrosine
- Tryptophan
- Prebiotics (ITF)
- Phenylalanine

**Gut epithelium**
- p-cresol
- indole
- indole-3-acetic acid
- LPS
- BAs
- SCFAs
- phenylacetic acid
- benzoic acid

**Portal blood**
- GLP-1
- GLP-2

**Liver**
- Inflammation
- Insulin resistance
- Steatosis
- Indoxyl-3-sulfate
- phenylacetylglutamine
- hippuric acid

**Liver inflammation**
- BAs
- GLP-1
- GLP-2
- Insulin resistance
- Steatosis

**Phenylacetic acid**
- SCFAs
- benzoic acid

**Gut epithelium**
- Phenylalanine
- Tryptophan
- Prebiotics (ITF)

**Portal blood**
- GLP-1
- GLP-2

**Liver**
- Inflammation
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Acknowledgments

M. Beaumont
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