Modulation of gut microflora by prebiotics: a new approach in the treatment of metabolic syndrome.

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The Metabolic Syndrome

Complex Dyslipidemia

Disordered Fibrinolysis

Hypertension

Steatosis NASH

Systemic Inflammation

Type 2 diabetes or Glucose intolerance/impaired fasting glucose

Visceral Obesity

Atherosclerosis

Adapted from Pradhan et al. *JAMA* 286:327-334 (2001)

Obesity is linked to division-wide shifts in the gut microbiota:

+ Firmicutes and – Bacteroidetes

Ley et al. 2005 PNAS, Ley et al 2006 Nature
The fermentation of prebiotics (inulin-type fructans) in the caeco-colon participates to the control of food intake, obesity and associated disorders (insulin resistance, dyslipidemia, hepatic steatosis...)

**Rationale**
- Association between gut microflora, obesity and/or metabolic response to nutrients (J. Gordon’s lab studies...)
- **Specific** modulation of gut microflora by prebiotics - fructans
   
   - Prospective study focusing on allergy
   - Analysis of fecal bacteria FISH/Q-rtPCR at 6 and 12 mois
   - Anthropometry at 8yrs
   - The number of Bifidobacteria is higher in children with normal weight and decreased in overweight children (opposite results with *Staphylococcus aureus*).

**Prebiotics like inulin-type fructans as model substances...**

**FIGURE 1** Chemical structure of the various fructo oligosaccharides. G, glucose; F, fructose; n or m indicate the number of fructose moieties in the molecules.

- **Gas**: +++ *Bifidobacteria* (Actinobacteria)
- **Prebiotic effect**
- **Short Chain Fatty Acids**
  - Acetate
  - Propionate
  - Butyrate
  - Lactate

OFS = Short chain
Prebiotics create a « gut-liver axis » ....

- decreased lipogenic gene expression
- decreased hepatic lipogenesis
- decreased hepatic triglycerides
- decreased serum triglycerides (VLDL)

OFS 10% versus cellulose10% in standard diet for 4 weeks

Potential mediators: propionate, decrease in glycemia...
Others?

... *with consequences on fat mass development*

- 4 weeks of treatment
- Male wistar rats with OFS vs standard diet

*Cani et al. Br J. Nutr 2007*
GENERAL HYPOTHESIS

The fermentation of fructans in the caeco-colon participates to the control of food intake, obesity and associated disorders.

Questions:
1. Implication of gastro-intestinal peptides?
2. Implication of inflammation?
3. Relevance for human health?
Study design

- 3 weeks of treatment
- 24 Wistar rats/4 groups:
  - CT (powdered diet AO4)
  - OFS 10% (oligofructose) (Mean DP 4)
  - SYN 10% (synergy 1) (mix oligofructose /inulin)
  - INU 10% (inulin) (Mean DP 25)

Food removal in the morning; samples taken 8 hours later

DP= mean number of fructosyl moieties
Hepato-Portal system major site of GLP-1 action on food intake

Holst and Deacon *Diabetologia* (2005)
### Gastrointestinal peptides


<table>
<thead>
<tr>
<th></th>
<th>Portal vein</th>
<th></th>
<th>Cava vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1</td>
<td></td>
<td>PYY</td>
<td>Acylated Ghrelin</td>
</tr>
<tr>
<td>(pM)</td>
<td>(pM)</td>
<td>(pM)</td>
<td>(pM)</td>
</tr>
<tr>
<td>CT</td>
<td>7.8 ± 0.7 (^a)</td>
<td>9.9 ± 1.9 (^a)</td>
<td>131.7 ± 7.4 (^a)</td>
</tr>
<tr>
<td>OFS</td>
<td>11.4 ± 1.2 (^b)</td>
<td>19.7 ± 2.4 (^b)</td>
<td>91 ± 7.2 (^b)</td>
</tr>
<tr>
<td>Syn</td>
<td>10.5 ± 1.7 (^b)</td>
<td>12.9 ± 2.6 (^a)</td>
<td>87.2 ± 12.6 (^b)</td>
</tr>
<tr>
<td>Inu</td>
<td>8.4 ± 1.3 (^a)</td>
<td>11.2 ± 3.2 (^a)</td>
<td>102.6 ± 12 (^{ab})</td>
</tr>
</tbody>
</table>

Intestinal GLP-1 (7-36) amide and PYY (3-36) amide

Intestinal GLP-1 (7-36) amide (panel A) and PYY (panel B) concentration of rats fed a control diet (CT) or a diet supplemented with Oligofructose (OFS), oligofructose-enriched inulin (Syn) or high molecular weight Inulin (Inu). For one organ, data with different superscript letters are significantly different, \( P<0.05 \)

Immunohistochemical detection of GLP-1

Implication of GLP-1 increase by fructans in improvement of satiety and glucose homeostasis

- Decrease in hyperphagia, glycemia, increase in insulinemia and of pancreatic b-cells in streptozotocin-diabetic rats (Cani et al J Endocrinol 185, 457 (2005))

- Decrease in food intake and body weight gain in high-fat fed rats (Cani et al Obes Res, 13, 1000 (2005))

- Increase in GLP-1 and decrease in caloric intake and of glucose response upon OGGT in JCR:LA-cp rats (Reimer and Russel Obesisty 16, 40-46 (2008))
Is GLP-1 a key peptide to explain the effect of OFS on obesity and diabetes?

Model of high fat fed mice (obese and diabetics)
With or without active GLP-1 receptor
We used two models of mice lacking GLP-1 receptor functionality (in collaboration with Pr R. Burcelin)

- Ex-9-39 (GLP-1 R antagonist) i.p. using mini-pump implanted intraperitoneally (28 days)

and

- GLP-1 R -/- mice

Dietary treatment inducing diabetes and obesity:

- High-Fat diet for 28 days +/- OFS
### Values versus CT

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>GLP-1R -/-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF-OFS</td>
<td>HF-OFS-Ex</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTT</td>
<td>⇑</td>
<td>=</td>
</tr>
<tr>
<td>Fasted</td>
<td>⇑</td>
<td>=</td>
</tr>
<tr>
<td>Fed</td>
<td>⇑</td>
<td>=</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>⇑</td>
<td>=</td>
</tr>
<tr>
<td>Fed</td>
<td>⇑</td>
<td>=</td>
</tr>
<tr>
<td>Pancreatic insulin</td>
<td>⇑</td>
<td>=</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>⇑</td>
<td>=</td>
</tr>
</tbody>
</table>

Insulin sensitivity

Data with different superscript letters are significantly different, $P<0.05$

Adapted from Delzenne NM, Cani PD and Neyrinck AM, Br J Nutr, 2005
GENERAL HYPOTHESIS

The fermentation of fructans in the caeco-colon participates to the control of food intake, obesity and associated disorders.

Questions:
1. Implication of gastro-intestinal peptides?
2. Implication of inflammation?
3. Relevance for human health?
Hypothesis: prebiotics, through the modulation of the flora, may counteract metabolic and inflammatory disorders associated to obesity induced by a high-fat diet.
High-Fat diet (70% fat in energy) increases endotoxemia in mice

1. **Bifidobacteria reduce intestinal or plasma LPS levels in models of enterocolitis**
   
   Caplan et al. Gastroenterology 1999
   Griffiths et al. Dig Dis Sci 2004

2. **Bifidobacteria improve mucosal barrier function**
   
   Wang et al. J Trauma 2006
   Wang et al. World J Gastroenterol 2004

3. **Prebiotics such as oligofructose (OFS) reproducibly increase gut bifidobacteria**
   

4. **Prebiotics improve glucose homeostasis and hepatic inflammation**
   
   Delzenne and Cani Curr Opin Clin Nutr Metab Care 2005
   Cani et al. Diabetes 2006
Material and methods

- 12 weeks-old C57bl6/J mice
- 4 groups
  - CT: A04 diet;
  - HF: high-fat diet (70% energy from fat);
  - HF-0FS: HF diet containing 10% Raftilose P95 (Orafti);
  - HF-CELL: HF diet containing 10% Avicel (microcristalline cellulose)
- Oral glucose tolerance test after 13 weeks
- End of experiment after 14 weeks
- Analysis: anthropometry; metabolism; gut microflora (caecal content; FISH analysis in Reading (UK)); cytokines/LPS;

_Cani et al. Diabetologia, 2007_
Prebiotics treated mice are protected against HF diet induced metabolic endotoxemia.
Body weight/fat mass positively correlate with serum endotoxin, and negatively correlate with Bifidobacteria

Same observation with glycemia and insulinemia
Relation between 1. cytokines and serum endotoxin
2. cytokines and bifidobacteria
Metabolic endotoxaemia initiates obesity and insulin resistance

Introduction

High-fat diet
Low fibres

1. Modulation of gut microbiota
2. Bifidobacterium spp.
3. LPS

Gut epithelium
Blood

- Lipogenesis
- Inflammation
- Steatosis
- Insulin sensitivity

- Inflammation
- Macrophages infiltration

- Insulin sensitivity

Legend

LPS: lipopolysaccharides
mCD14
TLR4
LPS

Bacteria induced metabolic disease hypothesis

Metabolic disorders
Inflammation
Increased endotoxemia
Increased LPS absorption
Increased permeability
Change Gut flora
High fat feeding

Prebiotic effect?

OFS improves biomarkers for gut permeability in Ob/Ob mice, an effect related to a lower endotoxemia and improvement of metabolic alterations (body weight, fat mass, glycemic response...).

Cani et al, manuscript in revision (Gut)
Conclusions and perspectives

Adapted from Delzenne NM, Cani PD and Neyrinck AM, Br J Nutr, 2005
The fermentation of fructans in the caeco-colon participates to the control of food intake, obesity and associated disorders.

Questions:
1. Implication of gastro-intestinal peptides?
2. Implication of inflammation?
3. Relevance for human health?
Fructans and metabolic diseases associated with obesity: experimental data

Complex Dyslipidemia

Addition of fructans in the diet of animals

Steatosis NASH

Decreases triglyceridemia (in fructose or high fat fed or genetically obese rats, and in lean and ob/ob mice)

Lessens hepatic steatosis (in high fat fed mice, obese zucker rats, fructose-fed rats); Effect related to a decrease in hepatic lipogenesis.


Fructans and metabolic diseases associated with obesity: human data.

Complex Dyslipidemia

Fructans supplementation in humans

Steatosis NASH

Decrease in blood lipids in humans receiving 8 to 15 g fructans (10/16 studies showing improvement)

Decreased hepatic lipogenesis and TG synthesis (stable isotopes) in inulin-fed volunteers
letexier et al am j clin nutr, 77:559 (2003)

Improvement of (NASH)
daubioul et al. eur j clin nutr 59:723-726 (2005)
Effect of Synergy 1 on BMI and fat mass

Table II. Effect of supplementation on body composition after 1 year*

<table>
<thead>
<tr>
<th></th>
<th>Prebiotic (n = 48)</th>
<th>Control (n = 49)</th>
<th>Difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI Z-score</td>
<td>0.25 ± 0.045</td>
<td>0.38 ± 0.044</td>
<td>0.13 ± 0.06</td>
<td>.048</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.52 ± 0.15</td>
<td>20.03 ± 0.15</td>
<td>0.52 ± 0.21</td>
<td>.016</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>47.7 ± 0.4</td>
<td>49.0 ± 0.4</td>
<td>1.3 ± 0.6</td>
<td>.048</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.7 ± 0.3</td>
<td>155.7 ± 0.3</td>
<td>0.0 ± 0.5</td>
<td>.99</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>23.3 ± 0.4</td>
<td>24.2 ± 0.4</td>
<td>−0.8 ± 0.6</td>
<td>.14</td>
</tr>
<tr>
<td>Total fat mass (kg)</td>
<td>11.24 ± 0.25</td>
<td>12.07 ± 0.25</td>
<td>0.84 ± 0.36</td>
<td>.022</td>
</tr>
</tbody>
</table>

Table III. Study year differences in change in BMI Z-score, BMI, total fat mass, and weight in subjects consuming a calcium intake below the cutoff point compared with those consuming calcium intakes above the cutoff point

<table>
<thead>
<tr>
<th>Calcium intake cut-off*†</th>
<th>700 mg/d‡</th>
<th>800 mg/d§</th>
<th>900 mg/d¶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>P value</td>
<td>Difference</td>
</tr>
<tr>
<td>BMI Z-score</td>
<td>0.20 ± 0.07</td>
<td>.008</td>
<td>0.17 ± 0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.67 ± 0.25</td>
<td>.006</td>
<td>0.54 ± 0.22</td>
</tr>
<tr>
<td>Total fat mass (kg)</td>
<td>1.02 ± 0.42</td>
<td>.016</td>
<td>0.58 ± 0.36</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>1.3 ± 0.7</td>
<td>.076</td>
<td>1.2 ± 0.6</td>
</tr>
</tbody>
</table>

8g/d prebiotic versus maltodextrin during 1 year
Abrams et al., J. Pediatrics 2007
Modulation of food intake in humans

- 16 g/j of OFS, single-blind study, cross-over, n=10. 2 x 2 wks R/ and 2wk wash-out

OFS increases satiety at breakfast and dinner.
Reduces Total Energy intake by about 10%

Cani et al, Eur J Clin Nutr, 2005
Obese and Type 2 diabetic patients exhibit metabolic endotoxemia

Creely et al. Am J Physiol Endocrinol Metab. 2007

Effect of prebiotics?
Conclusions, questions….

- Prebiotics decrease food intake, fat mass, and metabolic disorders in obese animals, by targeting endocrine cells and/or by modulating inflammation.
- Effect linked to modulation of gut microflora?
- Similar targets for probiotics?
- Relevance in humans?
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