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Il sottoscritto dichiara di non aver avuto negli ultimi 12 mesi conflitto d’interesse in relazione a questa presentazione

e

che la presentazione non contiene discussione di farmaci in studio o ad uso off-label
The Gut-Liver axis: a bidirectional relation in health and disease

Diagnostical and therapeutical issues

Diet, nutriceuticals, biotics therapies

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Department of Clinical and Experimental Medicine
Gastroenterology Unit
Data mining the human gut microbiota for therapeutic targets

Matthew Collison, Robert P. Hirst, Anil Wipat, Srintra Nakjang, Philippe Sanssea and James R. Brown

Exponential growth of research interest and publications opening the way to gut microbiome-targeting therapeutic strategies
Modulation of gut microbiome

Diet

Nutraceuticals

Biotics

Gut microbiota

Oxygen

STOP

LIVER HEALTH ???
Diet is the major driver for changes in gut bacterial diversity.

The intestinal microflora is both a target for nutritional intervention and a factor influencing the biological activity of foods.
At birth, the digestive tract of humans is sterile.

Colonization by microbes starts within the first few hours of life and depends on:
- delivery type
- maternal flora
- environment hygiene
- infant diet

Harmsen HJM et al. JPEN 2000
Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo, Duccio Cavalieri, Monica Di Paola, Matteo Ramazzotti, Jean Baptiste Poulet, Sebastien Massart, Silvia Collini, Giuseppe Pieraccini, and Paolo Lionetti

African Rural diet

European Western diet

Rural Diet = ▲ vegetable fibre:

Western diet = ▲ starch and protein:

▲ Bacteroidetes ▲ Prevotella

▲ Firmicutes, ▲ Proteobacteria
Influence of diet upon dominant human colonic bacteria determined by 16S rRNA gene sequencing

Bacterial change occurred within a few days, and were reversed equally quickly by a subsequent dietary switch

Flint HJ, Nat Rev Gastroenterol Hepatol 2012
Diet-driven changes in gut microbial community composition in humans

<table>
<thead>
<tr>
<th>Dietary Intervention</th>
<th>Duration (weeks)</th>
<th>Volunteers</th>
<th>Molecular profiling methods (16S rRNA)</th>
<th>Bacterial changes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controlled diet composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant starch (RS3)</td>
<td>3</td>
<td>14 obese, M</td>
<td>Sequencing; qPCR</td>
<td>† <em>Ruminococcus bromii</em>, <em>Eubacterium rectale</em>, <em>Roseburia</em> spp. and <em>Oscillibacter</em> spp.</td>
</tr>
<tr>
<td>Nonstarch polysaccharides (wheat bran)</td>
<td>3</td>
<td>14 obese, M</td>
<td>Sequencing; qPCR</td>
<td>No major changes</td>
</tr>
<tr>
<td>Weight-loss diet I</td>
<td>3</td>
<td>14 obese, M</td>
<td>Sequencing; qPCR</td>
<td>† <em>Collinsella aerofaciens</em>, <em>E. rectale</em>, and <em>Roseburia</em> spp.</td>
</tr>
<tr>
<td>Weight-loss diets II</td>
<td>4</td>
<td>18 obese, M</td>
<td>FISH</td>
<td>† <em>E. rectale</em>, <em>Roseburia</em> spp. and <em>Bifidobacterium</em> spp.</td>
</tr>
<tr>
<td>Weight-loss diets III</td>
<td>4</td>
<td>18 obese, M</td>
<td>FISH</td>
<td>† <em>E. rectale</em>, <em>Roseburia</em> and <em>Bifidobacterium</em> spp.</td>
</tr>
<tr>
<td><strong>Dietary supplementation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant starch (RS2)</td>
<td>3</td>
<td>10 healthy</td>
<td>Sequencing; qPCR</td>
<td>† <em>R. bromii</em> and <em>E. rectale</em></td>
</tr>
<tr>
<td>Resistant starch (RS4)</td>
<td>3</td>
<td>10 healthy</td>
<td>Sequencing; qPCR</td>
<td>† <em>Bifidobacterium</em> spp. and <em>Parabacteroides distasonis</em></td>
</tr>
<tr>
<td>Resistant starch (Hi Maize)</td>
<td>4</td>
<td>46 healthy</td>
<td>DGGE; qPCR</td>
<td>† <em>R. bromii</em></td>
</tr>
<tr>
<td>Inulin and oligofructose</td>
<td>2.3</td>
<td>12 healthy</td>
<td>qPCR</td>
<td>† <em>Faecalibacterium prausnitzii</em> and <em>Bifidobacterium</em> spp.</td>
</tr>
<tr>
<td>Inulin (long chain)</td>
<td>3</td>
<td>31 healthy</td>
<td>FISH</td>
<td>† <em>Bifidobacterium</em> spp., <em>Lactobacillus</em> spp. and <em>Atopobium</em> spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>† <em>Bacteroides</em> spp. and/or <em>Prevotella</em> spp.</td>
</tr>
<tr>
<td>Inulin</td>
<td>2</td>
<td>30 healthy</td>
<td>FISH</td>
<td>† <em>Bifidobacterium</em> spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>† <em>Bacteroides</em> and/or <em>Prevotella</em> and <em>Clostridium histolyticum</em></td>
</tr>
<tr>
<td>Galacto-oligosaccharides</td>
<td>3</td>
<td>18 healthy</td>
<td>Sequencing</td>
<td>† <em>F. prausnitzii</em> and <em>Bifidobacterium</em> spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>† <em>Bacteroides</em></td>
</tr>
<tr>
<td>Raffinose</td>
<td>3</td>
<td>12 healthy</td>
<td>Sequencing; qPCR</td>
<td>† <em>F. prausnitzii</em>, <em>Bifidobacterium</em> spp.</td>
</tr>
</tbody>
</table>

Flint HJ, Nat Rev Gastroenterol Hepatol 2012
Both obesity and diet may independently affect gut microbiome composition.

Therefore to better understand the weight of each factor it would be advantageous if one of the two variables be permanent.
RELMBβ knock out (lean independently of diet)

RELMBβ KO mice allow to study the effects of diet alone avoiding the bias of the obese state.

**Graph:**
- High fat diet vs. Standard chow for Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria.

**Proportion:**
- **High Fat Diet:**
  - Proteobacteria: ↑
  - Bacteroidetes: ↓
  - Firmicutes: ↑
  - Actinobacteria: ↓
- **Standard Chow:**
  - Proteobacteria: ↓
  - Bacteroidetes: ↑
  - Firmicutes: ↓
  - Actinobacteria: ↑

**Legend:**
- Yellow bar: High fat diet
- Blue bar: Standard chow

**Microbiome Changes:**
- **Firmicutes, Clostridiales, Proteobacteria**
- **Bacteroidetes**
Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice

Andrew L. Goodman, George Kallstrom, Jeremiah J. Faith, Alejandro Reyes, Aimee Moore, Gautam Dantas, and Jeffrey I. Gordon

Germ free mice

Human fecal flora from obese subjects

Low-fat or high-fat diet

Microbiome composition and mice phenotype were related to diet type, independently from the colonizing flora
Germ free mice
+ 10 human gut bacteria colonization
+ Diets with different concentration of:
  - Protein (casein)
  - Fat (corn oil)
  - Polysaccharide (cornstarch)
  - Simple sugar (sucrose)

= Casein and Sucrose  \(\uparrow\) E. Coli

Casein and Starch  \(\uparrow\) C. symbiosum

Casein  \(\uparrow\) Total fecal bacteria
How diet affect gut microbiome composition?

Maslowski KM, Nature Immunol 2011
Exercise and diet modification in non-obese non-alcoholic fatty liver disease: Analysis of biopsies of living liver donors

Comparison by liver biopsies of the hepatic steatosis between the initial and follow-up (mean 10 wks, range 1-39)

After exercise and diet modification, total macro- and microvesicular steatosis were significantly reduced.

120 subjects

25 (Cal/kg/day) x Ideal Body Weight

50% carbohydrates
30% fat
20% protein

Walking/jogging
20’ three times per week
Compositions of the gut microbiota changed specifically in each individual in relation to choline intake.

Choline depletion diet was directly associated with:
- higher liver fat
- increased levels of *Gamma-Proteobacteria* and *Erysipelotrichi*
### Proposed lifestyle modification guidelines for persons with Non-Alcoholic Fatty Liver Disease

<table>
<thead>
<tr>
<th>Modification</th>
<th>Guideline</th>
</tr>
</thead>
</table>
| Weight loss                        | Initial goal: 5-10% body weight lost over 1 y  
Long-term goal: ideal body weight  
Maintenance of weight loss                                      |
| Energy intake                      | 1,200-1,500 kcal/d                                                                                                                      |
| Total fat intake                   | <35% of total energy                                                                                                                    |
| Monounsaturated fatty acid intake  | Up to 25%                                                                                                                             |
| Polyunsaturated fatty acid intake  | Increase n-3 fatty acids                                                                                                                |
| Saturated fatty acid intake        | <7% of total energy                                                                                                                     |
| Carbohydrate intake                | ≥50% whole grain; avoid high-fructose corn syrup                                                                                         |
| Protein intake                     | Lean animal- or vegetable-based protein                                                                                                 |
| Antioxidant intake                 | Vitamin E 800 IU/d  
Fish oil 1 g/d (eicosapentaenoic + docosahexaenoic acids)                                                                                           |
| Physical activity                  | ≥150 min/wk at moderate-vigorous intensity  
Cardiovascular 5 times/wk  
Resistance training ≥2 times/wk                                                                                           |

McCarthy EM, Academy of Nutrition and Dietetics 2012
CONCLUSIVE REMARKS

There is no consensus as to which diet or lifestyle approach is the right one for NAFLD patients, largely because of a lack of scientific evidence.
Modulation of gut microbiome

Diet

Nutraceuticals

Biotics

Gut microbiota

LIVER HEALTH ???
The term “Nutraceutical” was coined from “Nutrition” & “Pharmaceutical” in 1989 by Stephen De Felice

**DEFINITION:** “Food extracts that have been demonstrated to produce a physiological benefit or provide some protection against chronic disease”

**GROUPS OF NUTRACEUTICALS**

- Antioxidants
- Flavonoids
- Allium compounds
- Digestive stimulants
- Glucosinolates
- Phytoestrogens
NUTRACEUTICALS: ECONOMIC AND SCIENTIFIC BURDEN

GLOBAL MARKET $ 105.9 BILLION

Market Capitalization for Nutraceuticals as of 2004
- Japan 18%
- Rest of the World 12%
- Europe 33%
- United States 37%

Federal spending on science and technology activities

1. Research and experimental development.
2. Related scientific activities.
Source: Statistics Canada, Catalogue no. 88-001-XE.
The potential prebiotic effects for both catechin and epicatechin were most notable at the lower concentration of 150 mg/l.
Effect of a Low Dose of Dietary Resveratrol on Colon Microbiota

Resveratrol used at doses (1mg/kg/day) achievable with enriched nutraceuticals

![Graph A](image1)

- ↑ **Lactobacilli**

![Graph B](image2)

- ↑ **Bifidobacteria**

A significant reduction of Enterobacteria was also observed

The potential influence of polyphenols on colonic microflora and human gut health

↓ Adhesion
Salmonella typhimurium

↑ Adhesion
Lactobacillus rhamnosus

Polyunsaturated fatty acids (ω-3) and gut microbiome

In vitro studies have shown PUFAs increased growth and adhesion of different Lactobacillus strains.
It is likely that the beneficial effect of omega 3 supplementation on AST-ALT levels and liver fat could be due not only to a direct anti-inflammatory action but also to their interaction with gut microbiome.
Nutraceuticals include various food- and herbal-derived products

Very often
- Dose,
- Duration of treatment,
- Drug-interactions
- Special warnings

are unclarified

Adverse events associated with the use of complementary medicine and health supplements: An analysis of reports in the Singapore Pharmacovigilance database from 1998 to 2009

- 627 adverse events associated with herbal derived products
- 80% be serious events
- Liver the main organ involved in the serious cases
- 22 fatalities reported
- 10 deaths due to hepatotoxicity
Modulation of gut microbiome

Diet

Nutraceuticals

Biotics

Gut microbiota

Liver health???
A century ago: the story begins........

Elie Metchnikoff, Russian Nobel Laureate, at Pasteur Institute in France used cultures of a lactic acid bacteria, the “Bacillus bulgaricus” to colonize the bowel and modify intestinal flora.
**...and the Business also!**

**GLOBAL MARKET FOR PROBIOTICS IN USA**

<table>
<thead>
<tr>
<th>Year</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>$14.9 billion</td>
</tr>
<tr>
<td>2008</td>
<td>$15.9 billion</td>
</tr>
<tr>
<td>2009</td>
<td>$17.8 billion</td>
</tr>
<tr>
<td>2011</td>
<td>$19.6 billion</td>
</tr>
</tbody>
</table>

**Common Probiotics for Human Use**

<table>
<thead>
<tr>
<th>Lactobacillus species</th>
<th>Bifidobacterium species</th>
<th>Other bacteria</th>
<th>Other microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>B. adolescentis</td>
<td>E. faecalis</td>
<td>B. cereus</td>
</tr>
<tr>
<td>L. paracasei</td>
<td>B. animalis</td>
<td>E. faecium</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>L. crispatus</td>
<td>B. bifidum</td>
<td>L. lactis</td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>L. gasseri</td>
<td>B. longum</td>
<td>P. acidilactici</td>
<td>S. boulardi</td>
</tr>
<tr>
<td>L. johnsoni</td>
<td>B. infantis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. reuteri</td>
<td>B. lactis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. salivarius</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria
DEFINITIONS

**PROBIOTICS:** Live microorganisms which, when administered in adequate amounts, confers a health benefit on the host.

**PREBIOTICS:** Non digestible substances that provide a beneficial physiological effect for the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria.

**SYNBIOTICS:** Products that contain both probiotics and prebiotics.

**POSTBIOTICS:** A metabolic product generated by a probiotic organism that influences the host's biological functions.
Probiotics: mechanisms of action

Preidis, Gastroenterology 2011
Probiotics as an emerging therapeutic strategy to treat NAFLD: focus on molecular and biochemical mechanisms
## Gut-liver axis: animal interventional studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Animal model</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li Z</td>
<td>2003</td>
<td>Ob/Ob mice</td>
<td>VSL#3</td>
<td>↓ Total hepatic fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ob/Ob mice + fat diet</td>
<td>Anti-TNFα Ab</td>
<td>↓ ALT, ↓ TNFα RNA</td>
</tr>
<tr>
<td>Ewaschuk J</td>
<td>2007</td>
<td>129Sv/Ev WT + LPS</td>
<td>VSL#3</td>
<td>↓ Liver damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>129Sv/Ev IL-10^- + LPS</td>
<td>anti-PPARg</td>
<td>↓ Intestinal damage</td>
</tr>
<tr>
<td>Ma X</td>
<td>2008</td>
<td>C57BL6 mice</td>
<td>VSL#3</td>
<td>↓ Liver NK cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ TNFα, ↓ IL-4</td>
</tr>
<tr>
<td>Velayudham A</td>
<td>2009</td>
<td>C57BL6 mice</td>
<td>VSL#3</td>
<td>↓ TGFβ receptor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C57BL6 mice + MCD diet</td>
<td></td>
<td>↓ Liver fibrosis</td>
</tr>
</tbody>
</table>

**VSL#3**: high concentration (450 billions colonies/sachet) of lyophilized *Bifidobacteria, Lactobacilli and Streptococcus thermophilus*
The supplementation of VSL#3 probiotics to a high fat diet
- ↑ liver NKT cells
- ↓ body weight
- ↓ steatosis
VSL#3 modulates liver fibrosis but does not protect from inflammation and steatosis

The mechanisms include modulation of impaired TGF signaling
Protective effects of *Lactobacillus paracasei* F19 in a rat model of oxidative and metabolic hepatic injury

Gerardo Nardone,1 Debora Compare,1 Eleonora Liguori,1 Valentina Di Mauro,1 Alba Rocco,1 Michele Barone,2 Anna Napoli,2 Dominga Lapl9, Maria Rosaria Iovene,4 and Antonio Colantuoni5

Hepatic microcirculation

Liver pro-inflammatory cytokines

Ileal flora

**Hepatic microcirculation**

**Liver pro-inflammatory cytokines**

**Ileal flora**

Lactobacyllus Paracasei F19 supplementation, by restoring gut microbiota, attenuated oxidative and metabolic liver injury
Probiotics for patients with hepatic encephalopathy (Review)


7 trials suitable for analysis
Each used different probiotics
Administration 10-180 days
The risk of bias was high

Probiotics VS. no treatment
- Significant difference in plasma ammonia after one and three months
- No advantage on symptom resolution, mortality, adverse events, quality of life
Prebiotics: mechanisms of action
The most important are

- Lactulose
- Fructo-Oligo Saccarides FOS
- Galacto-Oligo Saccarides GOS
- Inulin
Influence of dietary supplementation with dextrin or oligofructose on the hepatic redox balance in rats

Rats were fed either a 10% oligofructose (OFS) or a 10% dextrin (DEX) supplemented diet for 9 wk

**DEX** protects the liver from protein carbonylation

**DEX and OFC** improve glutathione/oxidized glutathione ratio

Antioxidative and hepatoprotective effects of fructo-oligosaccharide in D-galactose-treated Balb/cJ mice

Hsiao-Ling Chen¹,², Cheng-Hsin Wang³, Yi-Wen Kuo¹ and Chung-Hung Tsai⁴

**Hepatic antioxidant enzyme activities in D-galactose treated mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (IU/mg protein)</th>
<th>GPx (IU/mg protein)</th>
<th>Catalase (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>Control</td>
<td>80.0⁶ 5.7</td>
<td>46.2⁵ 4.2</td>
<td>844.7⁵ 53.9</td>
</tr>
<tr>
<td>DG</td>
<td>68.1⁶ 4.9</td>
<td>32.5⁵ 3.3</td>
<td>726.2⁵ 49.5</td>
</tr>
<tr>
<td>DG + FO</td>
<td>78.4⁶ 3.4</td>
<td>41.1⁵ 3.0</td>
<td>793.9⁵ 41.9</td>
</tr>
<tr>
<td>DG + vitamin E</td>
<td>82.4⁶ 3.5</td>
<td>37.5⁵ 4.5</td>
<td>871.2⁵ 49.8</td>
</tr>
</tbody>
</table>

**Fecal total and Bifidobacteria counts in D-galactose treated mice**

<table>
<thead>
<tr>
<th>Bifidobacterium (log₁₀ counts/g dry faeces)</th>
<th>Total bacteria (log₁₀ counts/g dry faeces)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>Control</td>
<td>6.65⁶ 0.02</td>
</tr>
<tr>
<td>DG</td>
<td>6.57⁶ 0.02</td>
</tr>
<tr>
<td>DG + FO</td>
<td>6.73⁶ 0.02</td>
</tr>
<tr>
<td>DG + vitamin E</td>
<td>6.66⁶ 0.02</td>
</tr>
</tbody>
</table>

During D-galactose induced liver damage both FOS and Vit E induce:
- ↑ antioxidant enzymes
- ↓ histological damage
- ↑ fecal Bifidobacteria
A randomized double-blind crossover design
16 g/day OFS or maltodextrine (placebo) for 8 weeks
7 patients with NASH confirmed by liver biopsy

## Table

<table>
<thead>
<tr>
<th>Tests</th>
<th>Normal range</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-peptide (pmol/l)</td>
<td>545–1605</td>
<td>985±178</td>
<td>1159±313</td>
<td>1204±372</td>
<td>1360±678</td>
<td>865±148</td>
<td>1227±955</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>&lt;180–250</td>
<td>127±26</td>
<td>148±19</td>
<td>111±18</td>
<td>130±20</td>
<td>124±13</td>
<td>99±12</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>&lt;190–250</td>
<td>196±9</td>
<td>195±13</td>
<td>184±13</td>
<td>190±12</td>
<td>202±7</td>
<td>186±12</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>&lt;115–160</td>
<td>127±9</td>
<td>125±13</td>
<td>117±12</td>
<td>122±11</td>
<td>131±10</td>
<td>121±10</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>&gt;55–35</td>
<td>43.3±5.0</td>
<td>40.6±4.6</td>
<td>44.9±5.4</td>
<td>41.5±5.2</td>
<td>46.2±4.9</td>
<td>45.9±5.3</td>
</tr>
</tbody>
</table>

Although many limitations (number of patients, follow-up, and endpoint) this is the first and unique human RCT assessing the effect of prebiotics on liver.

Oligofructose decreased significantly serum AST after 8 wks.
Take Home Message

**Gut microbiota** is not a bystander in the complex biological events regulating intestinal homeostasis, but it may be an active player implicated in pathogenesis of digestive and extradigestive diseases.

**Diet, nutriceuticals, and biotics** may change gut microbiota and affect its functional relationships with the host **opening the way to new therapeutic strategies**.
However the majority of available data come from animal model studies furnishing only biological plausible health benefits ... 

... there are limited clinical studies supporting systematic evidence-based recommendations
Diet, nutriceuticals, biotics therapies

Unmeet needs

Dietary intervention
Which is the best diet or dietary components

Probiotics-Prebiotics-Synbiotics-Nutraceuticals
Which is best type, dose, duration of treatment?
What outcome measures are appropriate?
What is the appropriate follow-up?

What’s needed

• Human Randomized controlled trials
• Larger population
• Longer duration of treatment and follow up
• Defined outcomes
"Let Food Be Your Medicine and Medicine Be Your Food"