Gut microbiome as predictors of obesity and addictive-eating behaviour

Patrizia Brigidi, UNIBO, Italy
Obese gut microbiota VS Lean gut microbiota

(Parekh et al., Frontiers in Endocrinology 2013)

Obese Gut Microbiota

- Lower Biodiversity
- Decreased Satiety
- Increased (LPS) Inflammation
- Increased SCFA (Lipogenesis)
- Decreased PYH Decreased GLP-1
- Decreased Fatty Acid Oxidation Decreased FAF/AMPK
- Decreased Butyrate Production

Lean Gut Microbiota

- Higher Biodiversity
- Increased Satiety
- Decreased (LPS) Inflammation
- Decreased SCFA (Lipogenesis)
- Increased PYH Increased GLP-1
- Increased Fatty Acid Oxidation Increased FAF/AMPK
- Increased Butyrate Production
The altered microbial profile occurring in obese people is considered as an extreme deviation from the microbiota-host mutualism (Candela et al., 2012).

RUPTURE OF THE MICROBIOTA-HOST MUTUALISTIC RELATIONSHIP AND COMPROMISED HOST ENERGY BALANCE AND IMMUNE HOMEOSTASIS
The obese-associated gut microbiome

(Ridaura et al., 2013, Turnbaugh et al., 2009, Cox et al., 2013)

- **Enterobacteriaceae**
- **Erysipelotrichaceae**
- **Bilophila wadsworthia**

**Biodiversity**

**Efficiency in energy extraction from the diet**

**Extra supply of calories to the host**

**Overload of pro-inflammatory and sulphate-reducing bacteria may consolidate the obesity-associated inflammation and insulin resistance**
Is the microbiome involved in the onset of obesity?
Timeline of recruitment and follow-up
IDFICS – I.Family cohort, starting with 2-10 year olds

Baseline 2006

16,228 children

Follow-up 1 2007

13,596 children

Transition: toddler \(\rightarrow\) child

T1

Follow-up 2 2008

9,617 children

Transition: child \(\rightarrow\) adolescent

T3

Contrasting groups


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Collection of stool samples in IDEFICS/ I.Family

- **T0 (baseline)** 2007-2008
- **T1 (follow up)** 2009-2010
- **I.Family** 2013-2014

Collection of stool samples (n=398)

Collection of stool samples (n=517)

Children with stool samples in IDEFICS (T1) and I.Family (T3) → n=97

<table>
<thead>
<tr>
<th>Weight development</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>stayed normal weight (controls)</td>
<td>34 (35.1%)</td>
</tr>
<tr>
<td>gained weight (cases)</td>
<td>36 (37.1%)</td>
</tr>
<tr>
<td>lost weight</td>
<td>11 (11.3%)</td>
</tr>
<tr>
<td>chronical overweight/obesity</td>
<td>11 (11.3%)</td>
</tr>
<tr>
<td>chronical underweight</td>
<td>5 (5.2%)</td>
</tr>
</tbody>
</table>
Study design

- **16S rRNA Sequencing of 140 samples** → $56,485 \pm 22,321$ reads/sample
- **20,360 OTUs**

**T1 Normal weight children (T1_N + T1_O)**
Age: 4-11y

**T3 OW/OB children (T3_O)**
Age: 8-15y

**T3 Normal weight children (T3_N)**
Huge variation of the major microbiota components in obese and normal weight children
(Rampelli, Guenther et al, Communications Biology, in press)

Firmicutes: 34-77%; Bacteroidetes: 1-51%; Actinobacteria: 0.2-31%; Proteobacteria 0-8%

(140 samples)
The gut microbiota of obese children is different from that of normal weight children.

### Differences between Children at T1

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Level</th>
<th>Mean T1_N</th>
<th>SEM T1_N</th>
<th>Mean T1_O</th>
<th>SEM T1_O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanobacteria</td>
<td>PHYLUM</td>
<td>0.19</td>
<td>0.07</td>
<td>0.14</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>524-7</td>
<td>FAMILY</td>
<td>0.7</td>
<td>0.25</td>
<td>0.11</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Slackia</td>
<td>GENUS</td>
<td>0.05</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.008</td>
</tr>
<tr>
<td>[Prevotella]</td>
<td>GENUS</td>
<td>0.3</td>
<td>0.18</td>
<td>0.0</td>
<td>0.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Lactococcus</td>
<td>GENUS</td>
<td>0.12</td>
<td>0.04</td>
<td>0.1</td>
<td>0.07</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### Differences between Normal weight Children at T1 and T3

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Level</th>
<th>Mean T1_N</th>
<th>SEM T1_N</th>
<th>Mean T1_O</th>
<th>SEM T1_O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>PHYLUM</td>
<td>1.51</td>
<td>0.19</td>
<td>1.08</td>
<td>0.11</td>
<td>0.04</td>
</tr>
<tr>
<td>Tenericutes</td>
<td>FAMILY</td>
<td>0.6</td>
<td>0.18</td>
<td>0.18</td>
<td>0.08</td>
<td>0.03</td>
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<tr>
<td>[Mogibacteriaceae]</td>
<td>FAMILY</td>
<td>0.14</td>
<td>0.02</td>
<td>0.18</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>FAMILY</td>
<td>0.32</td>
<td>0.17</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Catenibacterium</td>
<td>GENUS</td>
<td>0.07</td>
<td>0.04</td>
<td>0.23</td>
<td>0.1</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### Differences between children that become Obese

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Level</th>
<th>Mean T1_O</th>
<th>SEM T1_O</th>
<th>Mean T3_O</th>
<th>SEM T3_O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>PHYLUM</td>
<td>1.1</td>
<td>0.13</td>
<td>1.56</td>
<td>0.24</td>
<td>0.02</td>
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<tr>
<td>Lactobacillaceae</td>
<td>FAMILY</td>
<td>0.2</td>
<td>0.13</td>
<td>0.11</td>
<td>0.07</td>
<td>0.02</td>
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<tr>
<td>Clostridiaceae</td>
<td>FAMILY</td>
<td>1.5</td>
<td>0.2</td>
<td>1.14</td>
<td>0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Ruminococaceae</td>
<td>FAMILY</td>
<td>24.82</td>
<td>1.16</td>
<td>21.55</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td>Alcaligenaceae</td>
<td>FAMILY</td>
<td>0.66</td>
<td>0.09</td>
<td>0.86</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Ruminococcus</td>
<td>GENUS</td>
<td>5.92</td>
<td>0.86</td>
<td>3.88</td>
<td>0.47</td>
<td>0.05</td>
</tr>
<tr>
<td>Sutterella</td>
<td>GENUS</td>
<td>0.66</td>
<td>0.09</td>
<td>0.86</td>
<td>0.09</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Differences between Children at T3

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Level</th>
<th>Mean T3_N</th>
<th>SEM T3_N</th>
<th>Mean T3_O</th>
<th>SEM T3_O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidaceae</td>
<td>FAMILY</td>
<td>15.76</td>
<td>1.86</td>
<td>19.62</td>
<td>1.68</td>
<td>0.03</td>
</tr>
<tr>
<td>Prevotellaceae</td>
<td>FAMILY</td>
<td>6.16</td>
<td>1.45</td>
<td>5.23</td>
<td>1.74</td>
<td>0.02</td>
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<tr>
<td>Christensenellaceae</td>
<td>FAMILY</td>
<td>0.49</td>
<td>0.11</td>
<td>0.29</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>[Mogibacteriaceae]</td>
<td>FAMILY</td>
<td>0.18</td>
<td>0.02</td>
<td>0.11</td>
<td>0.02</td>
<td>0.001</td>
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<tr>
<td>[Tissierellaceae]</td>
<td>FAMILY</td>
<td>0.02</td>
<td>0.0</td>
<td>0.01</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Slackia</td>
<td>GENUS</td>
<td>0.05</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.008</td>
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<tr>
<td>Bacteroides</td>
<td>GENUS</td>
<td>15.76</td>
<td>1.86</td>
<td>19.62</td>
<td>1.68</td>
<td>0.03</td>
</tr>
<tr>
<td>Prevotella</td>
<td>GENUS</td>
<td>6.16</td>
<td>1.45</td>
<td>5.23</td>
<td>1.74</td>
<td>0.02</td>
</tr>
<tr>
<td>[Prevotella]</td>
<td>GENUS</td>
<td>0.26</td>
<td>0.12</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Lachnospira</td>
<td>GENUS</td>
<td>0.74</td>
<td>0.1</td>
<td>1.44</td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>Roseburia</td>
<td>GENUS</td>
<td>0.13</td>
<td>0.02</td>
<td>0.23</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Oscillospira</td>
<td>GENUS</td>
<td>0.8</td>
<td>0.05</td>
<td>0.66</td>
<td>0.05</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Clustering analysis identified 4 significantly different groups of children on the basis of microbiota data.

- 70% of the children in C1/C2 are T1_N or T3_N
- 70% of the children in C3/C4 are T1_O or T3_O
Differences in microbiota structures

**Enriched in C1/C2 (P<0.001)**

- **Akkermansia**
  Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae

- **Prevotella**
  Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae

- **Coprococcus**
  Firmicutes; Clostridia; Clostridiales; Lachnospiraceae

- **Phascolarctobacterium**
  Firmicutes; Clostridia; Clostridiales; Veillonellaceae

- **Lachnobody**
  Firmicutes; Clostridia; Clostridiales; Lachnospiraceae

- **Paraprevotella**
  Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae

- **Butyrivibrio**
  Bacteroidetes; Bacteroidia; Bacteroidales; Odoribacteraceae

- **Slackia**
  Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae

- **Odoribacter**
  Bacteroidetes; Bacteroidia; Bacteroidales; Odoribacteraceae

**Enriched in C3/C4 (P<0.001)**

- **Bacteroides**
  Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae

- **Enterococcus**
  Firmicutes; Bacilli; Lactobacillales; Enterococccaceae

- **Ruminococcus (Lachnospiraceae)**
  Firmicutes; Clostridia; Clostridiales; Lachnospiraceae

- **Coprobacillus**
  Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae

- **Eggerthella**
  Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae

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Microbiota – Health correlation

Inflammatory markers and other metadata

1. BMI
2. Weight
3. ISCED (educational level score)
4. Evenson MVPA (Moderate to Vigorous Physical Activity) score
5. Blood pressure (Systolic and Diastolic)
6. HOMA
7. HDL
8. CRP
9. TNF-α
10. IL-10
11. IL-8
12. IL-6
13. IL-15
14. IL-1

... 

49. Glucose level
50. Triglycerides

Adjust quantile regression model for:
- Age
- Gender
- Tanner’s classification of maturation stages

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Gut microbiome as predictors of obesity and addictive-eating behaviour

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Prospect

• The combination C3/C4 microbiota and D2/D5 diet is connected to the obese phenotype

• This combination is exclusively present in T1 normal weight children who showed excessive weight gain at T3

• Microbiome-host diet configuration possesses a sort of predictive potential of obesity, even if it can only partially explain the etiology of the obese phenotype
**IDEFICS/I.FAMILY: Metagenomics**

- Shotgun sequencing
  - Shotgun sequencing of 24 samples ➔ 9.5 Gbases
  - 1.4 M ± 0.6 M reads/sample

**HMP read processing for Human contamination and quality filtering**

**High quality reads**

**MetaCV pipeline**

*Patrizia Brigidi*

Gut microbiome as predictors of obesity and addictive-eating behaviour
C3/C4 and D2/D5

C1/C2 and D1/D3

RNA sequencing

• RNA sequencing of 24 samples ➔ 18 Gbases
• 6 M ± 2 M reads/sample

HMP read processing for Human contamination and quality filtering

High quality reads

HUMANn2

Kyoto Encyclopedia of Genes and Genomes

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Gut microbiome as predictors of obesity and addictive-eating behaviour

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Integration of the model with the metagenomic and metatranscriptomics data

New samples (11 lost weight, 11 chronical OW/OB, 5 chronical underweight, plus 107 children with stool samples recovered only at T1 but followed at T3: 16S rRNA analysis already done)
A **SPECIFIC SUBTYPE** of obesity results from an addiction to food that strongly resembles addiction to drugs, both behaviourally and in terms of underlying neural processes.

One of the aims of **MyNewGut** is to identify specific distinctive patterns of food addiction in the intestinal microbiome of obese women with or without food addiction.
The NEUROFAST cohort

- 36 overweight/obese women with FA
- 27 overweight/obese women without FA
- 39 normal-weight women

- Faeces, blood, urine
- Yale Food Addiction Scale (Questionnaire)
- Anthropometric data
Yale Food Addiction Scale (YFAS)
(Gearhardt et al., 2011)

**YFAS** is a questionnaire developed to operationalize FA by assessing signs of substance dependence symptoms (e.g. tolerance, withdrawal, loss of control) in eating behaviour. The questionnaire includes 25 sub-items that address eating habits over the past 12 months. Examples include “I eat to the point I feel physically ill” and “I have had withdrawal symptoms such as agitation, anxiety, or other physical symptoms when I cut down or stopped eating certain foods”.

To receive a diagnosis of FA, it is necessary to report experiencing three or more symptoms and clinically significant impairment or distress. Elevated YFAS scores have been associated with higher neural activation in reward circuitry in response to food cues and reduced activation of inhibitory regions in response to food intake.
16S sequencing of the Microbiome

- 16S rRNA Sequencing of 102 samples ➔ 73,152 ± 38,578 reads/sample
- 11,874 OTUs

UNPUBLISHED DATA
We found significantly lower values of $\alpha$-diversity in obese women compared to normal weight women.
Obese and normal weight women possess a different microbiome.
NEUROFAST: Metagenomics

- Shotgun sequencing of 45 samples ➔ ~15 Gbases
- 1 M ± 0.5 M reads/sample

15 overweight/obese women with FA
15 overweight/obese women without FA
15 normal-weight women

HMP read processing for Human contamination and quality filtering

High quality reads

MetaCV pipeline

Gut microbiome as predictors of obesity and addictive-eating behaviour
NEUROFAST: Metatranscriptomics

- RNA sequencing of 45 samples ➔ 70 Gbases
- 5 M ± 1 M reads/sample

15 overweight/obese women with FA
15 overweight/obese women without FA
15 normal-weight women

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Gut microbiome as predictors of obesity and addictive-eating behaviour
Conclusion

• The obesity is a complex mosaic of endogenous and exogenous determinants, and microbiome is only a single tile contributing to obesity development.

• **CHILDREN**: low diverse, dysbiotic microbiome configurations and unhealthy diets associated with an inflammation status were found in pre-obese children (predictive tool for the development of obesity in children: perspective of dietary recommendations tailored on the individual microbiome structure to reduce the obesity risk in children)

• **OBESE WOMEN**: In this mosaic, the single tile of the microbiome includes a possible role of gut microorganisms in eating behaviours, opening the way to further investigation on the role of *Ruminococcus* and *Dorea* in adults with food addiction.