Microbiota y Obesidad: Papel de los polifenoles

Prof. J. Alfredo Martínez
Microbiota and Obesity
Causes of Obesity

- Genetics
- Environment
- Lifestyle
- Neuroendocrine Factors

Energy Intake ↔ Energy Expenditure

Excessive Adiposity

Gut Microbiota

Question mark
Obesity comorbidities

Non-communicable diseases

Public health challenge

OBESITY

Environmental factors

Genetic factors

Diabetes
Cancer
CVDs
Chronic respiratory diseases

Unhealthy diet
Physical inactivity
Socioeconomic status
Neuroendocrine
Perinatal Nutrition
Microbiota

WHO, 2015; Ng et al., 2014; Bäckhed et al., 2004
Obesity consequences

↑ ADIPOSITY

- Insulin Resistance
  - Glucose Intolerance
    - Diabetes mellitus type II
    - Metabolic Syndrome

- Dyslipemia
  - Hypertension
    - Atherosclerosis

- Inflammation
Obesity complications: Inflammation

- Hypertension
- Diabetes Type 2
- Cardiovascular Disease
- Complex dyslipidemia
- Glucose homeostasis impairment
- Endothelial dysfunction
- Cancer
- CRP
- TNF-alpha
- IL-6
- IL-8

Low-grade systemic inflammation

Changes in Intestinal Microbiota

Spagnuolo et al., 2010
Cecal microbiota in lean vs obese mice
Ley et al., 2005 (PNAS)
Phylogenetic diversity curves for microbiota of lean vs obese individuals
Turnbaugh et al., 2009 (Nature)
INTRODUCTION

Changes in Intestinal Microbiota

CONNECTION

Low-grade systemic inflammation

Correlation of different bacterial groups with weight loss (kg)

Santacruz et al., 2009

Obesity and gut microbiota

- Increased B. fragilis
- Decreased C. coccoides
- Increased L. acidophilus
- Decreased E. coli

(weight loss > 2 Kg)

No significant differences
Interplay Between Weight Loss and Gut Microbiota Composition in Overweight Adolescents

Arlette Santacruz¹, Ascensión Marcos², Julia Wärnberg²³, Amelia Martí⁴⁵, Miguel Martin-Matillas⁶, Cristina Campoy⁶, Luis A. Moreno⁷, Oscar Veiga⁸, Carlos Redondo-Figuero⁹, Jesús M. Garagorri¹⁰, Cristina Azcona⁵, Manuel Delgado¹¹, Miguel García-Fuentes⁹, María C. Collado¹ and Yolanda Sanz¹; the EVASYON Study Group

The aim of this study was to determine the influence of an obesity treatment program on the gut microbiota and
Obesity and gut microbiota

INTENSIVE PHASE

WEIGHT LOSS < 2 KG

WEIGHT LOSS > 4 KG

2 MONTHS
Obesity and gut microbiota

**Bacterial counts in fecal samples of low-weight loss (<2 kg) group of adolescents, before and after intervention**

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Before intervention</th>
<th>After intervention</th>
<th>Mann-Whitney U-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pr²</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>13</td>
<td>13.2</td>
<td>12.9</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>13</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Clostridium coccoidei</td>
<td>13</td>
<td>10.0</td>
<td>10.0</td>
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<tr>
<td>Clostridium leptum</td>
<td>13</td>
<td>8.2</td>
<td>8.0</td>
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<tr>
<td>Lactobacillus</td>
<td>13</td>
<td>7.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13</td>
<td>6.7</td>
<td>6.5</td>
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<td>Analobacterium</td>
<td>13</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>Bifidobacterium longum</td>
<td>13</td>
<td>7.1</td>
<td>7.0</td>
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<tr>
<td>Bifidobacterium breve</td>
<td>13</td>
<td>4.8</td>
<td>4.8</td>
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<tr>
<td>Bifidobacterium bifidum</td>
<td>13</td>
<td>9.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis</td>
<td>13</td>
<td>8.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Bifidobacterium catenulatum</td>
<td>13</td>
<td>5.8</td>
<td>5.8</td>
</tr>
</tbody>
</table>

*Data are shown as medians and interquartile range (IQR) of cell number per gram of fecal samples. *Pr²* reflects the number of positive amplifications by quantitative real-time PCR from total samples (n = 13).

*Statistical differences between bacterial counts before and after intervention were calculated by using the Mann-Whitney U-test and established at P < 0.050.*
# Obesity and gut microbiota

## Bacterial Counts in Fecal Samples of Low-Weight Loss (>4 kg) Group of Adolescents, Before and After Intervention

**Table 5** Bacterial counts in fecal samples of high weight-loss (>4.0 kg) group of adolescents, before and after intervention

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Before Intervention</th>
<th>After Intervention</th>
<th>Mann–Whitney U-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pr</td>
<td>Mean</td>
<td>Median</td>
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<tr>
<td>Total bacteria</td>
<td>23</td>
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<tr>
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<td>23</td>
<td>7.5</td>
<td>7.6</td>
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<tr>
<td>Clostridium cocoides</td>
<td>23</td>
<td>8.7</td>
<td>8.6</td>
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<tr>
<td>Clostridium leptum</td>
<td>23</td>
<td>9.5</td>
<td>9.6</td>
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<tr>
<td>Lactobacillus</td>
<td>23</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>23</td>
<td>6.3</td>
<td>6.3</td>
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<tr>
<td><em>Bifidobacterium</em></td>
<td>23</td>
<td>8.3</td>
<td>8.1</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em></td>
<td>23</td>
<td>7.1</td>
<td>7.2</td>
</tr>
<tr>
<td><em>Bifidobacterium breve</em></td>
<td>15</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em></td>
<td>19</td>
<td>5.9</td>
<td>5.6</td>
</tr>
<tr>
<td><em>Bifidobacterium adolescentis</em></td>
<td>23</td>
<td>7.6</td>
<td>7.9</td>
</tr>
<tr>
<td><em>Bifidobacterium catenulatum</em></td>
<td>22</td>
<td>7.6</td>
<td>7.7</td>
</tr>
</tbody>
</table>

*Data are shown as medians and interquartile range (IQR) of cell number per gram of fecal samples. Prevalence (Pr) reflects the number of positive amplifications by quantitative real-time PCR from total samples (n = 23).

*Statistical differences between bacterial counts before and after intervention were calculated by using the Mann–Whitney U-test and established at P < 0.050.
### Obesity and gut microbiota

**Bacterial Counts in Fecal Samples of Low and High-Weight Loss Groups of Adolescents, Before and After Intervention**

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Low weight-loss group (&lt;2.0 kg)</th>
<th>High weight-loss group (&gt;4.0 kg)</th>
<th>Mann-Whitney U-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pr*</td>
<td>Mean</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td><strong>Total bacteria</strong></td>
<td>13</td>
<td>13.2</td>
<td>12.9</td>
<td>12.8-13.9</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>13</td>
<td>6.2</td>
<td>6.2</td>
<td>5.8-7.0</td>
</tr>
<tr>
<td><strong>Clostridium cocoelides</strong></td>
<td>13</td>
<td>10.0</td>
<td>10.0</td>
<td>9.8-10.2</td>
</tr>
<tr>
<td><strong>C. leptum</strong></td>
<td>13</td>
<td>8.2</td>
<td>8.0</td>
<td>7.9-8.5</td>
</tr>
<tr>
<td><strong>Lactobacillus</strong></td>
<td>13</td>
<td>7.9</td>
<td>7.8</td>
<td>7.6-8.1</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
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<td>6.7</td>
<td>6.5</td>
<td>6.0-7.7</td>
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<tr>
<td><strong>B. infantis</strong></td>
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<td>9.2</td>
<td>8.8-9.5</td>
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<tr>
<td><strong>B. longum</strong></td>
<td>13</td>
<td>7.1</td>
<td>7.0</td>
<td>6.8-7.4</td>
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<tr>
<td><strong>B. breve</strong></td>
<td>13</td>
<td>4.8</td>
<td>4.8</td>
<td>4.4-5.2</td>
</tr>
<tr>
<td><strong>B. bifidum</strong></td>
<td>13</td>
<td>9.1</td>
<td>9.0</td>
<td>8.8-9.5</td>
</tr>
<tr>
<td><strong>B. adolescens</strong></td>
<td>13</td>
<td>8.1</td>
<td>8.0</td>
<td>7.8-8.4</td>
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<tr>
<td><strong>B. catenulatum</strong></td>
<td>13</td>
<td>5.8</td>
<td>5.8</td>
<td>5.5-6.2</td>
</tr>
</tbody>
</table>

**Bacterial counts before intervention (log cells/g fecal sample)**

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Low weight-loss group (&lt;2.0 kg)</th>
<th>High weight-loss group (&gt;4.0 kg)</th>
<th>Mann-Whitney U-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pr*</td>
<td>Mean</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td><strong>Total bacteria</strong></td>
<td>13</td>
<td>13.2</td>
<td>13.1</td>
<td>12.8-13.4</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>13</td>
<td>6.3</td>
<td>6.2</td>
<td>5.8-6.9</td>
</tr>
<tr>
<td><strong>C. cocoelides</strong></td>
<td>13</td>
<td>9.9</td>
<td>10.0</td>
<td>9.7-10.2</td>
</tr>
<tr>
<td><strong>C. leptum</strong></td>
<td>13</td>
<td>8.4</td>
<td>8.3</td>
<td>7.9-8.8</td>
</tr>
<tr>
<td><strong>Lactobacillus</strong></td>
<td>13</td>
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<td>7.9</td>
<td>7.7-8.1</td>
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<tr>
<td><strong>E. coli</strong></td>
<td>13</td>
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<td>6.5</td>
<td>6.0-7.1</td>
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<tr>
<td><strong>B. infantis</strong></td>
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<td>8.9</td>
<td>9.0</td>
<td>8.4-9.6</td>
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<tr>
<td><strong>B. longum</strong></td>
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<td>6.3-7.7</td>
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<tr>
<td><strong>B. breve</strong></td>
<td>13</td>
<td>4.5</td>
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<td>4.3-4.7</td>
</tr>
<tr>
<td><strong>B. bifidum</strong></td>
<td>13</td>
<td>8.9</td>
<td>8.9</td>
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</tr>
<tr>
<td><strong>B. adolescens</strong></td>
<td>13</td>
<td>8.0</td>
<td>7.9</td>
<td>7.3-8.7</td>
</tr>
<tr>
<td><strong>B. catenulatum</strong></td>
<td>13</td>
<td>5.5</td>
<td>5.5</td>
<td>5.3-5.7</td>
</tr>
</tbody>
</table>

*Prevalence (Pr) reflects the number of positive amplifications by quantitative real-time PCR from total samples (n = 13 or 23). *Data are shown as medians and interquartile range (IQR) of cell number per gram of fecal samples. *Statistical differences between bacterial counts for each group (high- and low-weight adolescent groups) before and after intervention were calculated by using the Mann-Whitney U-test and established at P < 0.05.
Obesity and gut microbiota

(n=23; >4.0 kg weight lost)

LACTOBACILLUS GROUP

E. COLI GROUP

BACTEROIDES GROUP

\[ R = 0.55, P < 0.001 \]

\[ R = -0.37, P = 0.010 \]

\[ R = 0.27, P = 0.055 \]
Diet Induced Obesity: Microbiota & gut fermentation

Firmicutes/Bacteroidetes ratio
- Murphy et al., 2010

Erysipelotrichi class
- Turnbaugh et al., 2008

Bacterial species positively altered
- (E. cylindroides, E. ventriosum, B. wadsworthia, P. nanceiensis, L. reuteri)
- Turnbaugh et al., 2008
- Zhang et al., 2009
- Million et al., 2012
- Yin et al., 2012
- Lecomte et al., 2015

Bacterial species negatively altered
- (M. jalaludini, C. fusiformis)
- Turnbaugh et al., 2008
- Million et al., 2012
- Yin et al., 2012

SCFAs
- den Besten et al., 2013
Diet Induced Obesity and gut microbiota

Firmicutes/Bacteroidetes ratio

Ley et al., 2006
Turnbaugh et al., 2006
Bervoets et al., 2013

Healthy or Disease

Firmicutes/Bacteroidetes ratio

Low F/B ratio

High F/B ratio

Lean

Obese

Ley et al., 2006
Turnbaugh et al., 2006
Bervoets et al., 2013
Gut microbiota “new organ” within our organism

- $10^{11}$- $10^{12}$ cells/mL in colon
- ≥ 150 times more genes than the human genome

Classified from phylum to species levels
- Firmicutes (60-80%)
- Bacteroidetes (20-40%)

- Regulates key “host” functions
  - Immunity
  - Nutrient production/availability
  - Energy harvesting

Bäckhed et al., 2005

Obesity and gut microbiota
Obesity, microbiota and microbiome

SUPERORGANISM

90% Microbial cells

Bacterial genome ≥ 100-fold genes

Gut microbiota composition

Bacterial cells density
100 trillion \(10^{14}\)

Archaea
Micro-eukaryotes
Fungi
Protozoa

Virus

10% Human cells

HMP Consortium, 2012; Qin et al., 2010; Ley et al., 2006; Bäckhed et al., 2005
Microbiota in body Habitats

Primary clustering of the microbiota by body area.

- Stomach: $10^2$-$10^3$ cfu/mL
- Duodenum, jejunum: <$10^5$ cfu/mL
- Ileum: $10^3$-$10^7$ cfu/mL
- Large intestine: $10^9$-$10^{12}$ cfu/mL

Gut microbiota composition in healthy adults:

- Stomach: $10^2$-$10^3$ cfu/mL
- Duodenum, jejunum: <$10^5$ cfu/mL
- Ileum: $10^3$-$10^7$ cfu/mL
- Large intestine: $10^9$-$10^{12}$ cfu/mL

**Microbiome**

- **Firmicutes**
  - Bacteroidetes
  - Proteobacteria
  - Actinobacteria
  - Verrucomicrobia
Gut Microbiota and the host

**Metabolic functions**
- Production of vitamins
- Antimicrobial secretion
- Fermentation of non-digestible polysaccharides
  - Production of SCFAs
  - Energy source and energy harvest

**Structural & protective functions**
- Epithelial cell growth and differentiation regulation
- Intestinal villi and crypts development
- TJPs and mucus layer properties regulation
- Colonization resistance

**Immune functions**
- Innate and adaptive immunity activation
  - Inflammatory cytokine regulation
- Immune system development
  - B- and T-cell development

Gut microbiota exerts important functions for health

Villanueva-Millan et al., 2015; Prakash et al., 2011
Which microbes are there?

- 16 S rDNA based pyrosequencing
- RT-PCR

What are these microbes doing?

- Metabolomic analysis

CULTURE- INDEPENDENT MOLECULAR PHYLOGENETIC APPROACHES
Gut Microbiota and metagenomics

Dietary intervention → DNA extraction 
QIamp DNA Stool Mini Kit (Qiagen) → Amplification of 16S rDNA (hypervariable region, V4-V6) → RT-PCR → Gel electrophoresis and band purification (amplicons of ~560 bp)

Data interpretation → Statistical analysis → Taxonomic identification of bacterial species by 454 Roche GS FXL pyrosequencing

Pool each PCR amplicon
Shifts in microbiota species and fermentation products in a dietary model enriched in fat and sucrose

U. Etxeberria¹,², N. Arias³, N. Boqué⁴, M.T. Macarulla³,⁵, M.P. Portillo³,⁵, F.I. Milagro¹,²,⁵ and J.A. Martinez¹,²,⁵*

¹Department of Nutrition, Food Science and Physiology, University of Navarra, C/Irunlarrea s/n, 31008 Pamplona, Spain; ²Centre for Nutrition Research, University of Navarra. Irunlarrea St. E-31008 Pamplona, Spain; ³Nutrition and Obesity group, Department of Nutrition and Food Sciences, Faculty of Pharmacy, University of the Basque Country (UPV/EHU), Paseo de la Universidad 7, 01006 Vitoria, Spain; ⁴Nutrition and Health Research Group. Technological Center of Nutrition and Health (CTNS), TECNIO, CEIC S. Avinguda Universitat, 1, 43204 Reus, Spain; ⁵CIBERobn Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, 28029 Madrid, Spain; jalfmtz@unav.es

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Nutrition and the microbiota

Gut microbiota analysis by 16S rDNA based pyrosequencing

&

Short- chain fatty acid analysis by GC- MS metabolomic analysis

Control diet (Harlan, 2014)
In adaptation period

HFS diet (TD. 06415)

HFS diet intervention 6 weeks

Baseline faeces collection

n= 5

Final faeces collection

In collaboration with University of the Basque Country


<table>
<thead>
<tr>
<th></th>
<th>Control diet (2014)</th>
<th>High-fat sucrose diet (TD. 06415)</th>
</tr>
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<tbody>
<tr>
<td>Energy (Kcal/g)</td>
<td>2.9</td>
<td>4.6</td>
</tr>
<tr>
<td>Protein (Energy %)</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Carbohydrate (Energy %)</td>
<td>67</td>
<td>36</td>
</tr>
<tr>
<td>Starch* (g/kg)</td>
<td>480</td>
<td>85</td>
</tr>
<tr>
<td>Sucrose** (g/kg)</td>
<td>Traces</td>
<td>200</td>
</tr>
<tr>
<td>Fat (Energy %)</td>
<td>13</td>
<td>45</td>
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<tr>
<td>Saturated (%)</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>Monounsaturated (%)</td>
<td>20</td>
<td>47</td>
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<tr>
<td>Polyunsaturated (%)</td>
<td>62</td>
<td>17</td>
</tr>
<tr>
<td>Fiber content*** (g/kg)</td>
<td>221</td>
<td>58</td>
</tr>
</tbody>
</table>

* unrefined starch from wheat and corn in 2014; refined cornstarch in TD.06415
** no added sucrose in 2014; trace amounts of simple sugars from grain ingredients
*** calculated neutral detergent fiber in 2014 & cellulose in TD.06415

n= 5
Control diet (Harlan, 2014)
HFS diet (TD. 06415)

Baseline faeces collection

HFS diet intervention 6 weeks

Final faeces collection

Gut microbiota analysis by 16S rDNA based pyrosequencing

&

Short- chain fatty acid analysis by GC- MS metabolomic analysis

• In collaboration with University of the Basque Country
**Phenotypical-related measurements of male Wistar rats (n=5) fed a HFS diet for 6 weeks.**

(a) Weekly body weight changes of Wistar rats fed a HFS diet (n=5). (b) Weight-related parameters and plasma values after 6 weeks of HFS dietary intervention. All results are expressed as the mean± standard error of the mean. HFS, high-fat sucrose diet; WAT, white adipose tissue.

### Weight-related measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>194±2</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>376±7</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>176±7</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>17.0±0.2</td>
</tr>
<tr>
<td>Visceral WAT (%)</td>
<td>7.58±0.45</td>
</tr>
<tr>
<td>Subcutaneous WAT (%)</td>
<td>3.32±0.24</td>
</tr>
</tbody>
</table>

### Plasma values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose (mg/dL)</td>
<td>119.2±3.5</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>51.5±8.7</td>
</tr>
</tbody>
</table>
Diet Induced Obesity and gut microbiota

Principal component analysis (PCA) generated from 16S rDNA pyrosequencing bacterial taxa from control diet-fed lean rats and HFS diet-fed rats. PCA revealed grouping of samples as a result of the HFS dietary intervention. HFS, high-fat sucrose.
Diet Induced Obesity and gut microbiota

Principal component analysis (PCA) generated from 16S rDNA pyrosequencing bacterial taxa from control diet-fed lean rats and HFS diet-fed rats. Differentially identified bacterial families from faeces samples of control diet-fed lean and HFS diet-fed obese rats. HFS-b, standard diet-fed lean rats (baseline samples); HFS-f, high-fat sucrose diet-fed obese rats (final samples).

Figure 2. Faecal bacterial community at the Phylum level. (a) Relative abundance (% of total 16S rDNA) of significantly differed bacterial divisions (predominant phyla) in the faeces of five Wistar rats before and after the high-fat sucrose diet treatment. (b) Relative abundance (% of total 16S rDNA) of not significantly differed (non- predominant phyla) bacterial divisions in the faeces of five Wistar rats before and after the high-fat sucrose diet treatment. Results are expressed as the mean± SEM. Statistical analyses were performed by Paired- Samples T-Test. Variables presenting probabilities of p< 0.05 were applied the Benjamini-Hochberg multiple testing correction Unadjusted p values lower than the calculated critical values were considered statistically significant (*). HFS, high- fat sucrose.
**Figure 3.** *Firmicutes/Bacteroidetes* ratio in the faeces of five Wistar rats before and after the high-fat sucrose diet treatment. Results are expressed as the mean±SEM. Statistical analyses were performed by Paired Samples T-Test. Variables presenting probabilities of p<0.05 were applied the Benjamini-Hochberg multiple testing correction. Unadjusted p values lower than the calculated critical values were considered statistically significant (*). HFS, high-fat sucrose.
SCFAs in faeces of Wistar rats at baseline and after a 6-week HFS dietary intervention.

<table>
<thead>
<tr>
<th></th>
<th>Standard diet</th>
<th>High-fat sucrose diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (Kcal/g)</strong></td>
<td>2.9</td>
<td>4.6</td>
</tr>
<tr>
<td><strong>Protein (Energy %)</strong></td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td><strong>Carbohydrate (Energy %)</strong></td>
<td>67</td>
<td>36</td>
</tr>
<tr>
<td><strong>Starch (g/kg)</strong>$^1$</td>
<td>480</td>
<td>85</td>
</tr>
<tr>
<td><strong>Sucrose (g/kg)</strong>$^2$</td>
<td>traces</td>
<td>200</td>
</tr>
<tr>
<td><strong>Fat (Energy %)</strong></td>
<td>13</td>
<td>45</td>
</tr>
<tr>
<td><strong>Saturated (%)</strong></td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td><strong>Monounsaturated (%)</strong></td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td><strong>Polyunsaturated (%)</strong></td>
<td>62</td>
<td>17</td>
</tr>
<tr>
<td><strong>Fiber content (g/kg)</strong>$^3$</td>
<td>221</td>
<td>58</td>
</tr>
</tbody>
</table>

Composition of the diets.

$^1$ Unrefined starch from wheat and maize in standard chow diet; refined maize starch in HFS diet.

$^2$ No added sucrose in standard chow diet; trace amounts from grain ingredients.

$^3$ Calculated neutral detergent fibre in standard chow diet and cellulose in HFS diet.

Results are expressed as the mean ± SEM, n=5. P values were assessed using paired samples t-test.

Abbreviations: SCFA, short–chain fatty acid; HFS, high-fat sucrose.
Diet Induced Obesity and gut fermentation

What are these compounds/microbes doing?

• Non-Targeted metabolomic analysis

[Image of a graph showing metabolite production and gut microbiota modifications]
Short chain fatty acids (SCFA) in faeces of Wistar rats (n=5) at baseline and after the 6-week high-fat sucrose diet intervention. (a) Concentrations of SCFA levels detected in lyophilized samples (mg/g dry weight). (b) Absolute amounts of excreted SCFA in 24h (mg dry weight/24h). No statistical differences were observed in faecal moisture content (expressed as %) between baseline (63.2 ± 3.4) and the end of the study (50.6 ± 2.2). Results are expressed as the mean ± standard error of the mean. Statistical analyses were performed using Paired- Samples T-test. *p< 0.05, t< 0.1; HFS, high-fat sucrose.
THERAPEUTIC MODULATION OF GUT MICROBIOTA

INTRODUCTION

• “Living organisms that, when administered in sufficient numbers confer a benefit to the host”

“Classical” ones: Lactic acid bacteria and bifidobacteria

Next generation of probiotics:
- Faecalibacterium prausnitzii
- Clostridia clusters IV, XIVa, XVIII strains
- Akkermansia muciniphila
- Bacteroides uniformis

PREBIOTICS

• “Selectively fermented ingredients that allow specific changes in composition and/or activity of the gut
- Fructooligosaccharides
- Galactooligosaccharides
- Lactulose, inulin

Neef and Sanz, 2013
Obesity and gut microbiota modulation

POLYPHENOLS

OBESITY

WEIGHT LOSS

GUT MICROBIOTA

Wilson Tang et al., 2013
Mendelsohn and Larrick, 2013
Martínez et al., 2013
Obesity and gut microbiota modulation

Polyphehols

Gut microbiota
- Prevotella
- Bacteroides

Wilson Tang et al., 2013
Mendelsohn and Larrick, 2013
Martínez et al., 2013
INTRODUCTION

POLYPHENOLS

Del Rio et al., 2013
Tomé-Carneiro et al., 2013

FLAVONOIDS

Non-Flavonoids

NUMEROUS HEALTH EFFECTS:

- Anti-CVD/Diabetes/Obesity
- Inflammation response inhibition
- Vascular function improvement and oxidative stress reduction
- Apoptosis inhibition
- Metabolic modulation
- Signaling pathways, protein kinases and transcription factors modulation
- Anti-cancer
- Anti-aging

Phenolic acids
Stilbenes
Hydrolizable tannins
Hydroxicinammates
Hydroxibenzoic acids

RESHAPE GUT MICROBIOTA
OF DIET-INDUCED OBESITY??????

Quercitina

Flavonoids

Flavonols
Flavones
Isoflavones
Flavanones

Anthocyanidins
Flavan-3-ols
Dyhidrocalchones

Resveratrol
Impact of Polyphenols and Polyphenol-Rich Dietary Sources on Gut Microbiota Composition

Usune Etxeberria,† Alfredo Fernández-Quintela,*,‡,§ Fermín I. Milagro,†,§ Leixuri Aguirre,‡,§ J. Alfredo Martínez,†,§ and María P. Portillo‡,§

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‡Nutrition and Obesity Group, Department of Nutrition and Food Sciences, Faculty of Pharmacy, University of the Basque Country, Paseo de la Universidad 7, 01006 Vitoria, Spain
§CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, 28029 Madrid, Spain
**Metabolism modulation by Polyphenols**

Table 1. Body weight-related measurements and serum biochemical variables of obese Zucker (fa/fa) rats supplemented or not with pterostilbene.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=9)</th>
<th>PT15 group (n=10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight-related measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>156 ± 2</td>
<td>155 ± 1</td>
<td>ns</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>375 ± 10</td>
<td>340 ± 10</td>
<td>p= 0.024</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>220 ± 9</td>
<td>185 ± 9*</td>
<td>p= 0.017</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>24.7 ± 0.8</td>
<td>22.8 ± 0.2</td>
<td>ns</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue (% BW)</td>
<td>9.1 ± 0.3</td>
<td>6.4 ± 0.8*</td>
<td>p= 0.010</td>
</tr>
<tr>
<td>Visceral adipose tissue (% BW)</td>
<td>4.1 ± 0.1</td>
<td>3.6 ± 0.3</td>
<td>ns</td>
</tr>
<tr>
<td>Total adipose tissue (% BW)</td>
<td><strong>12.8 ± 0.3</strong></td>
<td><strong>10.0 ± 1.1</strong></td>
<td><strong>p= 0.035</strong></td>
</tr>
<tr>
<td>Liver weight (% BW)</td>
<td>4.9 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Serum biochemical variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>139 ± 11</td>
<td>119 ± 8</td>
<td>ns</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>246.6 ± 11.2</td>
<td>184.6 ± 17.9*</td>
<td>p= 0.011</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>967.3 ± 151.3</td>
<td>1119.2 ± 246.8</td>
<td>ns</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>196.6 ± 8.0</td>
<td>192.1 ± 16.3</td>
<td>ns</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>49.9 ± 0.6</td>
<td>48.8 ± 1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>154.1 ± 22.3</td>
<td>131.7 ± 17.4</td>
<td>ns</td>
</tr>
<tr>
<td>Phosphoglycerate kinase (U/L)</td>
<td>96.3 ± 7.0</td>
<td>92.6 ± 14.5</td>
<td>ns</td>
</tr>
</tbody>
</table>

All results are expressed as the mean ± SEM. Statistical analyses were performed using Student’s t-test. The level of probability was set up at p< 0.05 as statistically significant. PT, pterostilbene; BW, body weight.
# Gut Microbiota modulation by Polyphenols

**Table 1. Relative abundance of gut bacterial taxa in obese Zucker (fa/fa) rats supplemented or not with pterostilbene (15 mg/kg BW/day) at baseline and at the end of the 6 week dietary treatment period.**

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Control group (n=9)</th>
<th>PT 15 group (n=10)</th>
<th>( \Delta )</th>
<th>( p \text{ value} )</th>
<th>( \Delta )</th>
<th>( p \text{ value} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phyla</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>0.382 ± 0.029</td>
<td>0.264 ± 0.042</td>
<td>-0.119 ± 0.060</td>
<td>ns</td>
<td>0.497 ± 0.051</td>
<td>0.220 ± 0.026</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>0.373 ± 0.040</td>
<td>0.442 ± 0.051</td>
<td>0.069 ± 0.078</td>
<td>ns</td>
<td>0.417 ± 0.052</td>
<td>0.417 ± 0.029</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>0.003 ± 0.001</td>
<td>0.015 ± 0.006</td>
<td>0.012 ± 0.005</td>
<td>ns</td>
<td>0.003 ± 0.001</td>
<td>0.083 ± 0.027</td>
</tr>
<tr>
<td><strong>Class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridia</td>
<td>0.262 ± 0.018</td>
<td>0.194 ± 0.037</td>
<td>-0.069 ± 0.047</td>
<td>ns</td>
<td>0.385 ± 0.043</td>
<td>0.157 ± 0.019</td>
</tr>
<tr>
<td>Erysipelotrichia</td>
<td>0.018 ± 0.004</td>
<td>0.008 ± 0.002</td>
<td>-0.010 ± 0.005</td>
<td>ns</td>
<td>0.015 ± 0.004</td>
<td>0.010 ± 0.003</td>
</tr>
<tr>
<td>Negativicutes</td>
<td>0.004 ± 0.001</td>
<td>0.002 ± 0.000</td>
<td>-0.003 ± 0.001</td>
<td>0.034</td>
<td>0.004 ± 0.001</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td><strong>Family</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lachnospiraceae</td>
<td>0.150 ± 0.014</td>
<td>0.070 ± 0.015</td>
<td>-0.080 ± 0.021</td>
<td>0.005</td>
<td>0.289 ± 0.053</td>
<td>0.051 ± 0.008</td>
</tr>
<tr>
<td>Verrucomicrobiaceae</td>
<td>0.003 ± 0.001</td>
<td>0.013 ± 0.005</td>
<td>0.011 ± 0.005</td>
<td>ns</td>
<td>0.002 ± 0.001</td>
<td>0.076 ± 0.026</td>
</tr>
<tr>
<td>Rikenellaceae</td>
<td>0.005 ± 0.001</td>
<td>0.008 ± 0.001</td>
<td>0.004 ± 0.002</td>
<td>ns</td>
<td>0.002 ± 0.001</td>
<td>0.008 ± 0.001</td>
</tr>
<tr>
<td>Defluviitaleaceae</td>
<td>0.004 ± 0.001</td>
<td>0.000 ± 0.000</td>
<td>-0.004 ± 0.001</td>
<td>0.009</td>
<td>0.007 ± 0.002</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>Acidaminococcaceae</td>
<td>0.004 ± 0.001</td>
<td>0.002 ± 0.000</td>
<td>-0.003 ± 0.001</td>
<td>0.030</td>
<td>0.003 ± 0.001</td>
<td>0.002 ± 0.000</td>
</tr>
<tr>
<td>Desulfovibrionaceae</td>
<td>0.001 ± 0.000</td>
<td>0.002 ± 0.001</td>
<td>0.001 ± 0.000</td>
<td>0.035</td>
<td>0.002 ± 0.000</td>
<td>0.002 ± 0.000</td>
</tr>
<tr>
<td>Erysipelotrichaceae</td>
<td>0.018 ± 0.004</td>
<td>0.008 ± 0.002</td>
<td>-0.010 ± 0.005</td>
<td>ns</td>
<td>0.016 ± 0.005</td>
<td>0.011 ± 0.004</td>
</tr>
<tr>
<td>Christensenellaceae</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>ns</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
</tbody>
</table>
Table 1. Relative abundance of genera and identified gut bacterial species in obese Zucker (fa/fa) rats supplemented or not with pterostilbene (15 mg/kg BW/day) at baseline and at the end of the 6 week dietary treatment period.

<table>
<thead>
<tr>
<th>Genus level Bacterial species</th>
<th>Baseline</th>
<th>After treatment</th>
<th>∆</th>
<th>Unadjusted p value</th>
<th>Baseline</th>
<th>After treatment</th>
<th>∆</th>
<th>Unadjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erysipelotrichaceae</td>
<td>UI</td>
<td>0.002 ± 0.000</td>
<td>0.001 ± 0.000 -0.001±0.001 0.050</td>
<td>0.001 ± 0.000 0.000±0.001 ns 0.045</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lachnospiraceae inc. sed.</td>
<td>UI</td>
<td>0.010 ± 0.003</td>
<td>0.005 ± 0.002 -0.005 ± 0.003 ns 0.010 ± 0.004 0.003±0.001 -0.008 ± 0.003 0.050 0.040</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allobaculum</td>
<td>UI</td>
<td>0.000 ± 0.000</td>
<td>0.004 ± 0.002 0.004 ± 0.002 0.043</td>
<td>0.000 ± 0.000 0.006 ± 0.003 0.006 ± 0.003 ns ns ns</td>
<td></td>
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<tr>
<td>Blautia</td>
<td>UI</td>
<td>0.013 ± 0.002</td>
<td>0.001 ± 0.000 -0.012 ± 0.002 0.001 0.024 ± 0.006 0.002 ± 0.001 -0.022 ± 0.006 0.007 0.000</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Paraprevotella</td>
<td>UI</td>
<td>0.002 ± 0.001</td>
<td>0.000 ± 0.000 -0.002 ± 0.001 0.012</td>
<td>0.002 ± 0.001 0.000 ± 0.000 -0.002 ± 0.001 ns ns ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerostipes</td>
<td>UI</td>
<td>0.003 ± 0.001</td>
<td>0.001 ± 0.001 -0.002 ± 0.001 0.021</td>
<td>0.002 ± 0.001 0.000 ± 0.000 -0.002 ± 0.001 ns ns ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desulfovibrio</td>
<td>UI</td>
<td>0.001 ± 0.000</td>
<td>0.001 ± 0.000 0.001 ± 0.000 0.050</td>
<td>0.002 ± 0.000 0.000 ± 0.000 0.002 ± 0.000 0.000 ± 0.001 ns ns ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oscillibacter</td>
<td>UI</td>
<td>0.001 ± 0.000</td>
<td>0.001 ± 0.001 0.001 ± 0.001 0.050</td>
<td>0.002 ± 0.000 0.001 ± 0.000 0.002 ± 0.000 0.000 ± 0.001 ns ns ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinimonas</td>
<td>UI</td>
<td>0.002 ± 0.001</td>
<td>0.006 ± 0.002 0.004 ± 0.002 0.021</td>
<td>0.002 ± 0.001 0.000 ± 0.000 0.006 ± 0.002 0.005 ± 0.002 0.019 0.032</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasporebacterium</td>
<td>UI</td>
<td>0.002 ± 0.000</td>
<td>0.002 ± 0.001 0.000 ± 0.001 0.003</td>
<td>0.003 ± 0.000 0.002 ± 0.000 -0.001±0.000 0.003 0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alistipes Alistipes shahii</td>
<td>UI</td>
<td>0.004 ± 0.001</td>
<td>0.007 ± 0.001 0.003 ± 0.001 0.044</td>
<td>0.002 ± 0.001 0.008 ± 0.001 0.006 ± 0.001 &lt;0.001*** ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deflavitaleaceae Clostridium sp</td>
<td>UI</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000 0.000 ± 0.000 0.001</td>
<td>0.001 ± 0.000 0.000 ± 0.000 -0.001±0.000 0.010 ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turicibacter Turicibacter sp.</td>
<td>UI</td>
<td>0.009 ± 0.003</td>
<td>0.002 ± 0.001 -0.007 ± 0.003 0.035</td>
<td>0.006 ± 0.001 0.001 ± 0.000 -0.005 ± 0.001 0.008 ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phascolarctobacterium m succinatutens</td>
<td>UI</td>
<td>0.004 ± 0.001</td>
<td>0.002 ± 0.000 -0.002 ± 0.001 0.041</td>
<td>0.004 ± 0.001 0.002 ± 0.001 -0.002 ± 0.001 0.046 ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odoribacter Odoribacter splanchnicus</td>
<td>UI</td>
<td>0.001 ± 0.000</td>
<td>0.001 ± 0.000 0.000 ± 0.000 0.002</td>
<td>0.000 ± 0.000 0.002 ± 0.000 0.002 ± 0.000 0.002 ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudoflavonifractor Pseudoflavonifractor plautii</td>
<td>UI</td>
<td>0.001 ± 0.000</td>
<td>0.002 ± 0.000 0.001 ± 0.001 0.012</td>
<td>0.001 ± 0.000 0.002 ± 0.000 0.001 ± 0.000 0.023 ns</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Figure 1. Relative abundance percentage of bacterial species at baseline and after the 6-week dietary treatment. A) Relative abundance of *Alistipes shahii*, B) Relative abundance of *Akkermansia muciniphila*. Results are expressed as mean ± SEM. Comparisons between the relative abundance percentage of bacterial species at baseline and at the end of the study in each group were analysed by paired Student’s t-test and corrected with Benjamini-Hochberg procedure (**p ≤ 0.001**). PT, pterostilbene.
Figure 2. Correlations between the bacterial abundance detected in faecal samples of Zucker (fa/fa) rats treated with pterostilbene for 6 weeks and host variables. (A) Visceral adipose tissue percentage and the relative abundance of Verrucomicrobiaceae phylum. (B) Serum cholesterol levels and the relative abundance of Verrucomicrobiaceae phylum. Inserts correspond to Pearson’s correlation and the p value.
Reshaping faecal gut microbiota composition by the intake of trans-resveratrol and quercetin in high-fat sucrose diet-fed rats

U. Etxeberria¹,², N. Arias³,⁵, N. Boqué⁴, M.T. Macarulla³,⁵, M.P. Portillo³,⁵, J.A. Martínez¹,²,⁵,*, F.I. Milagro¹,²,⁵

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⁴Nutrition and Health Research Group, Technological Center of Nutrition and Health (CTINS), TECNIO, CEBIC S. Avinguda Universitat 1, 43204 Reus, Spain
⁵Physiopathology of Obesity and Nutrition, CIBERobn, Carlos III Health Research Institute, 28029 Madrid, Spain

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Polyphenols and fecal gut microbiota modulation

Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; BW, body weight; IL-18, interleukin 18, TNFα, tumor necrosis factor α; TLR, toll-like receptor; TJP, tight junction protein; MyD88, myeloid differentiation primary response 88; NFκβ, nuclear factor kappa β; LPS, lipopolysaccharide; LBP, LPS binding protein; NKGd2, natural killer; Ocln, occludin.
Principal Coordinate analysis (PCoA) graph showing faecal gut microbiota composition changes in HFS diet-fed rats supplemented or not with trans-resveratrol, quercetin or the combination of both polyphenols.

The distance measure was conducted by Sorensen method using Bray Curtis statistic (Courtesy of Dr. Sébastien Matamoros). Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin.

Etxeberria U et al. J Nutr Biochem, 2015; 26: 651-660
What are these microbes doing?

- Metabolomic analysis
  - Microbial metabolites

Microbial fermentation induced by polyphenols

**SCFA levels**

Short chain fatty acid (SCFA) concentrations in faeces of Wistar rats fed a HFS- diet and treated or not with polyphenols. Results are expressed as the mean± SEM. HFS, high-fat sucrose diet; RSV15, resveratrol (15 mg/kg BW/day); Q30, quercetin (30 mg/kg BW/day); RSV15+ Q30, a mix of resveratrol (15 mg/kg BW/day) and quercetin (30 mg/kg BW/day).
Gut metabolome modulation by polyphenols

Food Funct, (2015); 6: 2758-2767
doi: 10.1039/c5fo00473j

Metabolic faecal fingerprinting of \textit{trans}-resveratrol and quercetin following a high-fat sucrose dietary model using liquid chromatography coupled to high-resolution mass spectrometry

Usune Etxeberria,\textsuperscript{a,b} Noemi Arias,\textsuperscript{c,d} Noemí Boqué,\textsuperscript{e} Ana Romo-Hualde,\textsuperscript{b,d} M. Teresa Macarulla,\textsuperscript{c,d} María P. Portillo,\textsuperscript{c,d} Fermín I. Milagro\textsuperscript{a,b,d} and J. Alfredo Martínez\textsuperscript{*a,b,d}
Gut metabolome modulation by polyphenols

Wistar rats n=24

HFS diet

6-week dietary intervention

RSV n=6

RSV+Q n=6

Q n=6

15 mg/kg BW/day
Resveratrol

30 mg/kg BW/day
Quercetin

30 mg/kg BW/day
Resveratrol + Quercetin

HFS diet

Final faeces collection

1. Non-targeted metabolomic analysis

UHPLC-(ESI)-HRMS analysis

2. Serum measurements

Glucose
Insulin

Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; BW, body weight; UHPLC-(ESI)-MS, ultra-high performance liquid chromatography coupled to mass spectrometry.

Etxeberria U et al., Food Funct, 2015; 6: 2758-2767
Principal Component Analysis (PCA) graphs showing faecal metabolomic alterations in HFS diet-fed rats supplemented or not with trans-resveratrol, quercetin or the combination of both polyphenols.

Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; PCA, principal component analysis; EV, explained variability; ESI, electrospray ionization.
Correlations between the number of bacteria (taxa frequencies detected in faecal samples) and putative metabolites detected in ESI negative mode.

Putative metabolites detected in *trans*-resveratrol treated groups and *Clostridium* species levels. Abbreviations: HFS, high-fat sucrose; RSV, *trans*-resveratrol; Q, quercetin; RSV+Q, *trans*-resveratrol+quercetin.
Correlations between the number of bacteria (taxa frequencies detected in faecal samples) and putative metabolites detected in ESI positive mode.

Putative metabolites detected in quercetin treated groups and Clostridium species levels.
Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin.

Chapter 6- Results

Etxeberria U et al., Food Funct, 2015; 6: 2758-2767
Recent attention

Gut microbiota “new organ” within our organism

- $10^{11}$- $10^{12}$ cells/mL in colon
- ≥ 150 times more genes than the human genome

<table>
<thead>
<tr>
<th>Firmicutes</th>
<th>Bacteroidetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(60-80%)</td>
<td>(20-40%)</td>
</tr>
</tbody>
</table>

- Regulates key “host” functions
  - Immunity
  - Nutrient production/availability
  - Energy harvesting

Bäckhed et al., 2005
INTRODUCTION
Adapted from A.W. Walker et al., 2012

**DISBIOSYS** (Bacterial imbalance in the gut)
- Diversity reduced
- Elevated *Enterobacteriaceae* / opportunistic pathogens
- Skewed SCFA profile
- Disruption of mucosal barrier
- Host response inflammatory response initiated

**HEALTH**
- Diverse and abundant microbiota
- *Firmicutes, Bacteroidetes* and *Actinobacteria* dominant
- Healthy levels of SCFA production
- Intact mucosal barrier
- No overt inflammation

**THERAPEUTIC MODULATION**
Adapted from A.W. Walker et al., 2012
Gut Microbiota, Metagenomics and Metabolomics

CULTURABLE

Gut microbiota

Omics based approaches

Which microbes are there?
16 S gene sequencing

What are these microbes doing?
Metabolomics

Marcobal et al., 2013; Lamendella et al., 2012; Moco et al., 2012; Ley et al., 2005
Abbreviations: T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; IBD, inflammatory bowel disease; IBS, inflammatory bowel syndrome; CRC, colorectal cancer
METABOLIC DERANGEMENT

- Increased intestinal permeability
- Bacterial product and whole bacteria translocation
- Trigger immune response, inflammation, and immune cell infiltration (liver and AT)
- Induce food intake deregulation in the hypothalamus, and fat and glucose metabolism deregulation

Tsukumo et al., 2009
1. Modulation of inflammation and immune system
   - Lipopolysaccharide (Endotoxemia)

2. Regulate energy extraction and storage
   - Modulation of Fasting induced adipose factor (Fiaf), which inhibits the activity of lipoprotein lipase and thus, lipid storage.

3. Regulation of energy expenditure
   - Gut microbiota inhibits phosphorylation of in muscle and thus, fatty acid utilization.

Cani et al., 2007; Bäckhed et al., 2006; Bäckhed et al., 2004
Bioactive compounds

Resveratrol  Quercetin

Nutrition and Gut Microbiota

Abbreviations: LPL, lipoprotein lipase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; SREBP-1, sterol regulatory element-binding protein; PDE, phosphodiesterase; cAMP, cyclic adenosine monophosphate; PPAR, peroxisome proliferator–activated receptor; UCP, uncoupling protein; ACO, acyl CoA oxidase; CPT, carnitine palmitoyltransferase; BAT, brown adipose tissue; WAT, white adipose tissue.

Aguirre et al., 2014; del Rio et al., 2013; Aguirre et al., 2011
Manipulation of the gut microbiota

GUT MICROBIOTA

DIET (Polyphenols)

ANTIMICROBIALS

PREBIOTICS, PROBIOTICS & SYMBIOTICS

FAECAL MICROBIOTA TRANSPLANT

Scott et al., 2015; Aguirre et al., 2015

Non-obese microbiota vs Obese microbiota

Precision Nutrition and Gut Microbiota
Fecal transplants and Gut Microbiota

Administration of a faecal solution from a donor into the GI tract of a recipient

Figure 1. Disorders associated with alterations to the intestinal microbiota that could be treated by FMT. Green indicates disorders for which FMT has shown efficacy in randomized controlled trials (RCT), blue indicates disorders for which FMT has shown efficacy in case series studies, and black indicates disorders that have been associated with disruption of the intestinal microbiota.

Green: beneficial effect FMT in RCT
Blue: beneficial effect FMT in case series
Black: association between gut microbiota and disease from experimental/observational studies
Precision Nutrition and Gut Microbiota

Personalized Nutrition by prediction of metabolic responses.

Integration of Omics

- Metabolomics
- Proteomics
- Transcriptomics
- Genomics

Measure personal features for 800 people
Predict personal glycemic responses

Design personalized diet to lower glycemic responses

Vamanala et al., 2015; Zeevi et al., 2015
ACKNOWLEDGEMENTS
Reshaping faecal gut microbiota composition by the intake of trans-resveratrol and quercetin in high-fat sucrose diet-fed rats

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\textsuperscript{d}Nutrition and Health Research Group, Technological Center of Nutrition and Health (CTNS), TECNIO, CIBIC S. Avinguda Universitat 1, 43204 Reus, Spain
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INTRODUCTION

POLYPHENOLS

Del Rio et al., 2013
Tomé-Carneiro et al., 2013

FLAVONOIDS

NON-FLAVONOIDS

FLAVONOLS

Stilbenes

Phenolic acids

Hydrolizable tannins

Hydroxicinammates

Hydroxibenzoic acids

NUMEROUS HEALTH EFFECTS:

- Anti-CVD/Diabetes/Obesity
- Inflammation response inhibition
- Vascular function improvement and oxidative stress reduction
- Apoptosis inhibition
- Metabolic modulation
- Signaling pathways, protein kinases and transcription factors modulation
- Anti-cancer
- Anti-aging

RESHAPE GUT MICROBIOTA OF DIET-INDUCED OBESITY

Gut Microbiota modulation by Polyphenols

Del Rio et al., 2013
Tomé-Carneiro et al., 2013

Phenolic acids

Hydrolizable tannins

Hydroxicinammates

Hydroxibenzoic acids
Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; BW, body weight; IL-18, interleukin 18, TNFα, tumor necrosis factor α; TLR, toll-like receptor; TJP, tight junction protein; MyD88, myeloid differentiation primary response 88; NFKβ, nuclear factor kappa β; LPS, lipopolysaccharide; LBP, LPS binding protein; NKGd2, natural killer; OcI, occludin.

Etxeberria U et al. J Nutr Biochem, 2015; 26: 651-660
Figure 7. Phenotypical-related measurements of male Wistar rats fed a high-fat sucrose diet (HFS; n=6) supplemented or not with resveratrol (RSV, 15 mg/kg BW/day; n=6), quercetin (Q, 30 mg/kg BW/day; n=6) and a mix of resveratrol and quercetin (RSV+Q, 15 mg and 30 mg/kg BW/day; n=6) for 6 weeks. Weekly body weight changes of Wistar rats are represented. Results are expressed as the mean± SEM. Statistical analyses were performed using One-Way Anova followed by Newman-Keuls post-hoc test. A probability of p<0.05 was set up for determining statistically significant differences.
Polyphenols and metabolism modulation

**Results**

Body weight gain of Wistar rats fed a high-fat sucrose diet (HFS; n=6) supplemented or not with resveratrol (RSV, 15 mg/kg BW/day; n=6), quercetin (Q, 30 mg/kg BW/day; n=6) and a mix of resveratrol and quercetin (RSV+Q, 15 mg and 30 mg/kg BW/day; n=6) for 6 weeks. Results are expressed as the mean± SEM. Statistical analyses were performed using One-Way Anova followed by Newman-Keuls post-hoc test. A probability of p<0.05 was set up for determining statistically significant differences.
Polyphenols and metabolism modulation

Body weight-related measurements and serum biochemical parameters of animals fed with a HFS diet supplemented or not with trans-resveratrol and quercetin for 6 weeks.

<table>
<thead>
<tr>
<th></th>
<th>HFS (n=5)</th>
<th>RSV (n=6)</th>
<th>Q (n=6)</th>
<th>RSV + Q (n=6)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body- weight measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>194 ± 2</td>
<td>193 ± 1</td>
<td>191 ± 2</td>
<td>201 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>176 ± 7</td>
<td>169 ± 6</td>
<td>162 ± 7</td>
<td>144 ± 11</td>
<td>p= 0.070</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>17.0 ± 0.2</td>
<td>16.6 ± 0.4</td>
<td>17.0 ± 0.8</td>
<td>15.8 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Food efficiency (g/Kcal)</td>
<td>5.3 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>p= 0.076</td>
</tr>
<tr>
<td>Liver weight (% BW)</td>
<td>2.7 ± 0.1</td>
<td>2.7 ± 0.0</td>
<td>2.6 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>p= 0.056</td>
</tr>
<tr>
<td><strong>Serum biochemical variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>119 ± 4</td>
<td>105 ± 4</td>
<td>107 ± 3</td>
<td>110 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>51.5 ± 8.7^a</td>
<td>24.3 ± 4.8^b</td>
<td>24.6 ± 7.0^b</td>
<td>27.4 ± 2.6^b</td>
<td>p&lt; 0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>15.2 ± 2.6^a</td>
<td>6.0 ± 1.6^b</td>
<td>6.5 ± 1.9^b</td>
<td>8.3 ± 0.8^ab</td>
<td>p&lt; 0.05</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SEM, n=5/6. P values were assessed using one-way ANOVA followed by Dunnett post hoc test. Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; HOMA-IR, homeostasis model assessment of insulin resistance.
Faecal bacterial community representation at the Phylum level. Relative abundance (% of total 16S rDNA) of all detected bacterial divisions in the faeces of Wistar rats fed the high-fat sucrose diet (HFS; n=6) supplemented or not with resveratrol (RSV, 15 mg/kg BW/day; n=6), quercetin (Q, 30 mg/kg BW/ day; n=6) and a mix of resveratrol and quercetin (RSV+Q, 15 mg and 30 mg/kg BW/day; n=6) for 6 weeks.
Figure 10. Firmicutes/Bacteroidetes ratio in the faeces of Wistar rats fed the HFS- diet supplemented or not with polyphenols. Results are expressed as the mean± SEM. Statistical analyses were performed by Paired- Samples T-Test. Variables presenting probabilities of p< 0.05 were applied the Benjamini-Hochberg multiple testing correction Unadjusted p values lower than the calculated critical values were considered statistically significant (*). HFS, high-fat sucrose diet; RSV15, resveratrol (15 mg/ kg BW/day); Q30, quercetin (30 mg/ kg BW/ day); RSV15+ Q30, a mix of resveratrol (15 mg/kg BW/ day) and quercetin (30 mg/kg BW/ day).
Relative abundance (% of total 16S rDNA) of bacterial species significantly different from the HFS diet-fed control rats.

Results are expressed as the mean ± SEM, n=5/6. P values were assessed using Kruskal–Wallis followed by Mann-Whitney U test and p values corrected by Bonferroni test, *p < 0.05 HFS vs RSV; $ p < 0.05 HFS vs Q; # p < 0.05 HFS vs RSV+Q.

Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin.
Relative abundance (\% of total 16S rDNA) of *Akkermansia muciniphila* ATCC BAA-835 in faeces of HFS diet-fed rats supplemented or not with *trans*-resveratrol and quercetin for 6 weeks.

Results are expressed as the mean ± SEM, n=5/6. *P* values were assessed using Kruskal–Wallis followed by Mann-Whitney *U* test and *p* values corrected by Bonferroni test. Abbreviations: HFS, high-fat sucrose; RSV, *trans*-resveratrol; Q, quercetin; RSV+Q, *trans*-resveratrol+quercetin; T2DM, type 2 Diabetes Mellitus.
Principal Coordinate analysis (PCoA) graph showing faecal gut microbiota composition changes in HFS diet-fed rats supplemented or not with trans-resveratrol, quercetin or the combination of both polyphenols.

The distance measure was conducted by Sorensen method using Bray Curtis statistic (Courtesy of Dr. Sébastien Matamoros).

Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin.
Gut inflammation modulation by polyphenols

Relative mRNA expression of inflammation-related genes in colonic mucosa.

Results are expressed as the mean ± SEM, n=5/6. P values were assessed using Kruskal–Wallis followed by Mann-Whitney U test and p values corrected by Bonferroni test. * p< 0.05 HFS vs RSV; $ p< 0.05 HFS vs Q; # p< 0.05 HFS vs RSV+Q.
Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; ; IL-18, interleukin 18, TNFα, tumor necrosis factor α; TLR, toll-like receptor; MyD88, myeloid differentiation primary response 88; NFκβ, nuclear factor kappa β; LBP, lipopolysaccharide binding protein; NKGd2, natural killer.

Etxeberria U et al. J Nutr Biochem, 2015; 26: 651-660
Relative mRNA expression of tight-junction proteins and occluding in colon.

Results are expressed as the mean ± SEM, n=5/6. *p values were assessed using Kruskal–Wallis followed by Mann-Whitney U test and p values corrected by Bonferroni test. *p<0.05 HFS vs RSV; #p<0.05 HFS vs RSV+Q.

Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; TJP, tight-junction protein; Ocln, occludin.
What are these microbes doing?

- Non-Targeted metabolomic analysis
Short chain fatty acid (SCFA) concentrations in faeces of Wistar rats fed a HFS- diet and treated or not with polyphenols. Results are expressed as the mean±SEM. HFS, high-fat sucrose diet; RSV15, resveratrol (15 mg/kg BW/day); Q30, quercetin (30 mg/kg BW/day); RSV15+Q30, a mix of resveratrol (15 mg/kg BW/day) and quercetin (30 mg/kg BW/day).
Quercetin supplementation

Impact of polyphenols on gut microbiota dysbiosis

GUT MICROBIOTA COMPOSITION

Quercetin significantly altered gut microbiota composition

RSV  No relevant effects  Qiao et al., 2014

RSV+Q  Effects of Q were lessened

Quercetin led to a healthier gut microbial profile

Firmicutes/Bacteroidetes ratio
Erysipelotrichaceae
Eubacterium cylindroides
Akkermansia muciniphila

Ahnê et al., 2014

Abbreviations: RSV, trans-resveratrol; RSV+Q, trans-resveratrol+quercetin.
Gut metabolome modulation by polyphenols

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Metabolic faecal fingerprinting of trans-resveratrol and quercetin following a high-fat sucrose dietary model using liquid chromatography coupled to high-resolution mass spectrometry†

Usune Etxeberria,a,b Noemi Arias,c,d Noemí Boqué,e Ana Romo-Hualde,b,d M. Teresa Macarulla,c,d María P. Portillo,c,d Fermín I. Milagro,a,b,d and J. Alfredo Martínez*a,b,d
Gut metabolome modulation by polyphenols

Wistar rats n=24

HFS n=6

RSV n=6

Q n=6

RSV+Q n=6

HFS diet

15 mg/kg BW/day

6 week dietary intervention

During intervention

HFS diet

Sacrifice

Final faeces collection

1. UHPLC-(ESI)-HRMS analysis
   Non-targeted metabolomic analysis

2. Serum measurements
   Glucose
   Insulin

Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin; BW, body weight; UHPLC-(ESI)-MS, ultra-high performance liquid chromatography coupled to mass spectrometry.

Etxeberria U et al., Food Funct, 2015; 6: 2758-2767
Gut metabolome modulation by polyphenols

Principal Component Analysis (PCA) graphs showing faecal metabolomic alterations in HFS diet-fed rats supplemented or not with *trans*-resveratrol, quercetin or the combination of both polyphenols.

**Abbreviations:** HFS, high-fat sucrose; RSV, *trans*-resveratrol; Q, quercetin; RSV+Q, *trans*-resveratrol+quercetin; PCA, principal component analysis; EV, explained variability; ESI, electrospray ionization.

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ESI+ mode
PCA in ESI+ mode with an Evp 79.76% and PCA in ESI- mode with and Evn 83.82%.

---

ESI- mode

**ESI+ mode**
PCA in ESI+ mode with an Evp 79.76% and PCA in ESI- mode with and Evn 83.82%.

**Abbreviations:** HFS, high-fat sucrose; RSV, *trans*-resveratrol; Q, quercetin; RSV+Q, *trans*-resveratrol+quercetin; PCA, principal component analysis; EV, explained variability; ESI, electrospray ionization.

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**ESI- mode**

Etxeberria U *et al.*, Food Funct, 2015; 6: 2758-2767
Host-microbiota interactions

Correlations between the number of bacteria (taxa frequencies detected in faecal samples) and putative metabolites detected in ESI negative mode.

Putative metabolites detected in trans-resveratrol treated groups and Clostridium species levels. Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin.

Etxeberria U et al., Food Funct, 2015; 6: 2758-2767
Correlations between the number of bacteria (taxa frequencies detected in faecal samples) and putative metabolites detected in ESI positive mode.

Putative metabolites detected in quercetin treated groups and Clostridium species levels. Abbreviations: HFS, high-fat sucrose; RSV, trans-resveratrol; Q, quercetin; RSV+Q, trans-resveratrol+quercetin.
Non-targeted metabolomics in faeces

Identified marked *metabolome alterations* that were strong enough to *categorise* experimental groups into differentiated clusters

Animals supplemented with *trans-resveratrol* or *quercetin* were characterized by a *different* set of faecal compounds

Detected candidate *metabolites correlated* with specific *bacterial species*

Etxeberria U *et al.*, Food Funct, 2015; 6: 2758-2767
Aguirre et al., 2014; del Rio et al., 2013; Aguirre et al., 2011

Bioactive compounds

Resveratrol Quercetin

Nutrition and Gut Microbiota

Abbreviations: LPL, lipoprotein lipase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; SREBP-1, sterol regulatory element-binding protein; PDE, phosphodiesterase; cAMP, cyclic adenosine monophosphate; PPAR, peroxisome proliferator –activated receptor; UCP, uncoupling protein; ACO, acyl CoA oxidase; CPT, carnitine palmitoyltransferase; BAT, brown adipose tissue; WAT, white adipose tissue.
CONCLUSIONS

2. Quercetin supplementation to obese rats, relevantlycantly reversed high-fat sucrose diet-induced gut microbiota dysbiosis.

3. Neither resveratrol supplementation alone, nor the combination of resveratrol and quercetin significantly modified gut microbial ecosystem in obese animals.

GUT MICROBIAL FERMENTATION PRODUCTS

1. Significantly lower daily amounts of short chain fatty acids (acetate, propionate and butyrate) were excreted in faeces after a 6 week high-fat sucrose dietary treatment.

2. Supplementation with polyphenols did not produce statistically significant changes in the production of short chain fatty acids when compared to the high-fat sucrose diet fed control rats.
METABOLIC DERANGEMENT

- Increased intestinal permeability
- Bacterial product and whole bacteria translocation
- Trigger immune response, inflammation, and immune cell infiltration (liver and AT)
- Induce food intake deregulation in the hypothalamus, and fat and glucose metabolism deregulation

Tsukumo et al., 2009

Obesity and gut microbiota
Obesity epidemic

**WORLDWIDE PREVALENCE**

<table>
<thead>
<tr>
<th>Year</th>
<th>1998</th>
<th>2008</th>
<th>2030</th>
</tr>
</thead>
<tbody>
<tr>
<td>% OBESE ADULTS BMI (≥ 30.0 kg/m²)</td>
<td>5% Men, 8% Women</td>
<td>10% Men, 14% Women</td>
<td></td>
</tr>
</tbody>
</table>

Mean BMI 0.4 kg/m²

**PREVALENCE IN SPAIN**

<table>
<thead>
<tr>
<th>Year</th>
<th>1998</th>
<th>2008</th>
<th>2030</th>
</tr>
</thead>
<tbody>
<tr>
<td>% OBESE ADULTS BMI (≥ 30.0 kg/m²)</td>
<td>11% total</td>
<td>15.6% total</td>
<td></td>
</tr>
</tbody>
</table>

Source: Finucane et al., 2011

http://apps.who.int/bmi/index.jsp