

CATANIA  
3-4 OTTOBRE  
2014



# OBESITÀ E DIABETE

## Microbiota e suscettibilità al diabete



Gemelli

Andrea Giaccari

Patologia  
Endocrino-  
Metabolica

# Our Microbiome: A Hot Topic



2008



2010

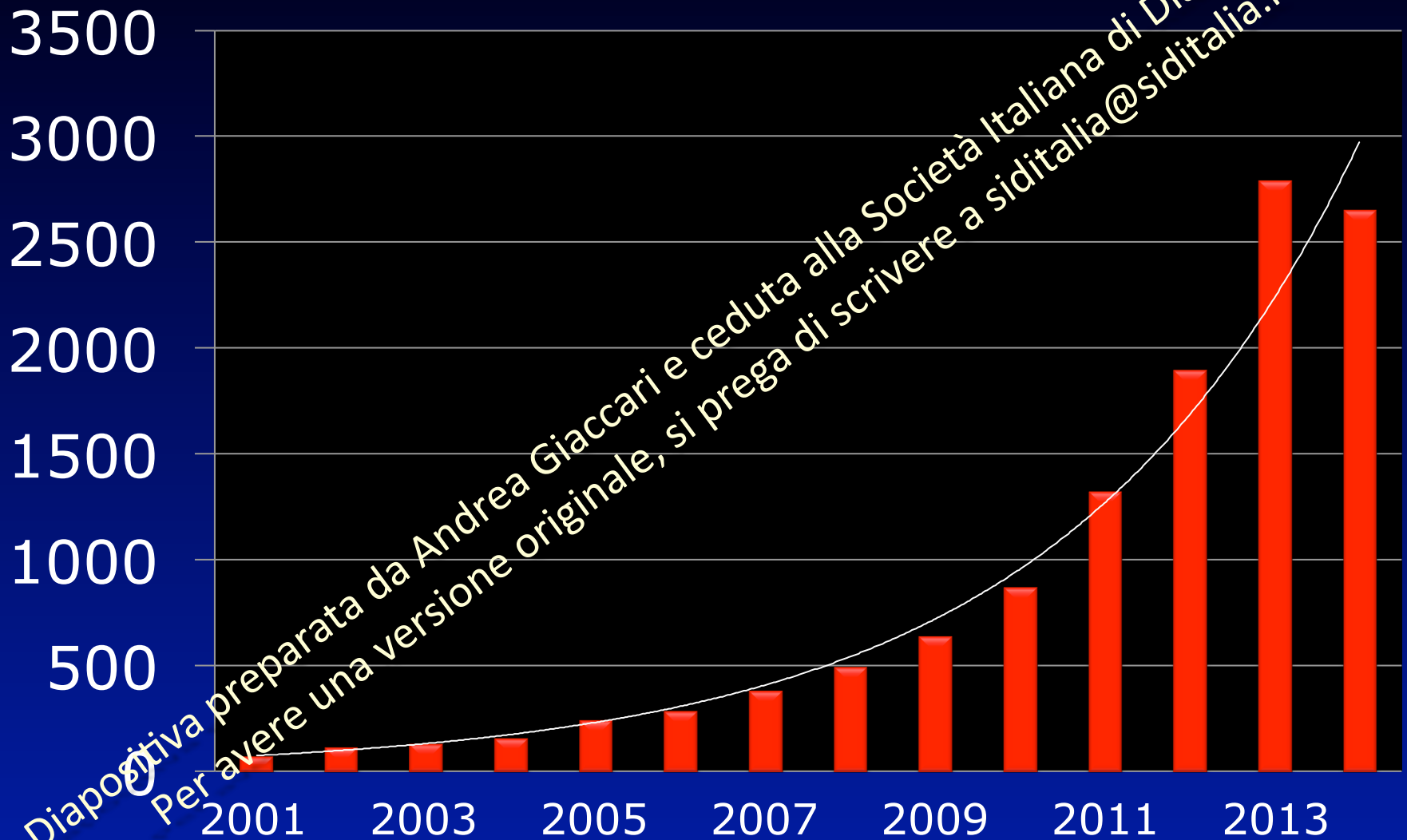


2012

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# PubMed items on «microbiota»



# microbiota, growing evidences (?)

## ARTICLE

doi:10.1038/nature13793

# Artificial sweeteners induce glucose intolerance by altering the gut microbiota

Jotham Suez<sup>1</sup>, Tal Korem<sup>2\*</sup>, David Zeevi<sup>2\*</sup>, Gili Zilberman-Schapira<sup>1\*</sup>, Christoph A. Thaiss<sup>1</sup>, Ori Maza<sup>1</sup>, David Israeli<sup>3</sup>, Niv Zmora<sup>4,5,6</sup>, Shlomit Gilad<sup>7</sup>, Adina Weinberger<sup>2</sup>, Yael Kuperman<sup>8</sup>, Alon Harmelin<sup>8</sup>, Ilana Kolodkin-Gal<sup>9</sup>, Hagit Shapiro<sup>1</sup>, Zamir Halpern<sup>5,6</sup>, Eran Segal<sup>2</sup> & Eran Elinav<sup>1</sup>

Non-caloric artificial sweeteners (NAS) are among the most widely used food additives worldwide, regularly consumed by lean and obese individuals alike. NAS consumption is considered safe and beneficial owing to their low caloric content, yet supporting scientific data remain sparse and controversial. Here we demonstrate that consumption of commonly used NAS formulations drives the development of glucose intolerance through induction of compositional and functional alterations to the intestinal microbiota. These NAS-mediated deleterious metabolic effects are abrogated by antibiotic treatment, and are fully transferrable to germ-free mice upon faecal transplantation of microbiota configurations from NAS-consuming mice, or of microbiota anaerobically incubated in the presence of NAS. We identify NAS-altered microbial metabolic pathways that are linked to host susceptibility to metabolic disease, and demonstrate similar NAS-induced dysbiosis and glucose intolerance in healthy human subjects. Collectively, our results link NAS consumption, dysbiosis and metabolic abnormalities, thereby calling for a reassessment of massive NAS usage.

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- lo scenario del microbiota
- come il nostro organismo può cambiare il microbiota
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- microbiota e obesità
- microbiota e diabete
- alcune considerazioni

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# *Human Gut Bacteriome*

## the second genome of the human body

However, most people share:

1. A core microbiota that comprises 50-100 bacterial species
2. A core microbiome harboring more than 6000 functional gene groups

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# Human Gut Bacteriome

**1.5 kg of bacteria, >3.300.000 genes**

**8 bacterial divisions (superkingdoms)**  
**95% genes identity**

**>1100 species**

**98% genes identity**

**>15000 strains**

**100% genes identity**

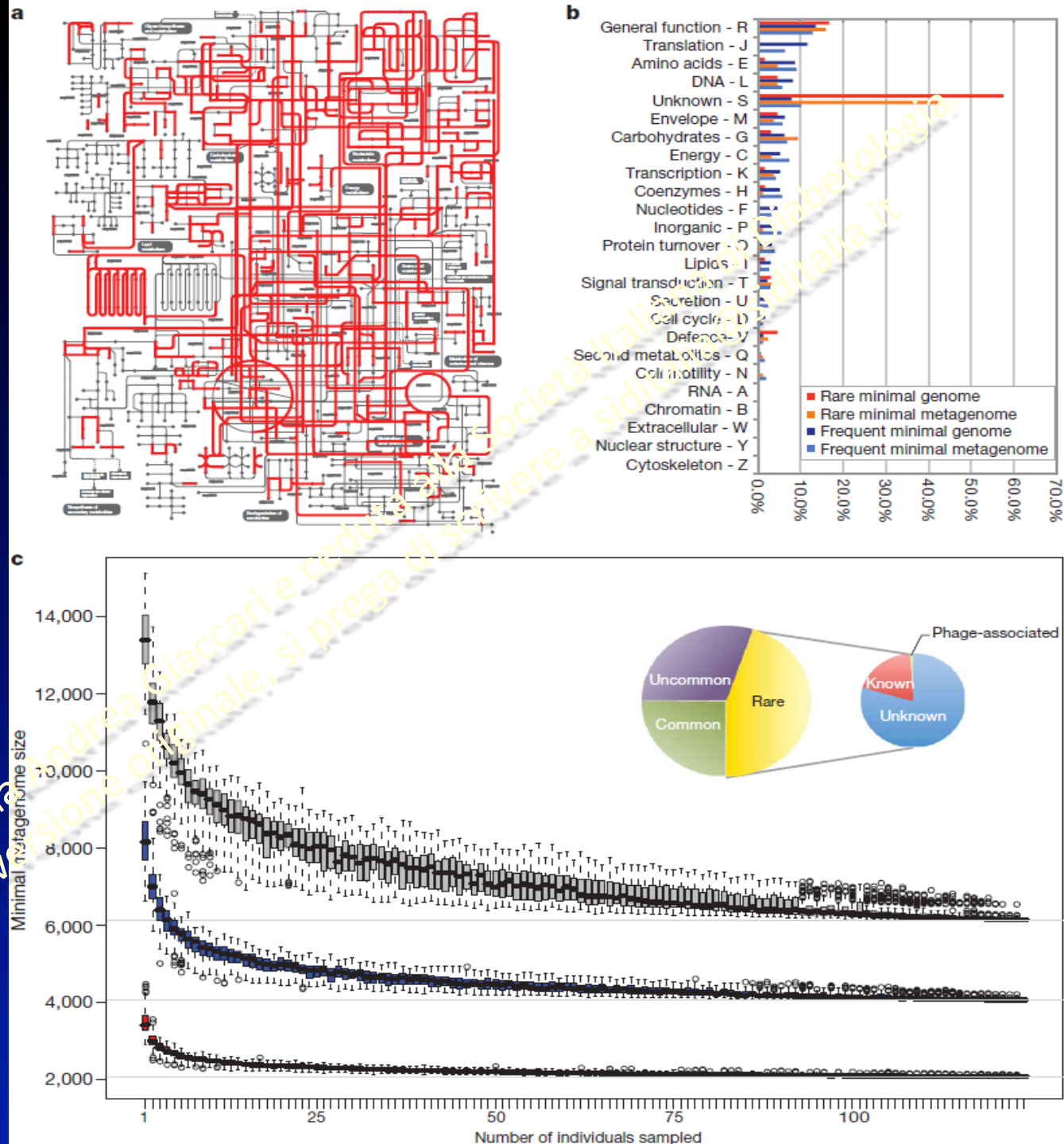
**Microbiome**



**Metabolome**

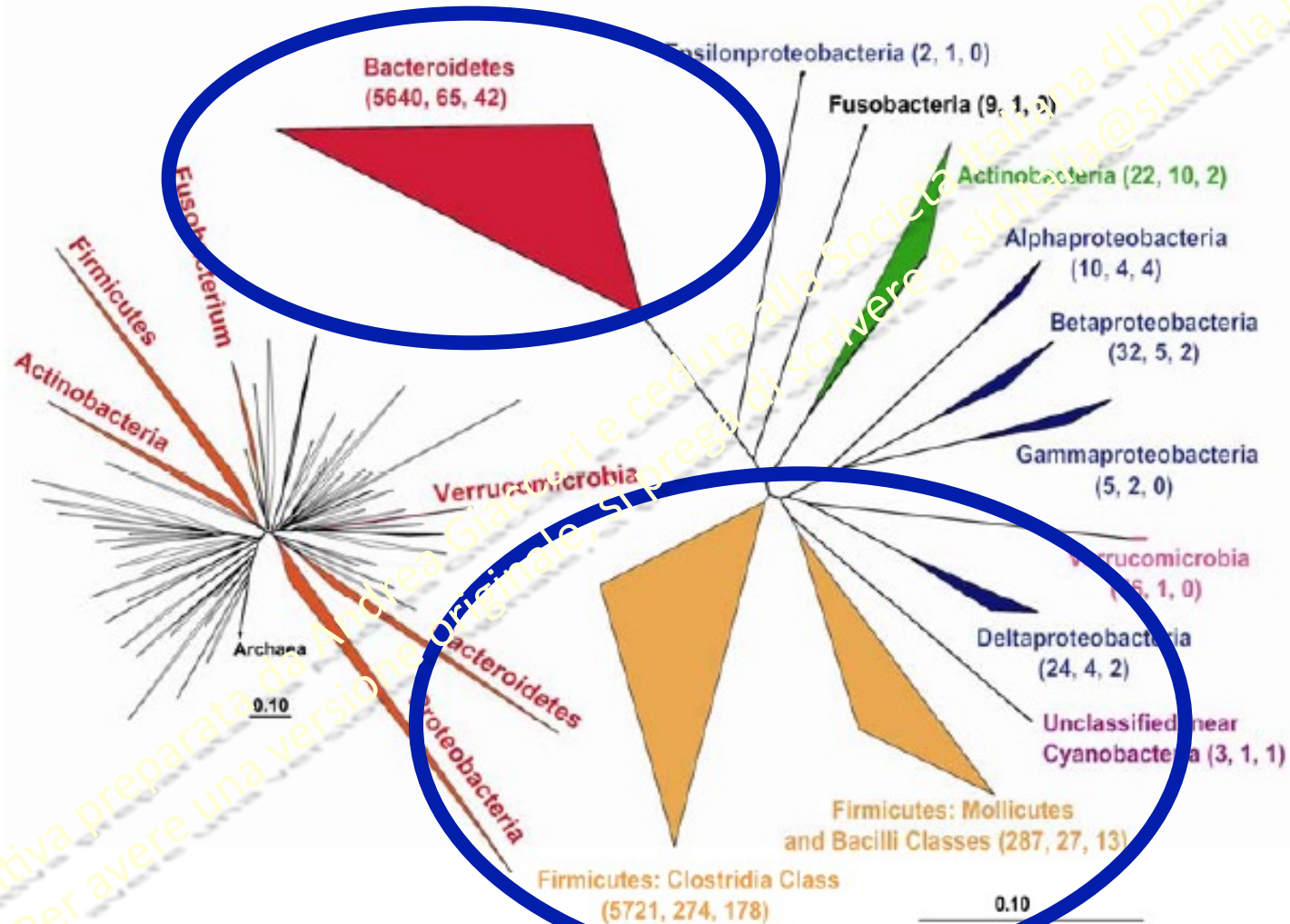
# The Minimal Core Gut Genome And Metagenome

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# COMPOSITION OF THE GUT MICROBIOTA: MOLECULAR APPROACH





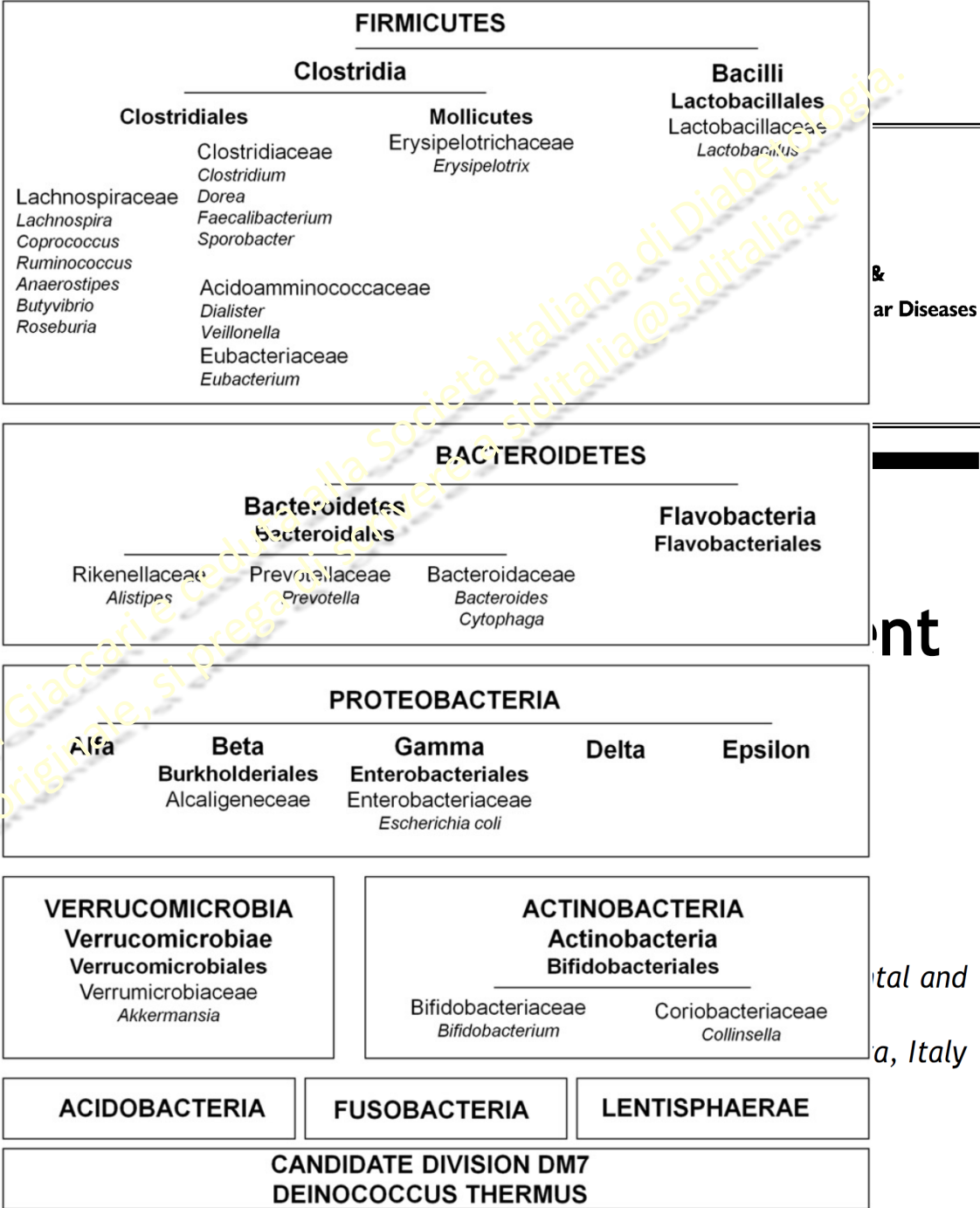
REVIEW

# The role of gut microorganisms in the pathogenesis of type 2 diabetes: findings and future perspectives

A. Tagliabue<sup>a,\*</sup>, M. Elli<sup>b</sup>

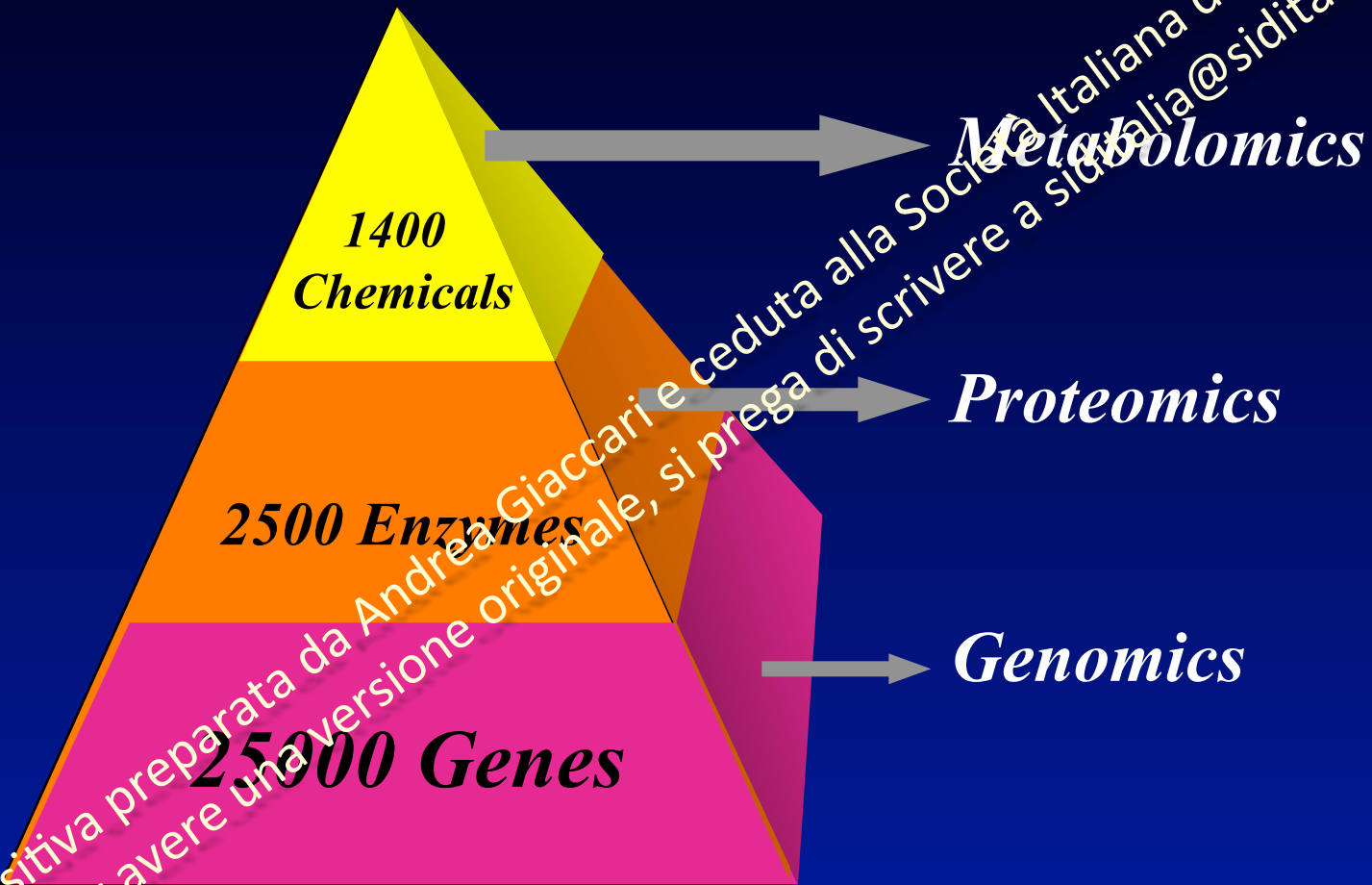
<sup>a</sup> Human Nutrition and Eating Disorders Research Unit, Department of Forensic Medicine, University of Pavia, Via A. Sarmiento 1, 27100 Pavia, Italy  
<sup>b</sup> AAT–Advanced Analytical Technologies Srl, Via S. Felice 1, 27012 Pavia, Italy

Received 3 April 2012; received in revised form 25 September 2012; accepted 10 October 2012  
Available online 10 November 2012



# ***THE PYRAMID OF LIFE: human body***

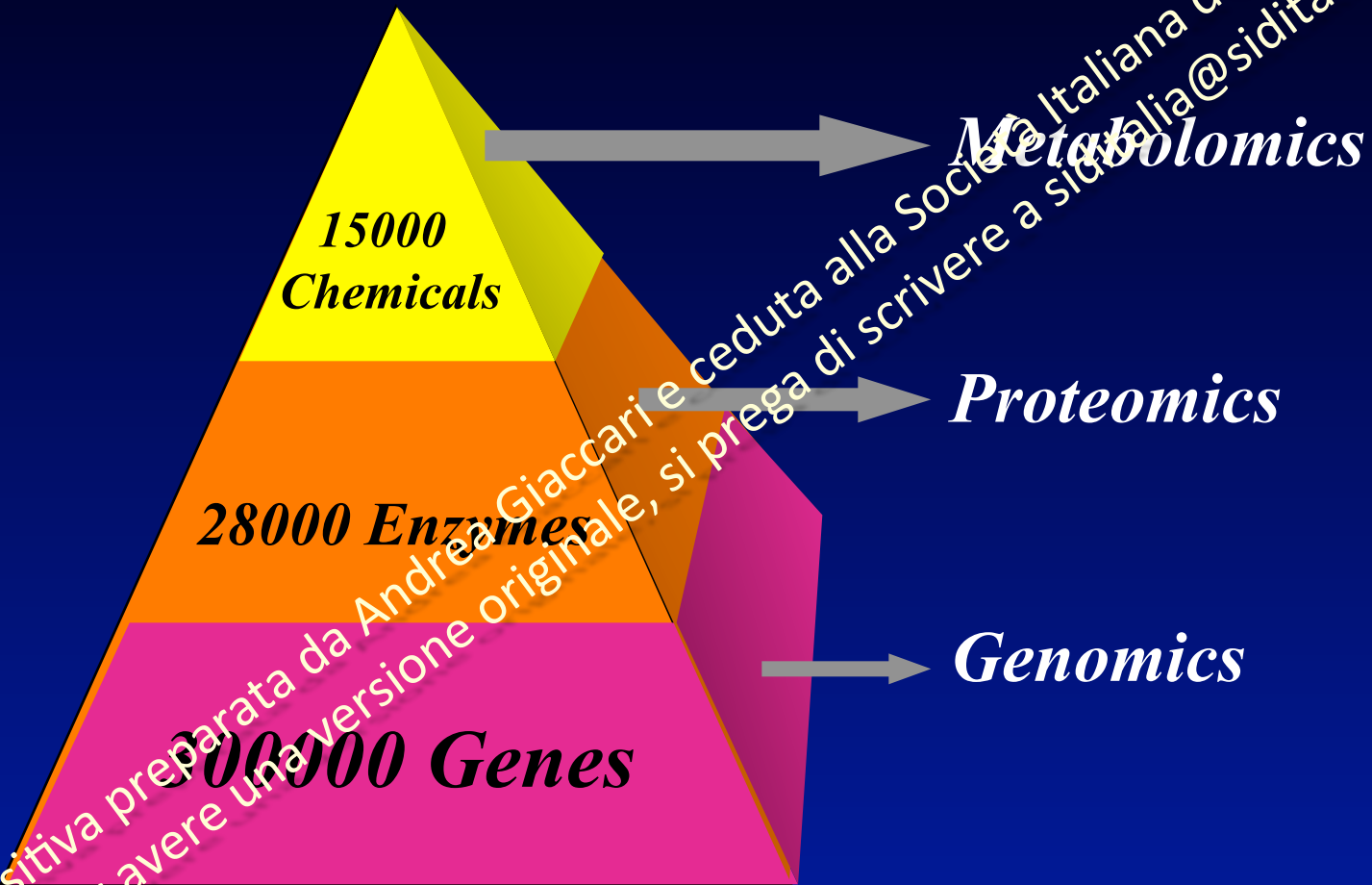
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# ***THE PYRAMID OF LIFE: human gut microbiota***

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# At birth the human body is sterile.

**Vaginal microbiota (mother)**

**Fecal microbiota  
(mother)**

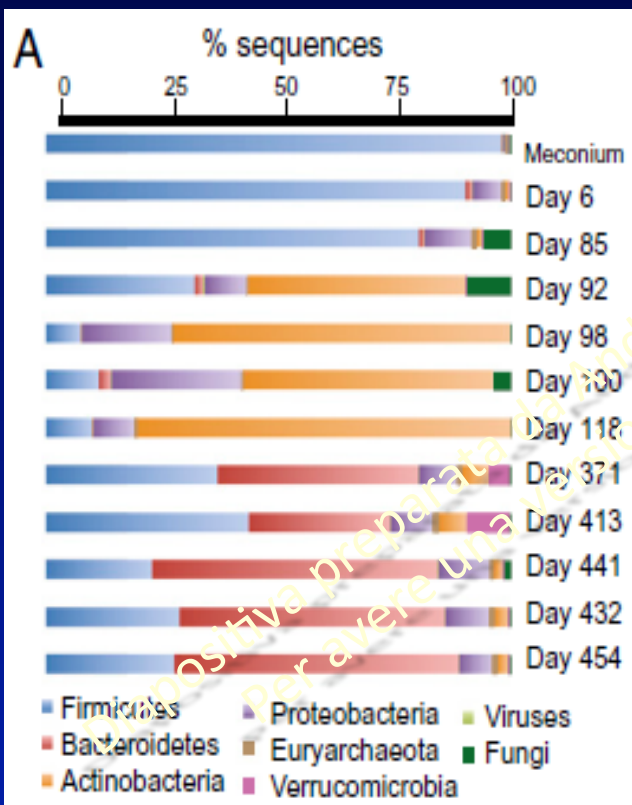
**Skin microbiota  
(mother)**

**Diet**

**Ambient**



**Native CORE microbiome  
(4-24 months of life)**

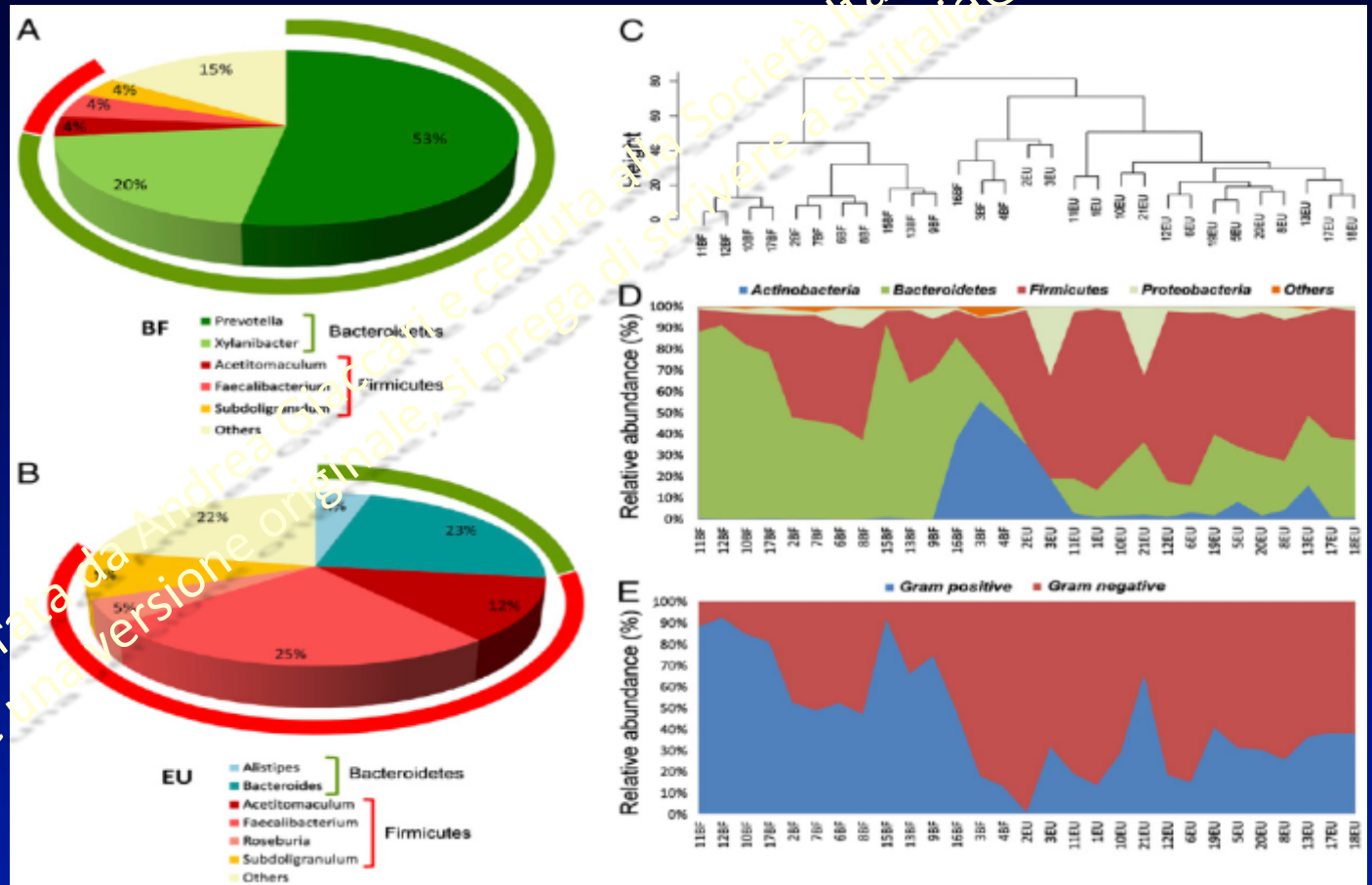


# Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

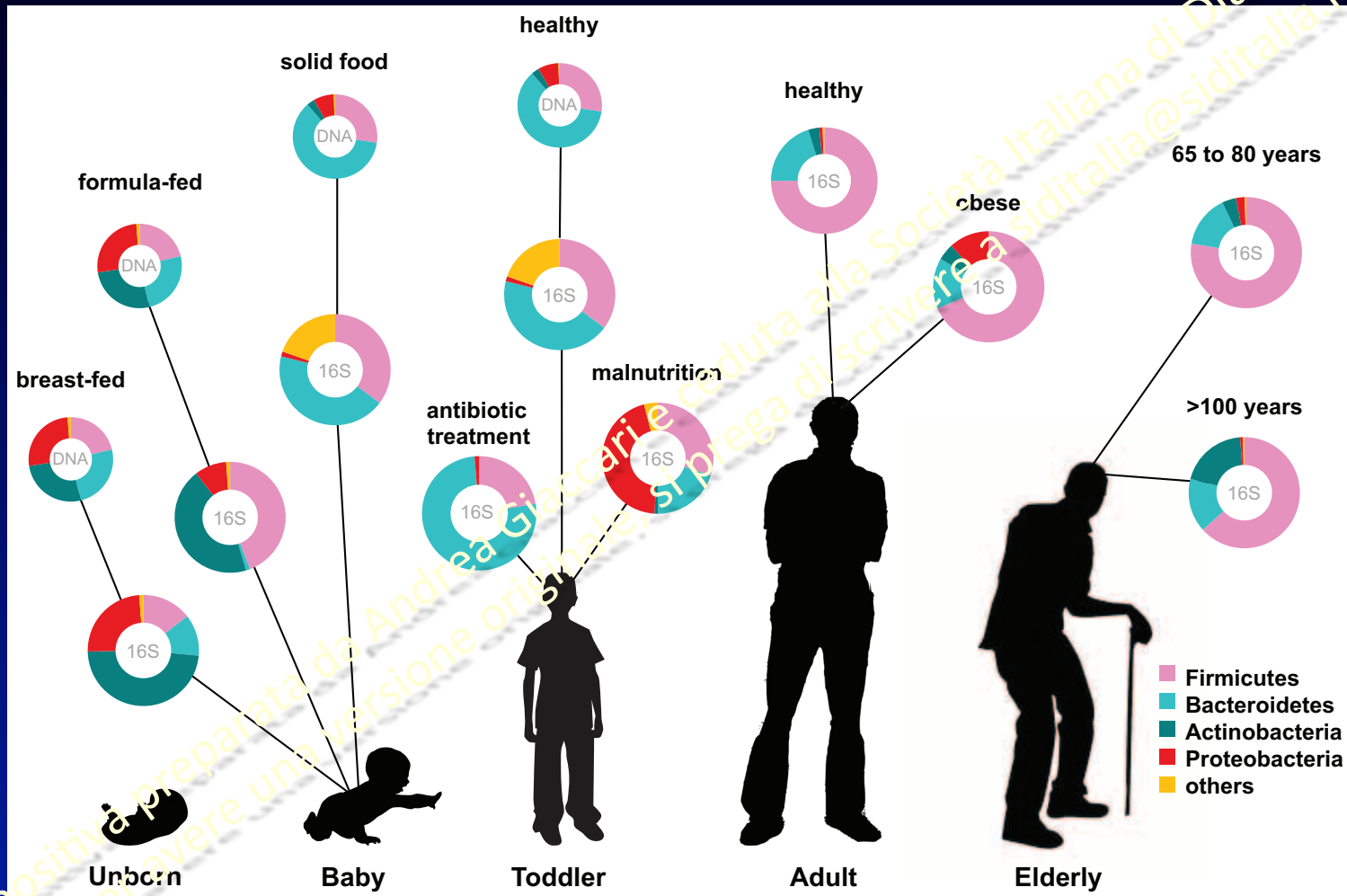
Carlotta De Filippo<sup>a</sup>, Duccio Cavalieri<sup>a</sup>, Monica Di Paola<sup>b</sup>, Matteo Ramazzotti<sup>c</sup>, Jean Baptiste Poulet<sup>d</sup>, Sebastien Massart<sup>d</sup>, Silvia Collini<sup>b</sup>, Giuseppe Pieraccini<sup>e</sup>, and Paolo Lionetti<sup>b,1</sup>

**BF**  
Bacteroidetes  
> Firmicutes

**EU**  
Firmicutes  
> Bacteroidetes



# Bacterial diversity is affected by ageing.



# DIET



# AGE



# ORIGIN

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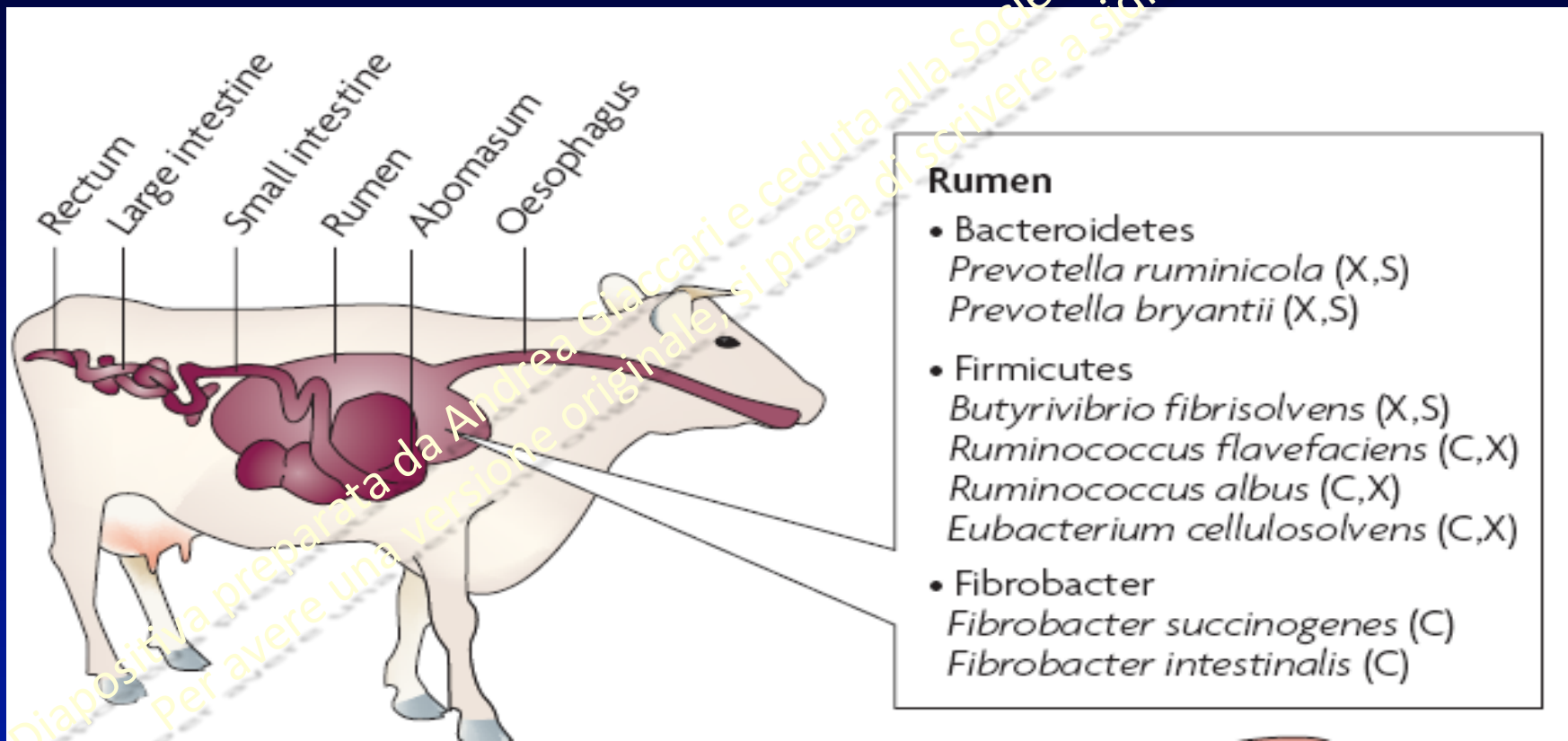
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**GUT microbiota has a powerful metabolic action in *ruminants*: herbivores derive 70% of their energy intake from microbial breakdown of dietary plant polysaccharides**





# Gut Micome

## Candida from commensal to pathogen

- Yeasts are commensal to the gut at low concentrations
- Candida overgrowth is a consequence of disturbances in the host's defense systems: *antibiotic therapy and change in physiological gut microbiota, pH, partial CO<sub>2</sub> pressure, amino acid availability, iron deficiency...*
- Yeast genome can be modified by repeated point mutations («microevolution») in order to overcome host protective measures



# HOW THE HOST-GUT MICROBIOTA BALANCE IS MAINTAINED?

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➤ Secretion of :

*Gastric acid*

*Mucus*

*Biliary salts*

*Mucosal Ig*

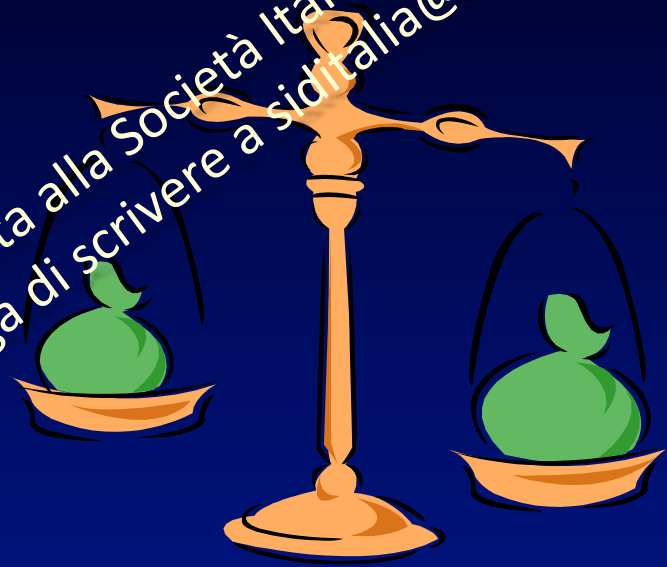
➤ Mucosal pH

➤ Mucosal barrier integrity

➤ Intestinal motility

➤ Local mucosal and systemic immunity

➤ Interactions among different bacteria species



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# GUT barrier

## *Gut Microbiota*

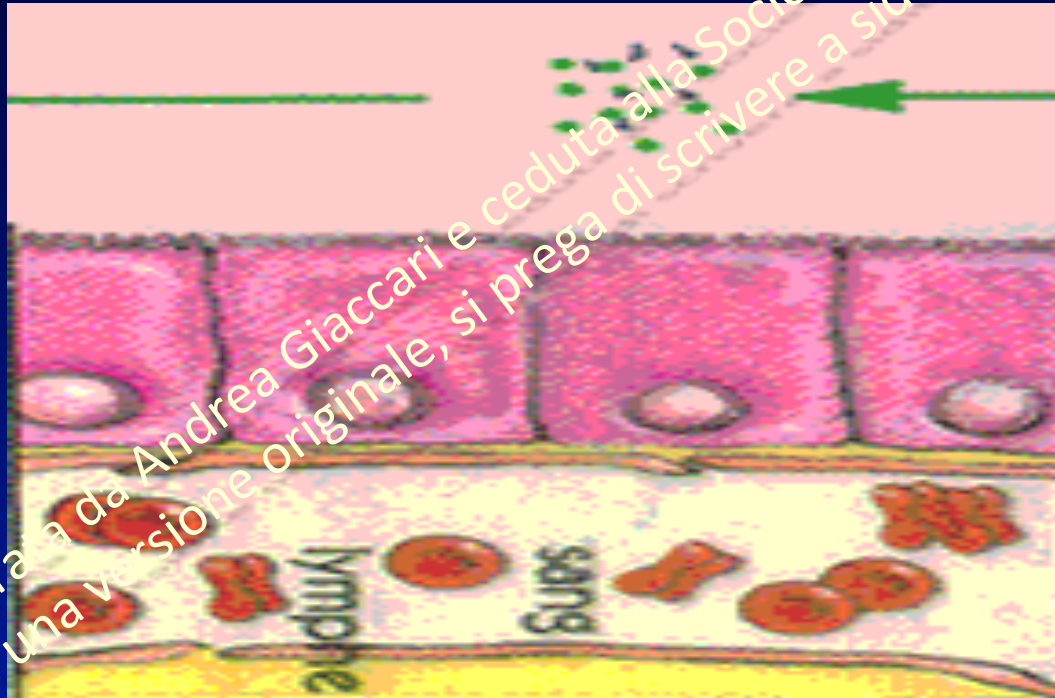
*(bacteria, yeasts, bacteriophages)*

*Mucosal  
Barrier*

*Epithelial  
barrier*

*Endocrine  
system*

*Vascular and  
lymphatic systems*



*Acquired  
and  
Innate  
immunity*

*Neuroenteric system*

*Digestive enzymes*

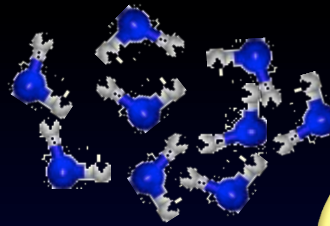
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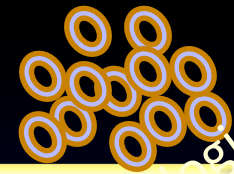
**Bad  
bacteria**



**Good  
bacteria**



**Water**



**Bile  
acids**



**Food  
antigens**

**Lumen**

**Colon**

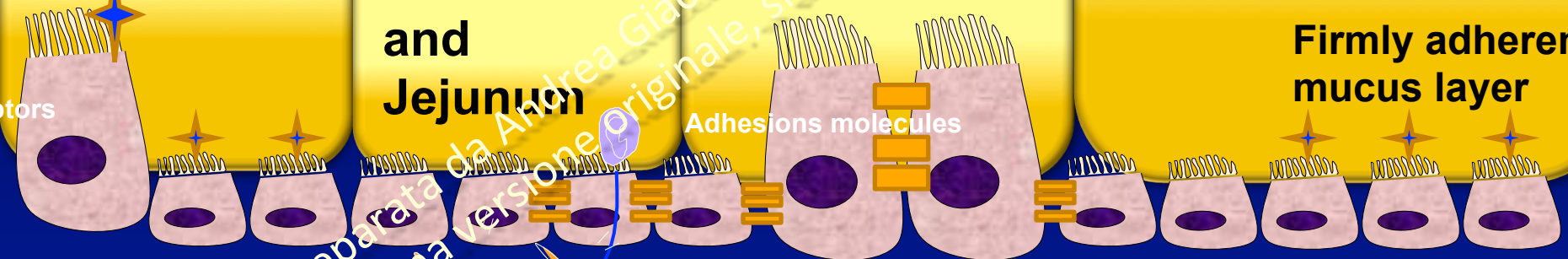
**Loosely  
adherent  
mucus layer**

**Stomach**

**Duodenum  
and  
Jejunum**

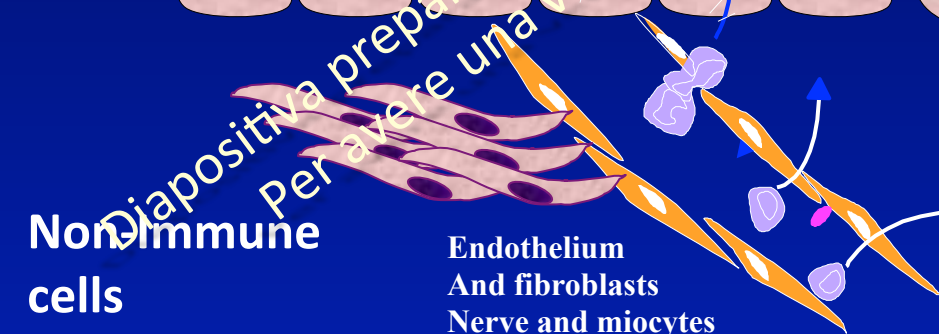
**Ileum**

**Firmly adherent  
mucus layer**

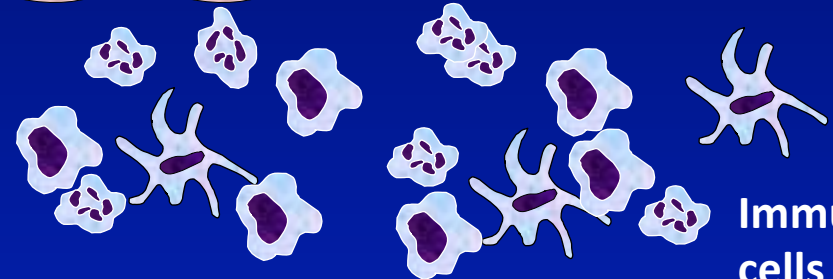


tors

Adhesions molecules

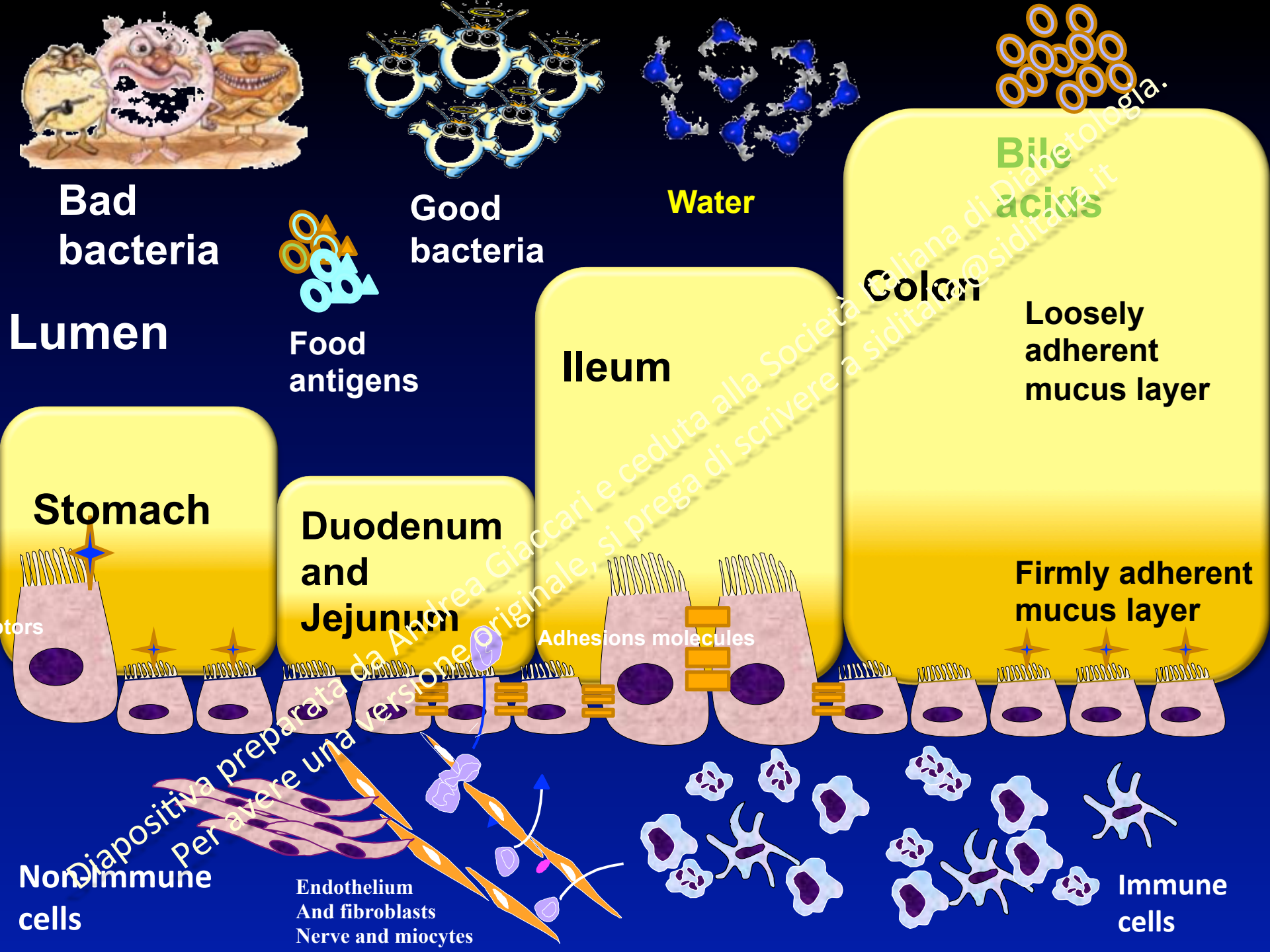


**Endothelium  
And fibroblasts  
Nerve and miocytes**



**Immune  
cells**

**Non-immune  
cells**





# ***EFFECTS OF GUT MICROBIOTA ON HOST HEALTH***

---

- **Barrier effect**
- **Immunocompetence/Tolerance**
- **Synthesis**
- **Metabolic/Trophic function**
- **Drug metabolism**
- **Behavior conditioning**

***But...specific effects in each GI tract!***



# WHEN DO THESE MECHANISMS FAIL?

---

Unbalanced DIET:

*High fats*

*Low fibers*

*High calories*

*High meat content*

...

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# WHEN DO THESE MECHANISMS FAIL?

---

Anti H2-PPI

Gastric atrophy

Surgery

Autoimmune disease

Aging

Selective IgA deficiency

HIV

Immunosenescence

Vascular diseases

Surgery

Diverticula/fistula

Neuromuscular diseases

Radiation/Inflammation

Infectious diseases

Connective diseases

Drugs

Endocrine disorders

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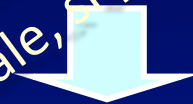
*When these mechanisms fail...*



***Quali-quantitative alterations of gastric, small bowel and/or colonic microbiota***



**Bacterial Overgrowth/Reduction:  
DYSBIOSIS**



***Bacteria or bacterial fragments cause inflammation or translocate in circulation***



***...Gut microbiota associated diseases***

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# ***Bacteroides Theta-iota-omicron***

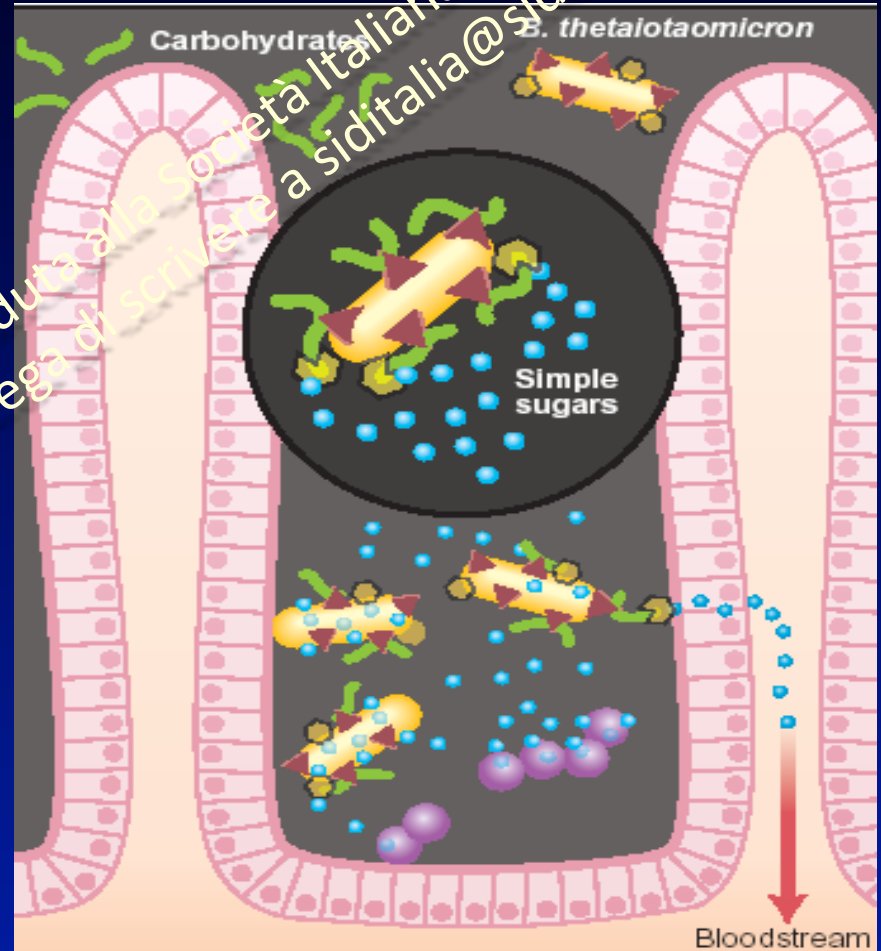
a key for host-microbial interactions understanding

⇒ Undigested complex carbohydrate polymers are bound to the *Bacteroides*' surface proteins

⇒ Surface localized glycohydrolases liberate simple sugars from complex sugar polymers

⇒ Simple sugars are partly used by *Bacteroides* itself, partly are absorbed through the intestinal villus into the host bloodstream

Sonnenburg JL, Nat Imm 2004



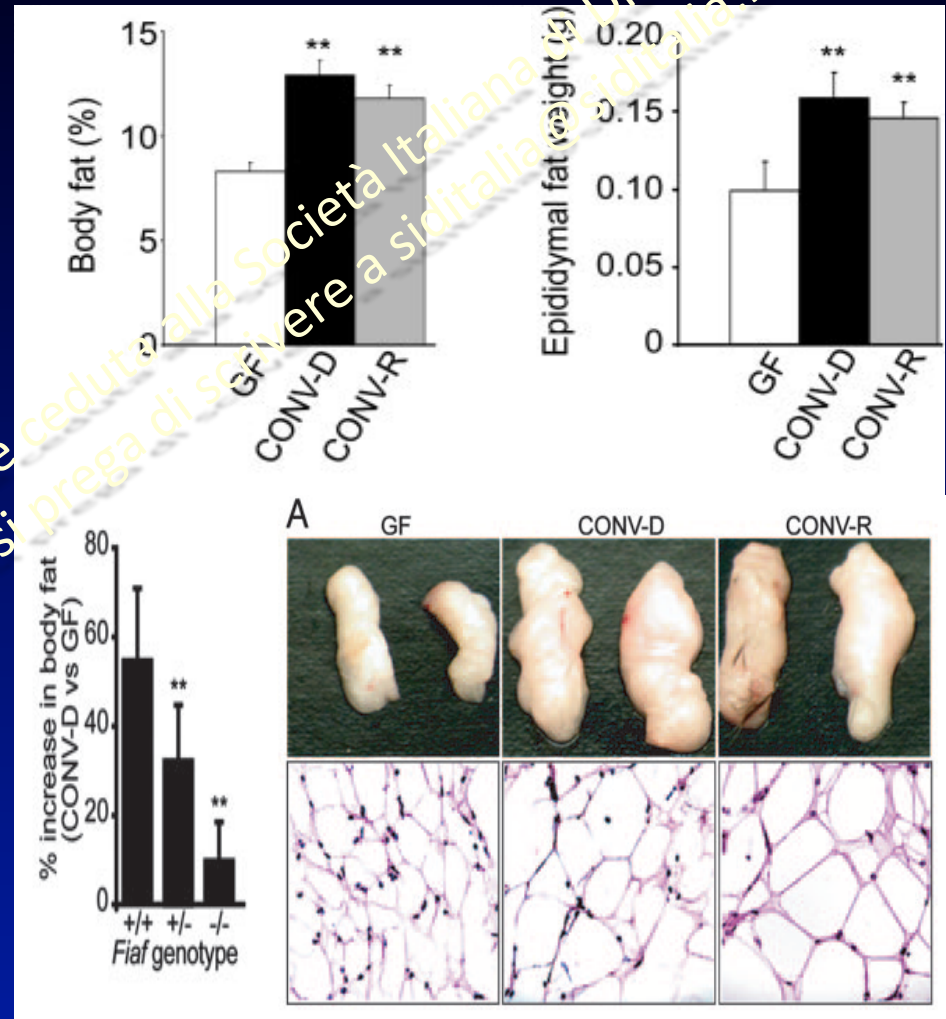
Gilmore, Science 2003



# GUT MICROBIOTA and REGULATION OF ENERGY STORAGE

WT mice have 42% more total body fat and 47% more gonadal fat than germ-free (GF) mice

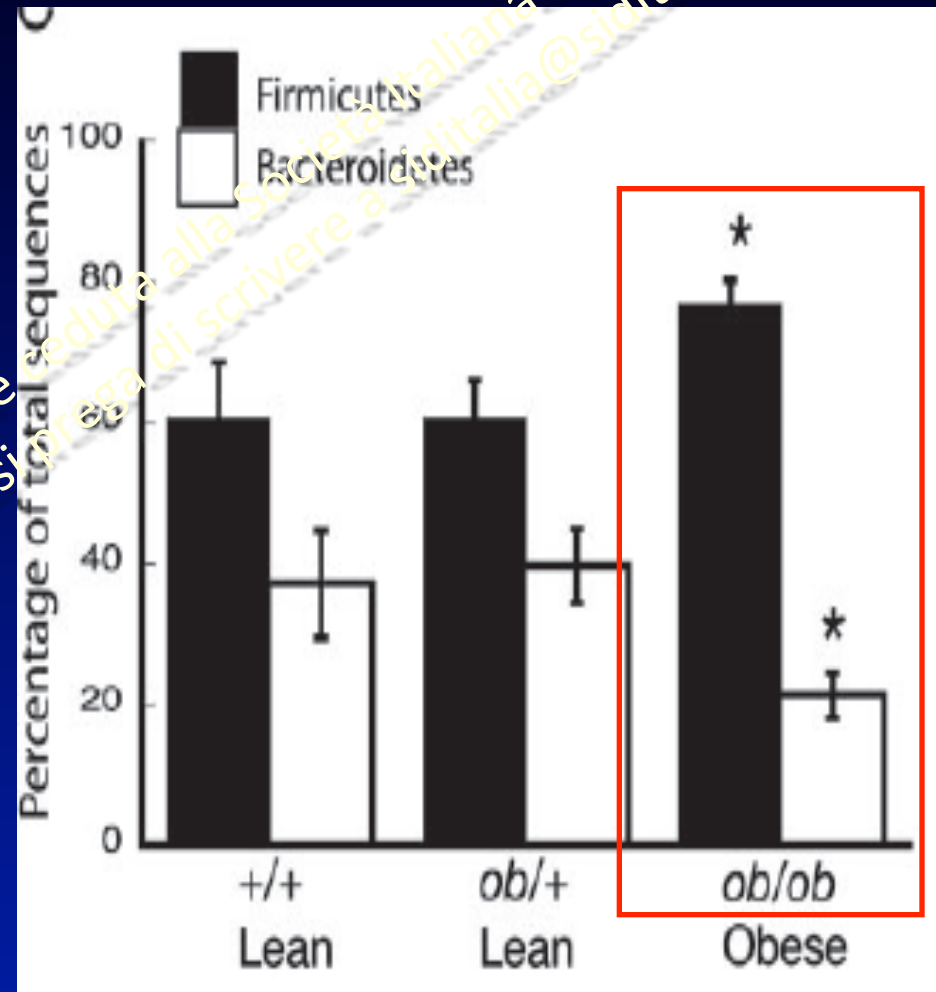
Colonisation of GF mice with microbiota from control mice produces a 60% increase in body fat mass, associated with increased insulin resistance



# Obesity alters gut microbial ecology

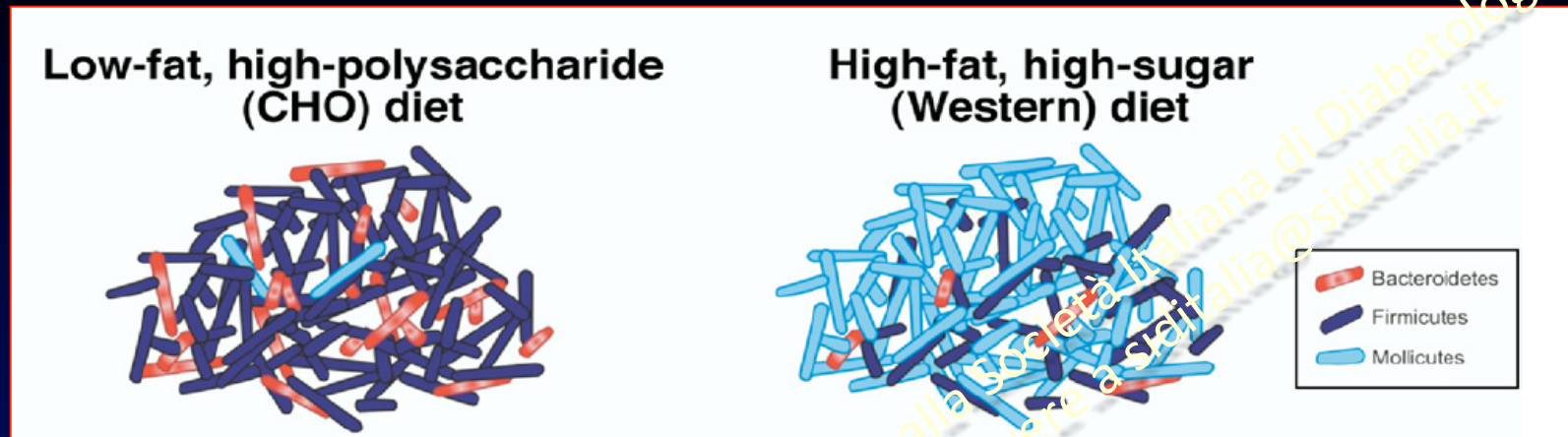
Ruth E. Ley<sup>†</sup>, Fredrik Bäckhed<sup>†</sup>, Peter Turnbaugh<sup>†</sup>, Catherine A. Lozupone<sup>‡</sup>, Robin D. Knight<sup>§</sup>, and Jeffrey I. Gordon<sup>†¶</sup>

Microbiota of obese mice have a **50% reduction in *Bacteroidetes* relative to lean mice, and a significantly greater proportion of *Firmicutes***



Ley RE, PNAS 2007

# Gut microbiota in obese humans



## *Changes in gut microbial ecology*

- Reduction in Bacteroidetes and proportional increase in Firmicutes
- Dramatic fall of overall diversity
- Bloom of a single class of Firmicutes: the Mollicutes

## *Alteration of metabolic potential*

- Enrichment for phosphotransferase systems
- Enrichment for genes encoding beta-fructosidases

## *Consequences*

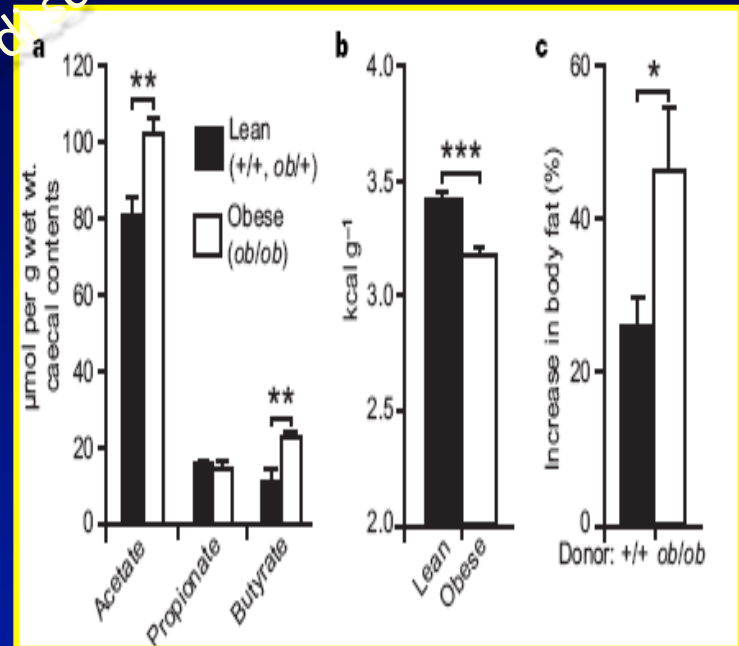
- Increased capacity to import “Western-diet”-typical carbohydrates
- Increased capacity to metabolize imported sugars

# An obesity-associated gut microbiome with increased capacity for energy harvest

Peter J. Turnbaugh<sup>1</sup>, Ruth E. Ley<sup>1</sup>, Michael A. Mahowald<sup>1</sup>, Vincent Magrini<sup>2</sup>, Elaine R. Mardis<sup>1,2</sup> & Jeffrey I. Gordon<sup>1</sup>

Ob/ob mice are enriched of genes able to harvest calories from complex polysaccharides in respect to their lean littermates

Transplantation of gut microbiota from ob/ob mice to germ free results in a greater increase in adiposity in the recipients than does a transplantation of microbiota from lean donors

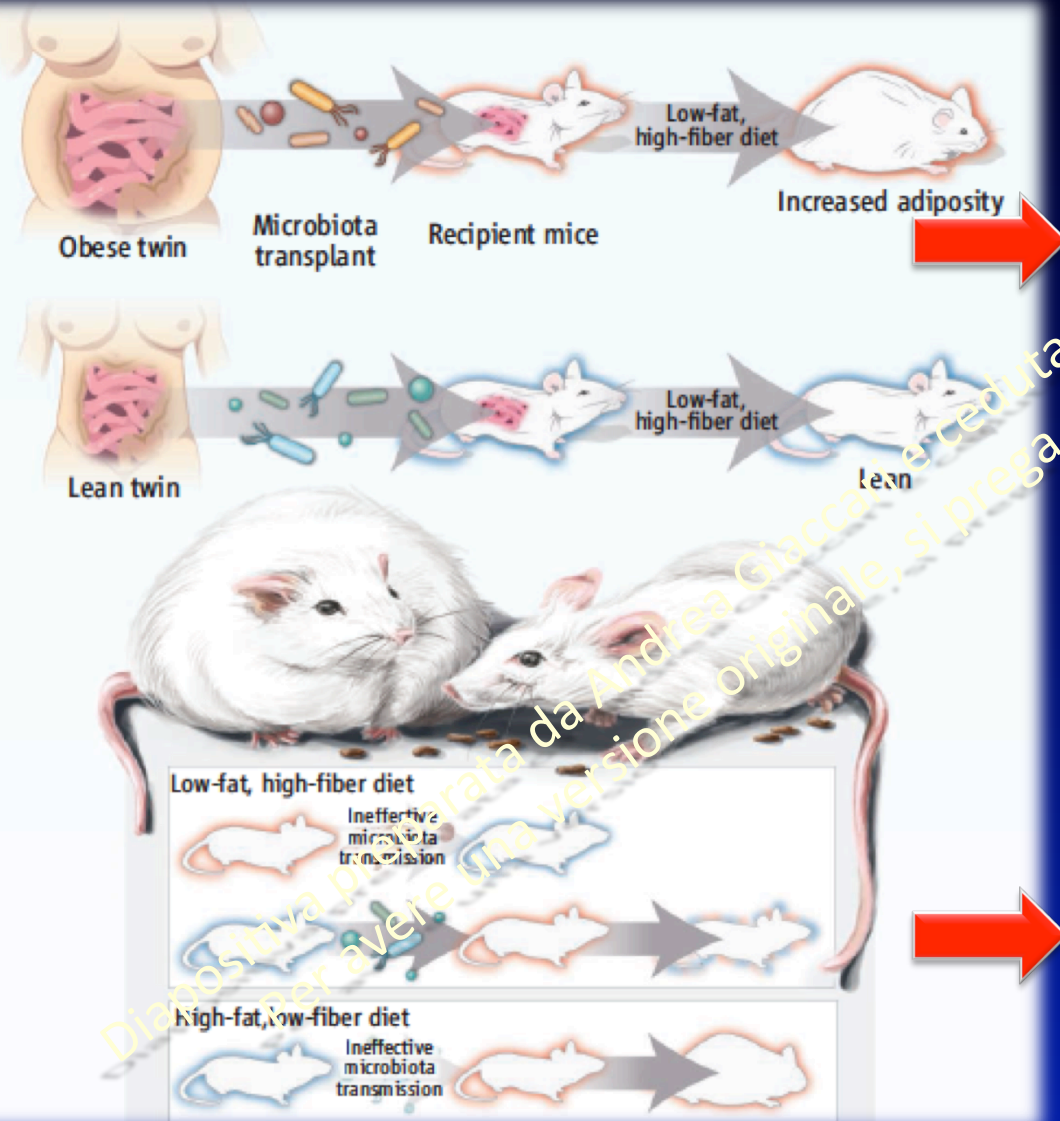


Turnbaugh et al, Nature 2006



# Microbiota transmit adiposity phenotype

*Fecal microbiota from 4 human female twin pairs discordant for obesity*



## TRANSFERRED INTO THE INTESTINES OF GERM-FREE MICE

(Ob) twin + mice =  $\uparrow$  adiposity  
(Ln) twin + mice =  $\downarrow$  adiposity



## TRANSMISSIBILITY OF INTESTINAL MICROBES AND ADIPOSITY PHENOTYPE ARE TIGHTLY LINKED



## COHOUSING

(Ob) twin transplanted mice + (Ln)  
twin transplanted mice =  
(Ob) mice became LEAN  
(Ln) mice remain LEAN

*Ridaura et al. Science 2013  
Walker AW et al. Science 2013*



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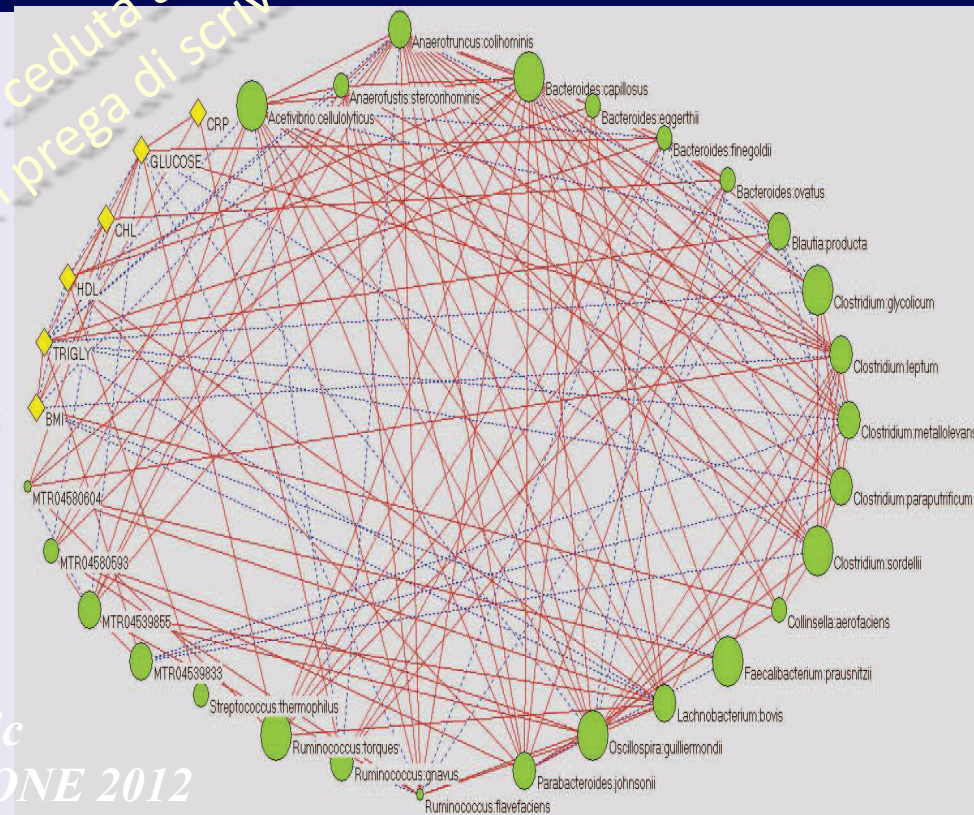
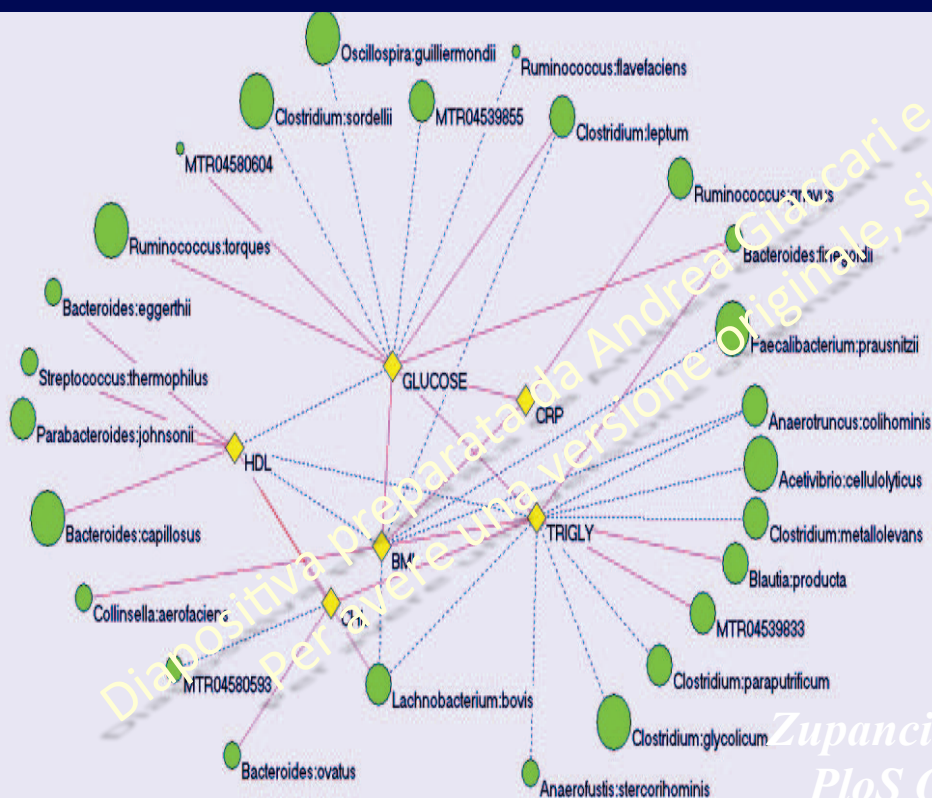
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# Analysis of the Gut Microbiota in the Old Order Amish and Its Relation to the Metabolic Syndrome

- 22 species of bacteria from the phyla Bacteroidetes, Firmicutes and Actinobacteria
- 4 OTUs from the order Clostridiales



Positive and negative correlations with BMI, triglycerides, HDL cholesterol, total cholesterol, fasting glucose levels, CRP



# GUT MICROBIOTA in TYPE 2 DIABETES

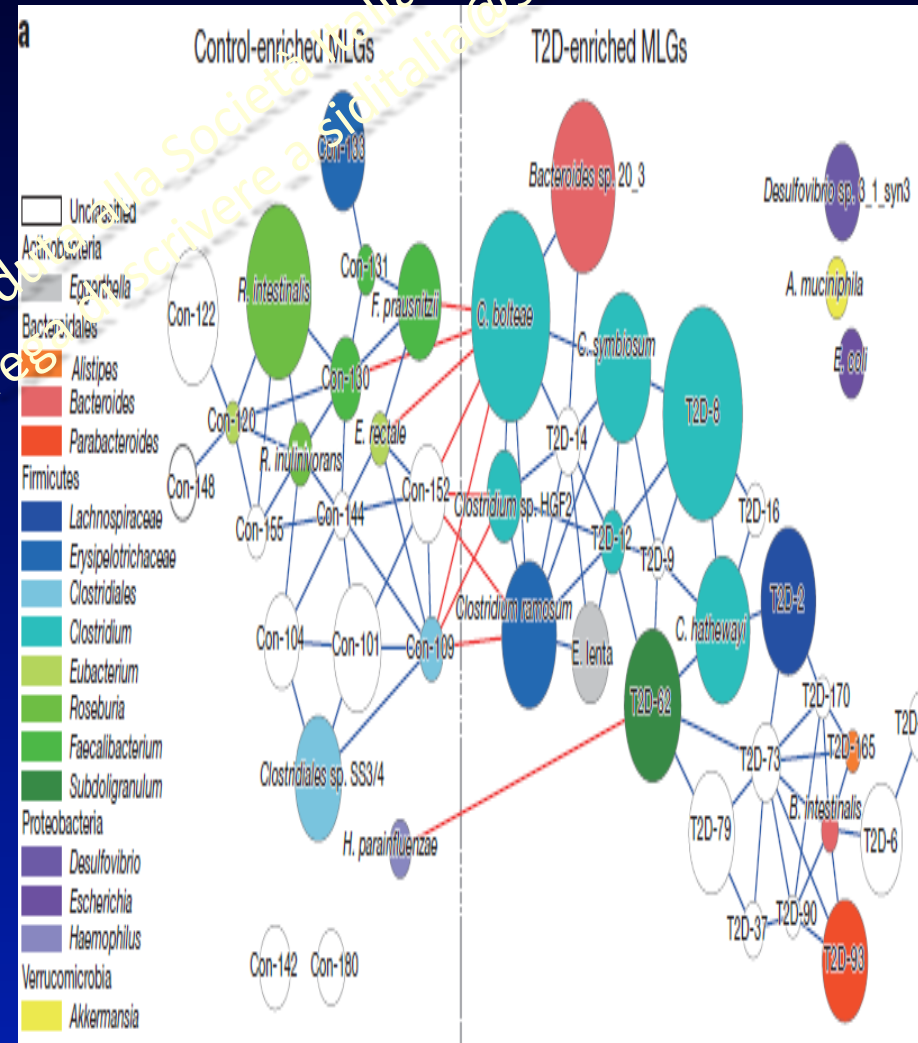
- ❖ Case-control metagenome-wide association study (MGWAS)
- ❖ Faecal DNA samples from 345 Chinese T2D patients and nondiabetic controls

## T2D vs Controls

Butyrate-producing bacteria  
(*E. rectale*, *F. prausnitzii*, etc.)

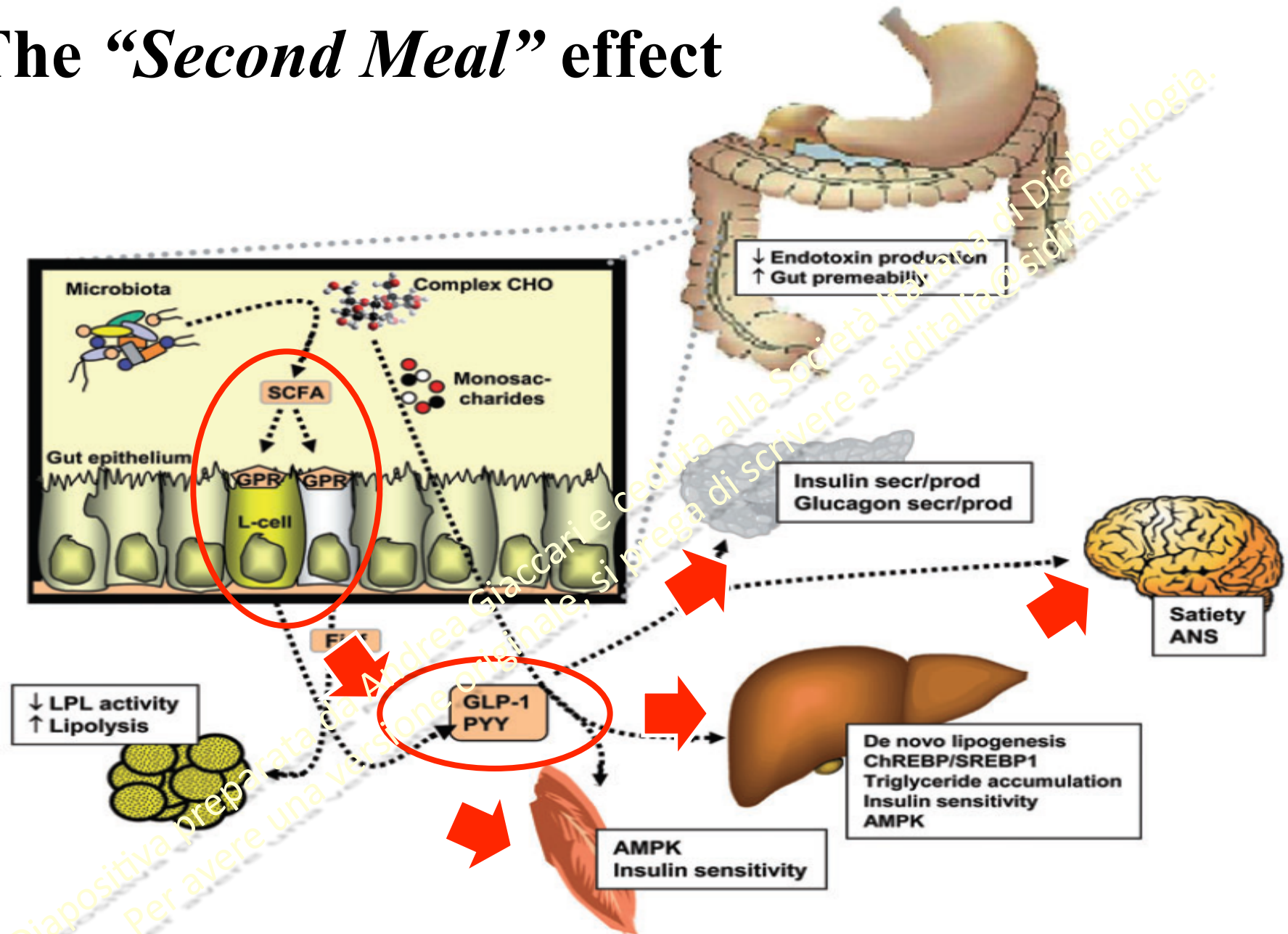
Opportunistic pathogens  
(*C. hathewayi*, *C. ramosum*, *E. coli*)

Mucin-degrading species  
Sulphate-reducing species

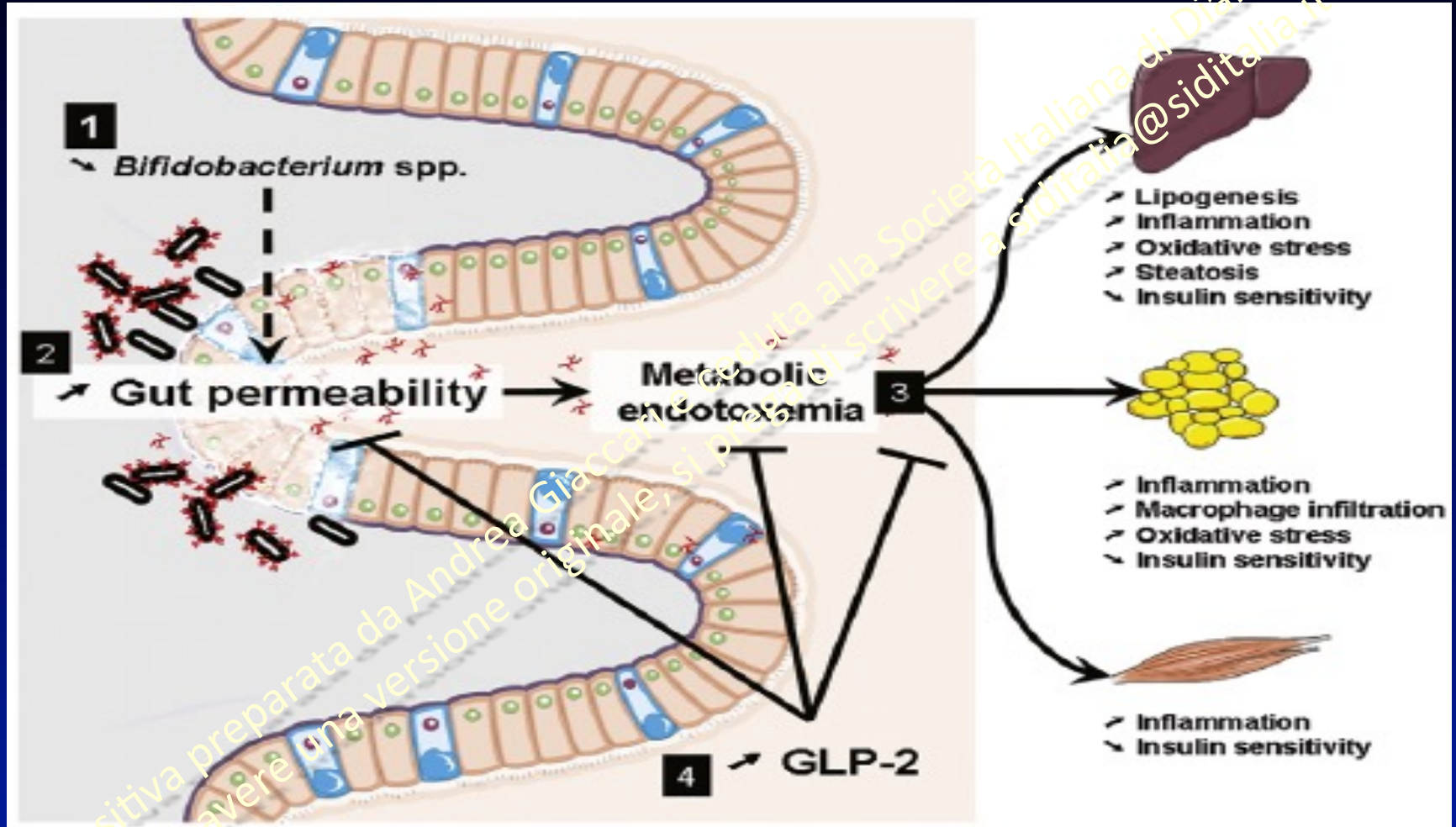




# The “*Second Meal*” effect



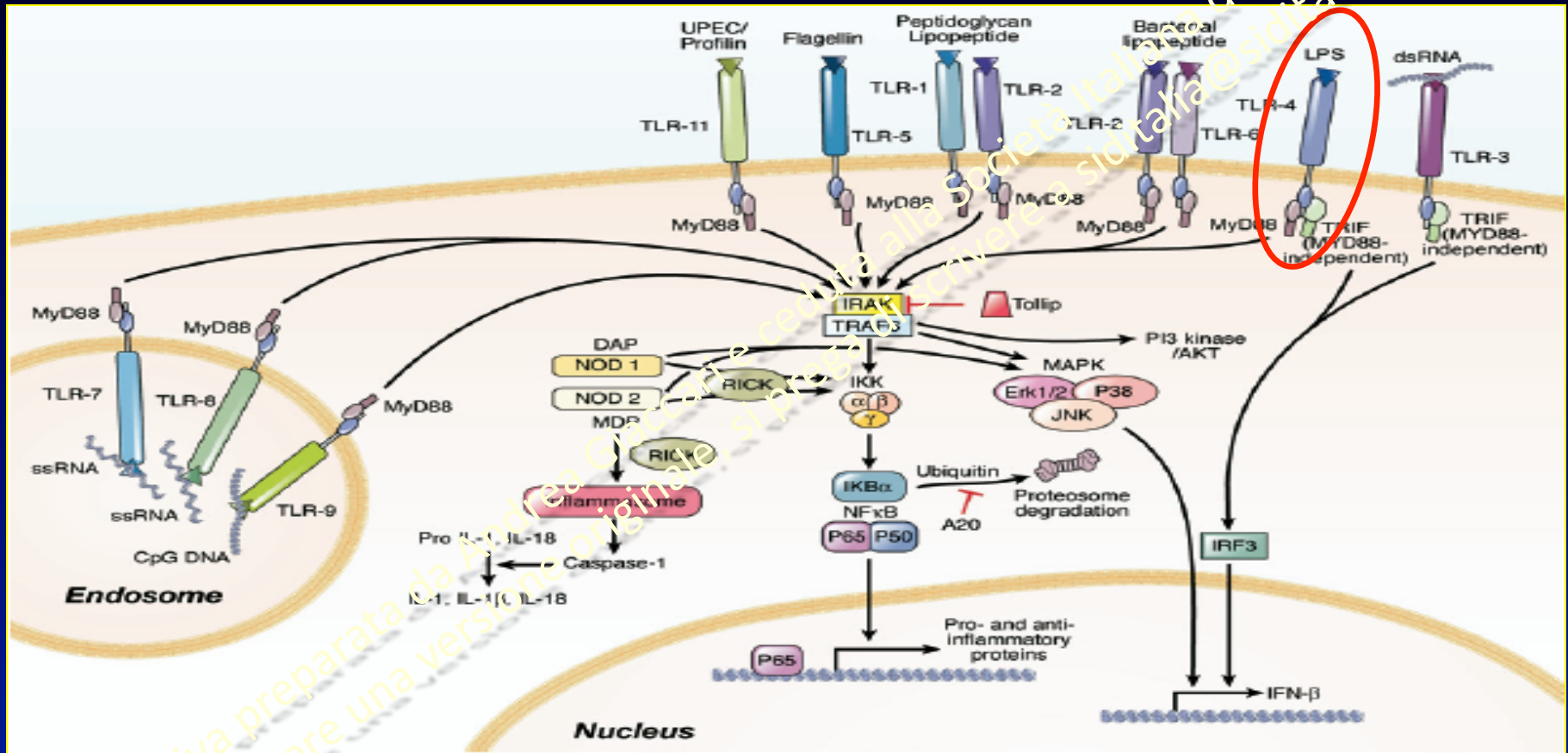
# GUT HYPERPERMEABILITY LEADS TO METABOLIC ENDOTOXEMIA

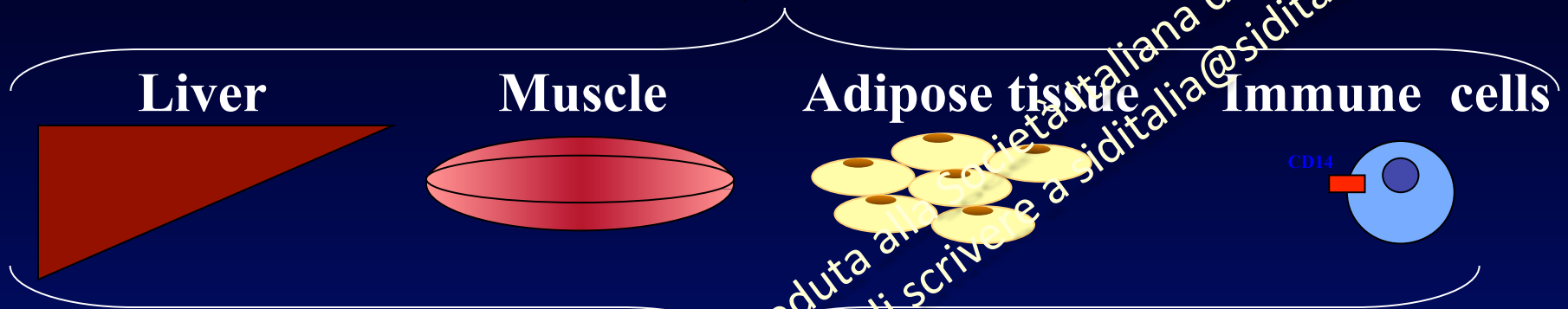
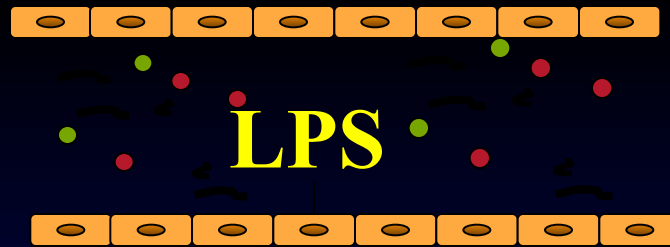


Cani et al, Pharm Ther 2010  
Miele and Gasbarrini, Hepatology 2009  
Scarpellini and Gasbarrini, Am J Gastro 2010



# LPS and inflammatory cascade



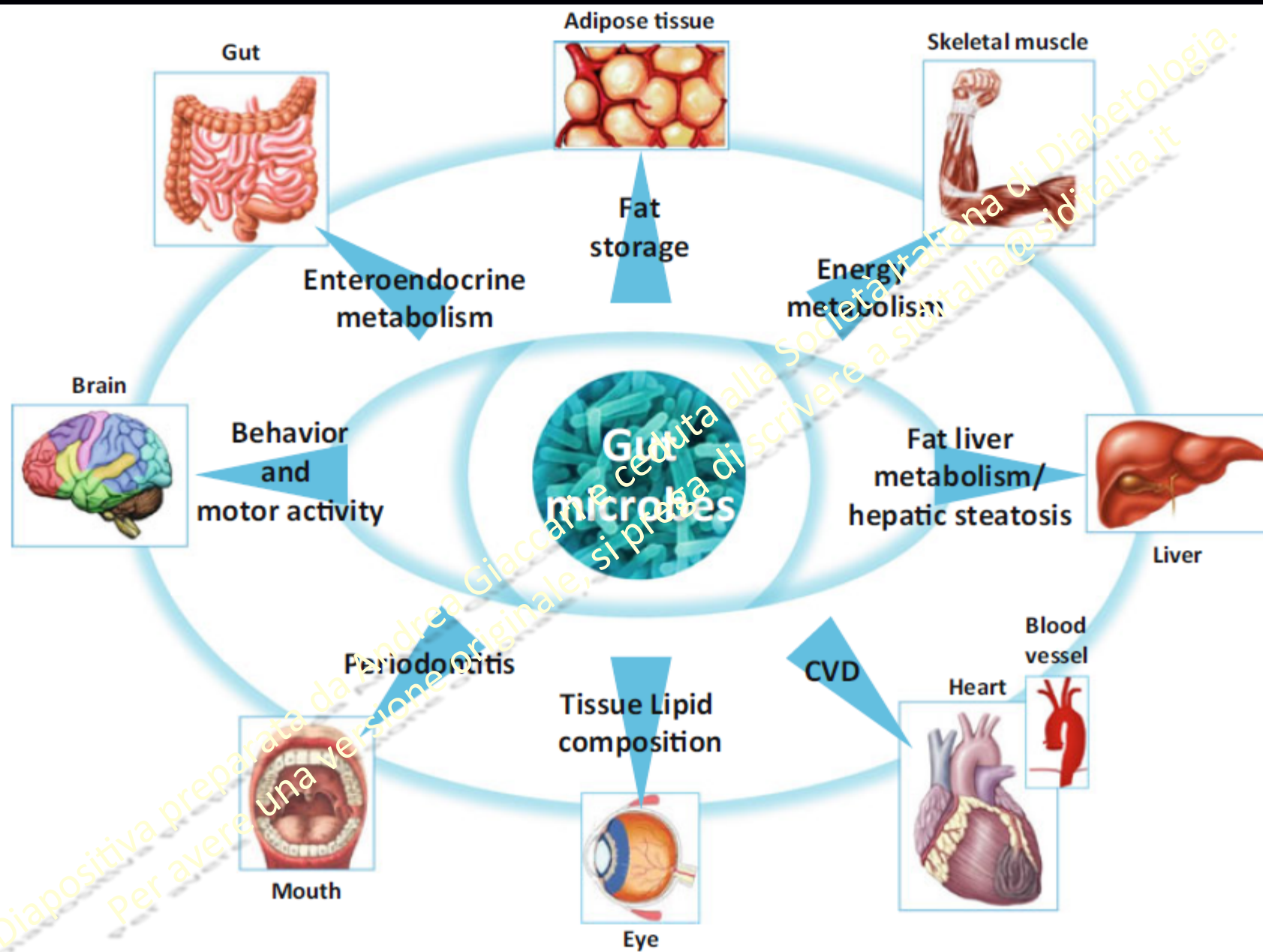


Increase mRNA expression of:

- IL1
- IL6
- Tumour Necrosis Factor alpha
- Plasminogen Activator Inhibitor-1

*Metabolic effects*

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# alcune considerazioni

- lo scenario è certamente complesso
- certamente esistono influenze reciproche, ma è difficile stimarle
- grandi quantità di dati permette alta potenza statistica
- reale ruolo deve essere confermato

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● erbivore  
● carnivore  
● onnivore



# alcune considerazioni

- l'approccio deve essere da astronomo, non da astrologo

● herbivore  
● carnivore  
● omnivore