



Congresso Regionale SINPIA



Caltanissetta 12-13 ottobre 2018
Sala Convegni Banca del Nisseno

*Save
the Date*

Caltanissetta, 13 Ottobre 2018

Simposio Satellite KOLFARMA SRL

**Il legame bidirezionale tra SNC e
microbiota intestinale
nell'epilessia e nelle altre
patologie neuropsichiatriche.**

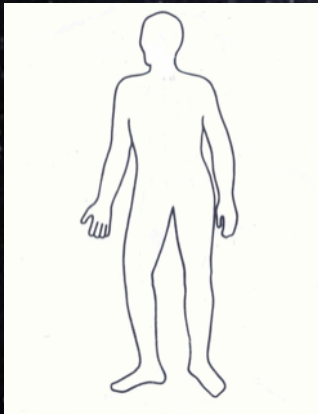
Paolo Mainardi

We Are Not Alone in This Endless Universe

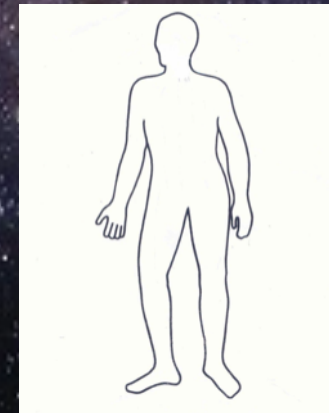
Human body



Cellular body



Microbial body



Shivaji S. **We are not alone**: a case for the human microbiome in extra intestinal diseases. Gut Pathog. 2017 Mar 7;9:13.

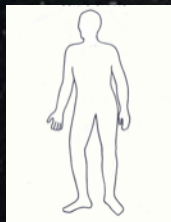
We Are Not Alone in This Endless Universe

10^{13} germ and somatic cells

≈ 80 kg

24,000 genes

Cellular body

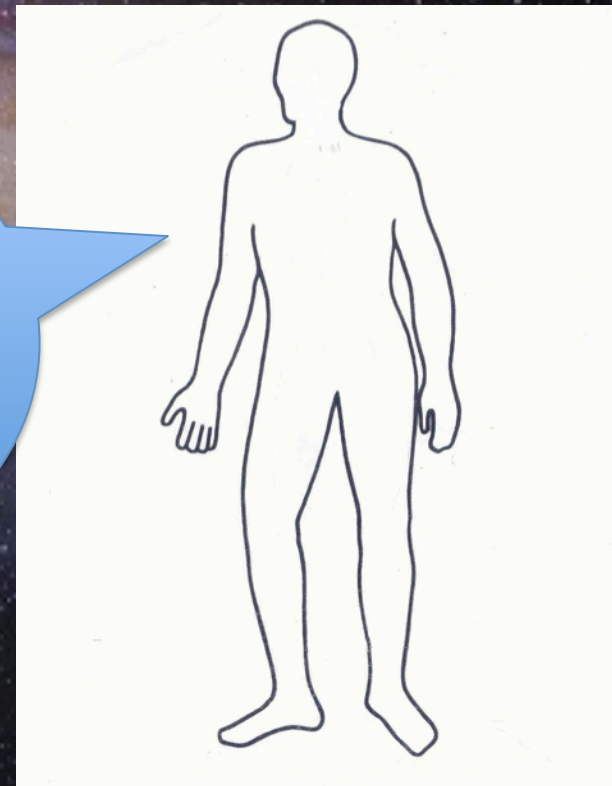


10^{14} bacteria

1.13 kg

3-4 million genes

Microbial body



Shivaji S. **We are not alone:** a case for the human microbiome in extra intestinal diseases. Gut Pathog. 2017 Mar 7;9:13.

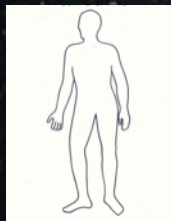
We Are Not Alone in This Endless Universe

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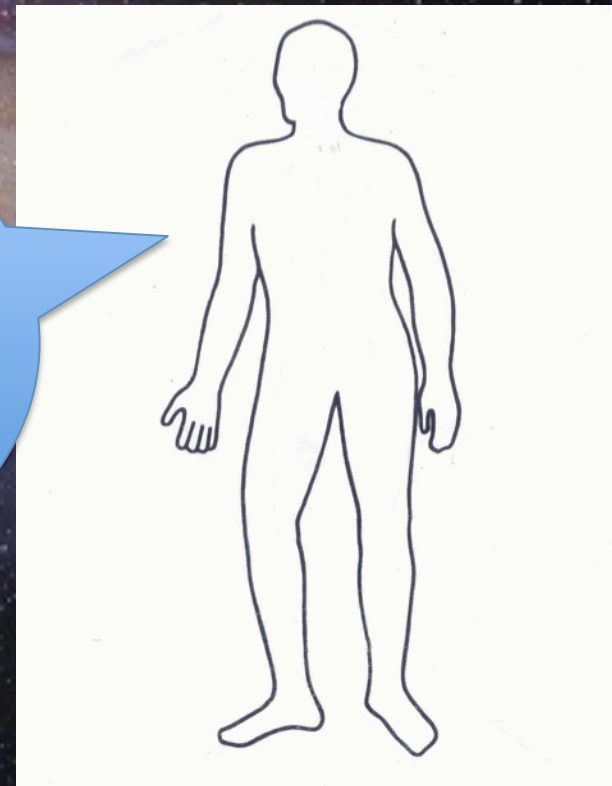


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1.13 kg

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Microbial body



Shivaji S. We are not alone: a case for the human microbiome in extra intestinal diseases. Gut Pathog. 2017 Mar 7;9:13.

R= Unità geniche/ massa

R= Energia/ massa

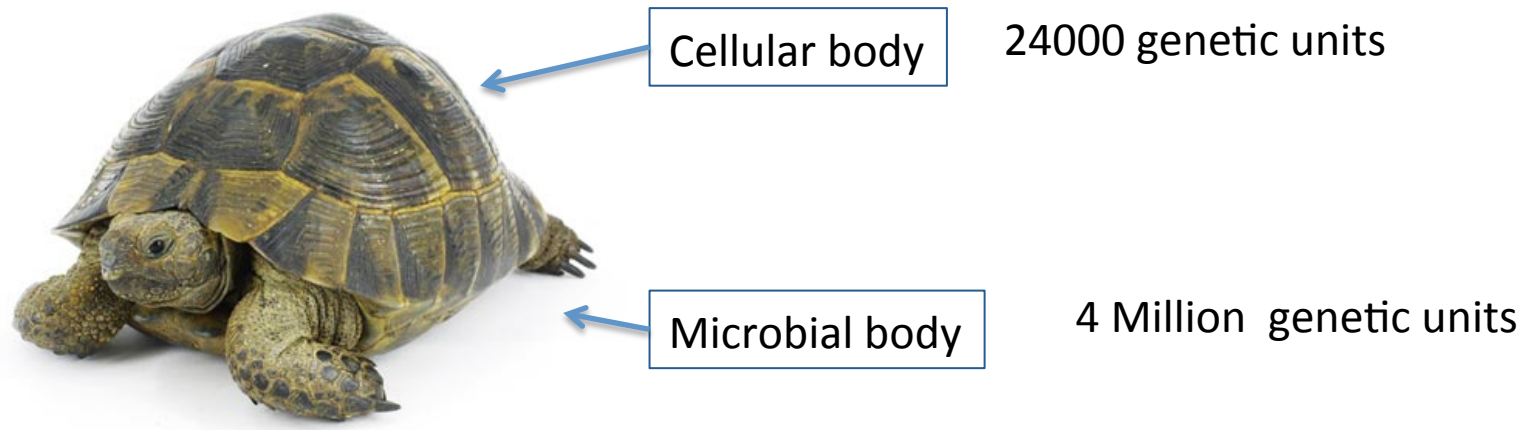
Fisica delle particelle \neq Fisica classica

R1= 24000/80

R2= 4000000/1.13



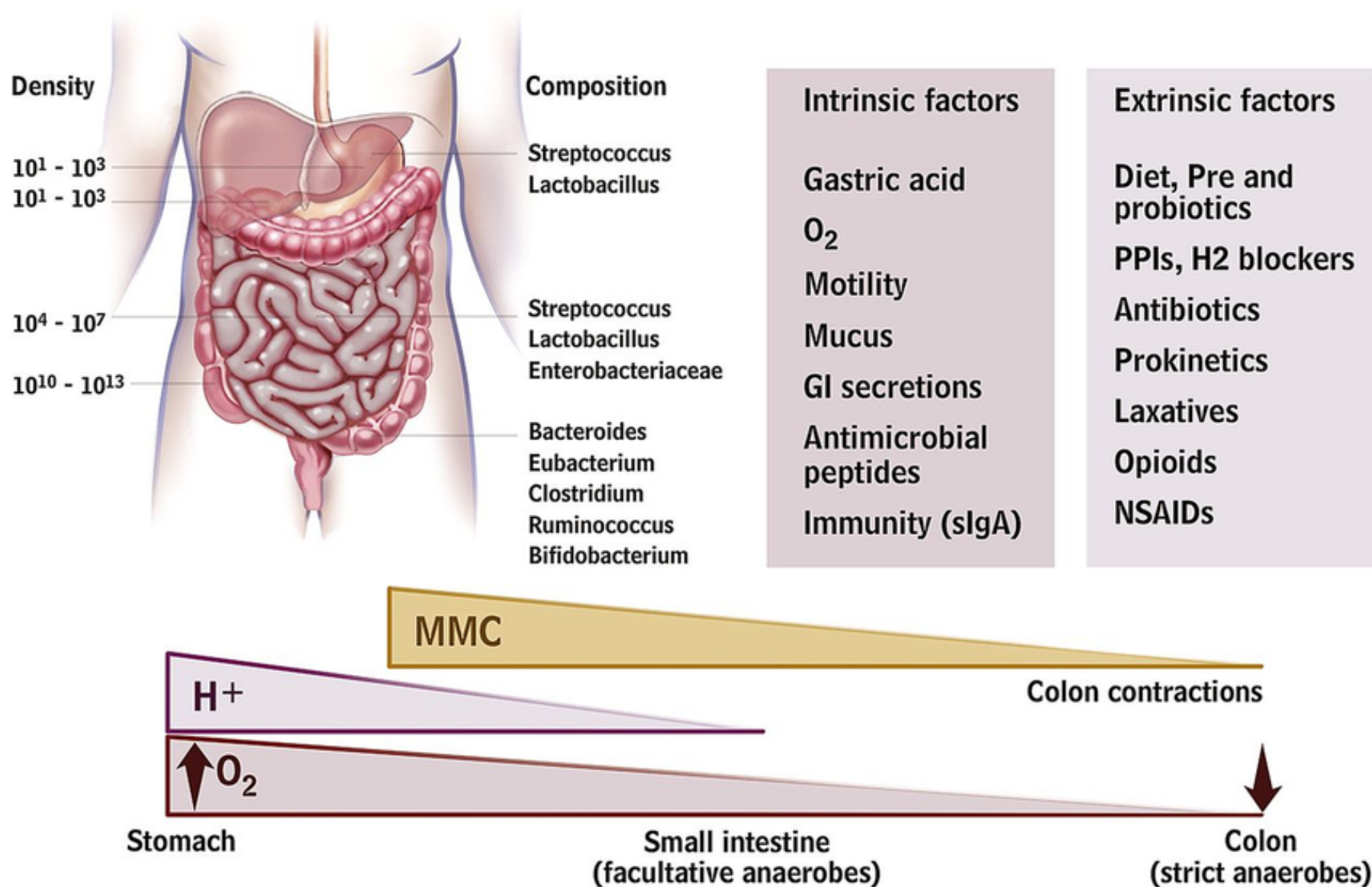
- **Microbial body is ten time cellular body**



“Surprisingly, each of us can be identified by the DNA of our gut microbes”.

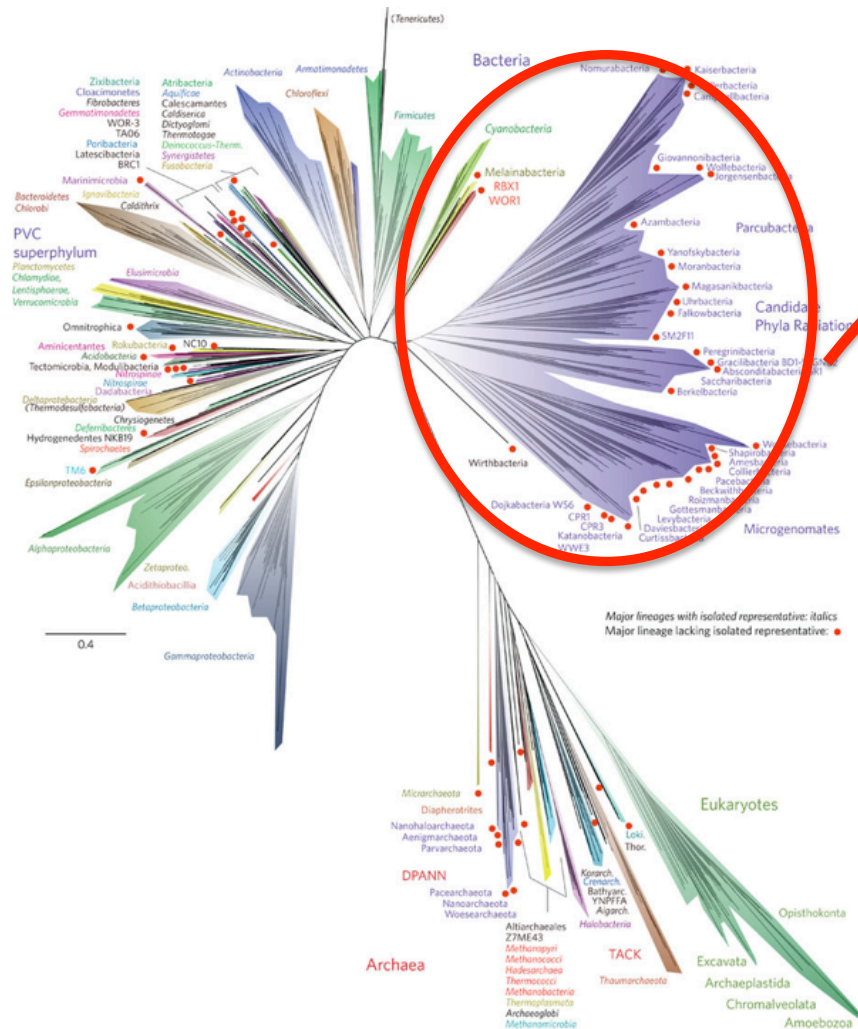
George Weinstock, Genoma Institute, Washington University

- **It compensates for our genetic damage**
- **It is able to repair our DNA**
- **It is able to modify our genetic expression**



A new tree of life:

grouped by evolutionary distance and not by taxonomy

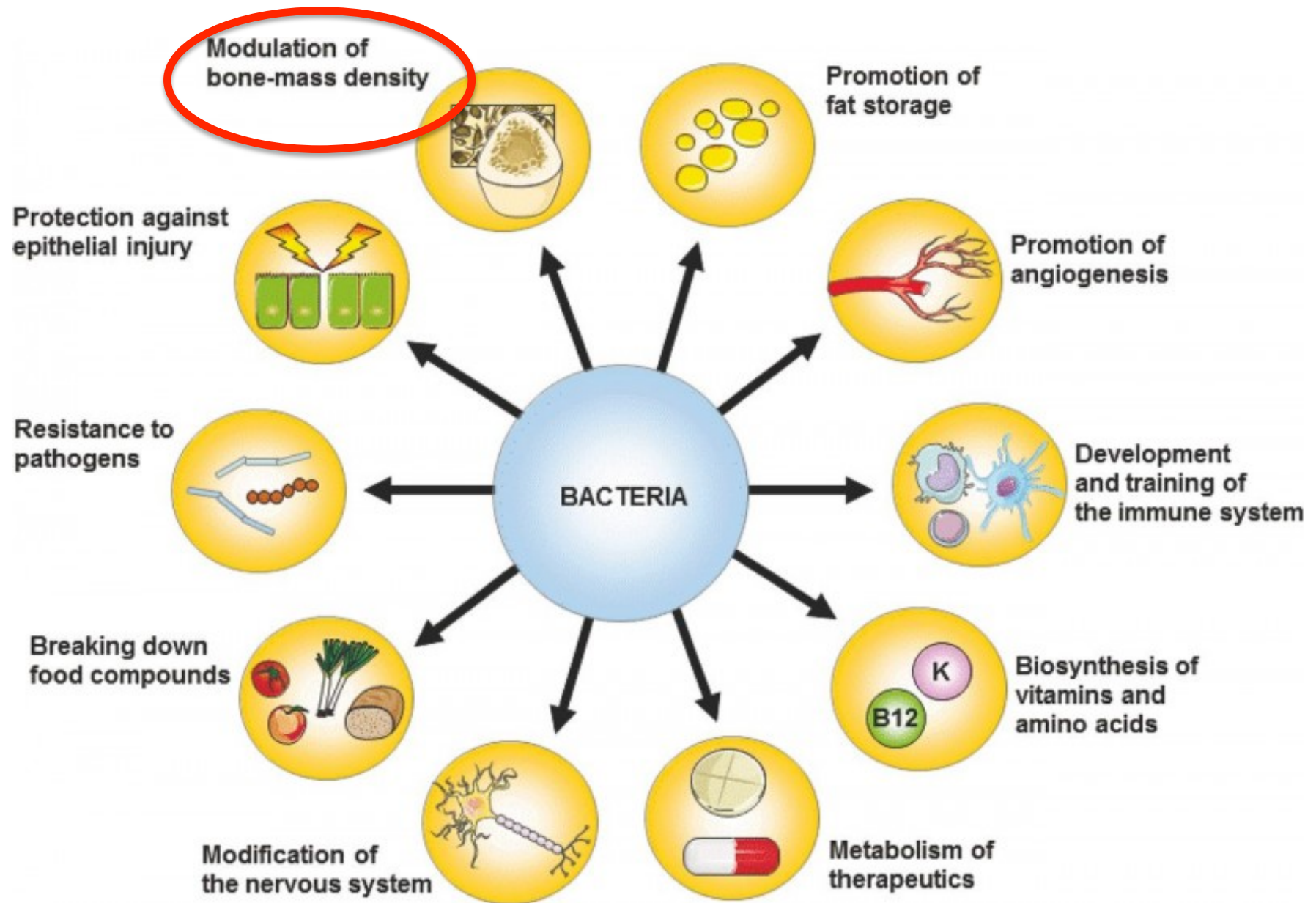


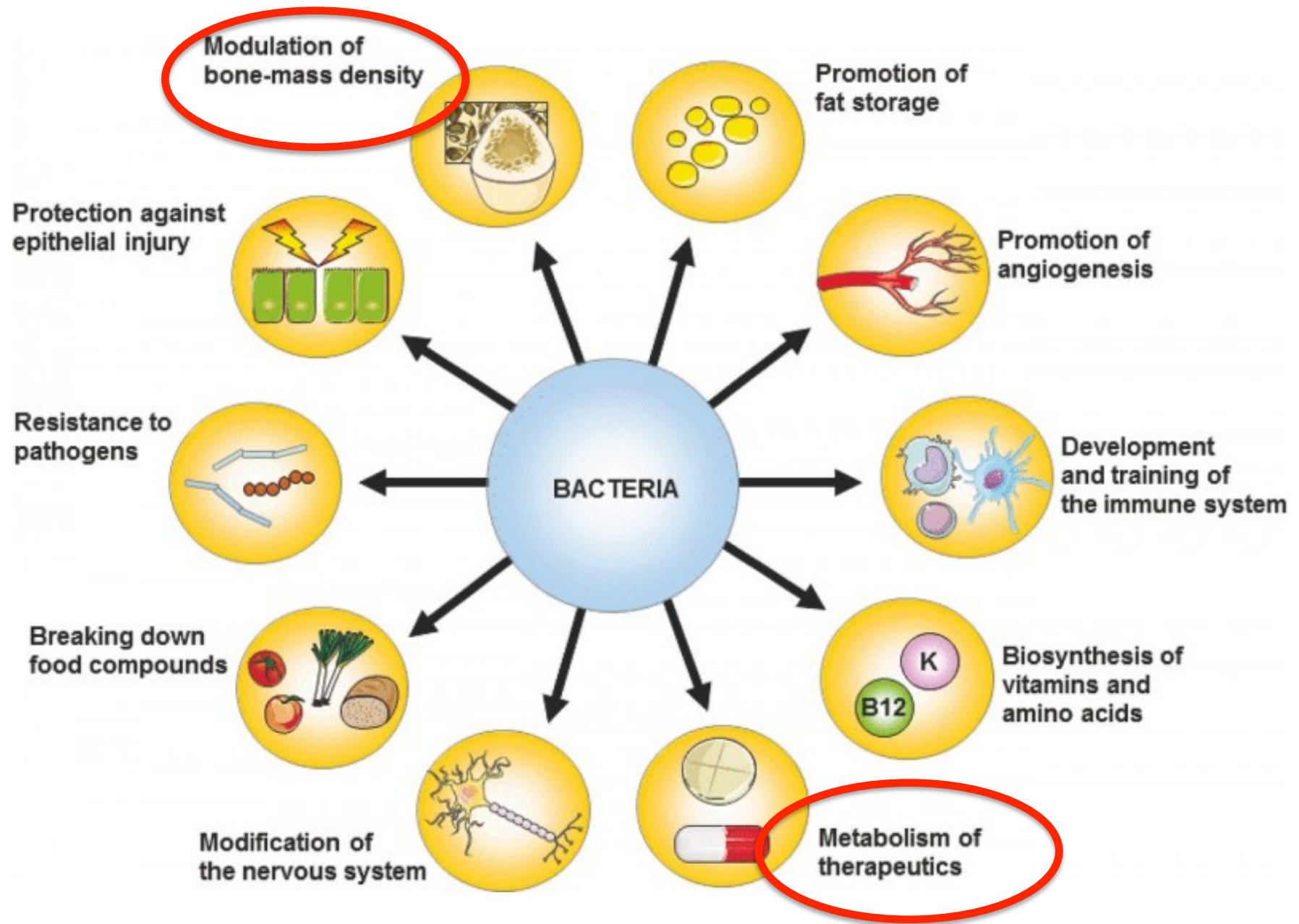
A new view of the tree of life

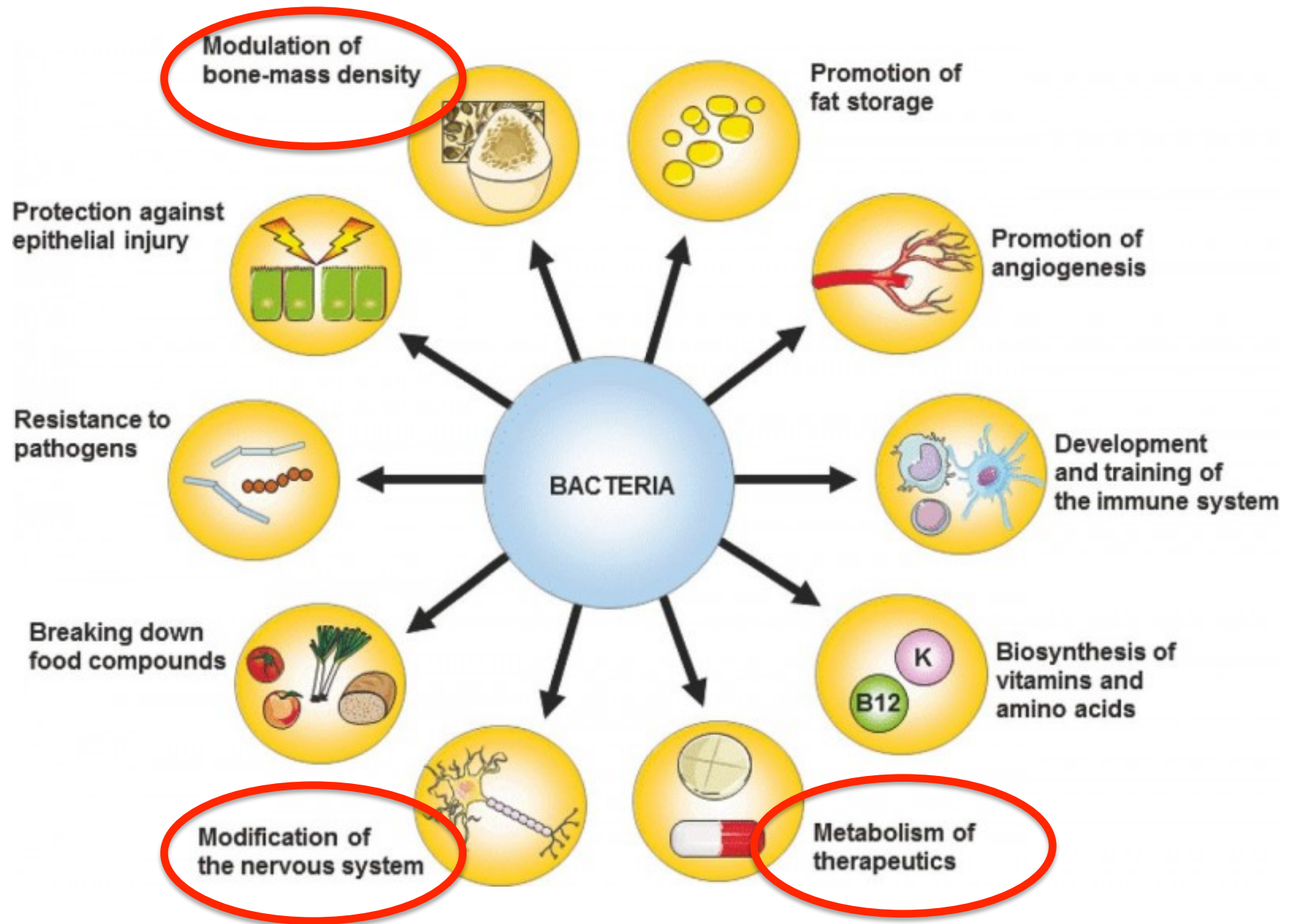
Laura A. Hug^{1†}, Brett J. Baker², Karthik Anantharaman¹, Christopher T. Brown³, Alexander J. Probst¹, Cindy J. Castelle¹, Cristina N. Butterfield², Alex W. Hernsdorf², Yuki Amano⁴, Kotaro Ise⁴, Yohey Suzuki⁵, Natasha Dudek⁶, David A. Relman^{7,8}, Kari M. Finstad⁹, Ronald Amundson⁹, Brian C. Thomas¹ and Jillian F. Banfield^{1,9*}

there is a large new branch, described as "candidate phyla radiation", only recently discovered and composed only of **symbiont extremophile bacteria**

"This incredible diversity means that there is a very incredible number of organisms we are just beginning to learn about the peculiarities, which could change our understanding of biology,"





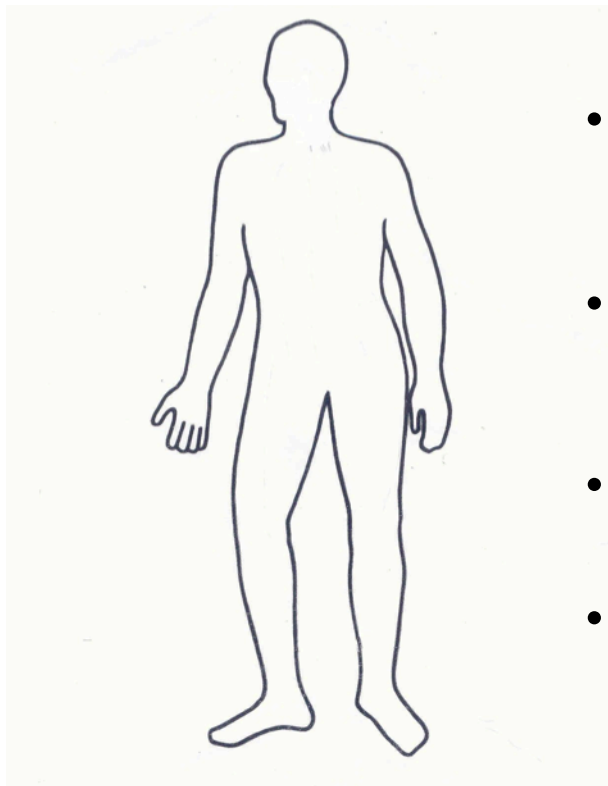


Physiological functions, i.e.:

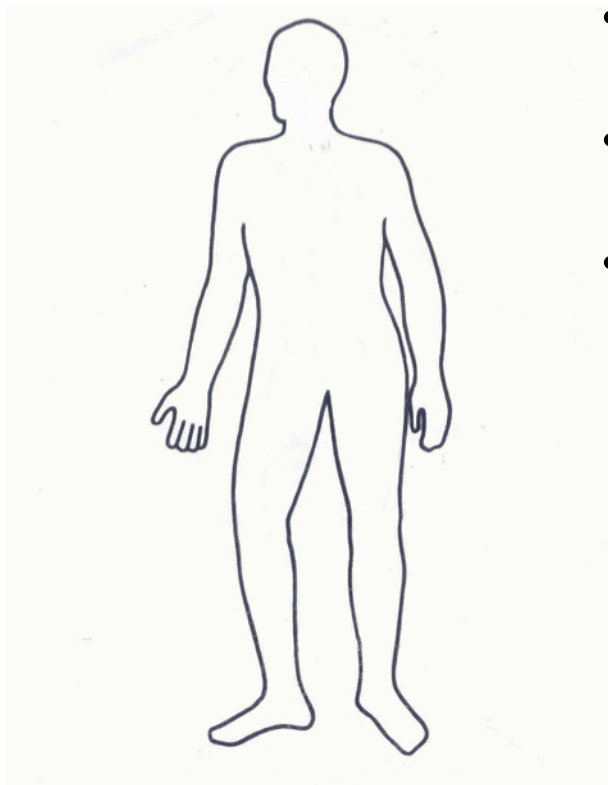
- **Blood pressure** (Yang T, Zubcevic J. Gut-Brain Axis in Regulation of Blood Pressure. Front Physiol. 2017 Oct 25;8:845).
- **Plasma levels of cholesterol** (Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens. 2013 Dec 30;3(1):14-24.)

Microbial body controls:

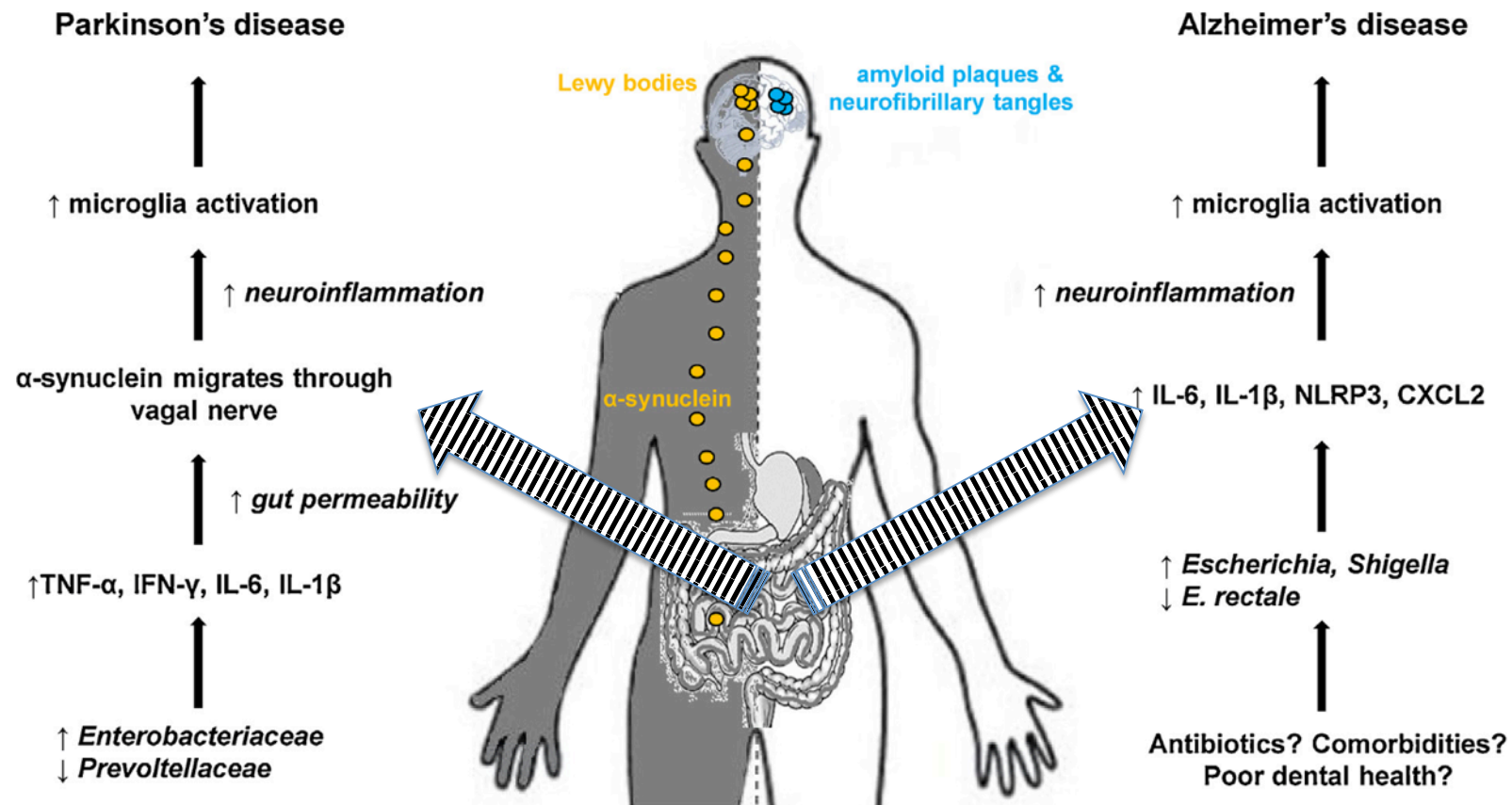
- **Metabolic diseases** (Clavel T, Desmarchelier C, Haller D, Gérard P, Rohn S, Lepage P, Daniel H. Intestinal microbiota in metabolic diseases: from bacterial community structure and functions to species of pathophysiological relevance. Gut Microbes. 2014 Jul 1;5(4):544-519)
- **Heart functions** (Luedde M, Winkler T, Heinsen FA, Rühlemann MC, Spehlmann ME, Bajrovic A, Lieb W, Franke A, Ott SJ, Frey N. Heart failure is associated with depletion of core intestinal microbiota. ESC Heart Fail. 2017 Aug;4(3):282-290)
- **Kidney functions** (Jiang S, Xie S, Lv D, Wang P, He H, Zhang T, Zhou Y, Lin Q, Zhou H, Jiang J, Nie J, Hou F, Chen Y. Alteration of the gut microbiota in Chinese population with chronic kidney disease. Sci Rep. 2017 Jun 6;7(1):287)0
- **Neuroendocrine system** (Farzi A, Fröhlich EE, Holzer P. Gut Microbiota and the Neuroendocrine System. Neurotherapeutics. 2018 Jan;15(1):5-22)
- ...



Altered Microbiota in:



- **Parkinson's**: Qian Y, Yang X, Xu S, Wu C, Song Y, Qin N, Chen SD, Xiao Q. Alteration of the fecal microbiota in Chinese patients with Parkinson's disease. Brain Behav Immun. 2018 Mar 2. pii: S0889-1591(18)30028-X.
- **Alzheimer's** (Di Sabatino A, Lenti MV, Cammalleri L, Corazza GR, Pilotto A. Frailty and the gut. Dig Liver Dis. 2018 Mar 16)
- **ALS** (Brenner D, Hiergeist A, Adis C, Mayer B, Gessner A, Ludolph AC, Weishaupt JH. The fecal microbiome of ALS patients. Neurobiol Aging. 2018 Jan;61:132-137.)
- **Huntington's**
- **MS** (Tremlett H, Waubant E. Gut microbiome and pediatric multiple sclerosis. Mult Scler. 2018 Jan;24(1):64-68).
- **Autism** (Campion D, Ponzo P, Alessandria C, Saracco GM, Balzola F. Role of microbiota in the autism spectrum disorders. Minerva Gastroenterol Dietol. 2018 Mar 30.)
- ...



Different microbiota alterations produce different pathologies. Microbiota alteration in childhood epilepsy remains unexplored.

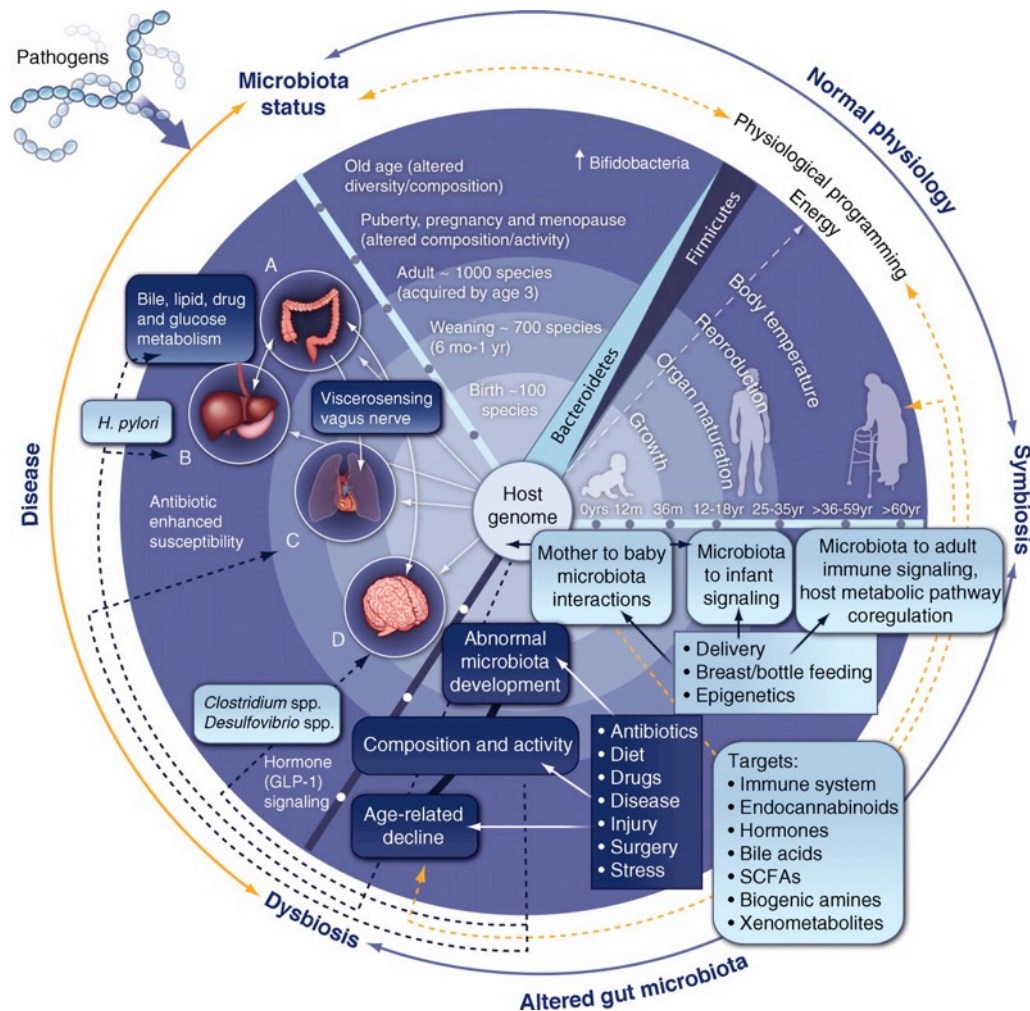
Intestinal Microbiota: A moderator in health and diseases.

4573 articles on pubmed

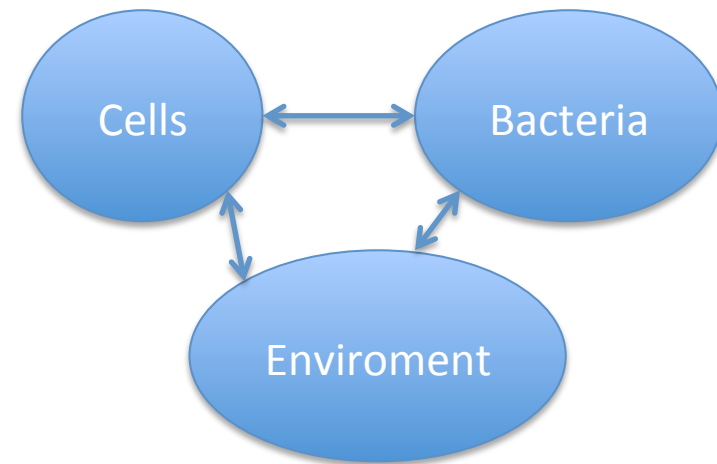


Feng Q, Chen W-D and Wang Y-D (2018) Gut Microbiota: An Integral Moderator in Health and Disease. Front. Microbiol. 9:151

THE GUT MICROBIOTA

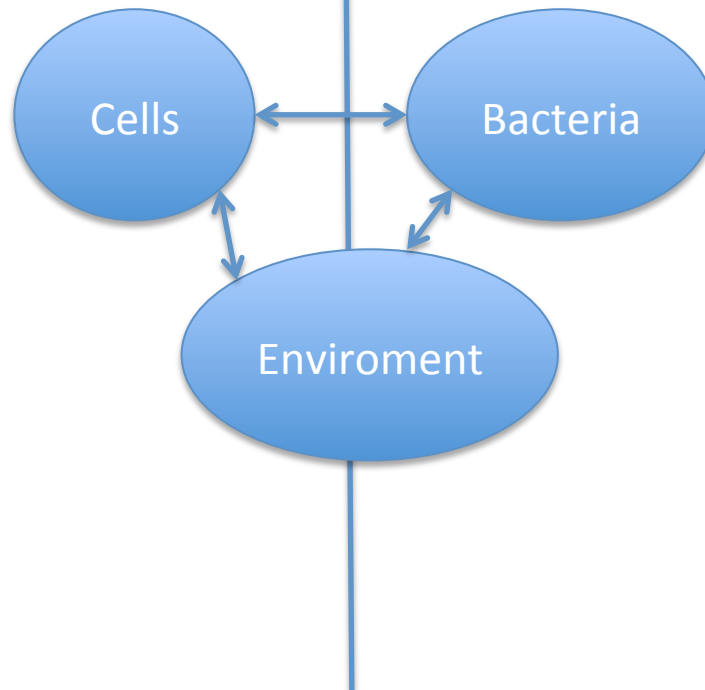


... Gut microecological imbalance caused by various biogenic and abiogenic agents and factors can produce different epigenetic abnormalities and the onset and progression of metabolic diseases associated ...



Malattia

Salute



Microbiota orchestrates inflammatory processes

Dysregulation of gut microbiota and **chronic inflammatory** disease

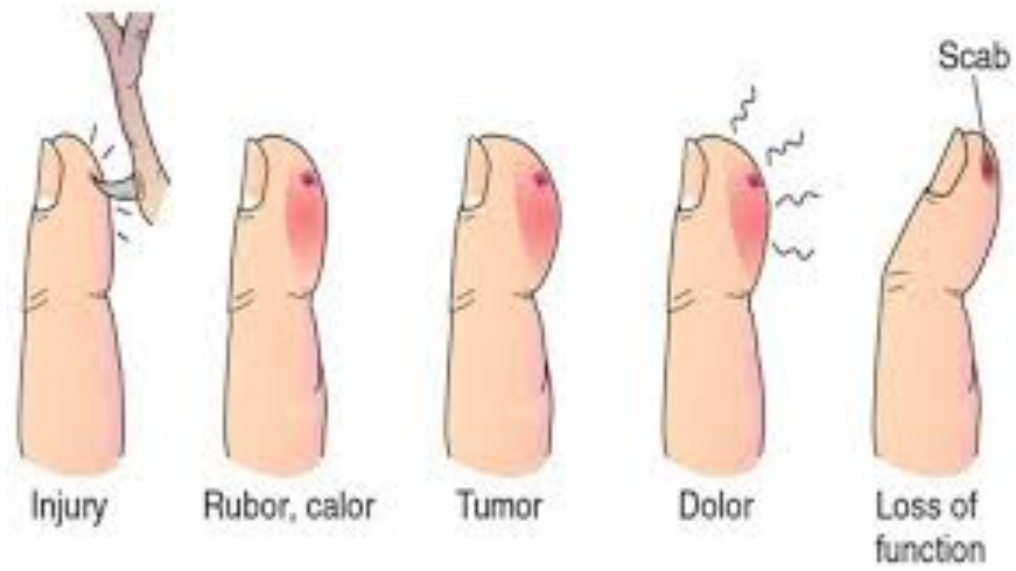
*In addition to their barrier function, Intestinal Epithelial Cells (IECs) serve as ‘mediators’, maintaining a **balanced relationship between gut microbes and the host immune system** by secreting **cytokines**, chemokines and hormones.*

Microbiota orchestrates inflammatory processes

Dysregulation of gut microbiota and **chronic inflammatory** disease

*In addition to their barrier function, Intestinal Epithelial Cells (IECs) serve as ‘mediators’, maintaining a **balanced relationship between gut microbes and the host immune system** by secreting **cytokines**, chemokines and hormones.*

*Thus, dysfunction of the IECs leads to perturbation of the gut microbiota and enhances susceptibility to **intestinal inflammation***



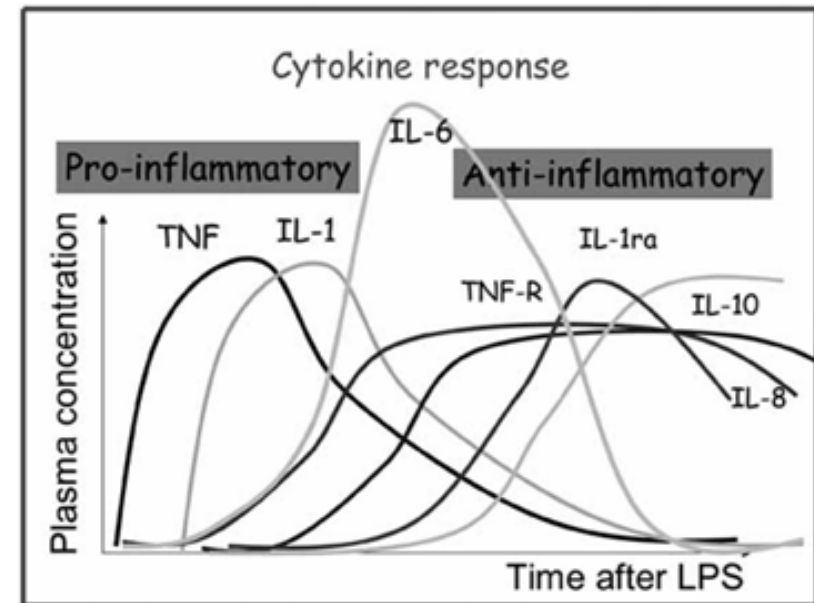
inflammatory processes to repair damaged tissues

Inflammatory cascade:

Pro- and anti-inflammatory Modulate from APR to CPR

APR: Acute Phase Reaction

CPR: Chronic Phase Reaction



- The Pro-Inflammatory Cytokines produce necrosis
- The Anti-inflammatory Cytokines rebuild tissues

In CNS:

- The Pro-inflammatory cytokines inhibit synaptogenesis and neurogenesis,
- The Anti-inflammatory cytokines restart Synpatogenesis and neurogenesis processes

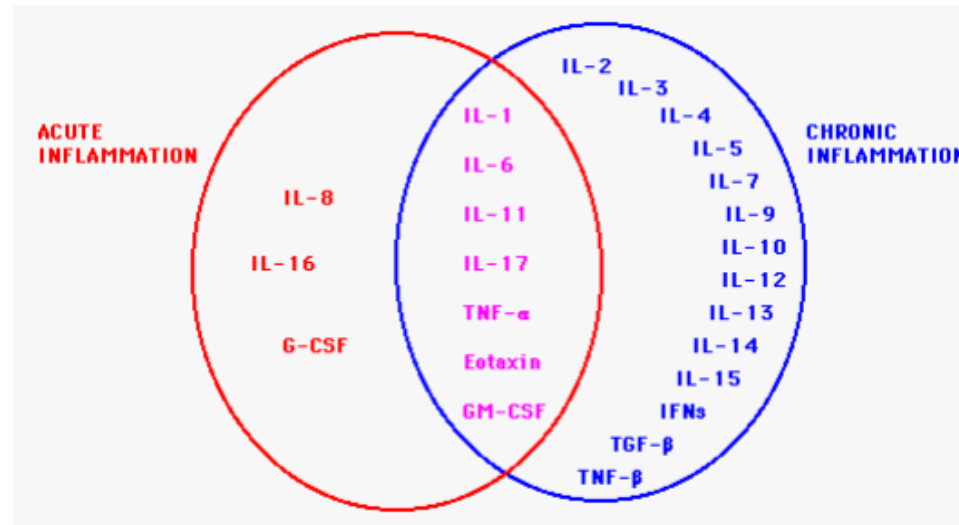


FIGURE 1: Cytokines involved in acute and chronic inflammatory responses.

In Chronic Inflammation are produced different anti-inflammatory cytokines: unable to rebuilt tissues, unble to restart synaptogenesis and neurogenesis processes in CNS

***Stig Bengmark, Acute and “chronic” phase reaction - a mother of disease.
Clinical Nutrition (2004) 23, 1256–1266***



Contributions of peripheral inflammation to seizure susceptibility: Cytokines and brain excitability

Kiarash Riazi*, Michael A. Galic, Quentin J. Pittman

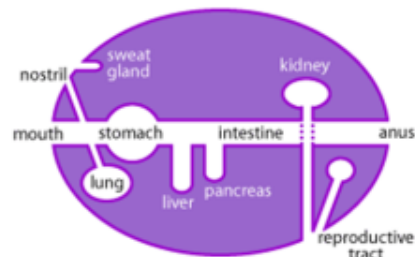


Figure 1: Schematic diagram of the mucous membranes of the body and surfaces with which they are in contact.

K. Riazi et al.

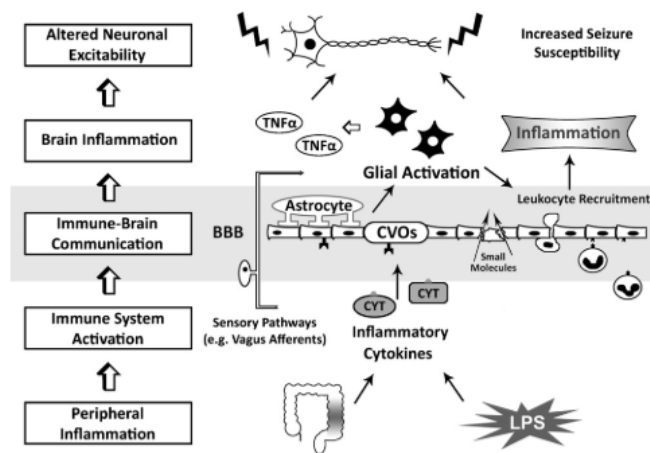


Figure 1 Schematic representation of the hypothetical cascade of events through which peripheral inflammation results in increased seizure susceptibility. Activation of a peripheral immune response, either by colitis or LPS, leads to increased cytokine signalling through neuronal or humoral routes triggering an inflammatory response within the CNS. This mirrored inflammation is likely mediated by cytokines (TNF α), along with interactions between leukocytes and glial cells across the blood–brain barrier. The culmination of an activated immune response within the brain may result in changes in neuronal excitability and ultimately a pro-convulsive tendency. BBB, blood–brain barrier; CVO, circumventricular organ; CYT, cytokine.

Chronic Inflammation is able to migrate from intestine to other tissues.

It start to repair tissues but it is able only to demolish them, not to rebuild them.

In the brain it is able to disconnect bad function neurons, but it is unable to substitute them with good function neurons (synaptogenesis) or to realize new neurons (neurogenesis)

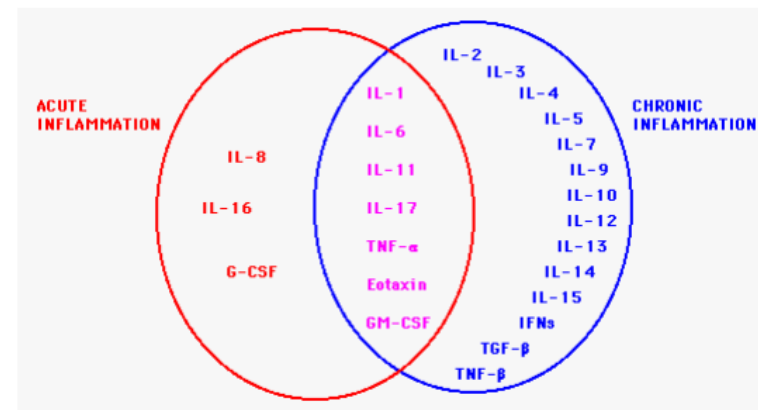
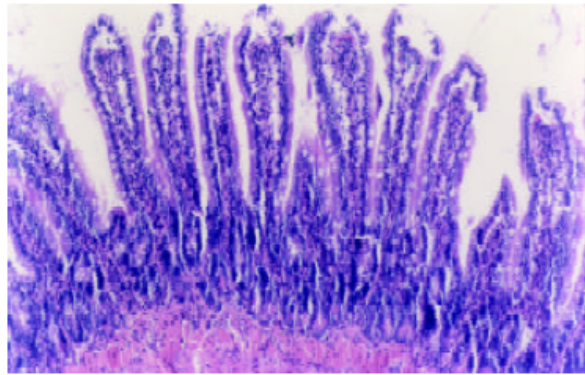
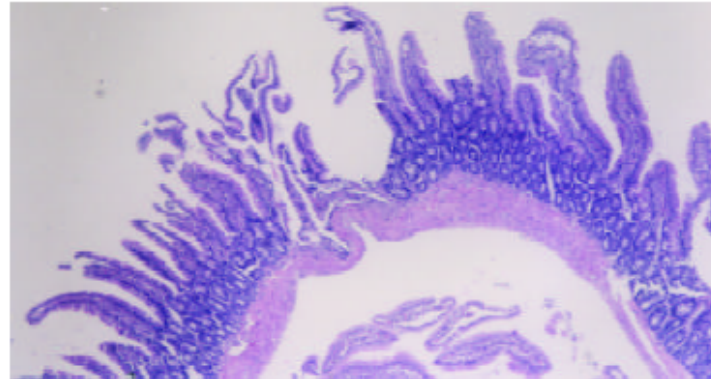


FIGURE 1: Cytokines involved in acute and chronic inflammatory responses.

3 days after TBI collapses the intestinal membrane

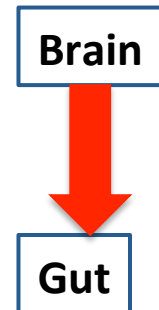


Before TBI



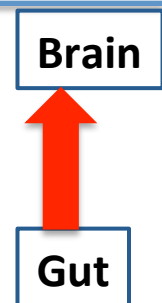
3 days After TBI

Hang Ch et al, Intestinal mucosa structure after TBI. World J Gastroenterol 2003,9 (12) 2776-2781



*IL-6 elevation in the brain is involved in the mediation of autism-like behaviors through **impairments of neuroanatomical structures** and neuronal plasticity*

Wei H, Alberts I, Li X. Brain IL-6 and autism. *Neuroscience*. 2013 Nov 12;252:320-5.



- Cytokines participate in neuronal development in brain functioning. Inappropriate activity can produce different neurological symptoms

Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. *Neurotoxicol Teratol*. 2013 Mar-Apr;36:67-81.

Brain, Behavior, and Immunity

**BRAIN,
BEHAVIOR,
and IMMUNITY**

The Official Journal of the Psychoneuroendocrinology Society

Diagram illustrating the relationship between Social Stress, HGF expression, SNS activation, IFN response, and Disease progression.

```

graph TD
    A([Social Stress]) --> B[↑ HGF expression]
    B --> C([SNS activation])
    C --> D[↑ IFN response]
    D --> E([Disease progression])
  
```

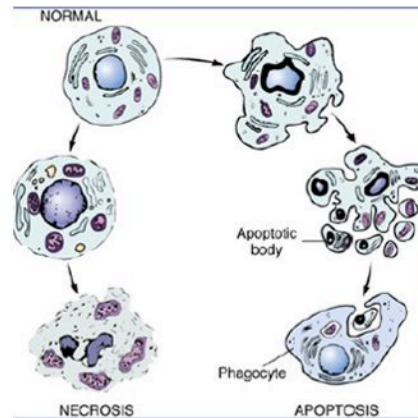
The role of cytokines in the pathophysiology of epilepsy

Mario Negri Institute for Pharmacological Research, Department of Neuroscience, Via G La Masa, 19 Milano, Italy

*Brain inflammation is not a predisposing factor for seizures, but it is the **physiopathogenetic cause** of them.*

Meccanismi di morte cellulare

- Apoptosi
 - Morte programmata
 - Contrazione
 - Corpi apoptotici
- Necrosi
 - Morte accidentale
 - Espansione
 - Infiammazione



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Cytokine-related Mechanisms of Apoptosis

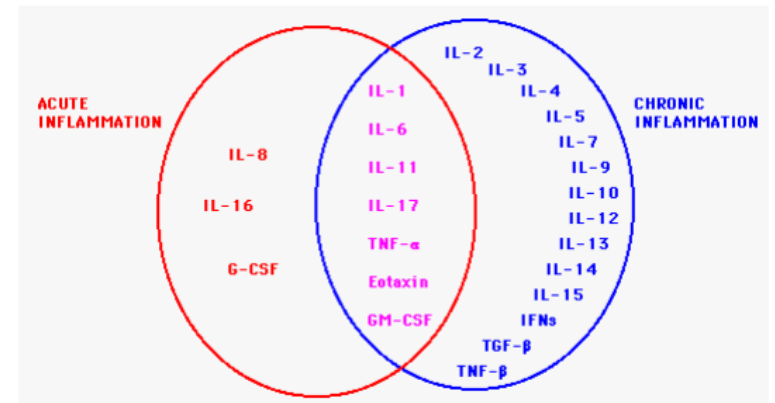
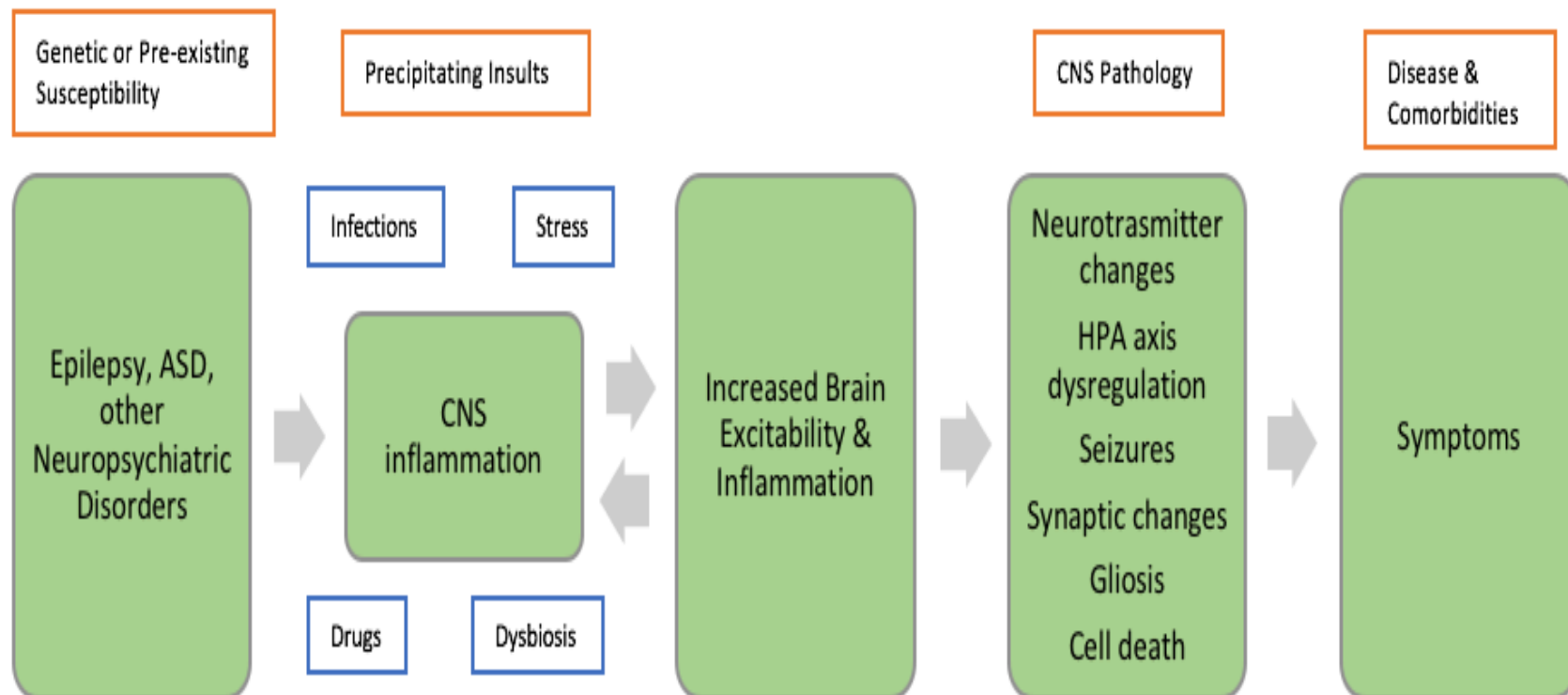


FIGURE 1: Cytokines involved in acute and chronic inflammatory responses.

IL RUOLO DEL MICROBIOTA NELLE MALATTIE NEUROPSICHIATRICHE

- ✓ Il microbiota influenza processi chiave del SNC
- ✓ Pazienti affetti da epilessia, ASD, ADHD, schizofrenia, ansia, depressione, malattie neurodegenerative hanno un microbiota alterato.



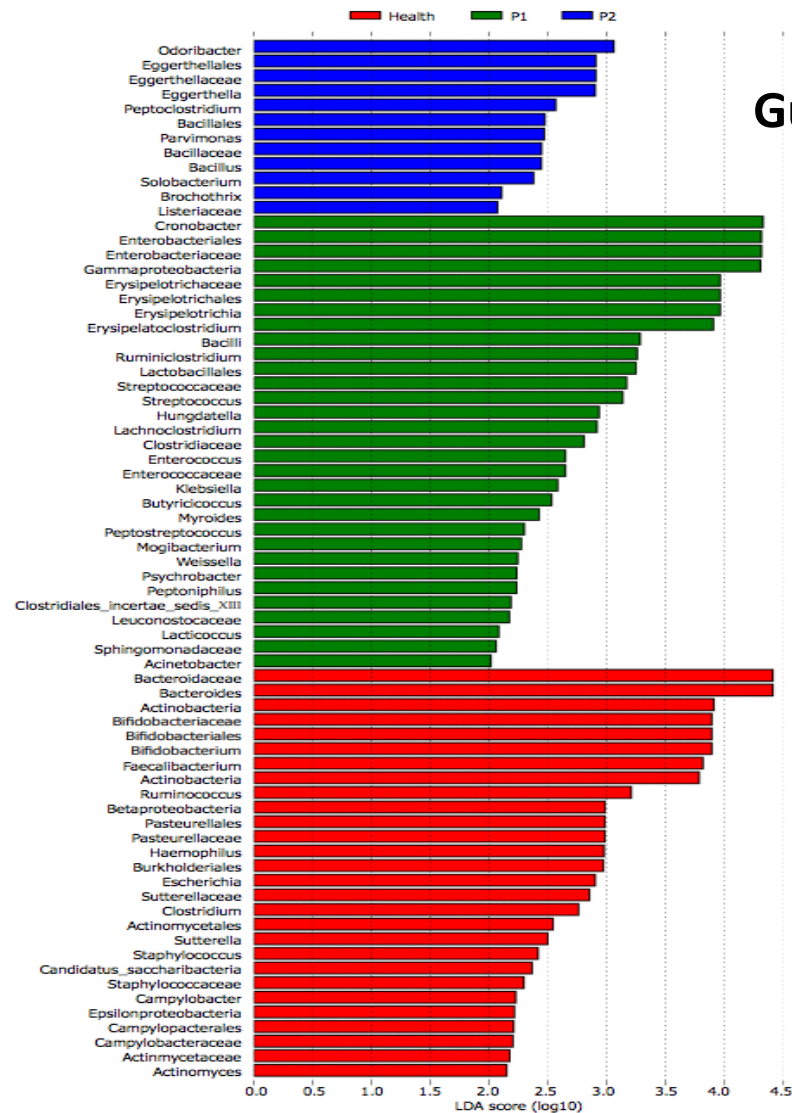


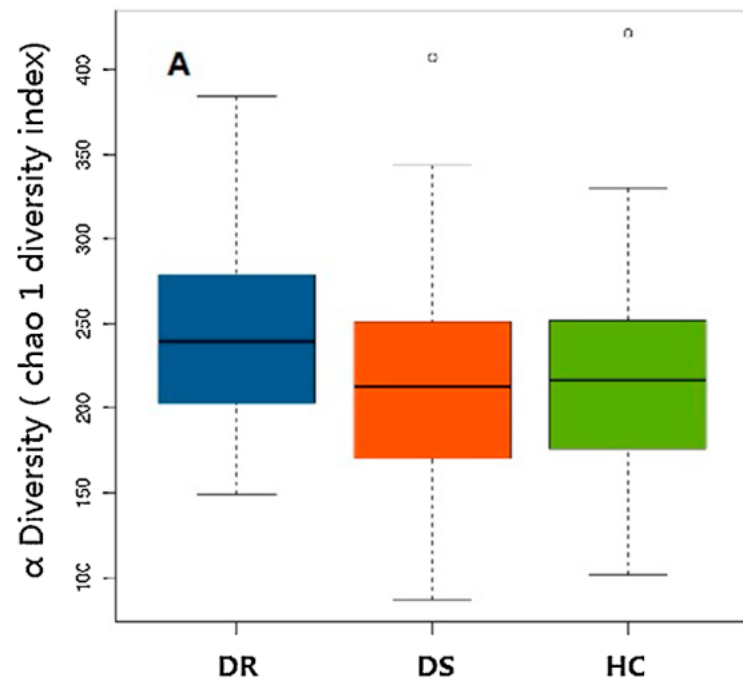
Figure 4 Significantly enriched gut microbiota components in the Health, P1 and P2 groups. LEfSe analysis was applied to detect the gut microbiota (GM) components in the three groups. Red, green, and blue represent the Health, P1 and P2 groups, respectively. The LDA score was set as ≤ 2 . The enrichment degree is proportional to the LDA score.

Gut microbiota is altered In epilepsy, too

- In conclusion, we found that GUT MICROBIOTA of infants with refractory epilepsy differed dramatically from that of healthy infants.
- Epileptic patients harbored significantly enriched pathogens and decreased beneficial bacteria.

Altered composition of the gut microbiome in patients with drug-resistant epilepsy

- ✓ patients with drug-resistant epilepsy (DR) are characterized by the over-expression of rare microbial lineages.



DR: drug resistant
DS: drug sensitive
HR: Health controls

Peng A, Qiu X, Lai W, Li W, Zhang L, Zhu X, He S, Duan J, Chen L. Altered composition of the gut microbiome in patients with drug-resistant epilepsy. *Epilepsy Res.* 2018 Sep 24;147:102-107.

OPEN

Analysis of gut microbiota profiles and microbe-disease associations in children with autism spectrum disorders in China

Mengxiang Zhang^{1,2}, Wei Ma³, Juan Zhang⁴, Yi He^{1,2} & Juan Wang^{1,2}

Received: 14 March 2018

Accepted: 24 August 2018

Published online: 18 September 2018

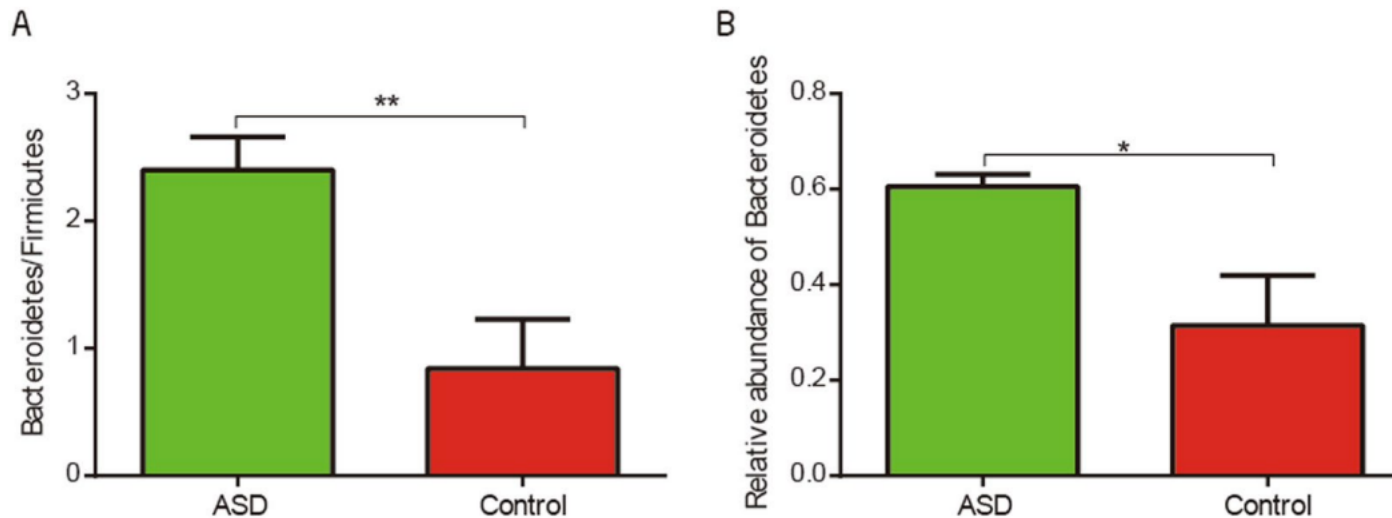
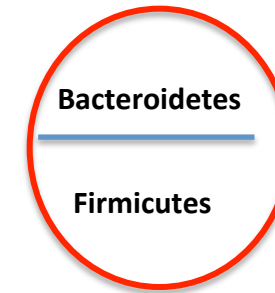
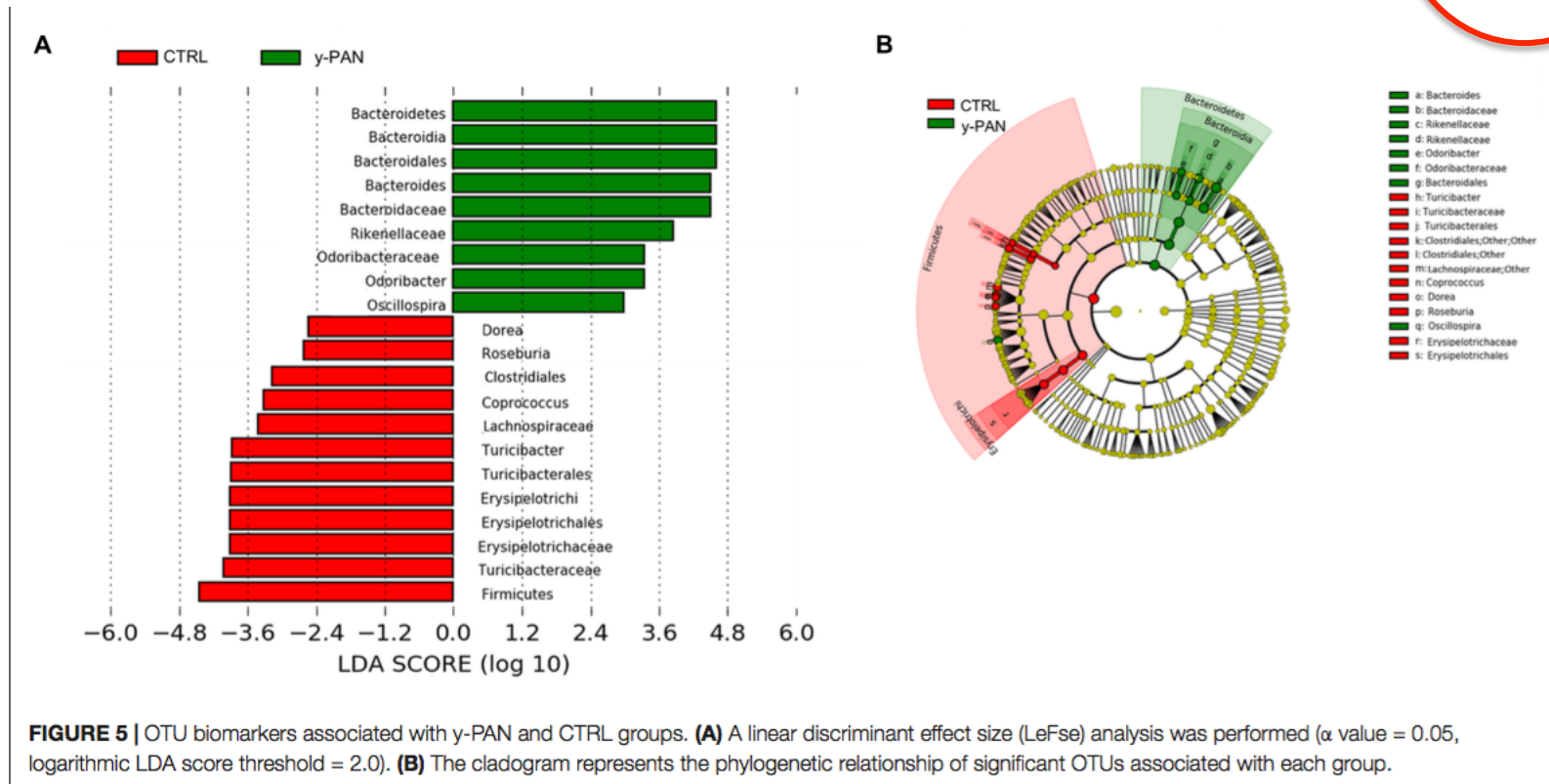
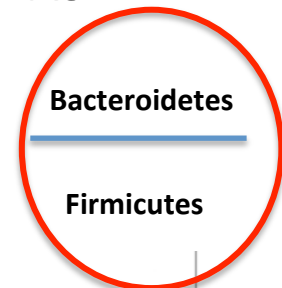


Figure 1. (A) Comparison of the ratio of *Bacteroidetes/Firmicutes* between ASD children and typical development children (** $p < 0.005$, Wilcoxon rank-sum test). (B) Box plot representation of the relative abundance of *Bacteroidetes* (*FDR-corrected $p < 0.05$, Wilcoxon rank-sum test). The boxes represent the mean \pm Standard Error of Mean (SEM).

Gut Microbiota Profiling and Gut-Brain Crosstalk in PANDAS

Disequilibrium between **Firmicutes** and **Bacteroidetes** (F/B)



Quagliariello A, et al. Gut Microbiota Profiling and Gut-Brain Crosstalk in Children Affected by Pediatric Acute-Onset Neuropsychiatric Syndrome and Pediatric Autoimmune Neuropsychiatric Disorders Associated With Streptococcal Infections. Front. Microbiol. 9:675.

An altered F/B ratio carry out to altered metabolism

$$F/B_{\gamma\text{-PAN}} < F/B_{\text{ctrl}}$$

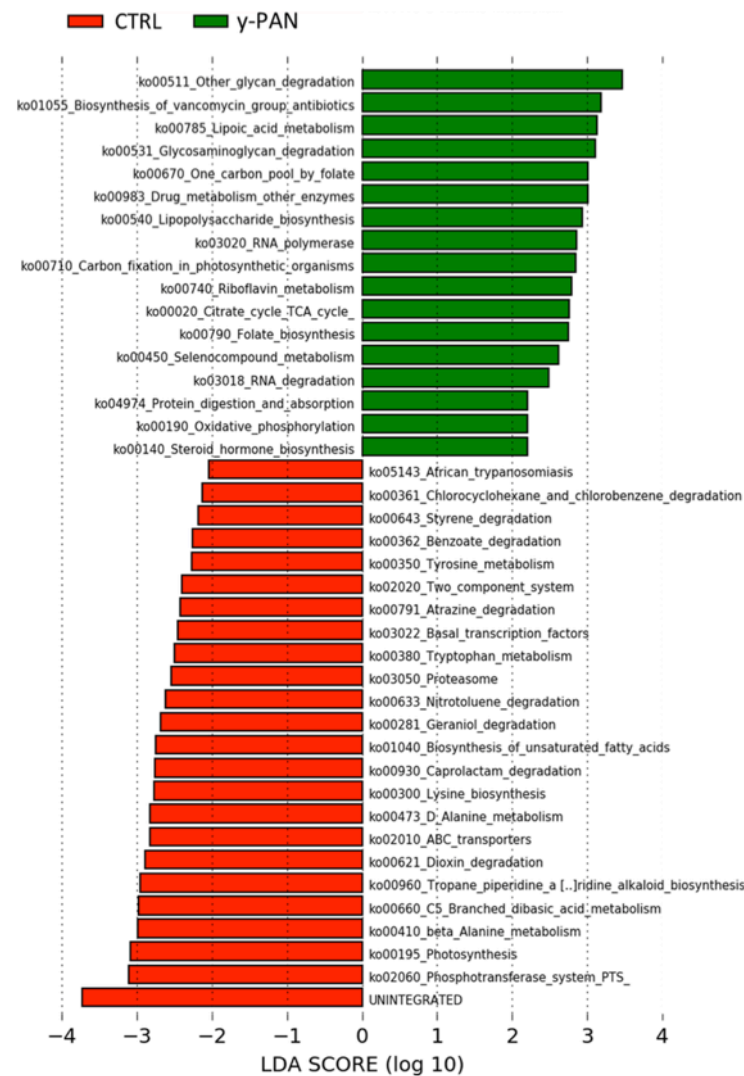
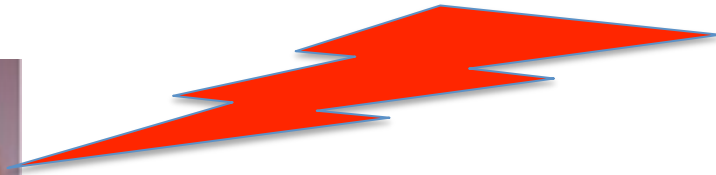


FIGURE 6 | KEGG biomarkers associated with the γ-PAN and CTRL groups. A linear discriminant effect size (LeFse) analysis has been performed (α value = 0.05, logarithmic LDA score threshold = 2.0).

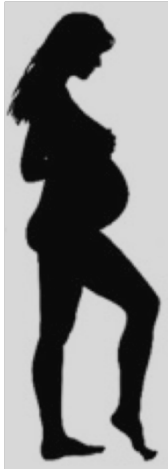
Quagliariello A, et al. Gut Microbiota Profiling and Gut–Brain Crosstalk in Children Affected by Pediatric Acute-Onset Neuropsychiatric Syndrome and Pediatric Autoimmune Neuropsychiatric Disorders Associated With Streptococcal Infections. *Front. Microbiol.* 9:675.

when does the problem originate?



- **Impact of Maternal Stress in Pregnancy on Brain Function of the Offspring**

Udagawa J, Hino K. Impact of Maternal Stress in Pregnancy on Brain Function of the Offspring. *Nihon Eiseigaku Zasshi*. 2016;71(3):188-194.



Metabolic changes during pregnancy:

- Same nutritional intake
- Increased energy extraction by foods:

Leptin (ng/ml) ^f	30.72 (±1.83)	37.58 (±2.47)	0.0008
Cholesterol (mmol/l) ^f	4.76 (±0.09)	6.37 (±0.12)	1.72 × 10 ⁻³³
Insulin (mU/l) ^f	6.48 (±0.59)	10.92 (±0.88)	1.01 × 10 ⁻⁸
Homeostatic model assessment (HOMA) ^f	1.35 (±0.12)	2.28 (±0.19)	1.93 × 10 ⁻⁷
Quantitative insulin sensitivity check index (QUICKI) ^f	0.39 (±0.01)	0.35 (±0.00)	2.39 × 10 ⁻⁹
Glucose (mmol/l)	4.65 (±0.03)	4.61 (±0.05)	0.5799
GHbA1c1 (%) ^f	5.01 (±0.03)	5.23 (±0.03)	9.92 × 10 ⁻¹⁰

Cell 150, 470–480, August 3, 2012 ©2012 Elsevier Inc.

Cell

Host Remodeling of the Gut Microbiome and Metabolic Changes during Pregnancy

Omry Korem,¹ Julia K. Goodrich,¹ Tyler C. Cullen,¹ Aydin Spon,^{1,11} Kari Laitinen,^{1,12} Helene Kling Bläckhed,^{1,3} Antonio Gonzalez,⁴ Jeffrey J. Warner,^{1,12} Liana T. Angerel,¹ Rob Knight,^{1,12} Fredrik Bläckhed,^{1,3} Erika Isaksson,⁵ Soope Sammes,⁶ and Ruth E. Ley^{1,12}

- the microbiota undergoes profound changes in T3 vs T1
- It is not due to nourish the fetus, but to realize his tissues by anti-inflammatory cytokines

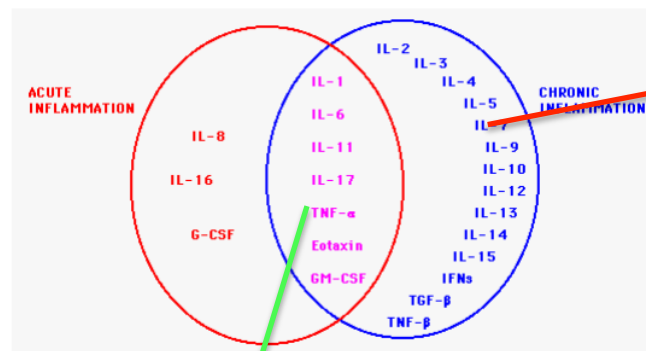


FIGURE 1: Cytokines involved in acute and chronic inflammatory responses.

Houston, we have a problem!

No-problems



when does the problem originate?



- **Neonatal inflammatory pain and systemic inflammatory responses as possible environmental factors in the development of autism spectrum disorder of juvenile rats.**

The first 1000 days of life set the tone for the whole of the lifespan.

May be linked with the bacterial phylogenetic diversity?

In the first 1000 days of life, new born arrives to have a microbiota like mother's one at T1

By Caesarean section or too medicalized deliveries risks taking the midwife's one

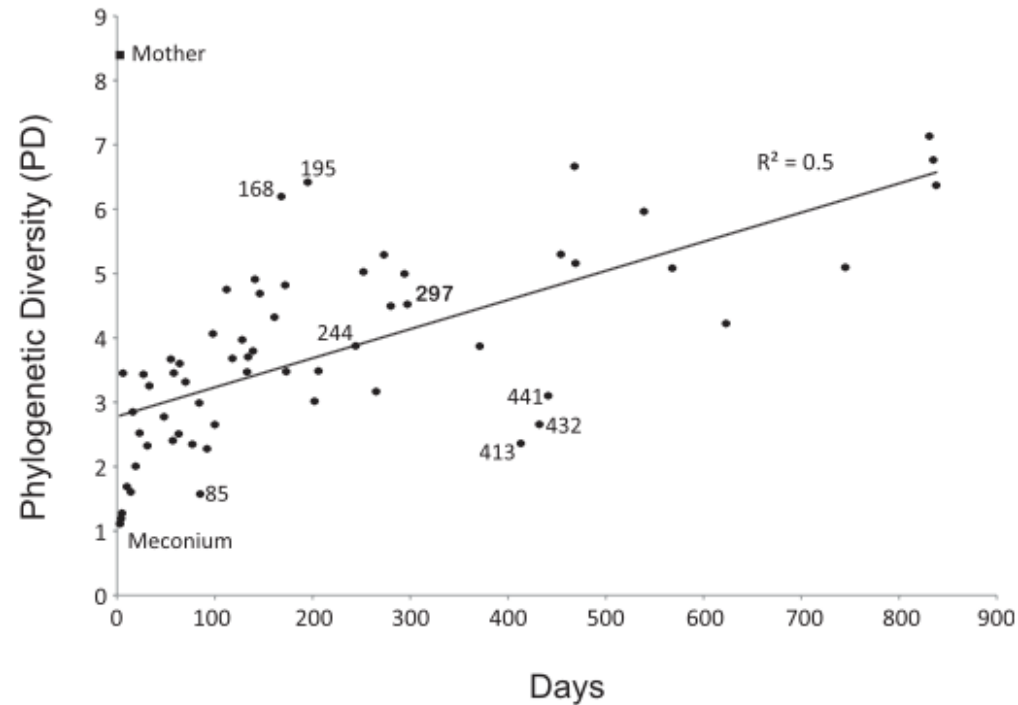
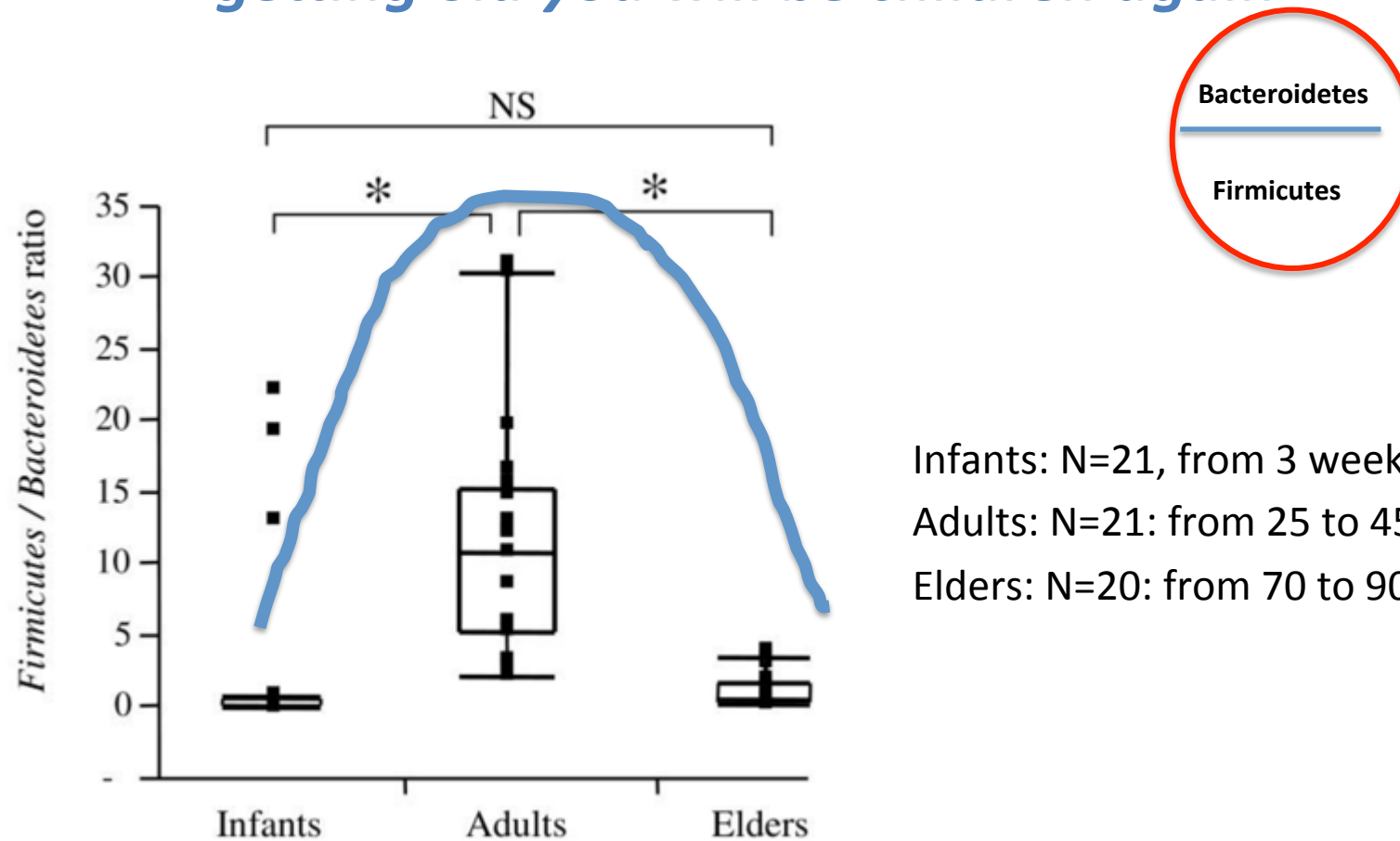


Fig. 1. Bacterial PD of the infant gut microbiota over time. PD provides a measure of the diversity within a community based on the extent of the 16S rRNA phylogenetic tree that is represented by that community. Symbols are fecal samples. The mother's fecal sample, collected at day 3, is denoted as a filled square.

Firmicutes/Bacteroidetes ratio vs ageing

getting old you will be children again



Infants: N=21, from 3 weeks to 10 months

Adults: N=21: from 25 to 45 years

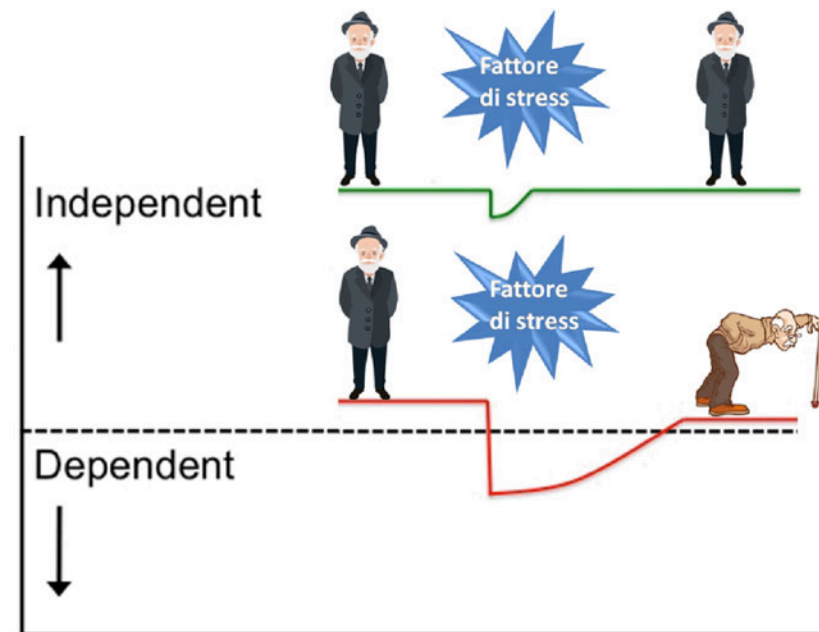
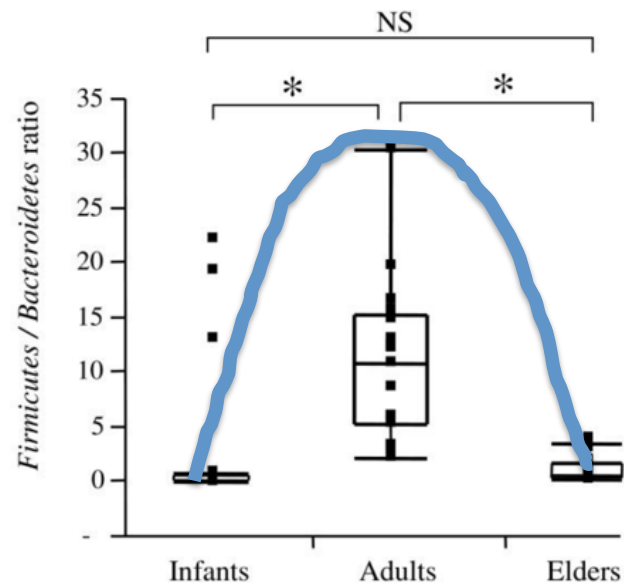
Elders: N=20: from 70 to 90 years

Mariat D, Firmesse O, Levenez F, Guimarães V, Sokol H, Doré J, Corthier G, Furet JP. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol. 2009 Jun 9;9:123

Frailty in older people

Frailty is the most problematic expression of population ageing.

It is a **state of vulnerability** to **poor resolution of homeostasis** following a stress and is a **consequence of cumulative decline in multiple physiological systems over a lifespan**.



The frail gut is characterised by a low-grade chronic mucosal inflammation, impaired immune response, increased permeability, and reduced microbiota diversity

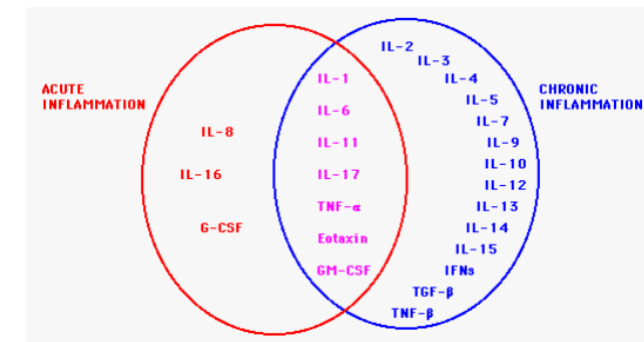
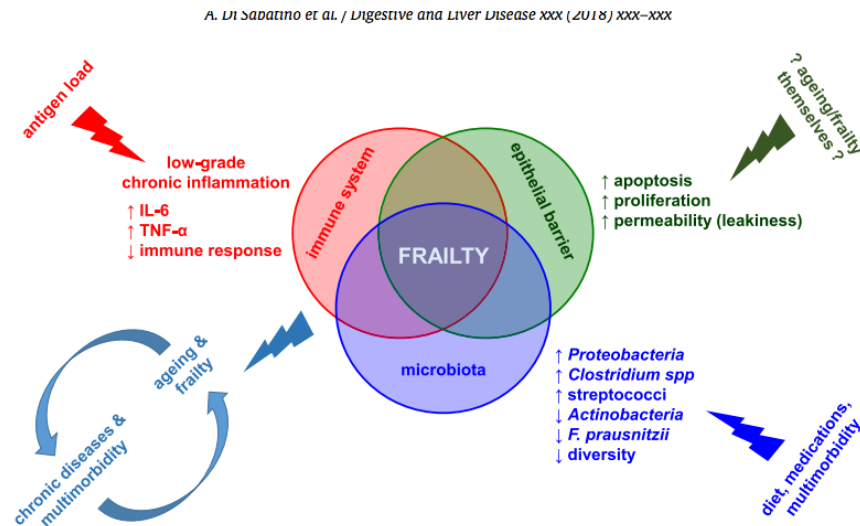


FIGURE 1: Cytokines involved in acute and chronic inflammatory responses.

A reduced microbiota diversity carry out to **Chronic Inflammation**:
A type of inflammation unable to repair tissues continually damaged by stressor

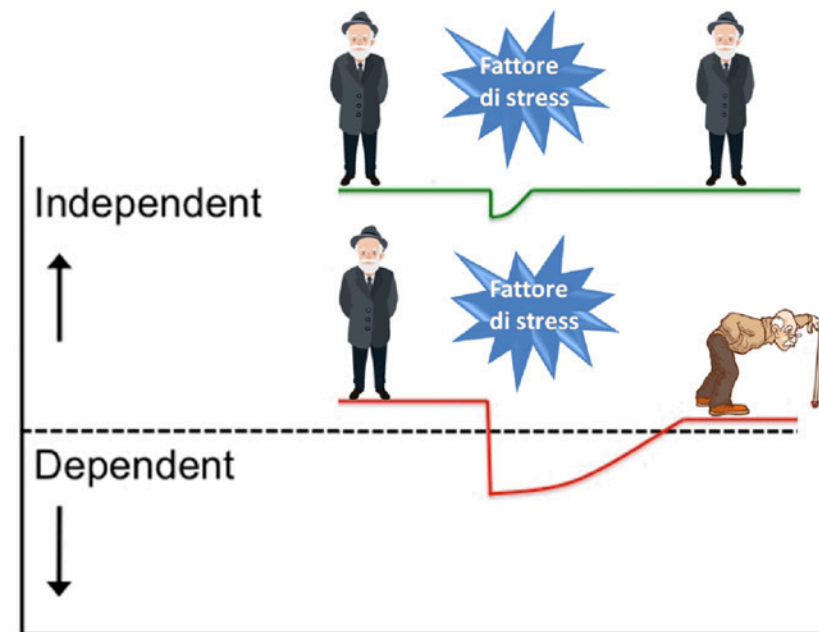
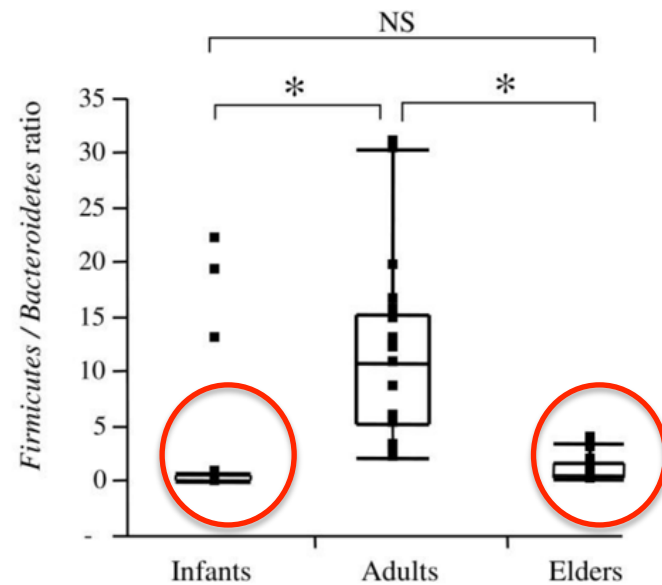
Fintanto che sono capace a riparmi, l'agente stressogeno non mi causa problemi

Di Sabatino A, Lenti MV, Cammalleri L, Corazza GR, Pilotto A. Frailty and the gut. Dig Liver Dis. 2018 Mar 16. pii: S1590-8658(18)30228-7.

Frailty in older people

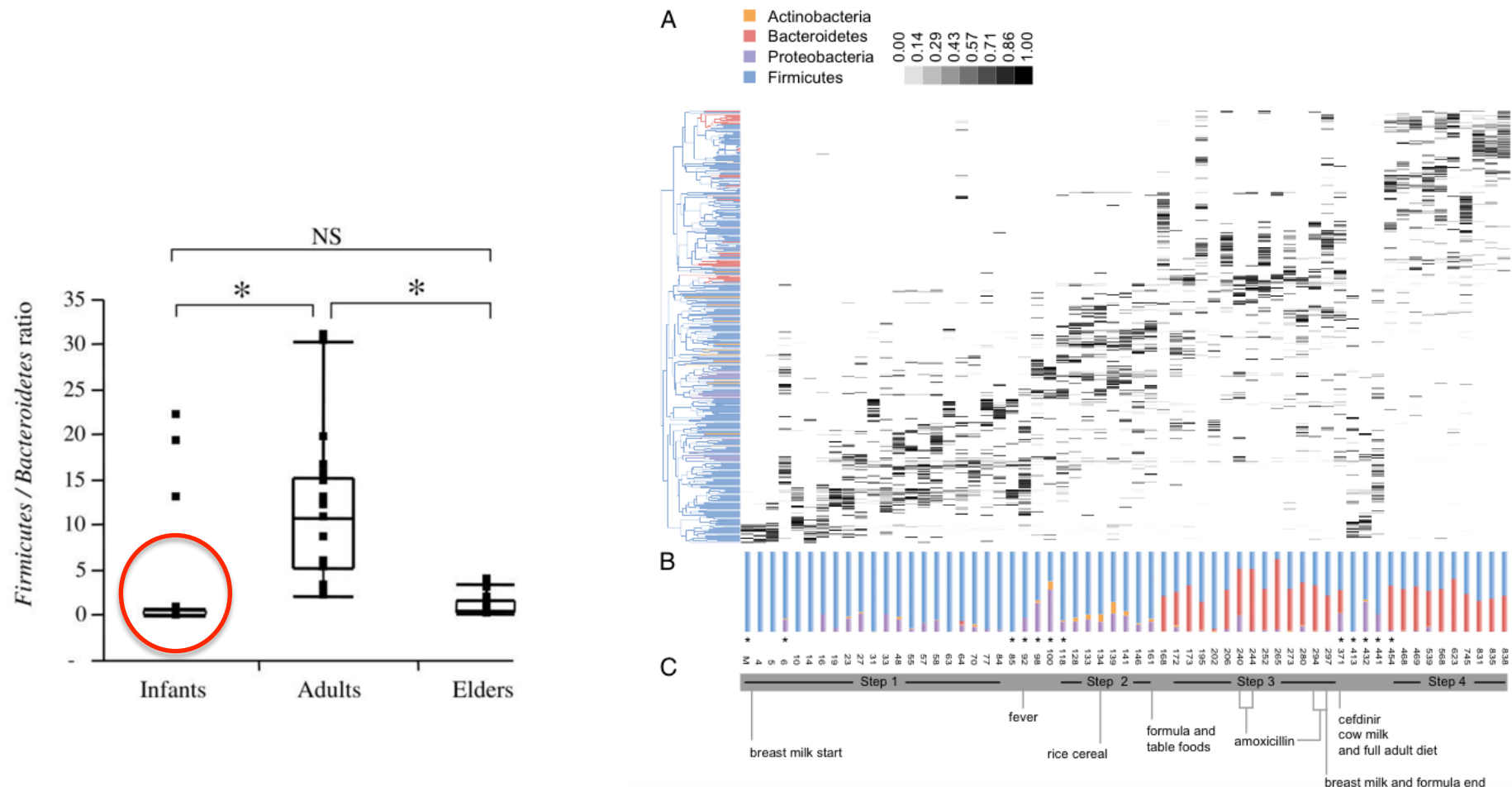
Frailty is the most problematic expression of population ageing. It is a state of vulnerability to **poor resolution of homeostasis following a stress** and is a consequence of cumulative decline in multiple physiological systems over a lifespan.

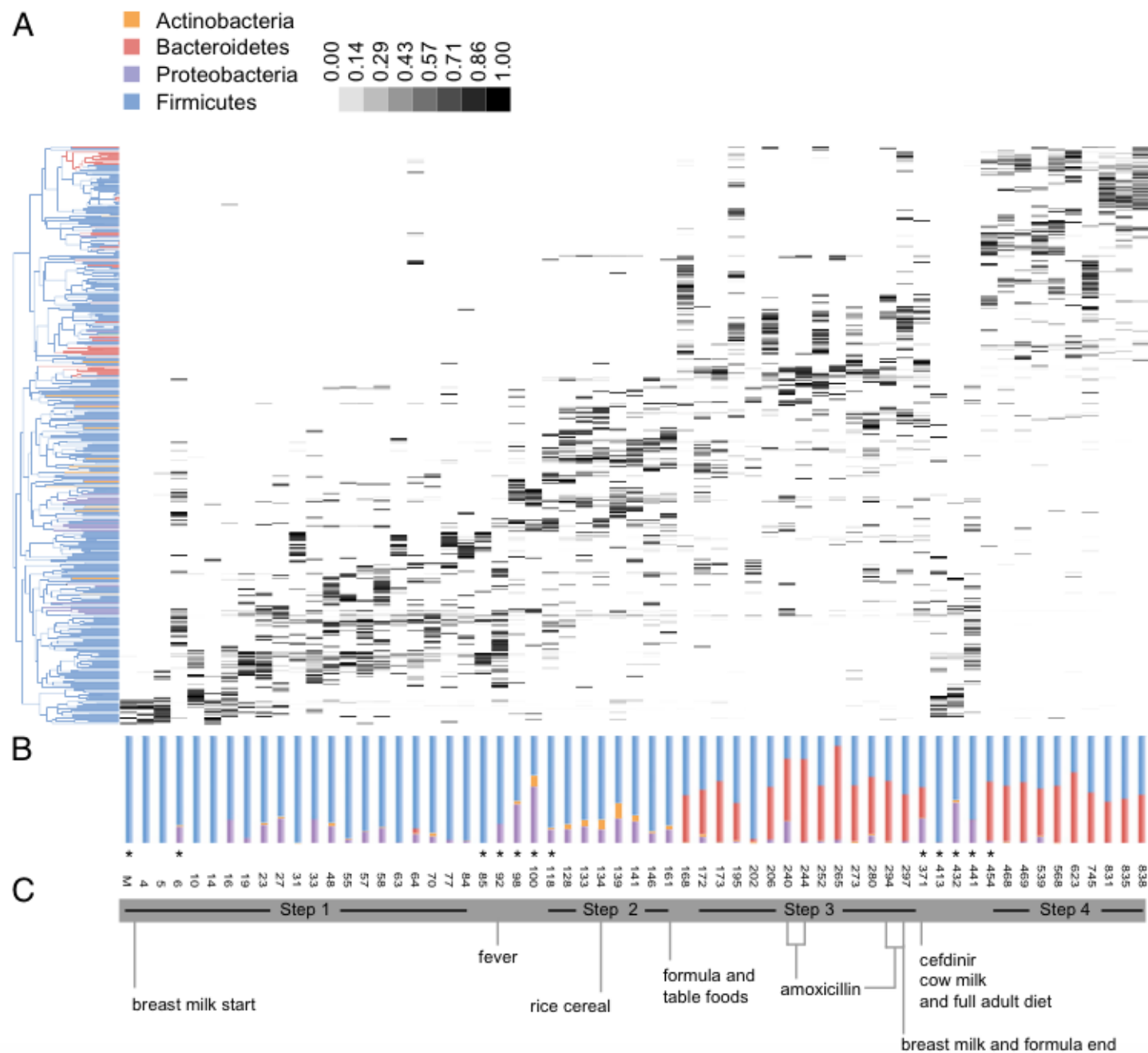
LIKE VULNERABILITY IN THE FIRST 1000 DAYS OF LIFE



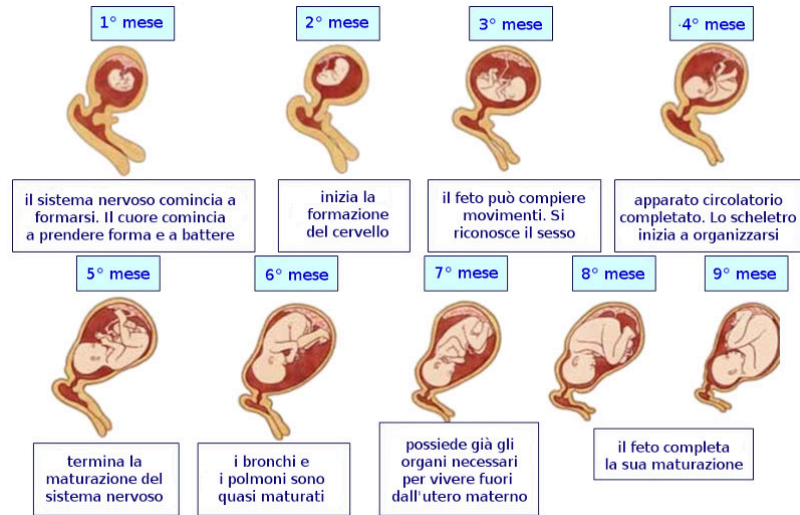
Frailty in newborns and in infants

- Weaning ends at 7 years
- It is equivalent to bring the digestive system to gym: it has to be gradual and progressive.
- Microbiota has to be perturbed by stressful agents, but the strength of stress must be comparable with that of the microbiota.





PROGRAMMA DI MATURAZIONE FETALE



the neonatologist, and then the pediatric neurologist, must know the entire clinical history of the child.



The clinical history begins on the day of conception and must take into account the intestinal health of the mother.

There can not be common protocols:

- weaning,
- therapies,
- vaccines

Molto fragile

Fragile

Robusto








Molto robusto

We can not only look at those who arrive first,



but we have to help the last ones to reach the first ones.

Bristol Stool Test to explore the Gut Microbiota

BRISTOL STOOL CHART			
	Type 1	Separate hard lumps	SEVERE CONSTIPATION
	Type 2	Lumpy and sausage like	MILD CONSTIPATION
	Type 3	A sausage shape with cracks in the surface	NORMAL
	Type 4	Like a smooth, soft sausage or snake	NORMAL
	Type 5	Soft blobs with clear-cut edges	LACKING FIBRE
	Type 6	Mushy consistency with ragged edges	MILD DIARRHEA
	Type 7	Liquid consistency with no solid pieces	SEVERE DIARRHEA

Gut

Gut microbiota composition associated with stool consistency

Authors: G. First

Gut

Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates

Doris Vandeputte,^{1,2,3} Gwen Falony,^{1,2} Sara Vieira-Silva,^{1,2} Raul Y Tito,^{1,2,3}
Marie Joossens,^{1,2,3} Jeroen Raes^{1,2,3}

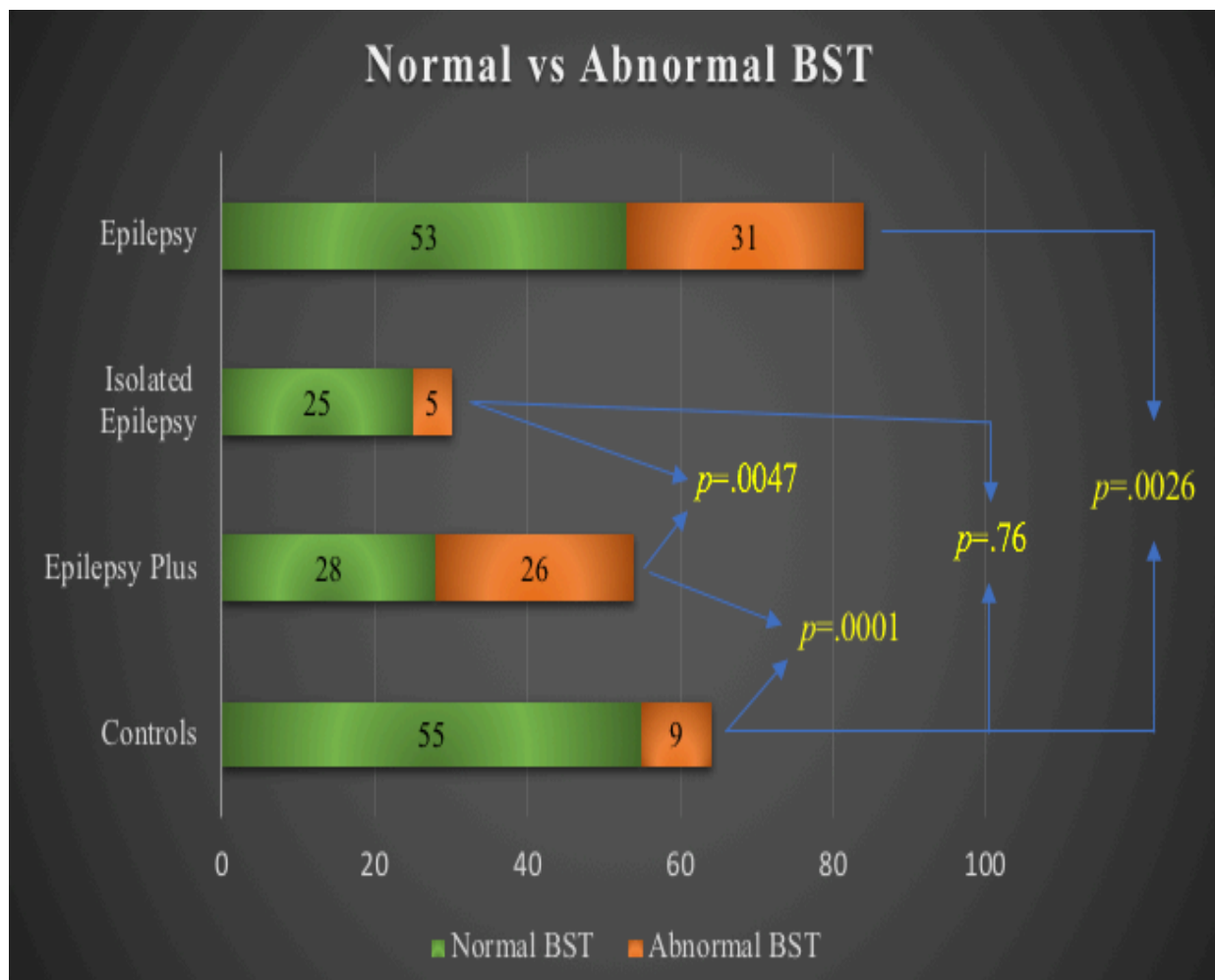
GUT

Gut microbiota composition associated with stool consistency

E F Tigchelaar, M J Bonder, S A Jankipersadsing, J Fu, C Wijmenga and A Zhernakova

Gut published online August 14, 2015

RISULTATI



Preda A, Striano P, Mainardi P. unpublished

LETTER TO THE EDITORS

Can epilepsy be treated by antibiotics?


Hilde M. H. Braakman¹  · Jakko van Ingen²

Table 1 Baseline patient characteristics

Case	Age	Gender	Epilepsy etiology	Current AED treatment	Average SF	SF during antibiotic	Antibiotic	Indication
1	13	Female	Angelman syndrome	VPA, CLB, LEV	10/day	0	Piperacillin/tazobactam, ciprofloxacin and clindamycin	Pneumonia
2	13	Male	<i>FGF12</i> gene truncation, 3q28q29 duplication	ZNS, LTG, CLB, TPM	6/day	0	Azithromycin	Pneumonia
3	10	Male	Perinatal asphyxia	LEV, VPA, NTZ, KD	3/day	0	Amoxicillin/clavulanic acid	Pneumonia
4	13	Female	<i>GNAIA</i> gene mutation	TPM	1/day	0	Clarithromycin	Pertussis
5	16	Female	Down syndrome	LEV, CLB	2/week	0	Amoxicillin/clavulanic acid	Pneumonia
6	10	Male	Absence epilepsy, genetic	TPM, CLB	> 300/day	30/day	Amoxicillin	Otitis media

AED antiepileptic drug, SF seizure frequency, VPA valproic acid, CLB clobazam, LEV levetiracetam, ZNS zonisamide, LTG lamotrigine, TPM topiramate, NTZ nitrazepam, KD ketogenic diet

LETTER TO THE EDITORS

Can epilepsy be treated by antibiotics?

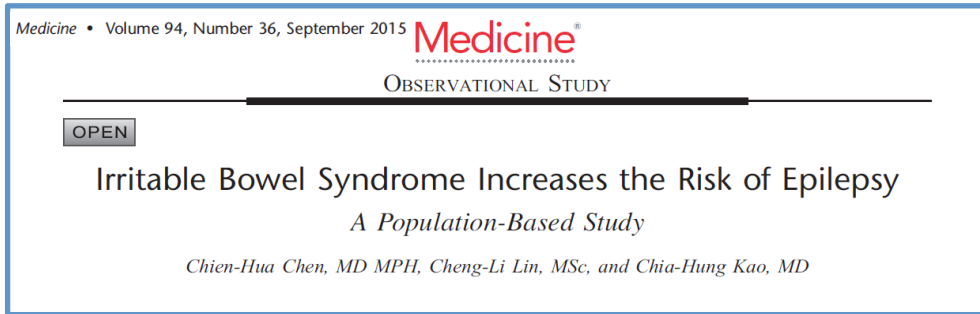
Hilde M. H. Braakman¹  · Jakko van Ingen²

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AED antiepileptic drug, SF seizure frequency, VPA valproic acid, CLB clobazam, TPM topiramate, NTZ nitrazepam, KD ketogenic diet

Average SF	SF during antibiotic
10/day	0
6/day	0
3/day	0
1/day	0
2/week	0
> 300/day	30/day



IBS increases risk of epilepsy

- Fecal microbiota transplantation cured epilepsy in a case with Crohn's disease

He, Z., et al. (2017). Fecal microbiota transplantation cured epilepsy in a case with Crohn's disease: The first report. World Journal of Gastroenterology, 23(19), 3565–3568.

- Fecal microbiota transplantation in Tourette Syndrome .

Huijun Zhao, et al. "The Effect of Fecal Microbiota Transplantation on a Child with Tourette Syndrome," Case Reports in Medicine, vol. 2017, Article ID 6165239, 3 pages, 2017

- Fecal microbiota transplantation in autism.

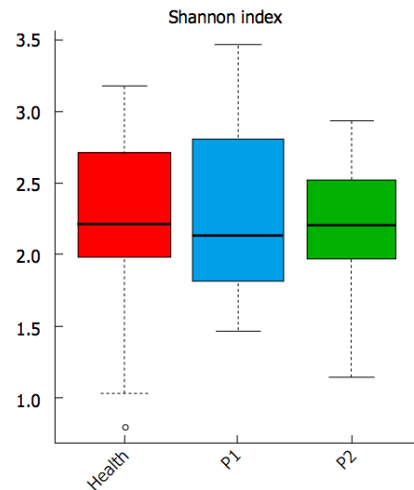
Mangiola, F., Ianiro, G., Franceschi, F., Fagiuoli, S., Gasbarrini, G., & Gasbarrini, A. (2016). Gut microbiota in autism and mood disorders. World Journal of Gastroenterology, 22(1), 361–368.

CURARE = GUARIRE

Una terapia che non posso sospendere,

mi sta curando?

Ketogenic diet modifies gut microbiota of epileptic pts



P1: before KD
P2: after 1 w in KD

Xie G, Zhou Q, Qiu CZ, Dai WK, Wang HP, Li YH, Liao JX, Lu XG, Lin SF, Ye JH, Ma ZY, Wang WJ. Ketogenic diet poses a significant effect on imbalanced gut microbiota in infants with refractory epilepsy. World J Gastroenterol. 2017 Sep 7;23(33):6164-6171

Figure 1 Gut microbial diversity of the three groups. Distribution of Shannon index (evenness) is shown. Red, blue, and green represent the Health, P1 and P2 groups, respectively. The gut microbiota (GM) of the healthy infants was more stable than that of the other two groups.

Is the antiepileptic effect of the ketogenic diet due to ketones?

Mainardi, P. et al. Medical Hypotheses. 2008, Volume 70 , Issue 3 , 536 - 539

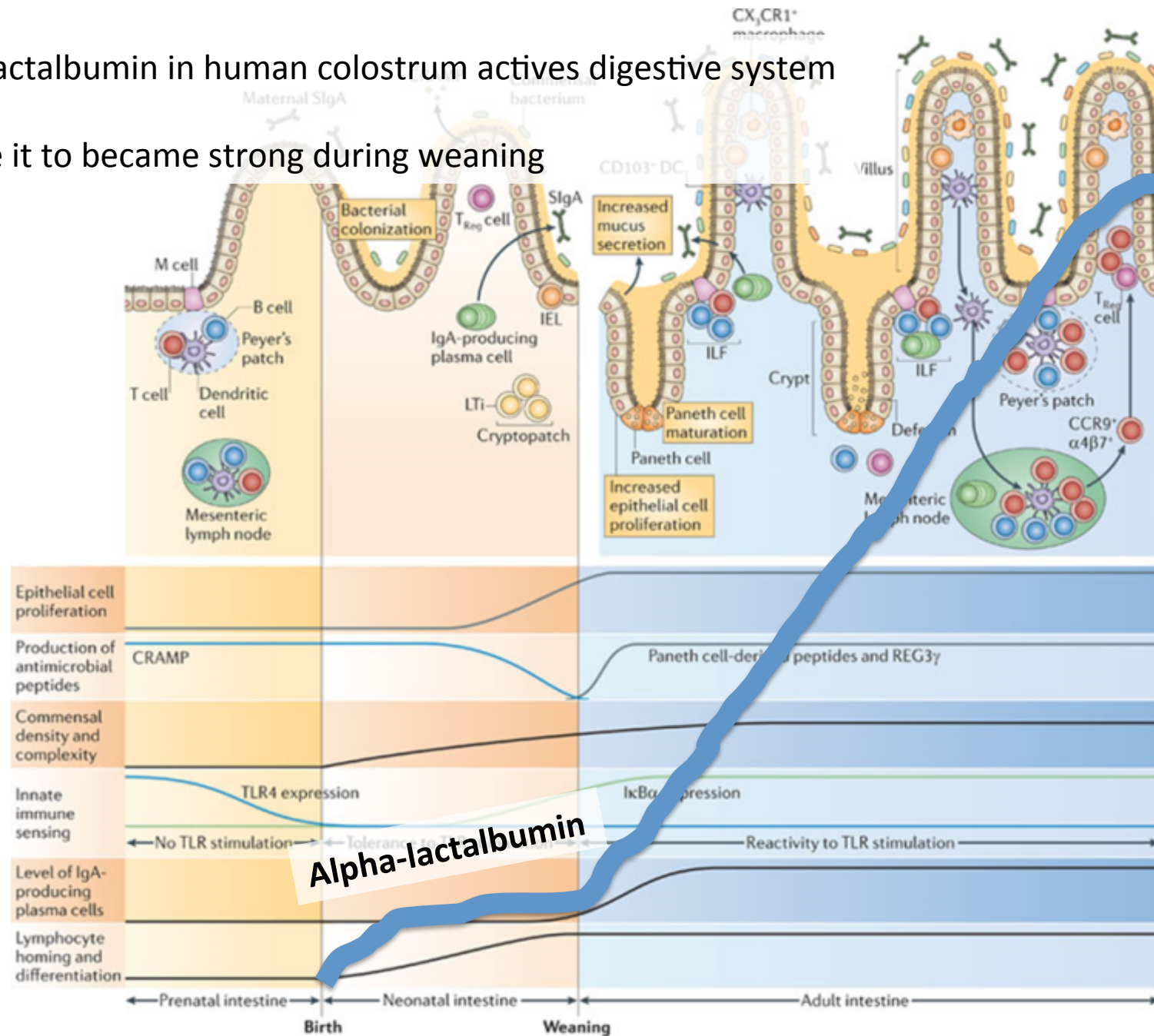
Dear Paolo,

I actually read your article a few weeks ago and agree. We have one of our researchers, dr Adam Hartman, investigating KD in a mouse model and he agrees (as do I in my clinical research) that ketosis is probably over-rated.

Erik Kossoff

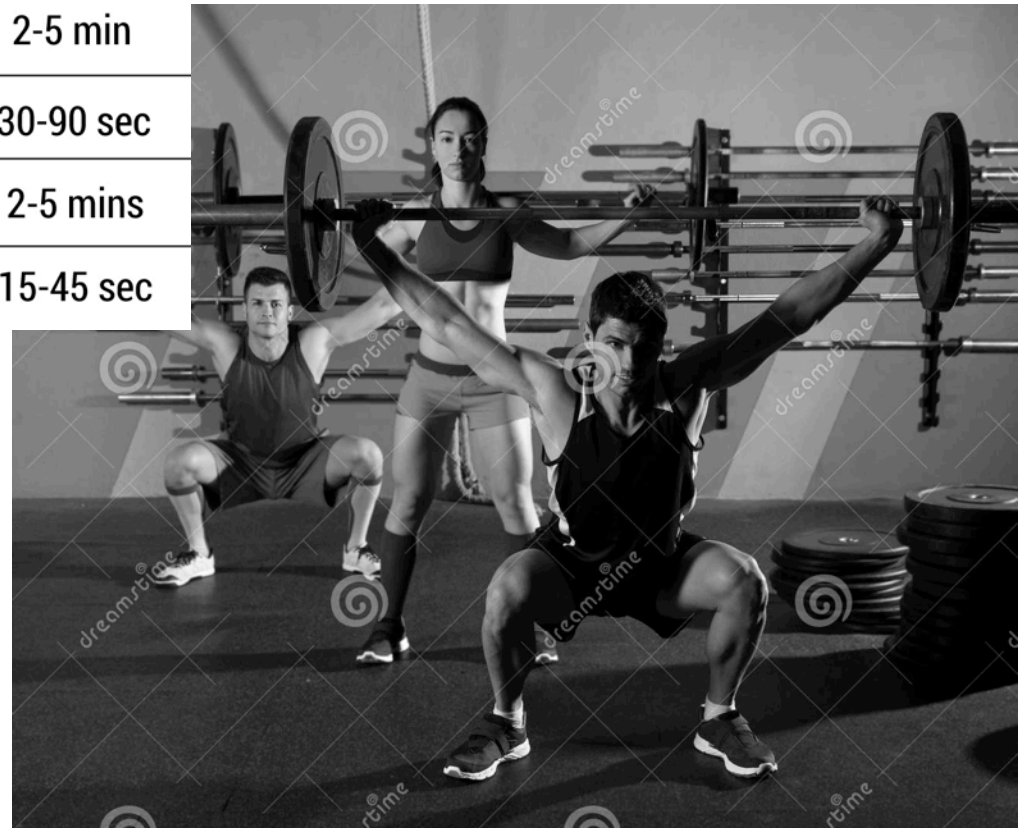
Alpha-lactalbumin in human colostrum activates digestive system

Prepare it to become strong during weaning



Svezzamento= tabella di allenamento

TIPO	Ripetizioni	Set	Intensità	Riposo
Forza massima	1-6	3-4	85-100%	2-5 min
Potenza	1-5	3-9	90+%	2-5 min
Ipertrofia	8-12	3-6	65-80%	30-90 sec
Forza base	8-12	2-4	85-100%	2-5 mins
Resistenza	15-50+	2-4	40-70%	15-45 sec



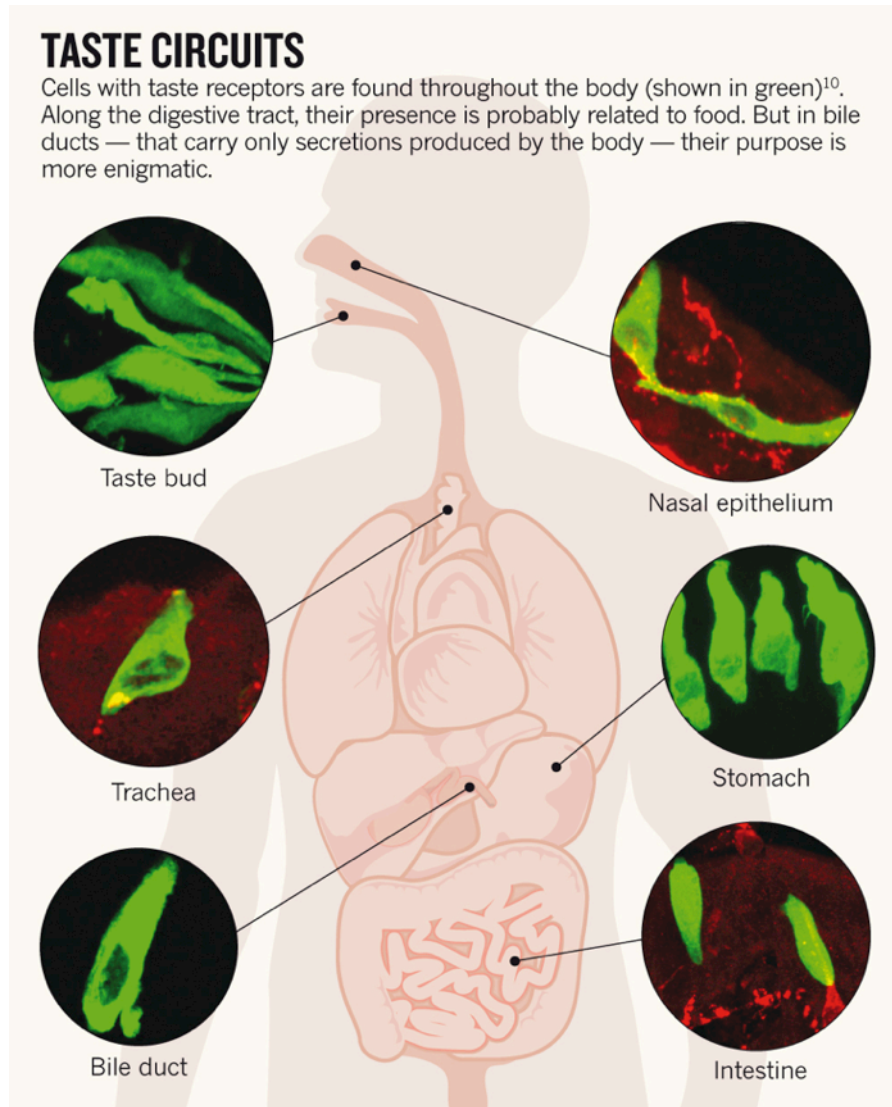
Download from
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ID 39663826

Lunamarina | Dreamstime.com

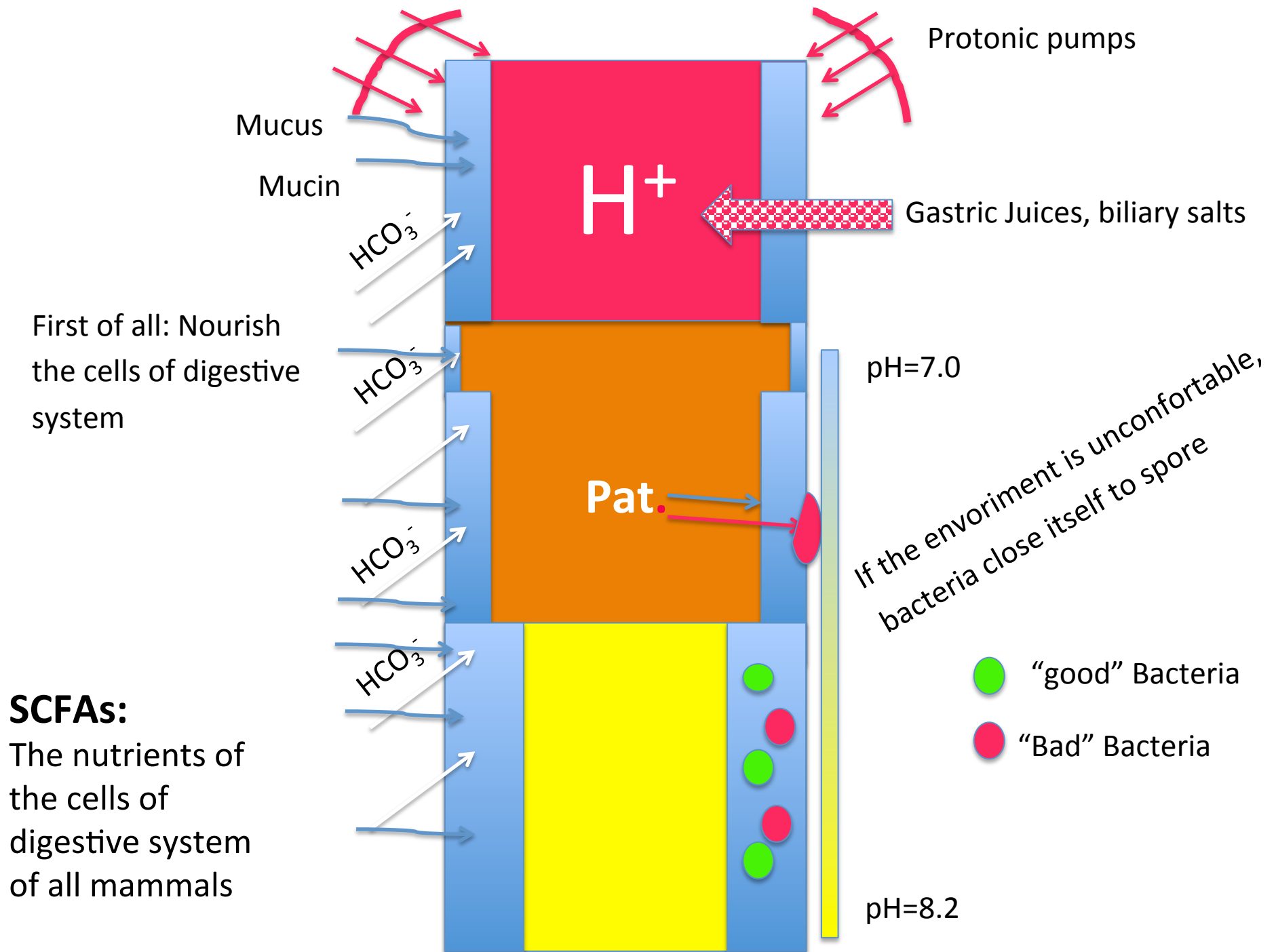
Taste receptors to produce anticipatory response to foods or pathogens.



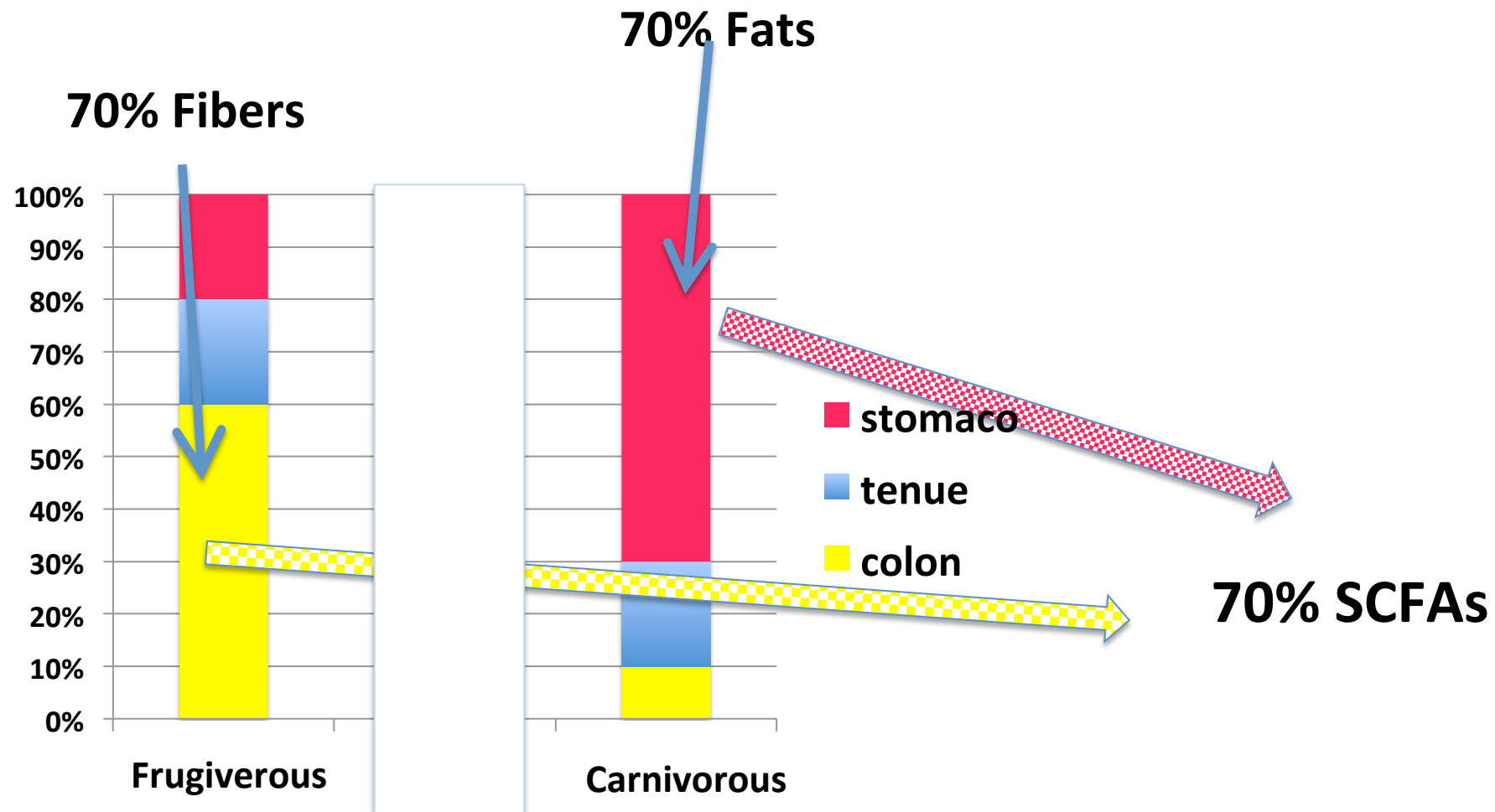
- They recognize food
- Memorized it
- To produce fast response

- ✓ **The main role of digestive system is to protect gut ecosystem**
- ✓ **By the food we can talk with microbiota**
- ✓ **Food information goes beyond the nutritional aspect**

*Let the food to be the medicine
and the medicine to be the food.
Hippocrates*



Different digestive systems on the basis of different nutritional preferences, to obtain the same nutrients from the diet





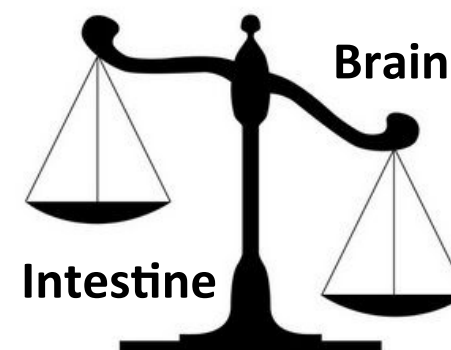
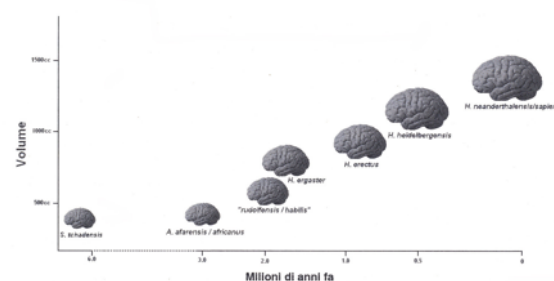
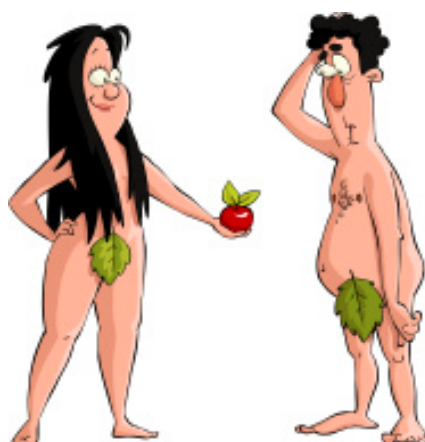
From the Ancient Diets to the Recent Acquisitions on the Role of Brain Inflammation in Epilepsy, Are there Any Links?

Paolo Mainardi^{1*}, Paolo Carta², Pasquale Striano³, Michele Mainardi⁴ and Massimo Montinari⁵

Became omnivorous shortens intestine to
reduce contact time of aggressive foods

Theories of Human Evolutionary Trends in Meat
Eating and Studies of Primate Intestinal Tracts

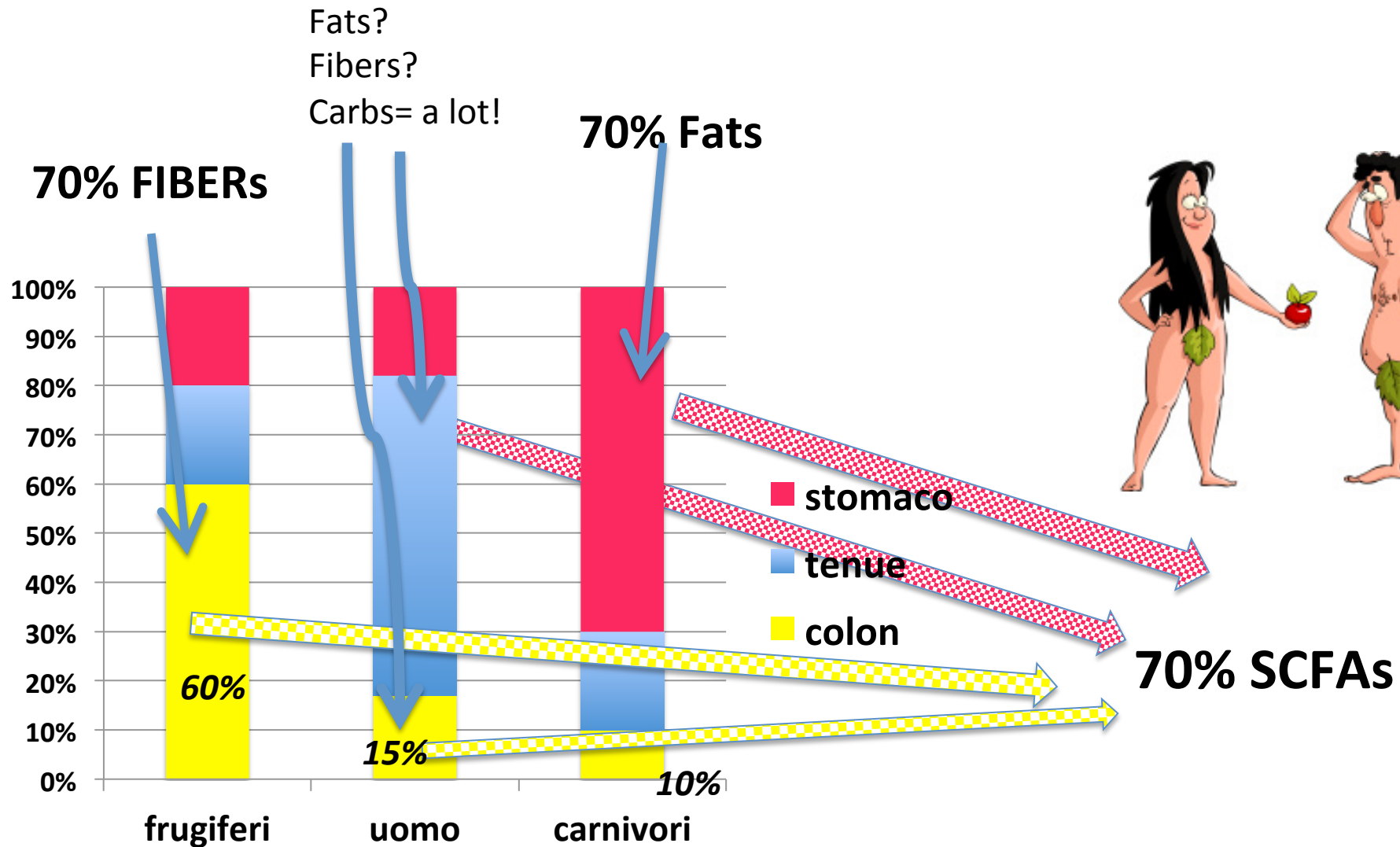
Patrick Pasquet
Centre National de la Recherche Scientifique, France
Claude-Marcel Hladik
Museum d'Histoire Naturelle, France

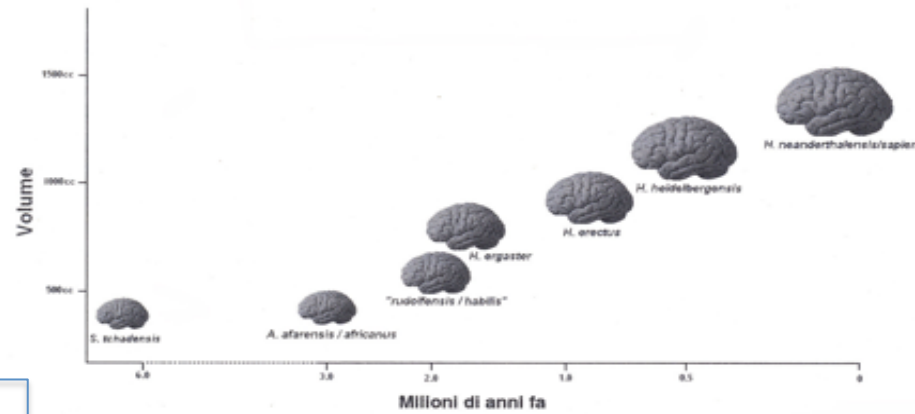


During evolution time human learn to obtain SCFAs from fats, too.

They can obtain SCFAs both from Fibers and from Fats, but he prefers the new way.

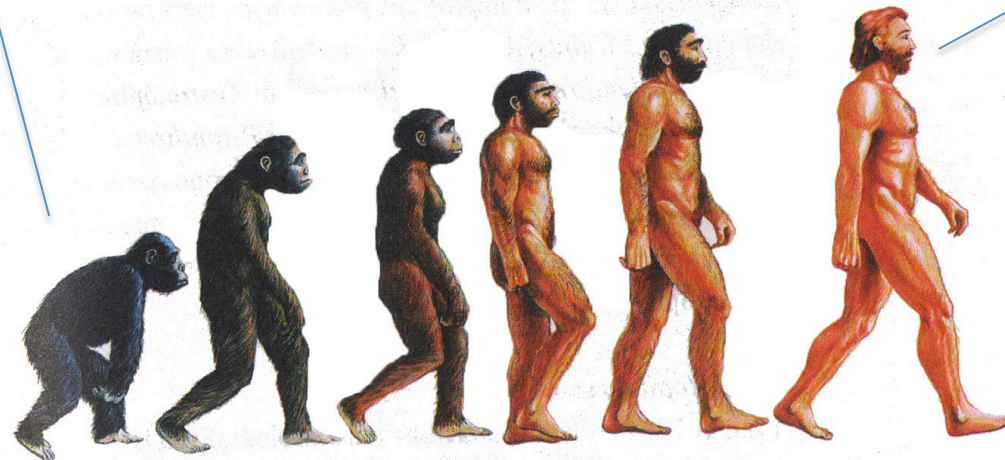
In fact, large intestine decreases from 60% to 15% of the entire length of gut





SCFAs from Fibers

SCFAs from Fats



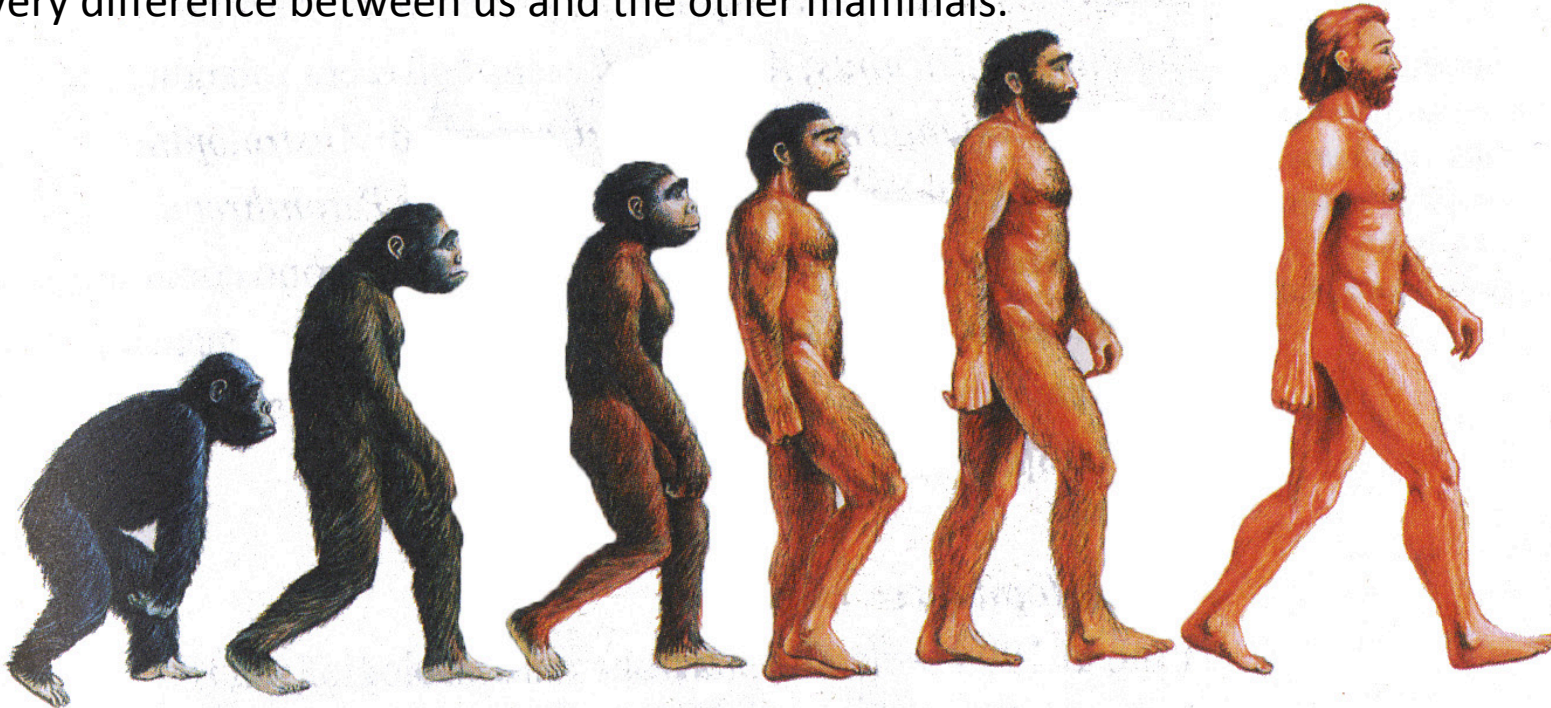
Large intestine: from 60% of length to 15%

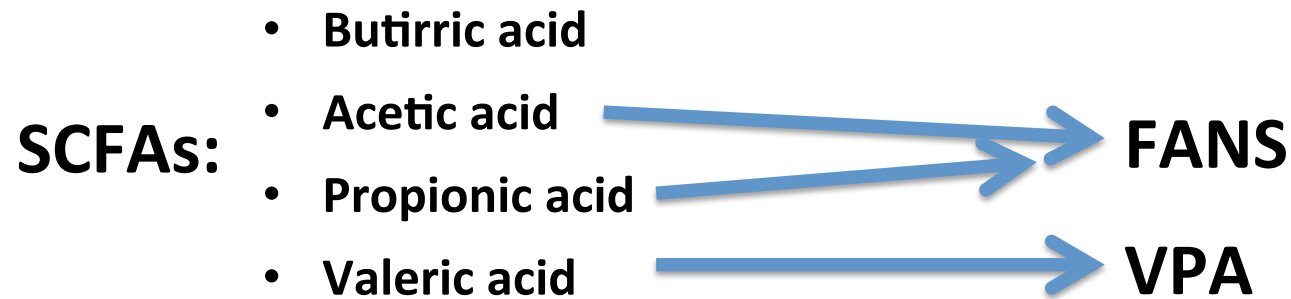
**During evolution time human learn to obtain SCFAs from fats, too.
It forces digestive system to became able to process complex foods**

To an increasing in intestinal inflammation corresponds an increasing in anxiety.

In this way, in human start **the anxiety of knowledge**.

The very difference between us and the other mammals.





SCFAs= Histone-deacetylase inhibitor = **Intestinal disinflammation**

Saturated fats in the diet allow us to obtain SCFAs.

Ketogenic diet: Fats/Carbohydrates ratio 4:1 or 3:1

Then, rich in saturated fats and butter

KD nourishes and disinflames intestine

Does the butter hurt?

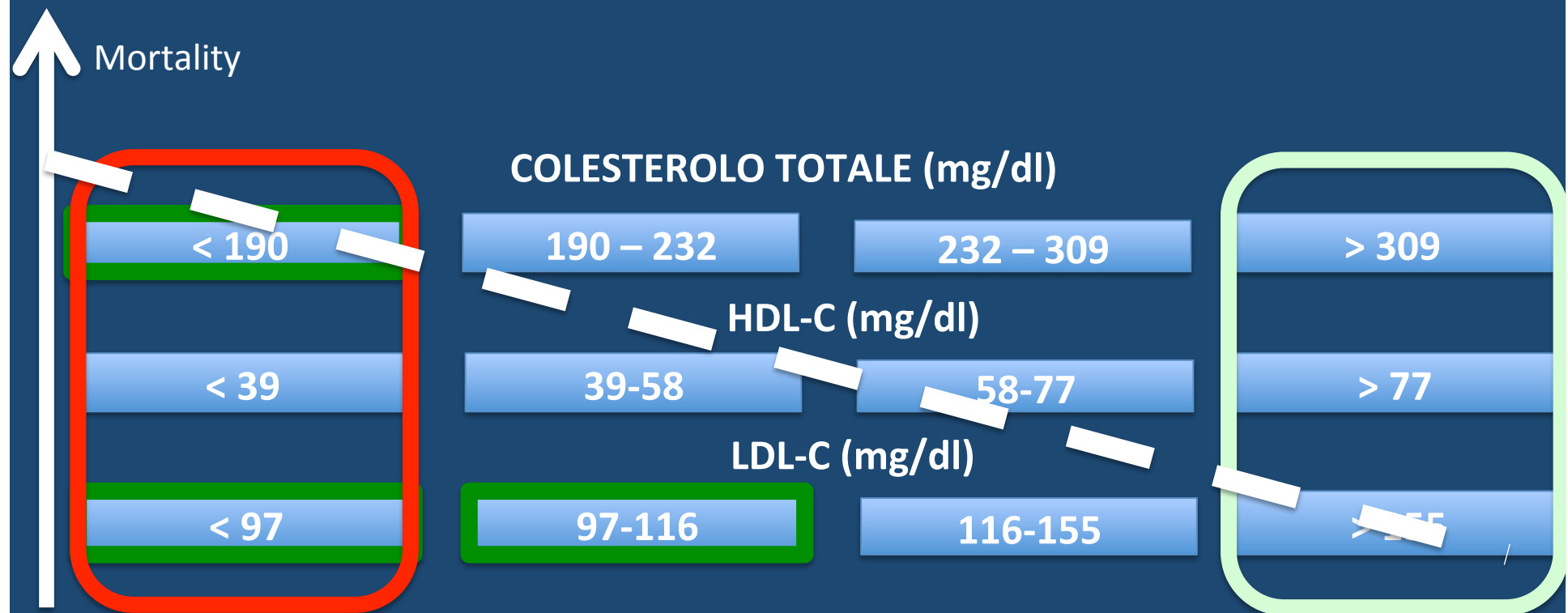
Kim CH, Park J, Kim M. Gut Microbiota-Derived Short-Chain Fatty Acids, T Cells, and Inflammation. Immune Network. 2014;14(6):277-288. doi:10.4110/in.2014.14.6.277.

Waldecker M(1), Kautenburger T, Daumann H, Busch C, Schrenk D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. J Nutr Biochem. 2008 Sep;19(9):587-93.

Butyric acid has been suggested:

- as an alternative approach in **autoimmune and inflammatory diseases**,
- against **bacterial infections**,
- reduce cell proliferation in **colon cancer**,
- **reduce blood sugar**,
- **insulin resistance**,
- **dyslipidemia and gluconogenesis** comparable to metformin.
- has protective action in experimental models of **muscular spinal atrophy**,
- as well as reducing **muscular atrophy from aging**,
- has therapeutic action on **allergic rhinitis**,
- improves **cardiac functions**,
- **reduces alcohol intake in dependent animals**,
- protects against severe **acute lung injury at a distance caused by burns**.
- ...

Study on **118.160** patients >50 from 1999 to 2007 without precedent cardiovascular pathologies or diabete (2013)



High levels of TC, HDL-C, or LDL-C were associated with minor mortality, also in subject with very elevated levels.

Bathum L, et al. Association of lipoprotein levels with mortality in subjects aged 50 + without previous diabetes or cardiovascular disease: a population-based register study. Scand J Prim Health Care. 2013 Sep;31(3):172-80.

Alpha-lactalbumin in epilepsy

Preclinical activity profile of α -lactalbumin, a whey protein rich in tryptophan, in rodent models of seizures and epilepsy

Rita Citraro¹, Francesca Scicchitano¹, Salvatore De Fazio¹, Riccardo Raggio², Paolo Mainardi³, Emilio Perucca⁴, Giovambattista De Sarro^{1,*}, Emilio Russo¹.



More than 400 animals, experimental models:

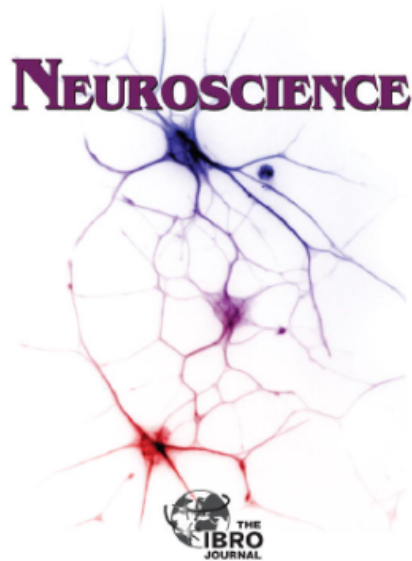
- GEPR-9 rats
- Pilocarpine
- MES test
- WAG Rij rats

Main results:

- Repeated daily administrations are needed
- **It is able to control both types of seizures induced by Pilocarpine (*)**

(*) Mainly for this result, ALAC entered in the NIH new drug program screening

Alpha-lactalbumin in epilepsy



Neuroscience 226 (2012) 282–288

PROTECTIVE ACTIVITY OF α -LACTOALBUMIN (ALAC), A WHEY PROTEIN RICH IN TRYPTOPHAN, IN RODENT MODELS OF EPILEPTOGENESIS

E. RUSSO,^{a*} F. SCICCHITANO,^a R. CITRARO,^a
R. AIELLO,^a C. CAMASTRA,^a P. MAINARDI,^b
S. CHIMIRRI,^a E. PERUCCA,^c G. DONATO^a AND
G. DE SARRO^a

- Adigenic mice: after 3 weeks of daily administrations, the seizures control last for at least one month since the end of treatment..

ALAC increases epileptogenic threshold

Alpha-lactalbumin in epilepsy



A Proof-of-Concept Trial of the Whey Protein Alfa-Lactalbumin in Chronic Cortical Myoclonus

	m	f	
sex	7	6	13
age	29±12		

Luca Errichiello, MD,¹ Marianna Pezzella, MD,²
Lia Santulli, MD,¹ Salvatore Striano, MD,¹
Federico Zara, PhD,² Carlo Minetti, MD, PhD,²
Paolo Mainardi, PhD,³ and
Pasquale Striano, MD, PhD,^{2*}

EPILEPSY	N
Unverricht-Lundborg disease	6
benign adult familial myoclonic epilepsy	6
Lafora disease	1

- Seizure control doesn't reach statistical significance
- Improvement in sleep disorder is statistically significant

6 out of 13 patients are affected by Unverricht-Lundborg epilepsy:

Unverricht-Lundborg disease would begin to progress early and lack of effective treatment meant a quick progression. In many cases the patient would require a wheelchair for mobility, and would die at a young age.

Chew NK, et al. 2008. The natural history of Unverricht-Lundborg disease: A report of eight genetically proven cases. Movement Disorders 23:107-13

- Role of serotonin in Unverricht-Lundborg epilepsy

Striano P, D'Amato E, Pezzella M, Mainardi P, Zara F, Striano S. Sudden death in Unverricht-Lundborg patients: is serotonin the key? Neurol Sci. 2010 Feb;31

Alpha-lactalbumin in autism



Master in "NUTRIZIONE CLINICA"

ASSE INTESTINO-CERVELLO E PATOLOGIE COLLEGATE:

l'importanza dell'alimentazione e dell'integrazione
prebiotica e probiotica per la riduzione dei sintomi nei
bambini affetti da disturbo dello spettro autistico

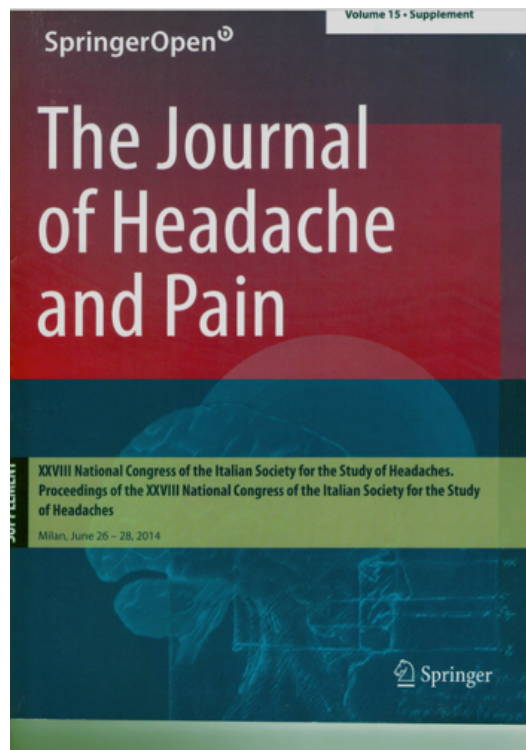
Candidato
DR. ANDREA TOSATTO

Relatore
PROF. CARMELO RIZZO

Alpha-lactalbumin is reported in a Thesis of Master of Nutrition on the basis of results on autism.

Alpha-lactalbumin in Headache

- After 3 months: MoA (da 9.81 ± 2.44 a 6.18 ± 1.73 attacks/month)
- Bristol stool test: type 2 stool (from 64% to 38%)
- The decrease in MoA correlates with decreasing in intestinal inflammation



The gut brain connection in pediatric migraine: an open label trial

M. Carotenuto, E. Corvino, M. Esposito

Clinic of Child and Adolescent Neuropsychiatry, Department of Mental Health, Physical and Preventive Medicine, Second University of Naples, Italy;
e-mail: marco.carotenuto@unina2.it






Introduction The aims of the present study were to assess the efficacy and safety of dietary supplement of Alpha Lactalbumin with FOS and inulin, specifically designed for pediatric age on frequency, severity and grade of disability related to migraine, in a population of children affected by MoA.
Methods Study population comprised 42 children (22 males) 6-10 years aged (mean 8.97 ± 1.93 years) consecutively referred for MoA to the Childhood Headache Center of the Child and Adolescent Neuropsychiatry Clinic of the Second University of

Naples. The monthly migraine frequency was assessed from daily headache diaries kept by all the children. MoA intensity was assessed on a VAS (visual analogue scale), and grade of disability linked to migraine attacks was assessed using PedMIDAS questionnaire. The whole population was assessed according to the Bristol Stool Chart in order to define the presence of stool problems, and underwent a haematological evaluation in order to survey inflammatory indexes such as ESR and CRP. All parameters were assessed at the beginning of the study (T0) and after 3 months of treatment with the nutraceutical complex.
Results At baseline (T0) MoA children showed normal values of all inflammatory indexes examined (ESR: mean 0.63 ± 0.48 mm; CRP: 0.29 ± 0.14 mg/dL) and not significantly different values at T1 (ESR: mean 0.57 ± 0.33 mm, $p=0.506$; CRP: 0.33 ± 0.12 mg/dL, $p=0.164$).

After 3 months of treatment (T1) the studied population showed a significant reduction in MoA frequency (9.81 ± 2.44 vs 6.18 ± 1.73 attacks/month; $p<0.001$) with no significant reduction in MoA severity (8.03 ± 1.97 vs 7.91 ± 2.15 ; $p=0.790$) and PedMIDAS score (26.52 ± 11.43 vs 22.48 ± 14.63 ; $p=0.162$). According to the Bristol Stool Test evaluation, at T1 MoA children showed a significant higher prevalence of type 2 stool (commonly considered as "normal stool") compared to T0 evaluation (64.28% vs 38.09%; $p=0.029$).

Conclusions The present open trial suggests the potential safety and efficacy for MoA pediatric brief prophylaxis of the Alpha Lactalbumin plus FOS and inulin complex.

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on the surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. Entirely Liquid



✓ L'aspirina controlla le crisi in tipi di epilessia fortemente farmacoresistenti

Ma L, et al. Aspirin attenuates spontaneous recurrent seizures and inhibits hippocampal neuronal loss, mossy fiber sprouting and aberrant neurogenesis following pilocarpine-induced status epilepticus in rats. Brain Res. 2012 Aug 21;1469:103-13.

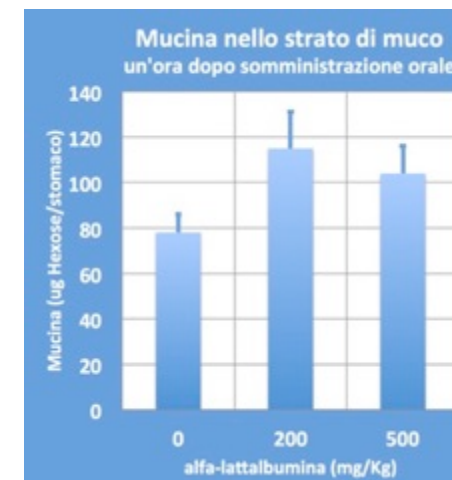
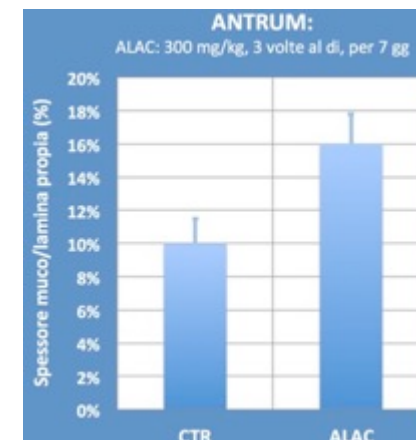
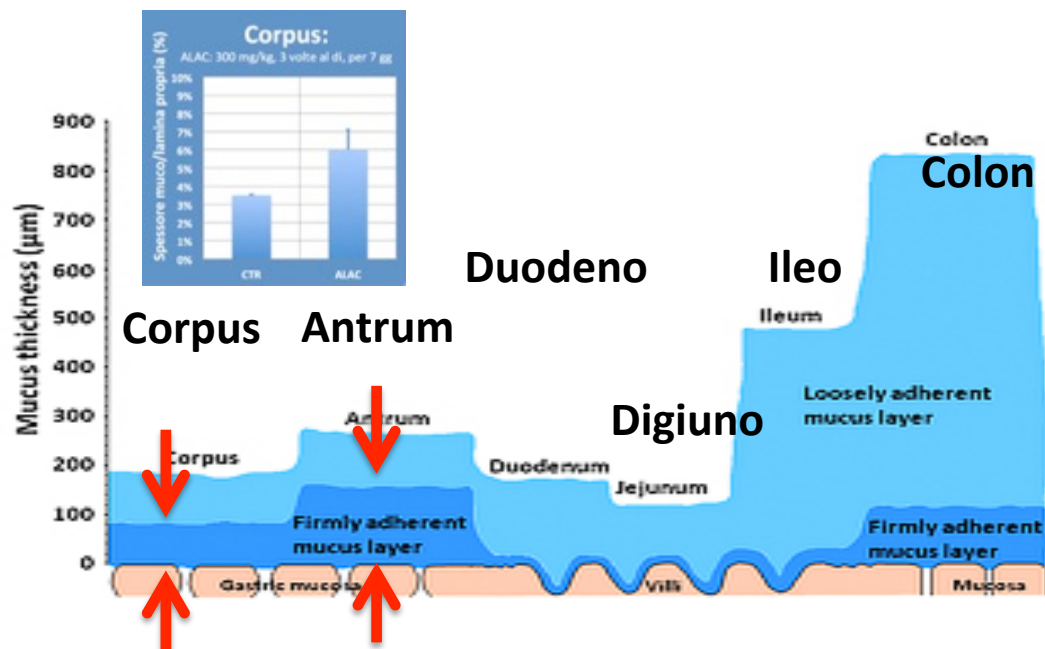
✓ Animali sottoposti a stress (acuto) hanno meno crisi se sottoposti a modello sperimentale

Oliviero A et al. Anticonvulsant effects of stress. Brain Res. 1983; 18: 271 (1):193-5

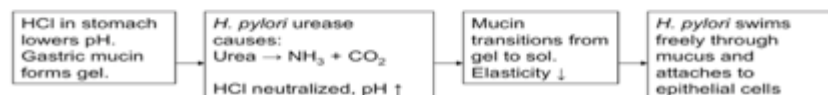
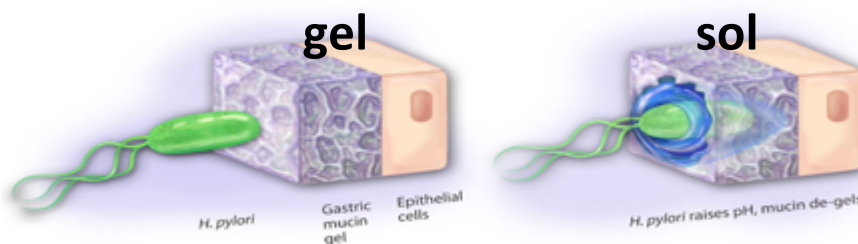
✓ Curcumina definita agente neuroprotettivo

✓ Alfa-lattoalbumina specifico prebiotico intestinale.

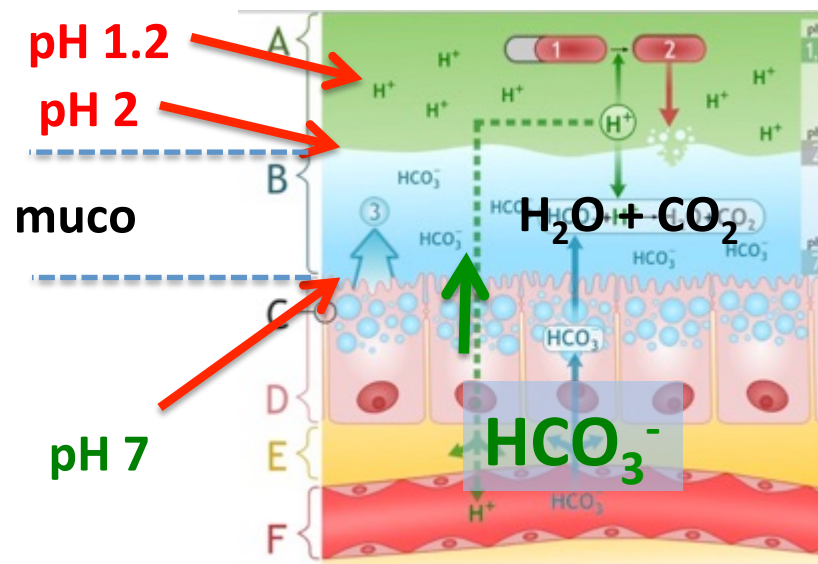
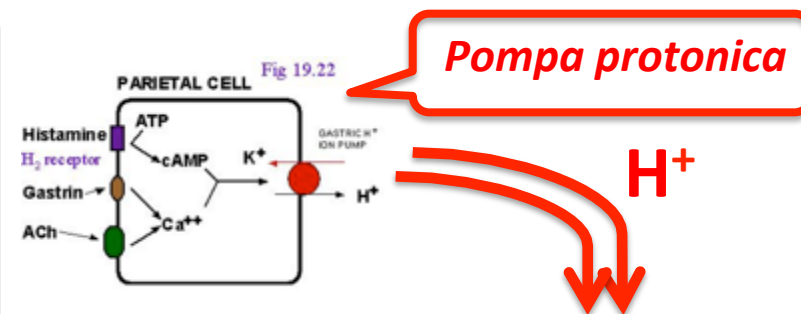
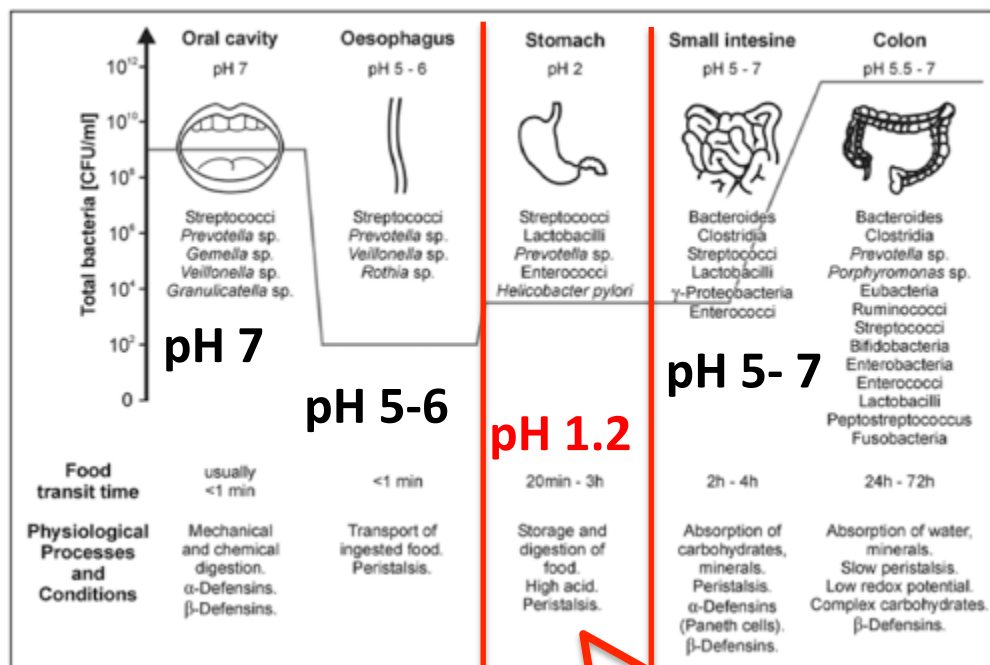
Azioni intestinali dell'alfa-lattoalbumina



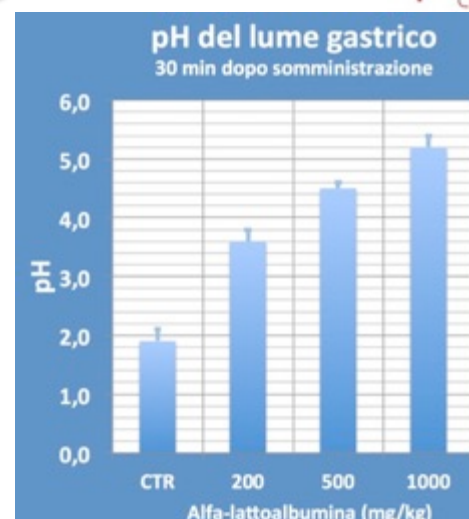
H. PYLORI CROSSING MUCUS LAYER OF STOMACH



- gastrite cronica
- atrofia gastrica
- ...



Demolisce cibo e Agenti patogeni



Alpha-lactalbumin protects in a dose-dependent manner from gastric ulcers induced by alcohol or stress

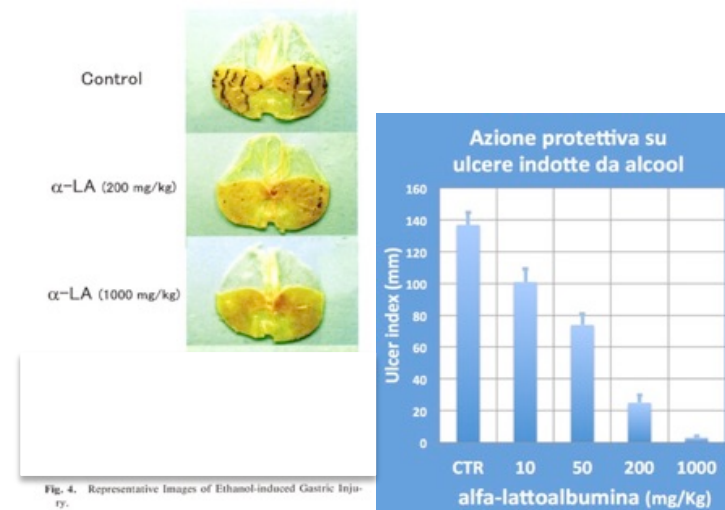


Fig. 4. Representative Images of Ethanol-induced Gastric Injury.

Biosci. Biotechnol. Biochem., 65 (5), 1104–1111, 2001



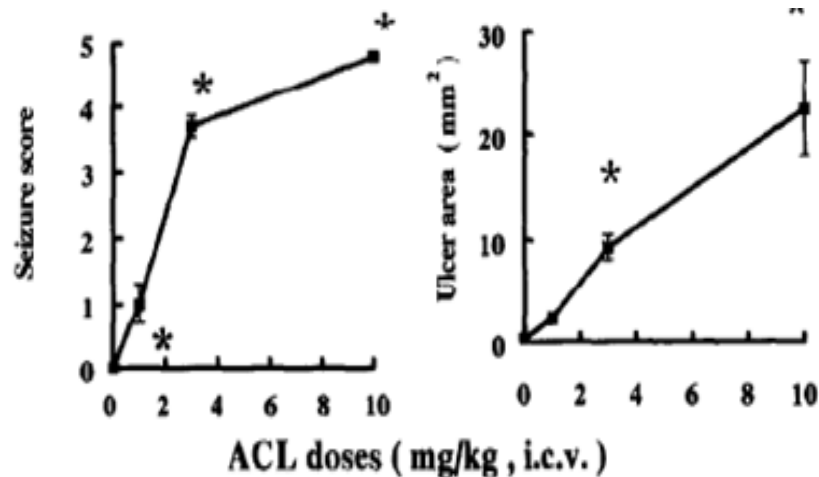
New Biological Function of Bovine α -Lactalbumin: Protective Effect against Ethanol- and Stress-induced Gastric Mucosal Injury in Rats

Hiroshi MATSUMOTO,[†] Yukiko SHIMOKAWA, Yoshihiko USHIDA, Tomohiro TOIDA, and Hirotohi HAYASAWA

Biochemical Research Laboratory, Morinaga Milk Industry Co. Ltd., Zama, Kanagawa 228-8583, Japan

- The amount of gastric ulcers depend in a dose-dependent manner on the amount of epileptogenic agent injected i.c.v.

Hung CR, Cheng JT, Shih CS. Gastric mucosal damage induced by arecoline seizure in rats. *Life Sci.* 2000 May 5; 66 (24): 2337-49.



Alpha-lactalbumin protects in a dose-dependent manner from gastric ulcers induced by alcohol or stress

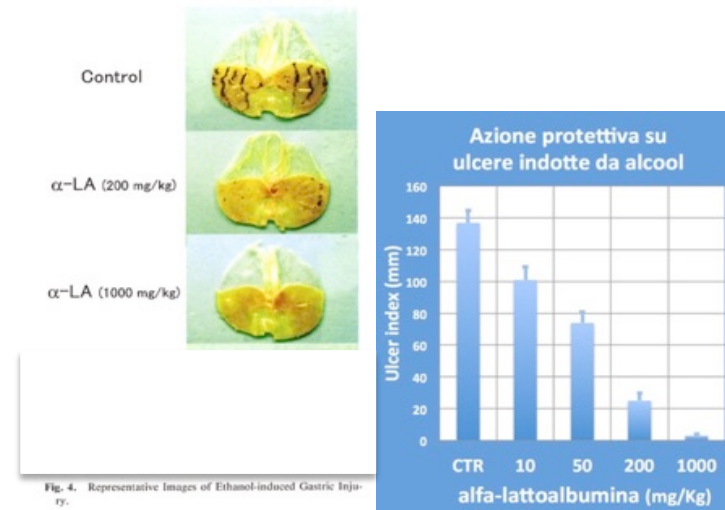


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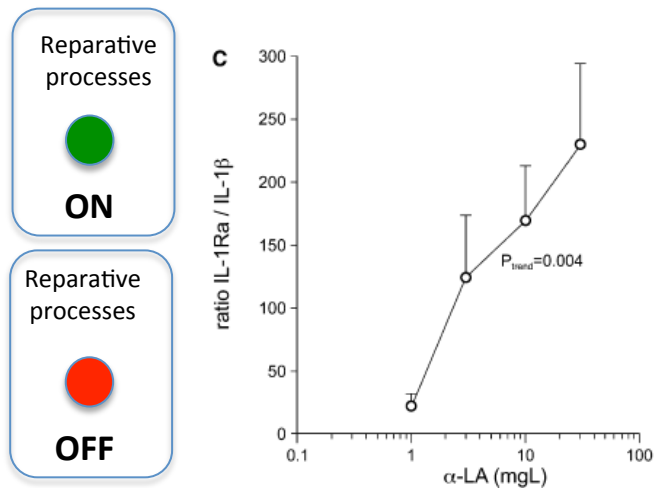
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- Bovine milk-derived α -lactalbumin inhibits colon inflammation and carcinogenesis in azoxymethane and dextran sodium sulfate-treated mice. Yamaguchi M, Takai S, Hosono A, Seki T.. Biosci Biotechnol Biochem. 2014;78(4):672-9.
- Anti-inflammatory mechanisms of bioactive milk proteins in the intestine of newborns Chatterton DE, Nguyen DN, Bering SB, Sangild PT.. Int J Biochem Cell Biol. 2013 Aug;45(8):1730-47.

L'alfa-lattoalbumina esplica azioni SPECIFICHE nel digerente dell'uomo, in quanto selezionata dalla Natura ad attivarlo dopo la nascita

Alpha-lactalbumin increases IL-1ra/IL-1 ratio:



- brings out chronic inflammation,
- Restore endogenous reparative processes
- **MORE POWERFUL THAN ANY DRUGS**

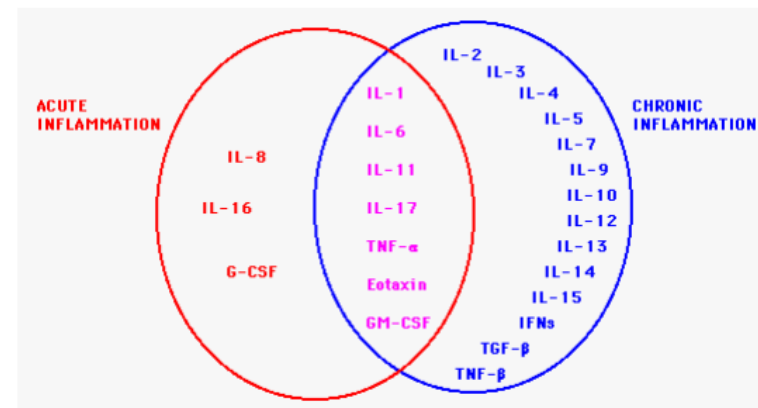


FIGURE 1: Cytokines involved in acute and chronic inflammatory responses.

It becomes **REALLY INTERESTING** to correlate the ability to act on intestinal inflammation with to control epileptic seizures.

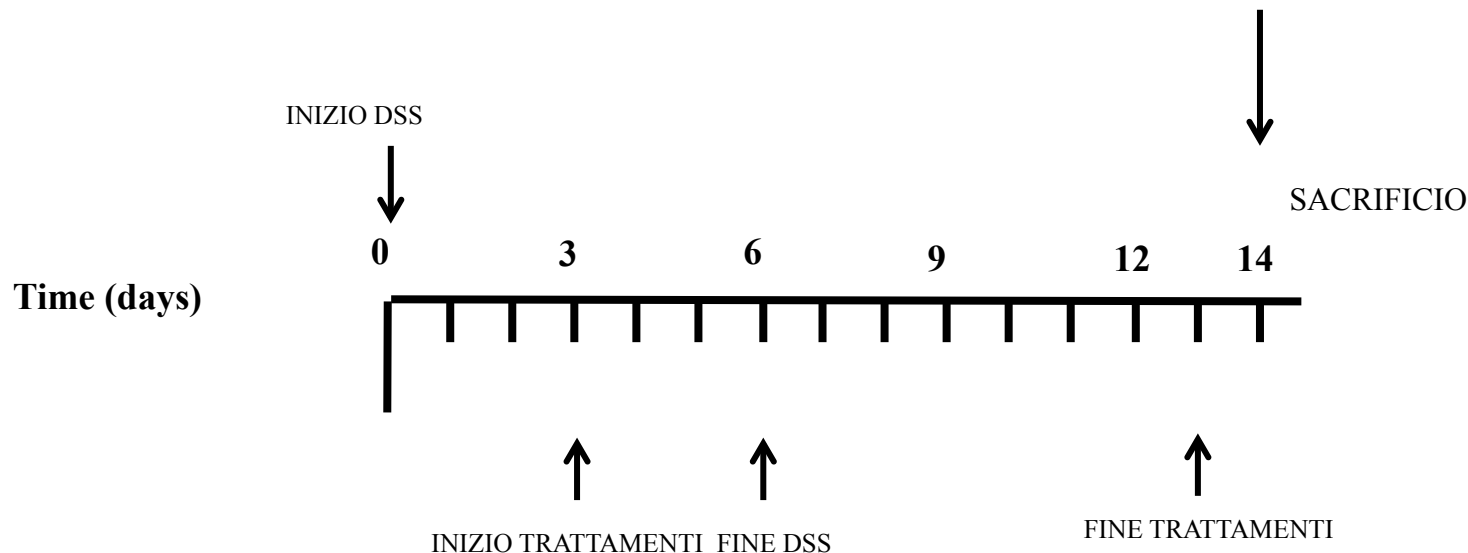
Intestinal inflammation is linked to anticonvulsant activity in a mouse model

Experimental colitis will be induced in mice BALB/c by Dextran Sulfate Sodium (DSS)

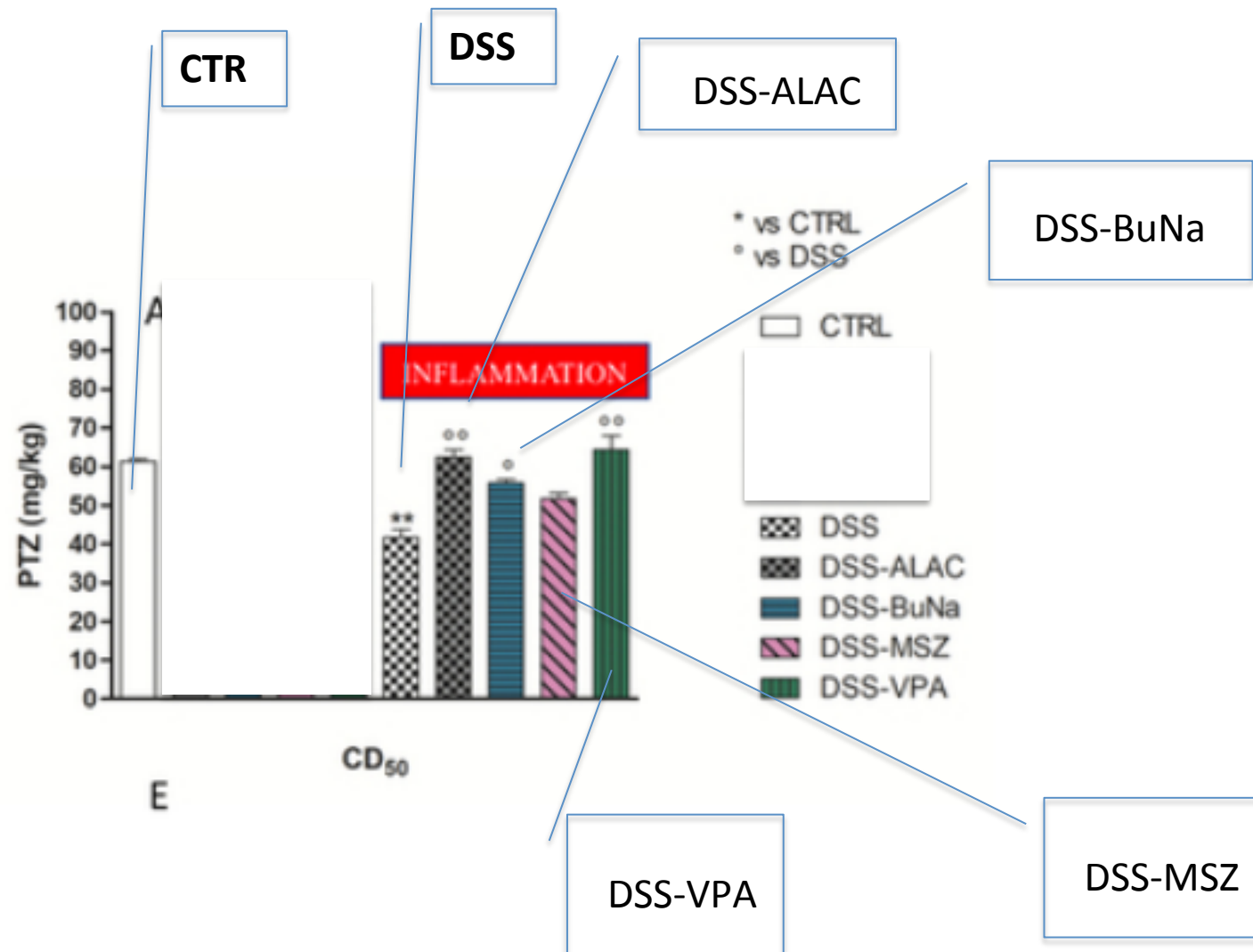
Experimental test drugs will be administered as follows:

- ALAC (375 mg/kg)
- Valproic Acid (600 mg/kg)
- Sodium Butyrate (100 mg/kg)
- Mesalazine (15mg/kg)

Anticonvulsant activity in all groups and for all drugs will be evaluated as previously reported for ALAC in the PTZ model



Intestinal inflammation is linked to anticonvulsant activity in a mouse model



Looking towards a new medicine:



- MORE POWERFUL THAN ANY DRUGS

restore the powerful and endogenous controller and self-repair mechanisms

Take – home message:

Cure the microbiota to allow it to take care of you.

Looking for the One Medicine

