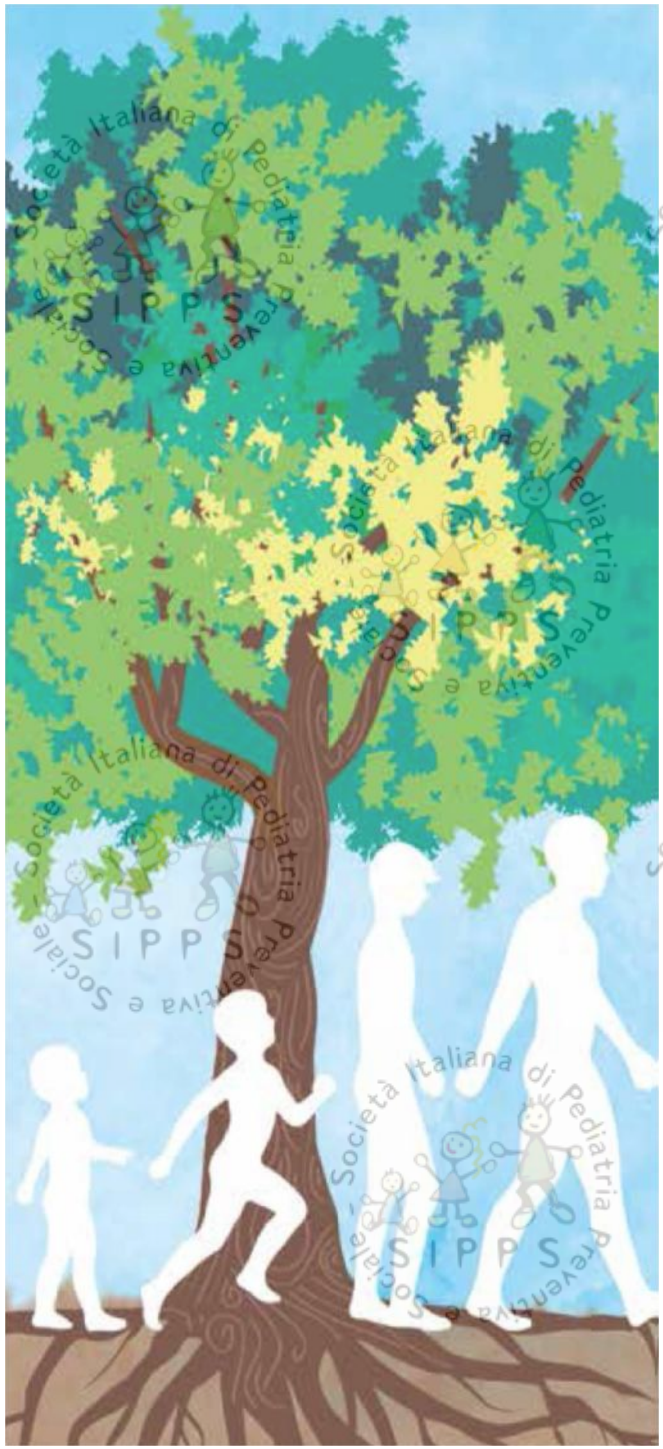


Società Italiana di Pediatria Preventiva e Sociale
SIPPS

# Microbiota Intestinale: vulnerabilità e opportunità

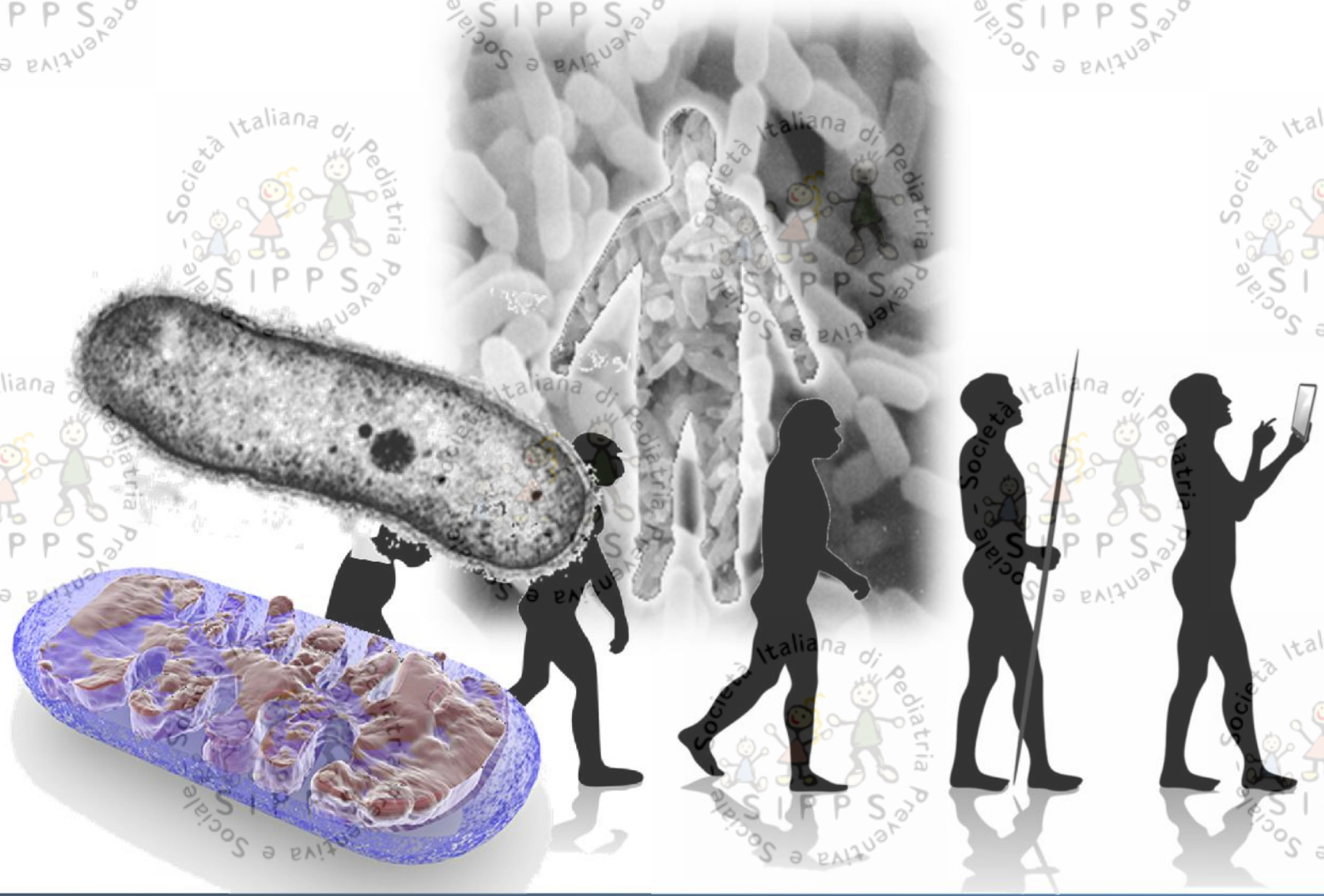

Vito Leonardo Miniello

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

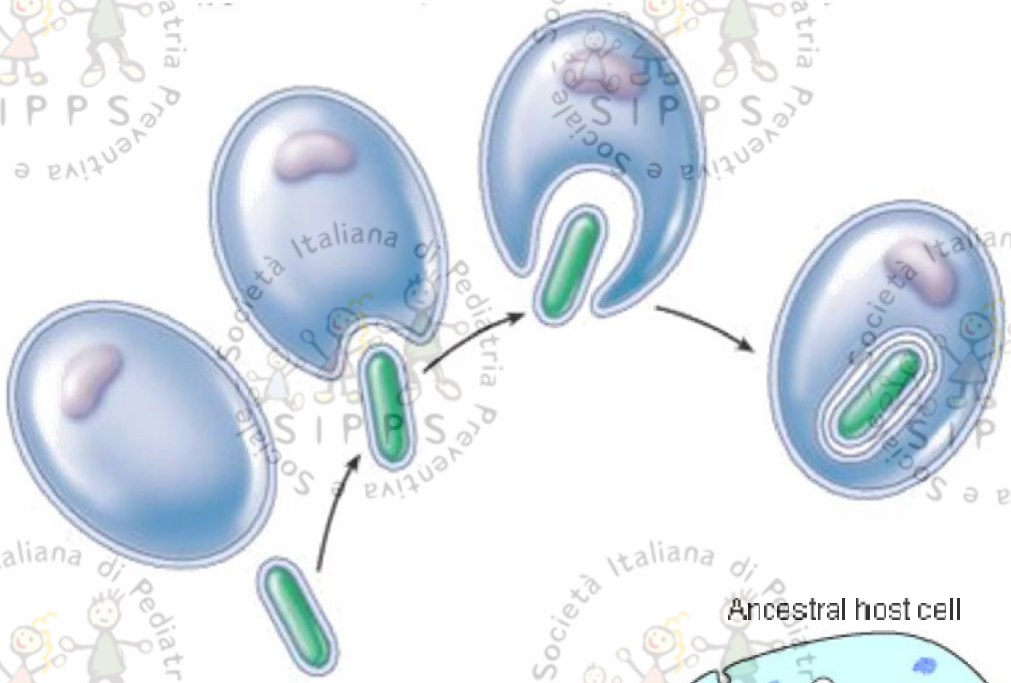
Policlinico di Bari - Ospedale Giovanni XXIII

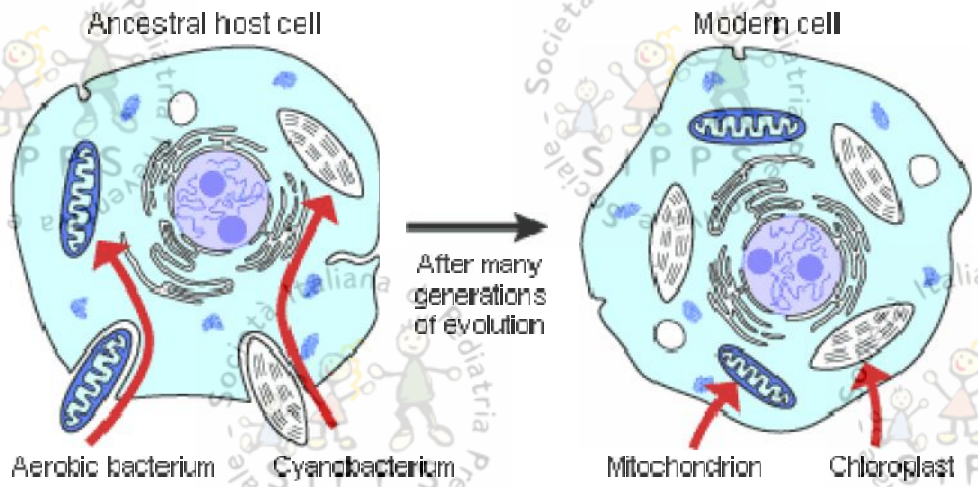
# *Homo bacteriens* and a network of surprises

B. HENDERSON



# The Endosymbiosis theory






“La vita conquistò la Terra
non con la lotta,
ma attraverso la cooperazione”
Lynn Margulis. Marvellous microbes in Resurgence

P.D. Cani, N.M. Delzenne / Pharmacology & Therapeutics 130 (2011) 202–212

# «The small world with in the gut»

We are composed of several species:

- Eucaryotic
- Bacterial
- Archaea

As adults our microbial census exceeds the total number of our own human cells

- By about 10 fold

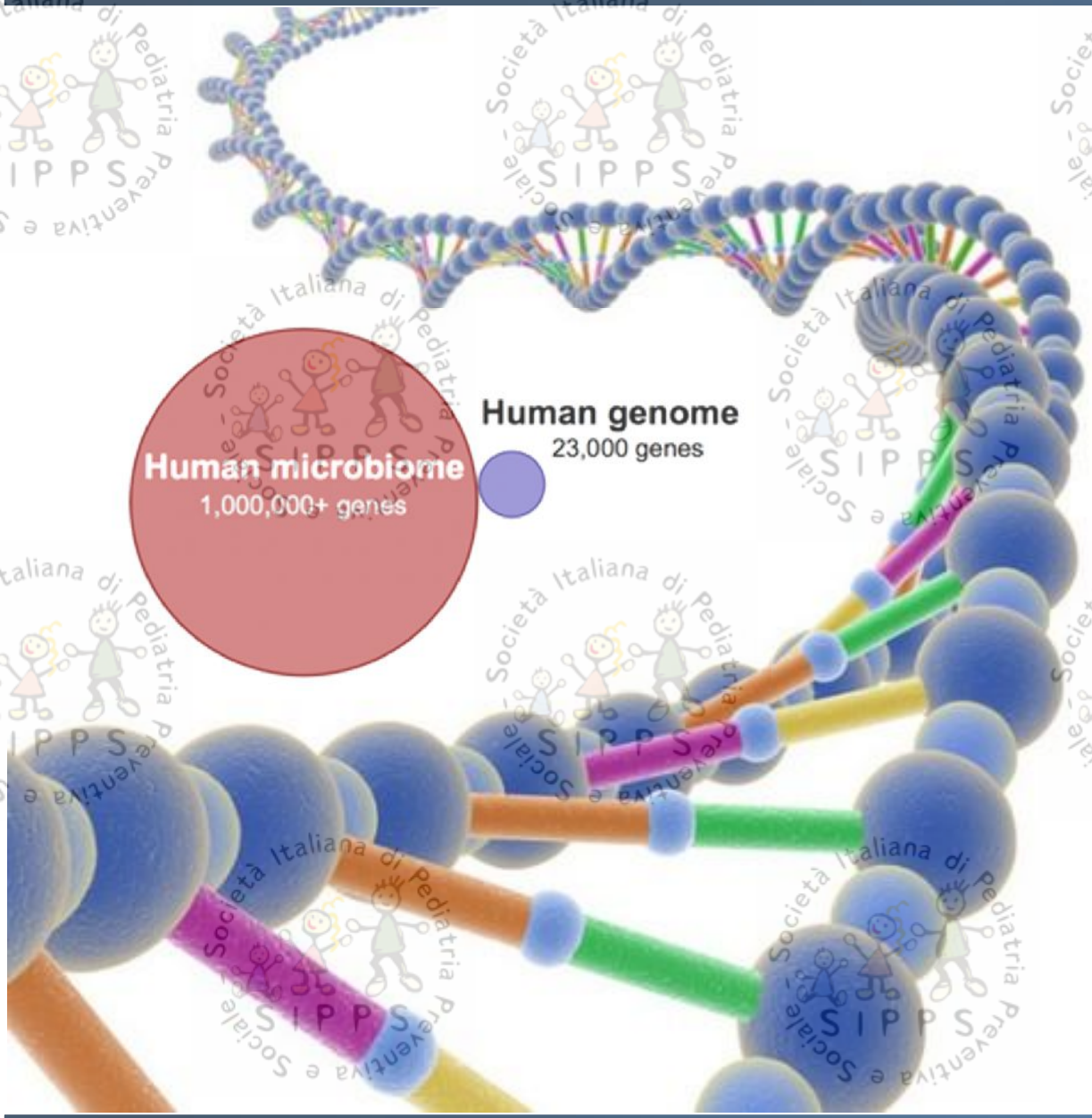
The largest collection of microbes resides within the intestine

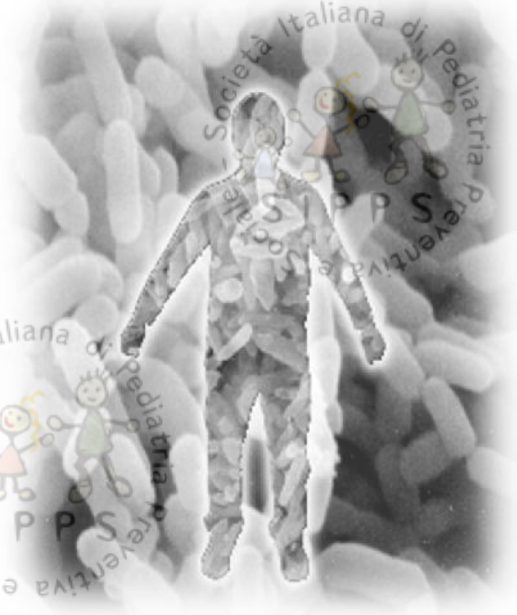
- With $10^{13-14}$ cells!!!!
- Several hundreds of species
- «The GUT MICROBIOTA»

100 % Human?

90% microbes

10% human cells


Human genome
23,000 genes
Human microbiome
1,000,000+ genes




nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
First results from the Human Microbiome Project highlight the healthy variation in our microbial selves
PAGES 194, 207 & 215
FELLOW TRAVELLERS
MATERIALS
FEELING THE PRESSURE
The quest for convincingly metallic hydrogen
PAGE 174
CLIMATE CHANGE
GET USED TO UNCERTAINTY
Climate modelling faces its limits
PAGE 183
SOLAR SYSTEM
TITAN'S ELUSIVE METHANE
Tropical lakes on Saturn's enigmatic moon?
PAGE 237
NATURE.COM/NATURE


The Economist
The Catholic church's unholy mess
Paul Ryan: the man with the plan
Generation Xhausted
China, victim of the Olymp
On the origin of specie
Microbes maketh man
AUGUST 18TH - 24TH 2012
Worldwide cover

# Microbiota Intestinale

**Azione microbiologica**

1. Modulazione della composizione del Microbiota
2. Adesione competitiva ai recettori con prevenzione di invasione di patogeni
3. Produzione di Batteriocina con prevenzione di crescita di patogeni

**Azione epiteliale**

1. Modulazione delle cellule della barriera epiteliale
2. Espressione di proteine delle Tight Junction
3. Produzione di SCFA (Acidi Grassi a catena breve) con miglioramento della barriere epiteliale e azione antiinfiammatoria

**Azione immunologica**

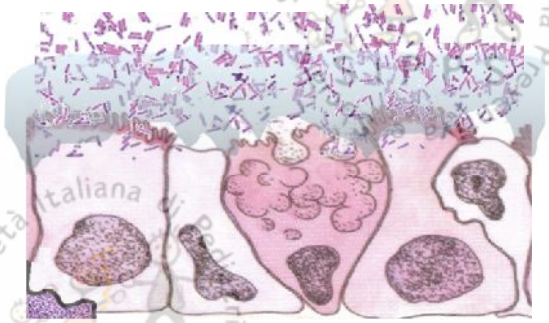
Durata della **gestazione**

Modalità del **parto**

**Antibiotici** perinatali

Modalità di **allattamento**





Eubiosi

Microbiota bilanciato
e diversificato

Disbiosi

Ridotta diversificazione e
ritardata colonizzazione

# Cesarean Section

World Health Organization

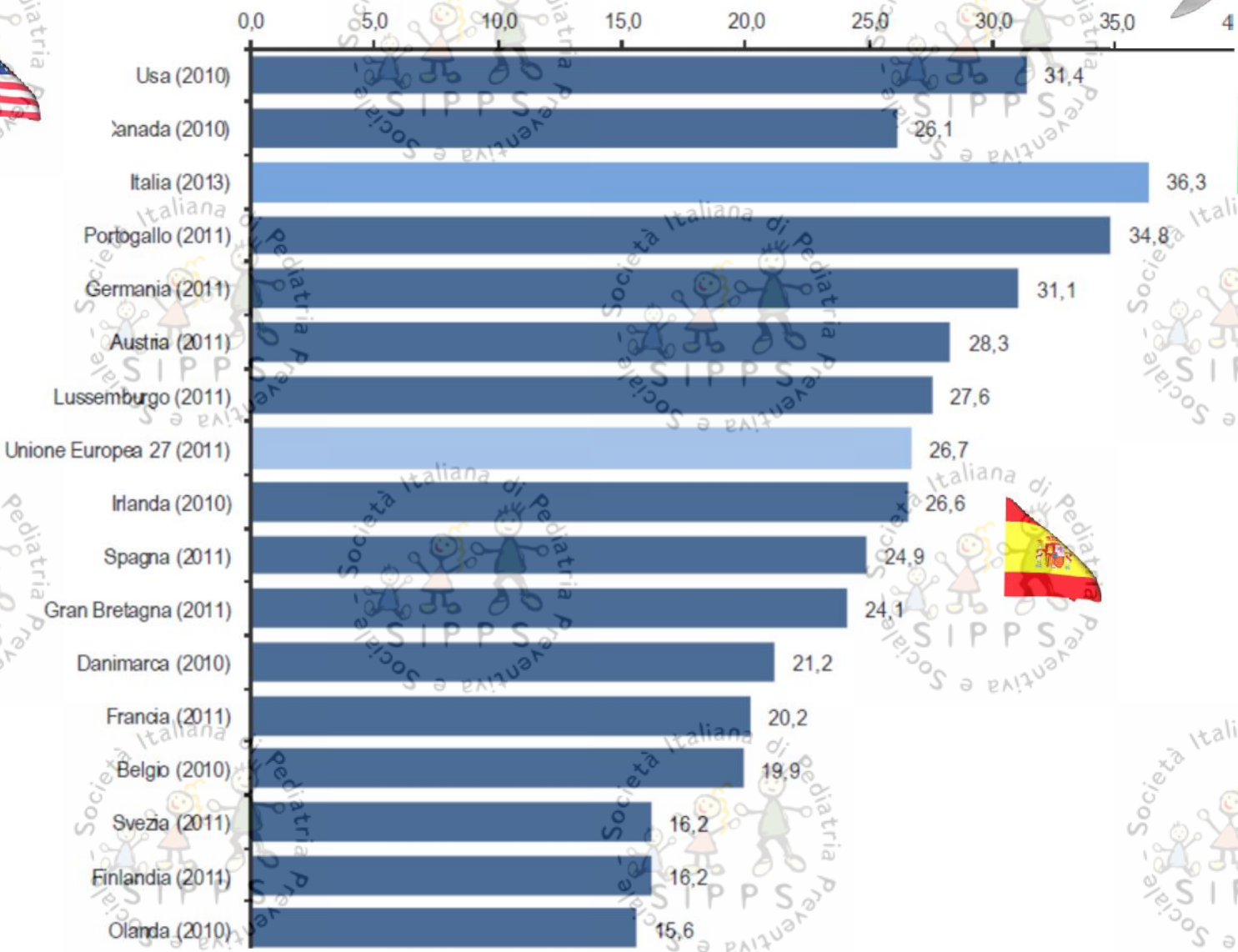
in 1985 the World Health Organization (WHO) stated:

"There is no justification to have CS rates higher than 10-15%"

Updated December 2012

More recent studies reaffirm earlier World Health Organization recommendations about optimal rates of cesarean section. The best outcomes for women and babies appear to occur with cesarean section rates of 5% to 10%. Rates above 15% seem to do more harm than good

# Cesarean Section Rates




Ministero della salute - Schede di dimissione ospedaliera 2013

# Prevalenza di parto cesareo nelle Regioni Italiane


**Percentuale di taglio cesareo nelle regioni italiane**

Ministero della salute

# Cesarean section and disease associated with immune function

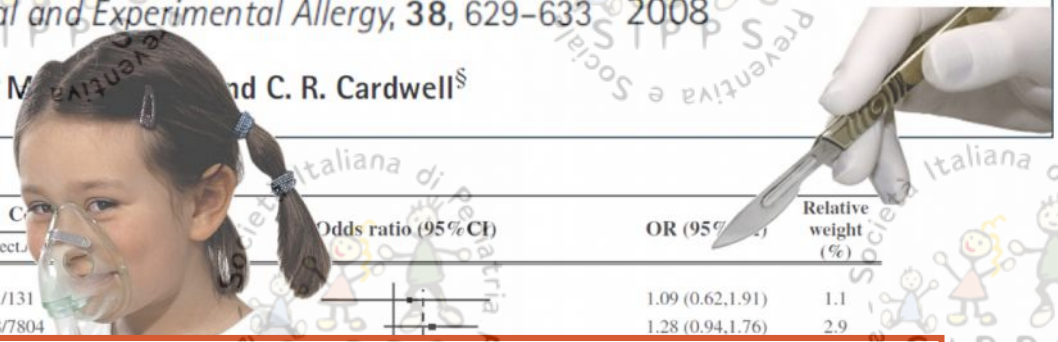
Kim Kristensen J Allergy Clin Immunol 2016;137:587-90

**Children delivered by CS are at increased risk of disease associated with immune function.**

# Asthma

**A meta-analysis of the association between Caesarean section and childhood asthma** *Clinical and Experimental Allergy*, **38**, 629–633 2008

S. Thavagnanam*, J. Fleming†, A. Bromley‡, M[illegible] and C. R. Cardwell§



| First author | Asthma C-sect./tot | (%) | C[illegible] C-sect. | | Odds ratio (95% CI) | OR (95%[illegible]) | Relative weight (%) |
|---|---|---|---|---|---|---|---|
| Oliveti | 33/131 | (25) | 31/131 | | | 1.09 (0.62,1.91) | 1.1 |
| Xu | 49/282 | (17 | 1098/7804 | | | 1.28 (0.94,1.76) | 2.9 |
| Rusconi | 247/844 | (29) | 3916/14 765 | (27) | | 1.15 (0.98,1.34) | 7.5 |
| | | | Overall[a] | | | **1.22 (1.14, 1.29)** | |
| | | | Childhood studies[b] | | | **1.20 (1.14, 1.26)** | |

0.2 0.5 1 2 5

*Reduced risk of asthma after Caesarean* *Increased risk of asthma after Caesarean*

Planned Cesarean Delivery and Adverse Outcomes in Childhood Health

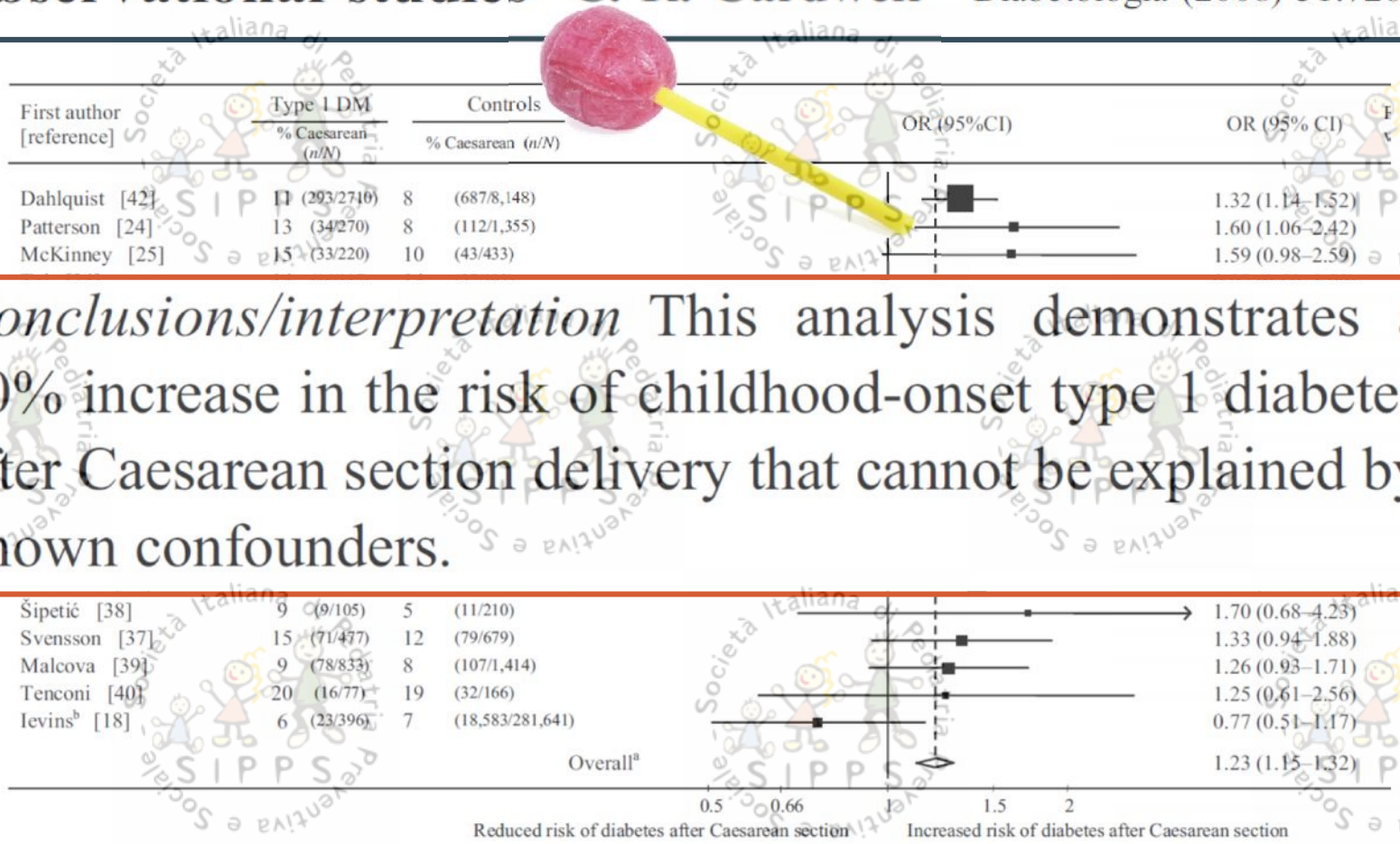
JAMA December 1, 2015

Co[illegible] [illegible]ease

ch[illegible] associated with a small absolute increased risk of asthma requiring hospital admission, salbutamol inhaler prescription at age 5 years [illegible]n.

# Tipe 1 diabetes

**Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies** C. R. Cardwell Diabetologia (2008) 51:726–735

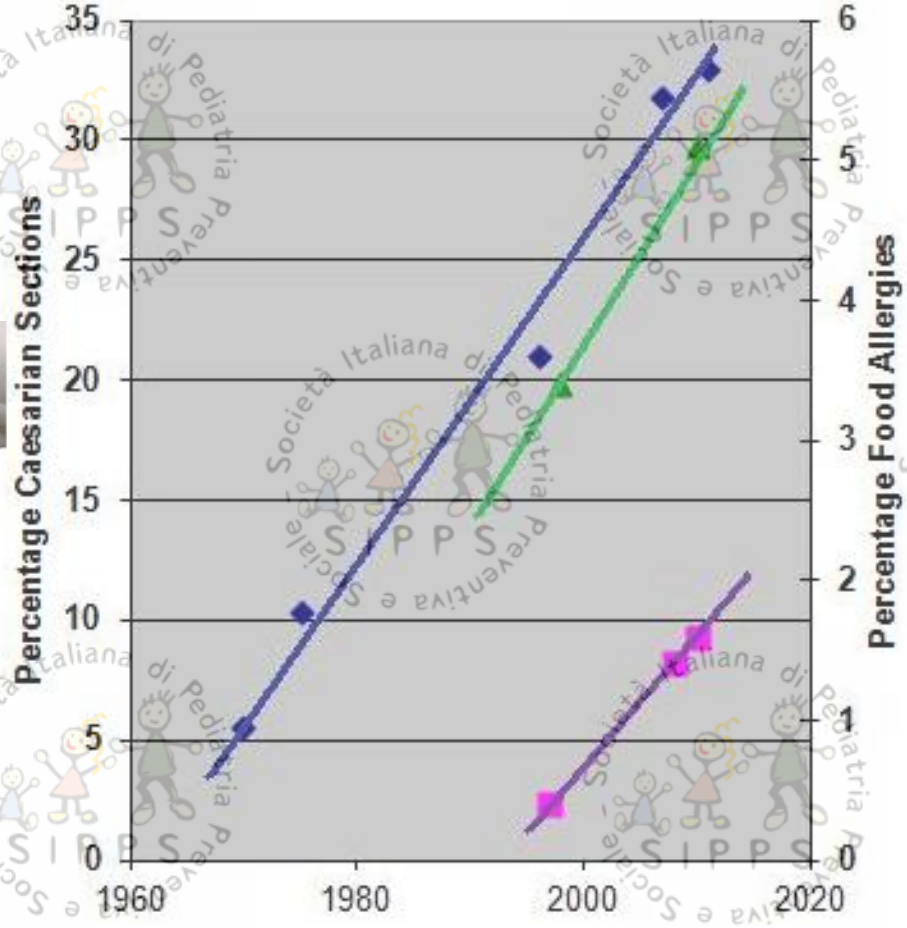


| First author [reference] | Type 1 DM % Caesarean (n/N) | Controls % Caesarean (n/N) | OR (95%CI) | OR (95% CI) |
|---|---|---|---|---|
| Dahlquist [42] | 11 (293/2710) | 8 (687/8,148) | | 1.32 (1.14–1.52) |
| Patterson [24] | 13 (34/270) | 8 (112/1,355) | | 1.60 (1.06–2.42) |
| McKinney [25] | 15 (33/220) | 10 (43/433) | | 1.59 (0.98–2.59) |
| Šipetić [38] | 9 (9/105) | 5 (11/210) | | 1.70 (0.68–4.23) |
| Svensson [37] | 15 (71/477) | 12 (79/679) | | 1.33 (0.94–1.88) |
| Malcova [39] | 9 (78/833) | 8 (107/1,414) | | 1.26 (0.93–1.71) |
| Tenconi [40] | 20 (16/77) | 19 (32/166) | | 1.25 (0.61–2.56) |
| Ievins[b] [18] | 6 (23/396) | 7 (18,583/281,641) | | 0.77 (0.51–1.17) |
| Overall[a] | | | | 1.23 (1.15–1.32) |



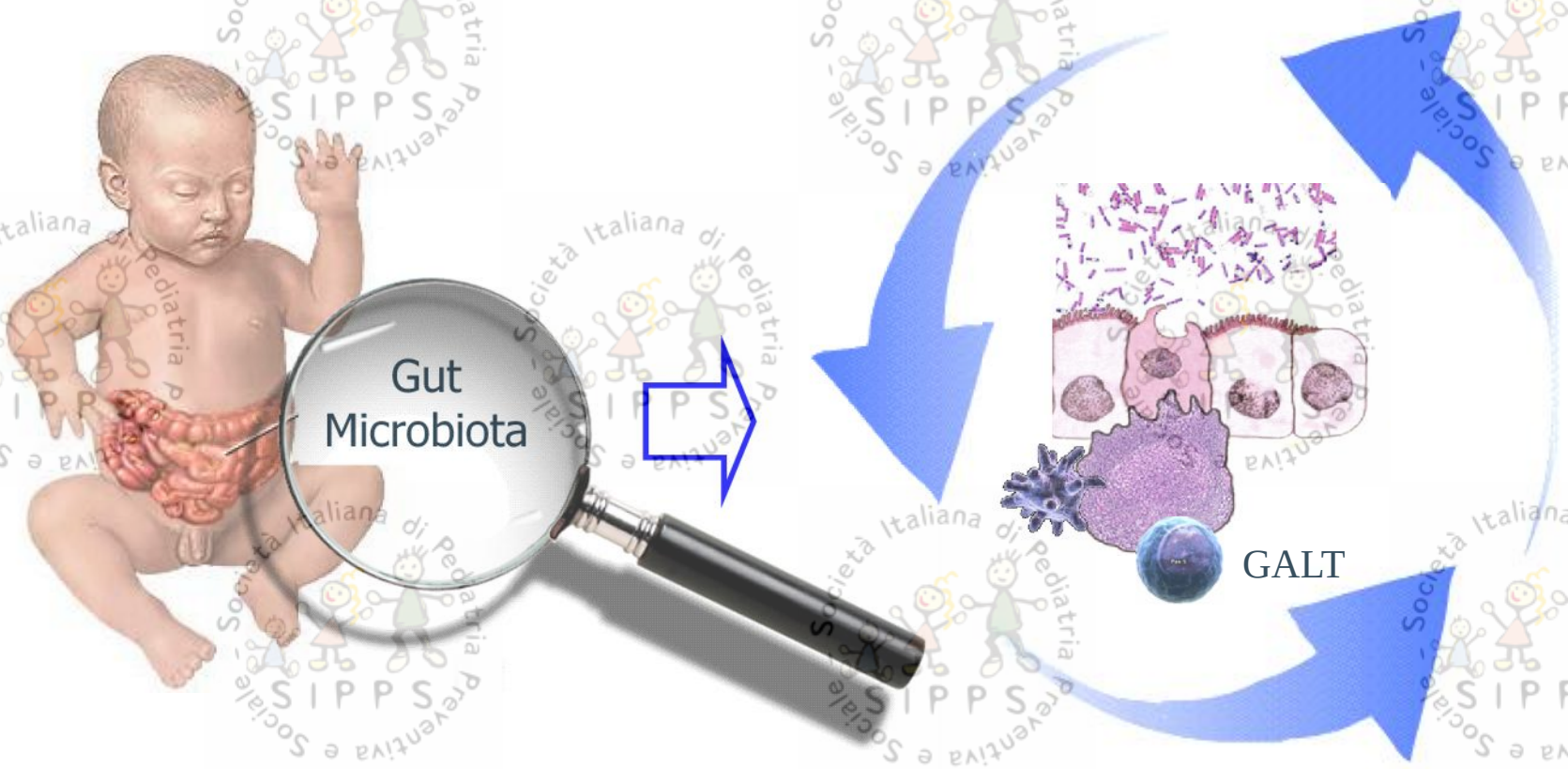
*Conclusions/interpretation* This analysis demonstrates a 20% increase in the risk of childhood-onset type 1 diabetes after Caesarean section delivery that cannot be explained by known confounders.

# Allergie Alimentari




Food Allergies

# Il microbiota intestinale è un 'organo' immunologicamente attivo



# The hygiene hypothesis

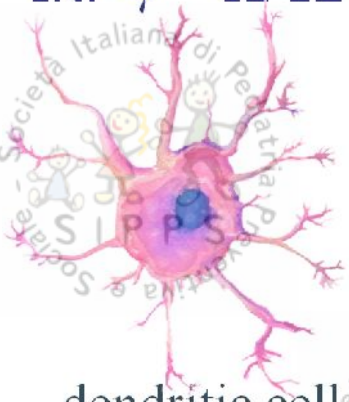

Th2

Th1

infezioni

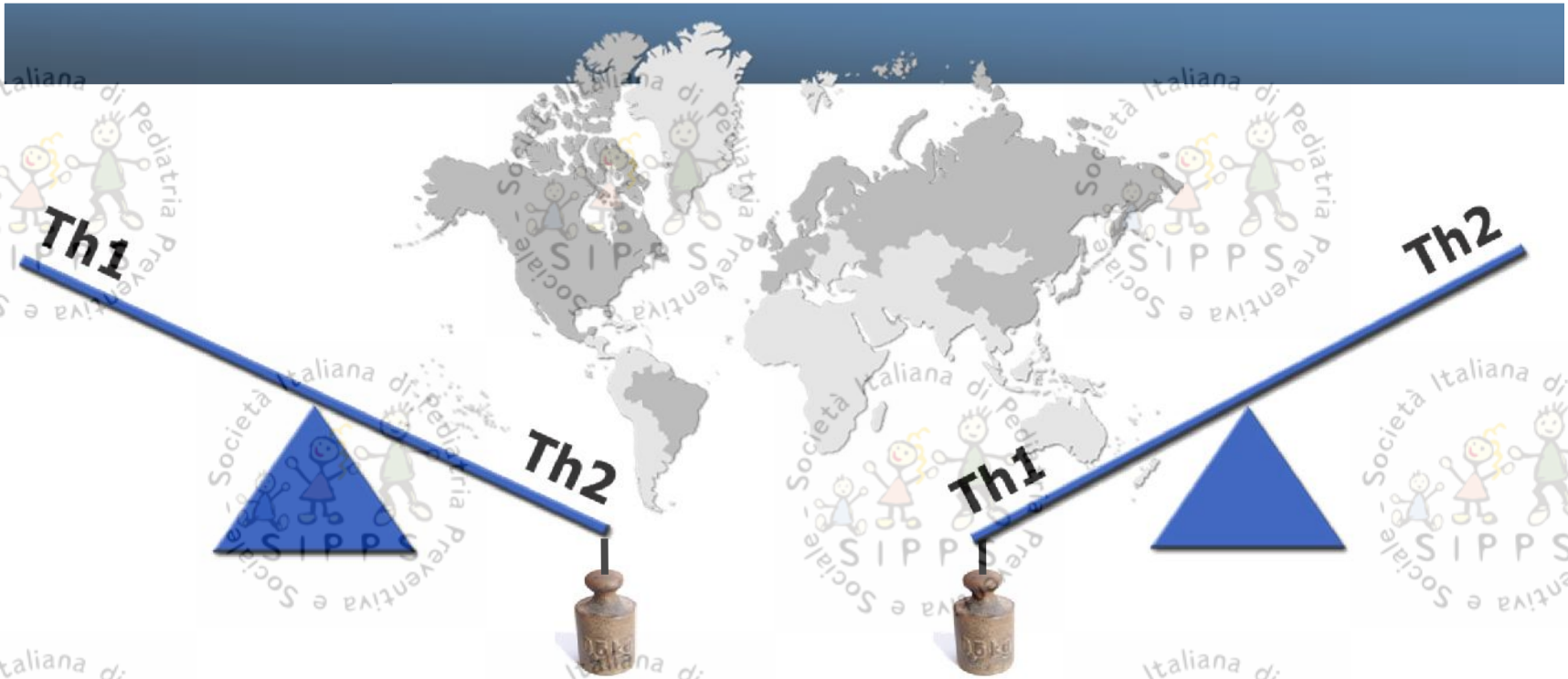
Strachan DP, 1989

INF-γ IL-12

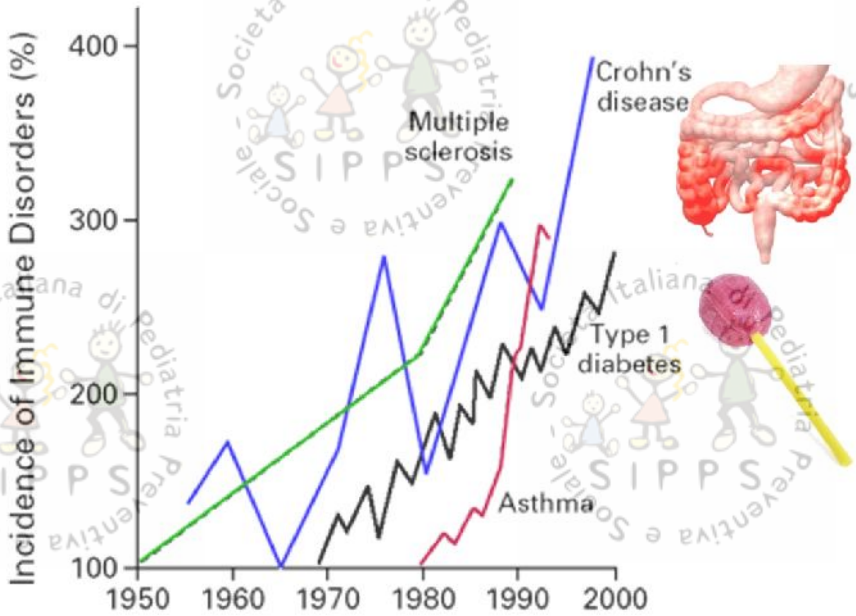


dendritic cell
(MHCII$^{+}$ CD11C$^{+}$)

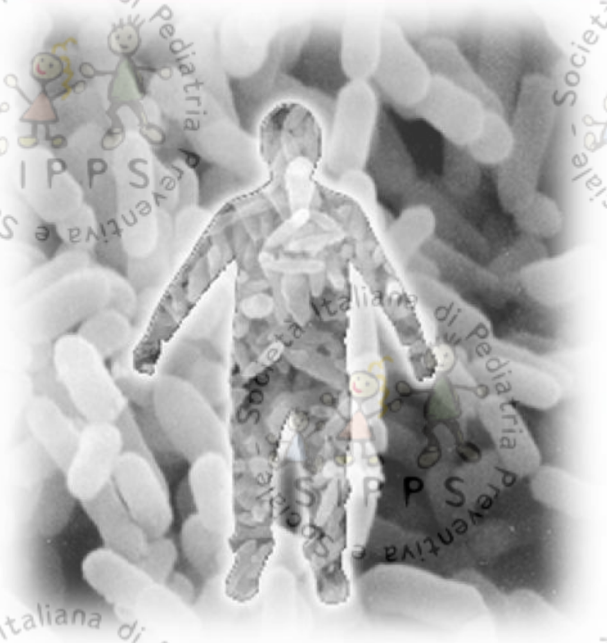
WA Walker, 2009


Th1
Th2
Th1
Th2


Eczema
Food allergy
Wheeze asthma
Rhinitis
Birth
Childhood
Adult


Incidence of Immune Disorders (%)
400
300
200
100
1950
1960
1970
1980
1990
2000
Crohn's disease
Multiple sclerosis
Type 1 diabetes
Asthma

# The microbiota hypothesis



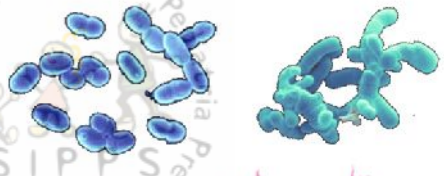
Th2

Th1

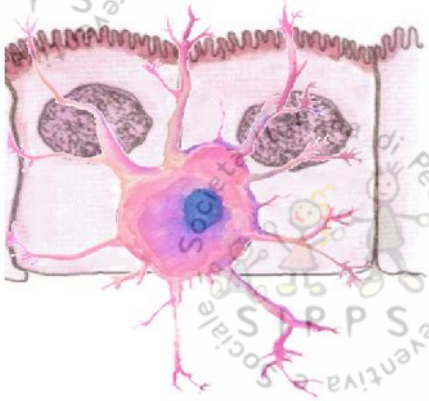
INF-γ IL-12

dendritic cell
(MHCII$^{+}$ CD11C$^{+}$)

# The biodiversity hypothesis



microbiota bilanciato
e diversificato



tolerogenic
dendritic cell

IL-10

TGF β

Treg

Regulatory CD4+T { Foxp3- (Tr1) / Foxp3+ ($T_{reg}$)

Th1

Th2

network regolatore

~~Autoimmunità~~

~~Allergia~~

risposte Th1/Th2 balanciate

# Disrupted gut immune homeostasis

## Initial Intestinal Colonization in the Human Infant and Immune Homeostasis

W. Allan Walker Ann Nutr Metab 2013;63

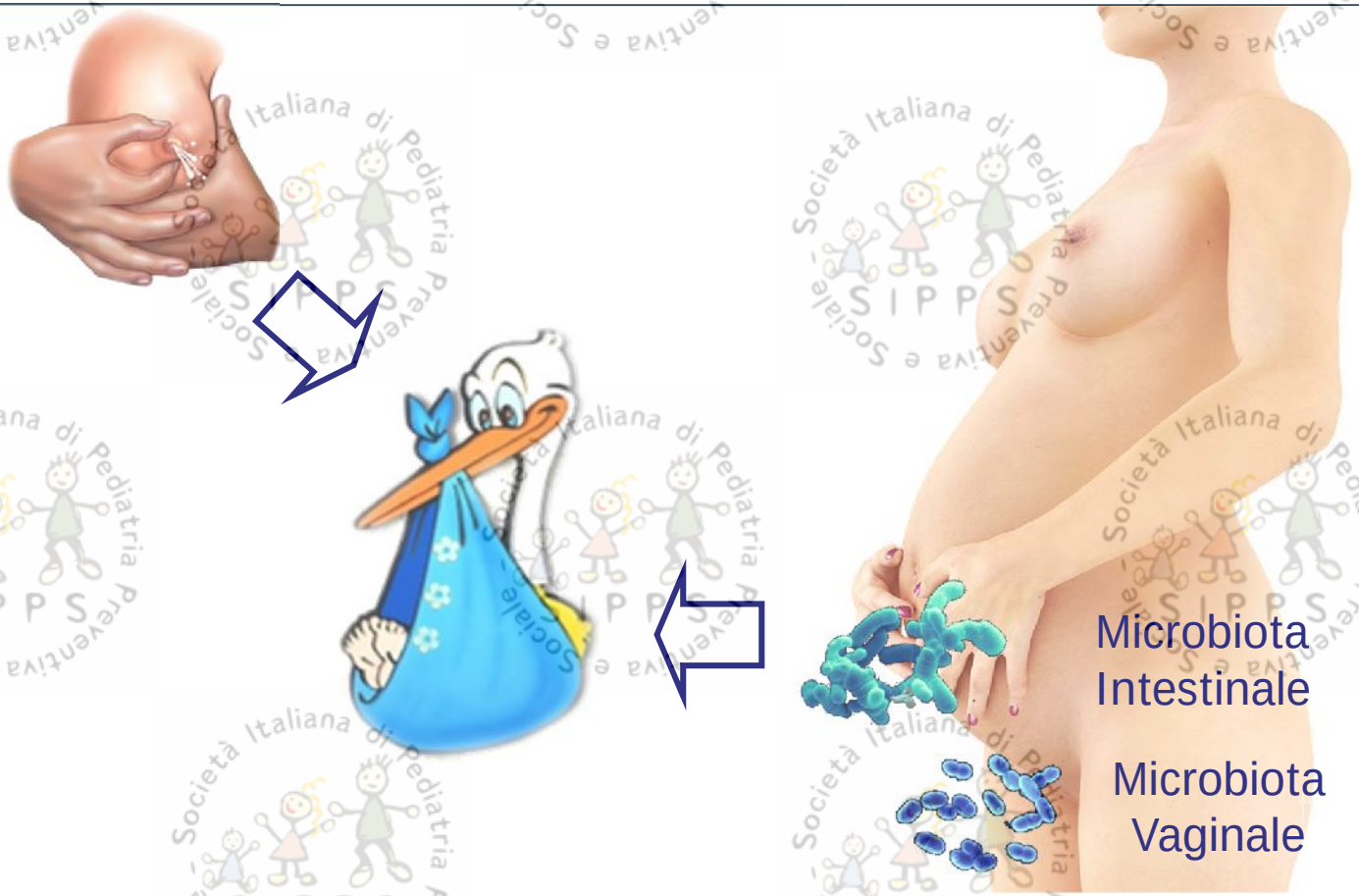
There is strong evidence that disruption of the normal colonization process can lead to alterations in the important symbiotic relationship that is necessary for immune homeostasis. For example, infants born by cesarean section or receiving excessive perinatal antibiotics have inadequate initial colonization and aberrant mucosal immune function.

# Eredità Microbica

**Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates**

J Allergy Clin Immunol 2013;131:1453-63

Petra Ina Pfefferle, PhD, DrPH,[a,b] Susan L. Prescott, MD, PhD,[b,c] and Matthias Kopp, MD[d] *Marburg and Lübeck, Germany.*




mechanisms of prenatal and postnatal induction of tolerance by microbial components.

# Eredità Microbica

**Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates**

J Allergy Clin Immunol 2013;131:1453-63

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mechanisms of prenatal and postnatal induction of tolerance by microbial components.

**Colonisation of the gut by bifidobacteria is much more common in vaginal deliveries than Caesarean sections** Musilova et al. Acta Pædiatrica 2015.

In conclusion, colonisation of the gut by bifidobacteria in vaginally delivered infants is much more common than in infants delivered by Caesarean section.

However, if infants born by Caesarean section did not have bifidobacteria in their gut microbiota, *E. coli* was relatively dominant along with clostridia and gram-negative bacteria.

**Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy** J Allergy ClinImmunol 2011;128:948-55

Frederika A. van Nimwegen, MSc,[a] John Penders, PhD,[a,b] Ellen E. Stobberingh, PhD,[b] Dirkje S. Postma, MD, PhD,[c]

colonization rates

vaginally home-born } 19.1%

vaginally hospital-born } 27.2%

cesarean section–delivered 43.4%

($P_{trend} < .001$)

# Disbiosi ed eubiosi

Parto vaginale
Latte materno

Antibiotici

Parto Cesareo

Prematurità

- Ottimale composizione del Microbiota Intestinale
- Prevalenza di Bifidobatteri

↓

## Eubiosi

↓

Interazione tra Microbiota e tessuto linfoide intestinale (GALT)

↓

**Omeostasi Immunitaria**
Protezione per infezioni, malattie allergiche e malattie autoimmuni

## Disbiosi

- Ritardata colonizzazione con ridotta diversità microbica
- Anomala composizione del Microbiota (prevalenza di *Clostridia* ed *E. coli* con deficit di *Bacteroidetes*, Bifidobatteri e Lattobacilli)

↓

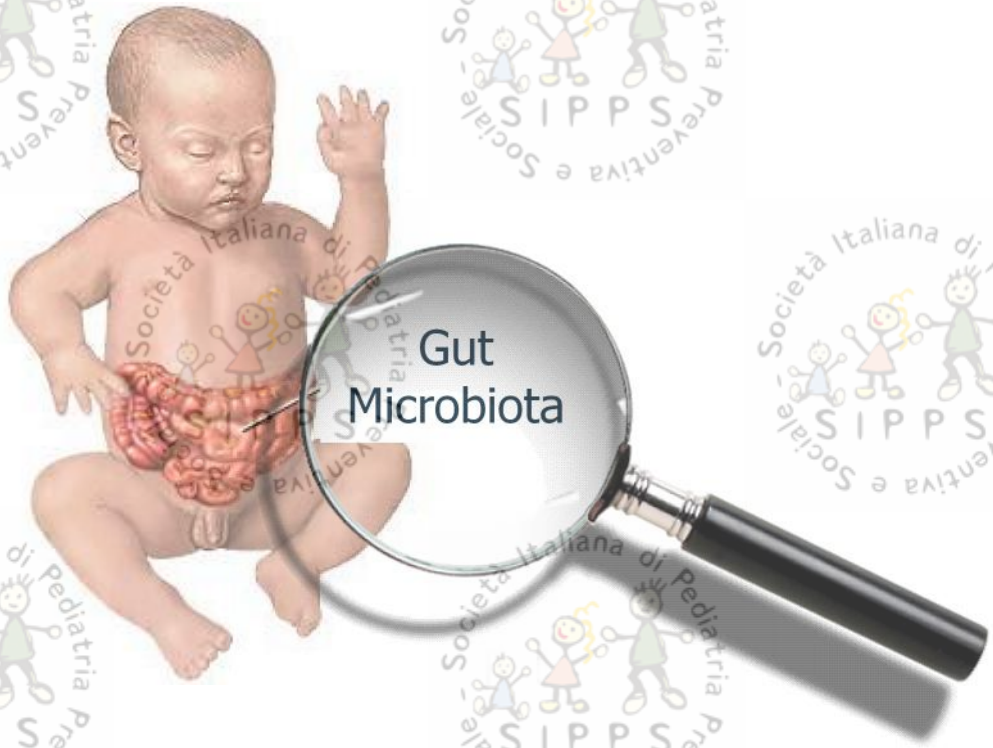
**Alterata omeostasi immuno-metabolica**

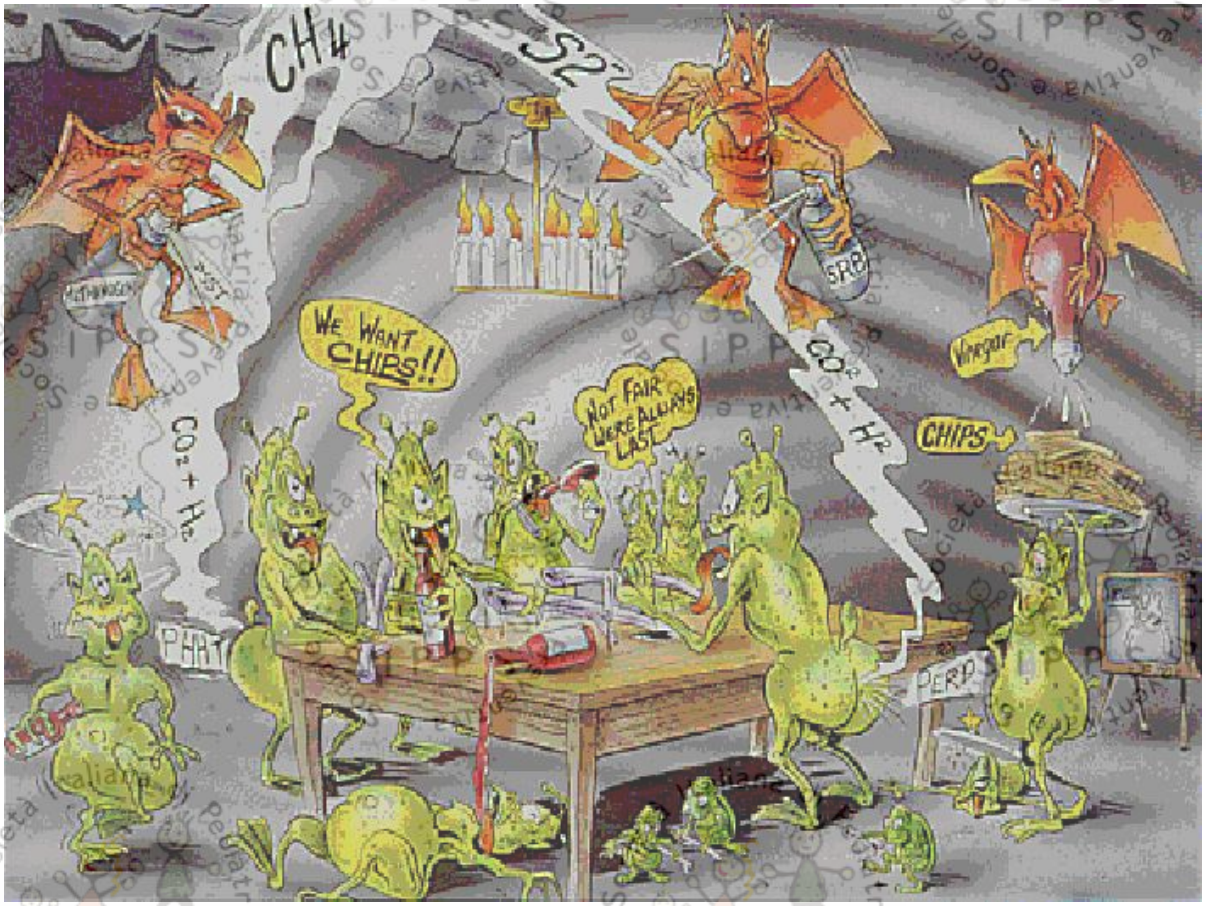
↓

Aumentata prevalenza malattie allergiche e malattie autoimmuni

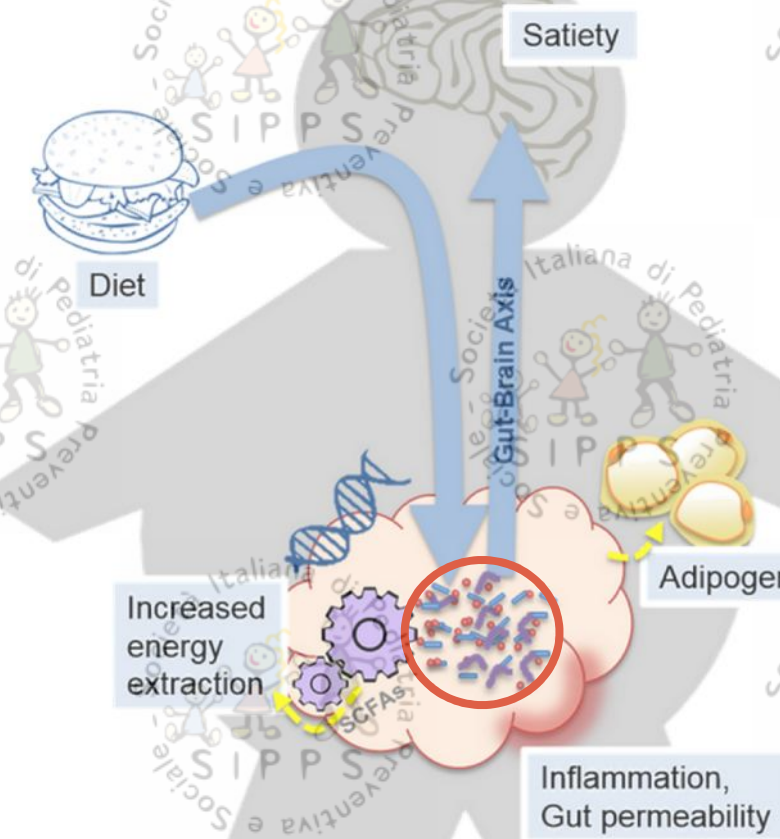
Miniello VL, Diaferio L, Verduci E

# Il microbiota intestinale è un 'organo' metabolicamente attivo




CH4
S2
WE WANT CHIPS!!
NOT FAIR WE'RE ALWAYS LAST
CO2 + H2
CO2 + H2
CHIPS
Vinegar
SRB
PERD
PERD

# Obesity


Satiety
Diet
Gut-Brain Axis
Adipogenesis
Increased energy extraction
SCFAs
Inflammation, Gut permeability

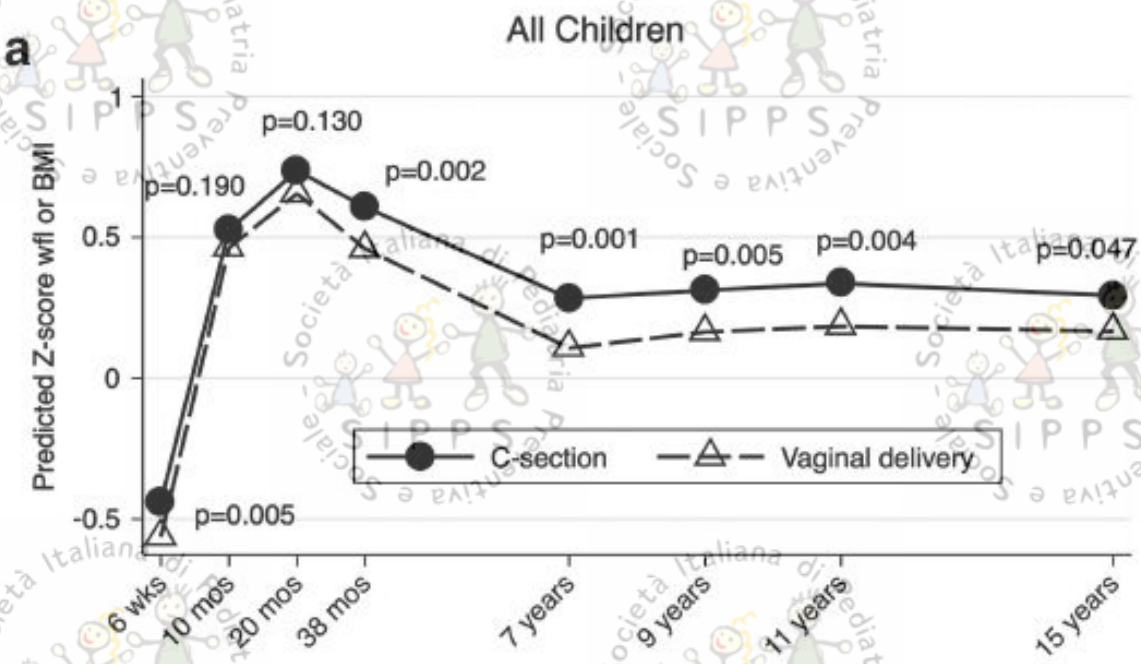
PEDIATRIA PREVENTIVA & SOCIALE

# Malattie croniche non trasmissibili: complice il microbiota intestinale?

Miniello VL, Diaferio L, Verduci E

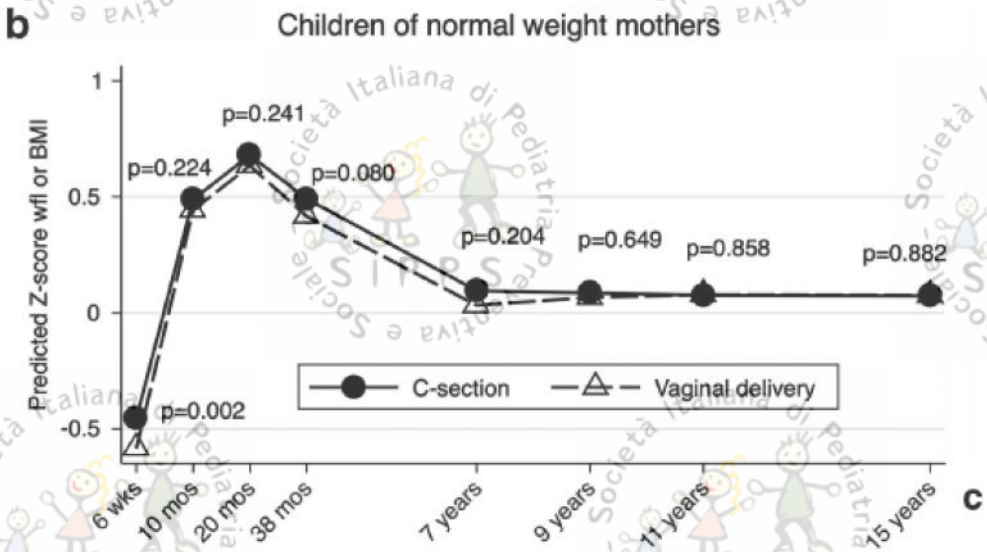
# Obesity

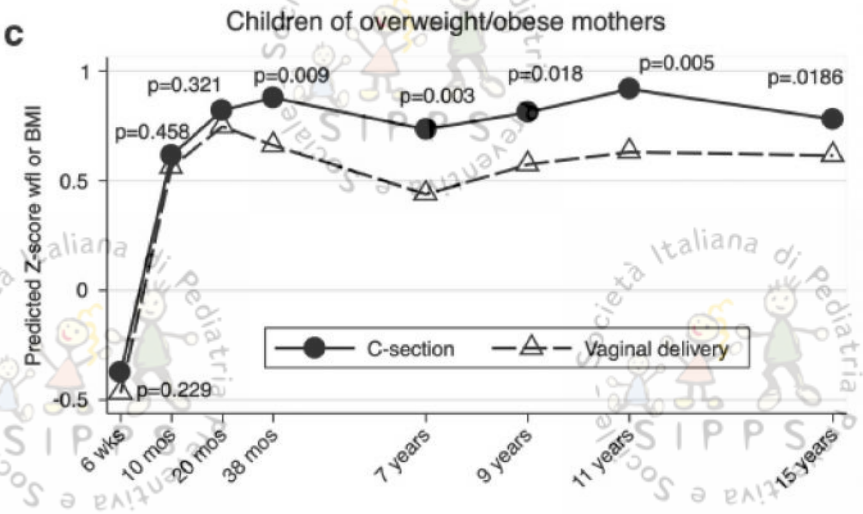
Association of caesarean delivery with child adiposity from age 6 weeks to 15 years J Blustein International Journal of Obesity (2013) **37**, 900–906




# Obesity

Association of caesarean delivery with child adiposity from age 6 weeks to 15 years J Blustein International Journal of Obesity (2013) **37**, 900–906



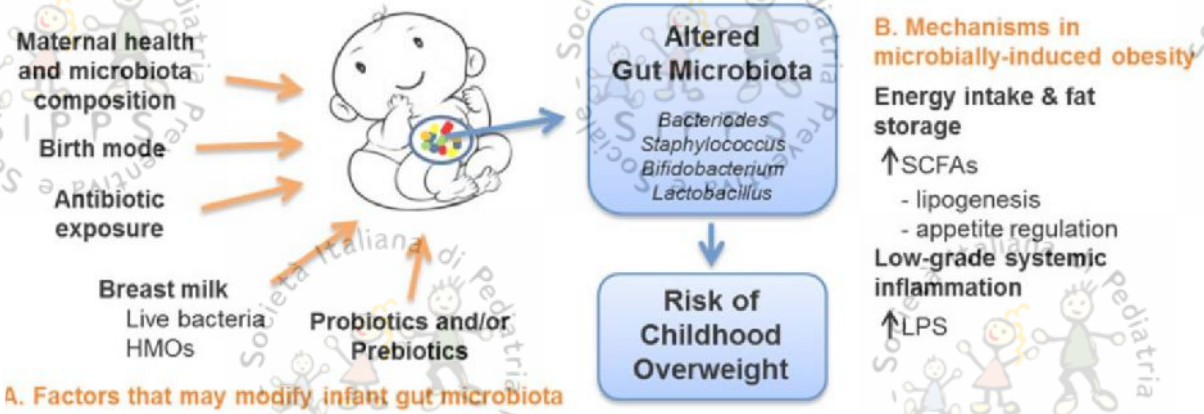
# Obesity

Childhood Health and Developmental Outcomes After Cesarean Birth in an Australian Cohort

Stephen J. Robson

PEDIATRICS Volume 136, number 5, November 2015

It has also been postulated that differences in gut microbiota may regulate obesity.




*Nutrients* **2015**, *7*

# Permeabilità Intestinale

JPGN
Journal of Pediatric Gastroenterology and Nutrition

# INTESTINAL PERMEABILITY IN HEALTHY BREAST-FED INFANTS, DURING THE POSTNATAL PERIOD

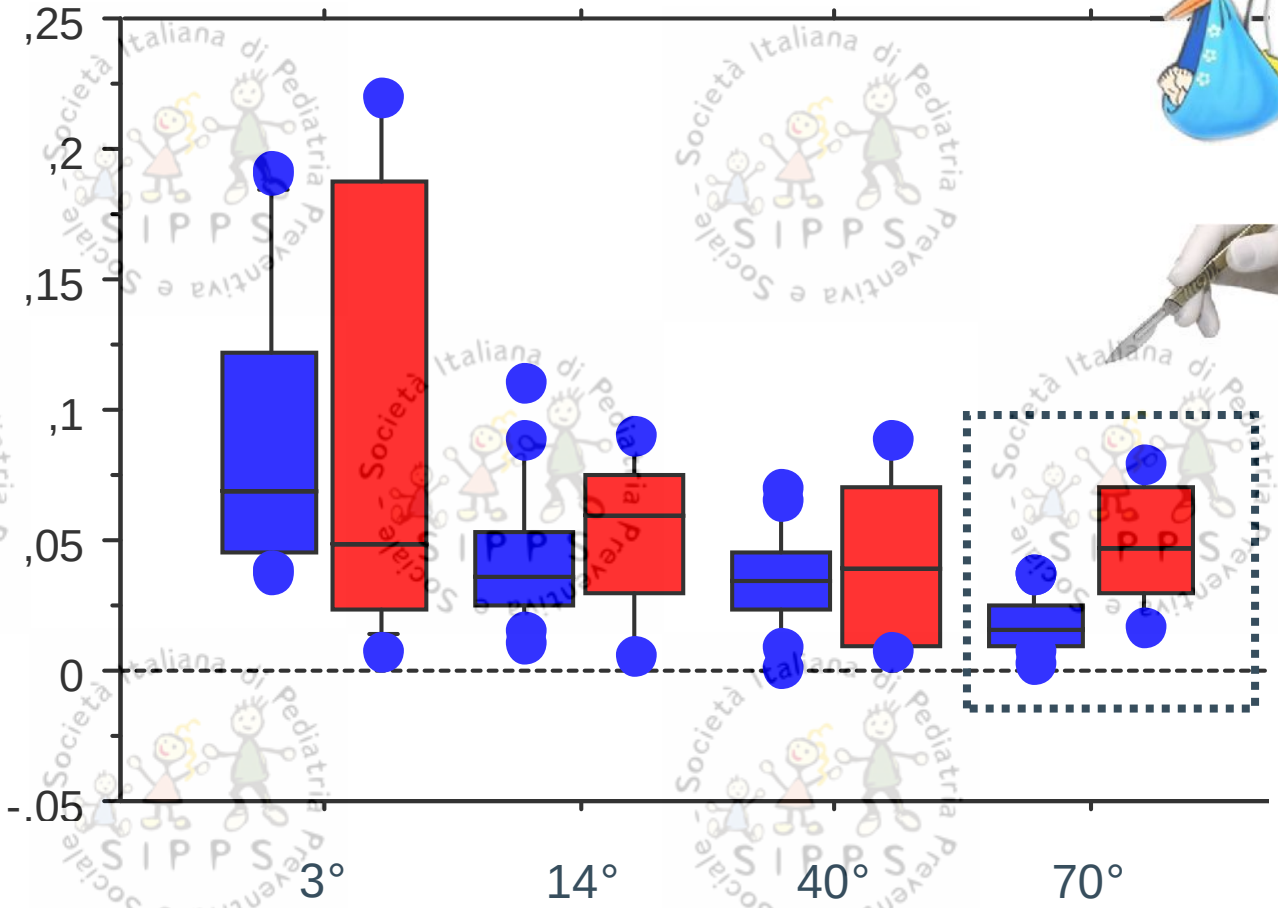
SP Castellaneta[1], A Masciale[1], A Zaccaro[1], S Straziuso[2], V Miniello[2], F Gatti[3], L Polimeno[3], R Francavilla[3].

*Journal of Pediatric Gastroenterology and Nutrition*
**40**:616–706 © May 2005 Lippincott Williams & Wilkins

**Background and Aim:** The maturation of the intestinal epithelial barrier play an important role during the postnatal period and few data are available on the exact timing of gut closure in neonates. The aim of our study was to assess the timing of maturation of the gut barrier by the measure of the intestinal permeability (IP) in full term infants.

**Summary and conclusion:** Our study show that the IP to sugar probes in healthy breast fed infants is high at birth and progressively decrease during the first two months of life; however yet at 20 days the maturation of the IP and the gut closure seems to be completed.

**Box Plot**
**Grouping Variable(s): CRF**
**Split By: Parto**



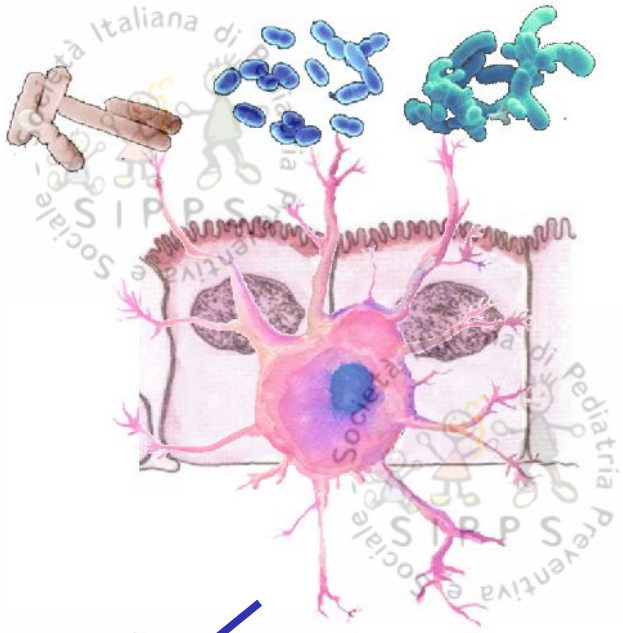

# Reciprocal interactions of the intestinal microbiota and immune system

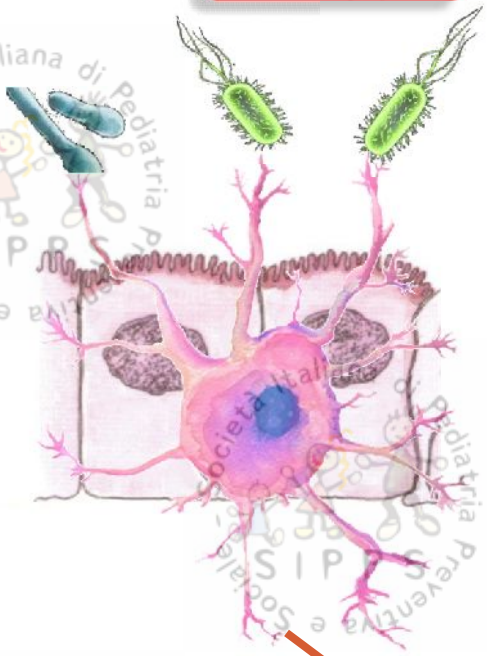
2012 | VOL 489 | NATURE

Craig L. Maynard[1], Charles O. Elson[2], Robin D. Hatton[1] & Casey T. Weaver[1]

Eubiosi

Disbiosi

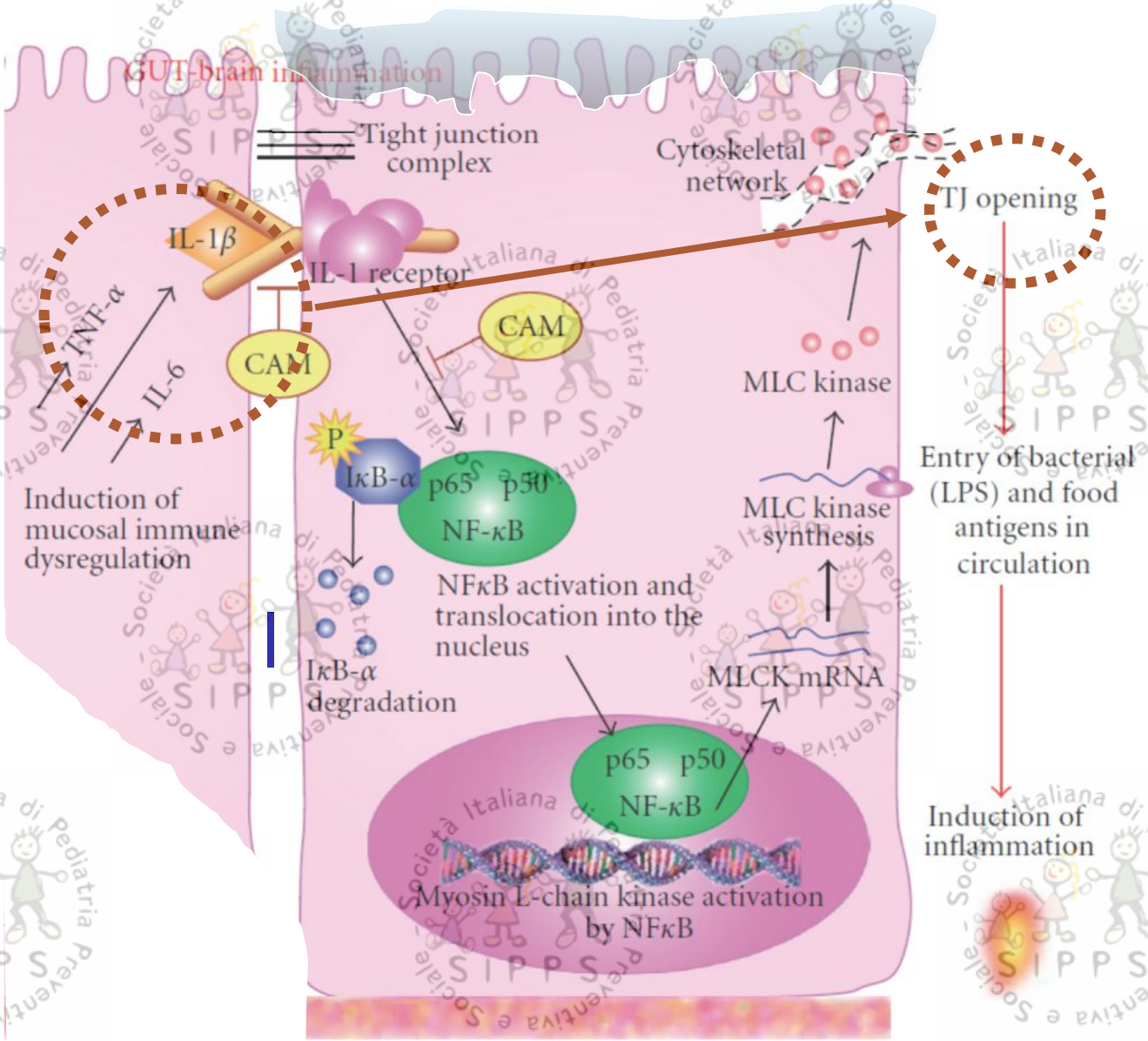




TGF-β

IL-1β IL-6 TNFα


Alterata colonizzazione

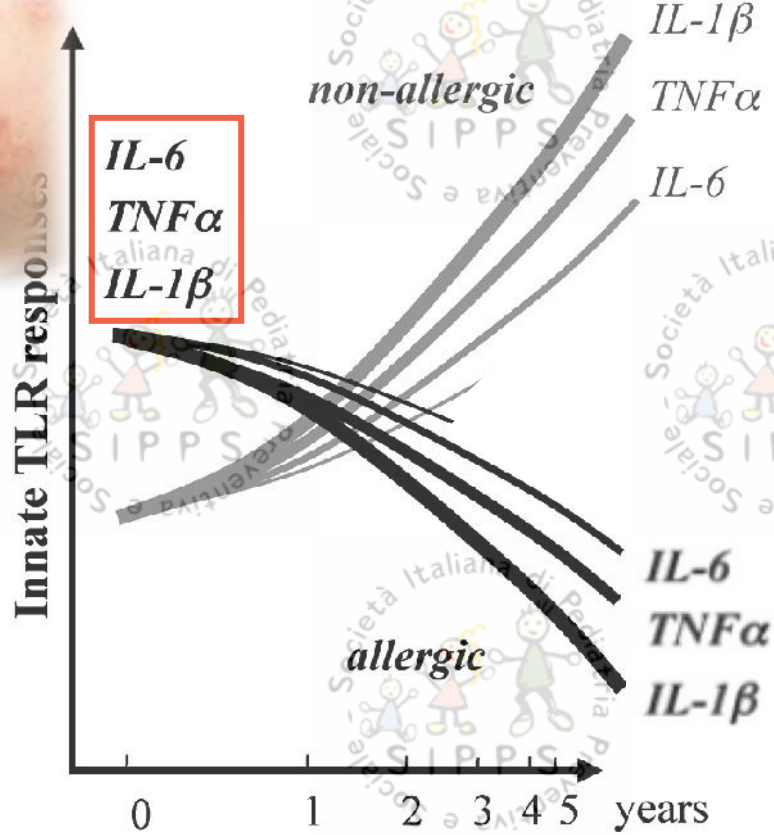


# Differences in innate immune function between allergic and nonallergic children: New insights into immune ontogeny

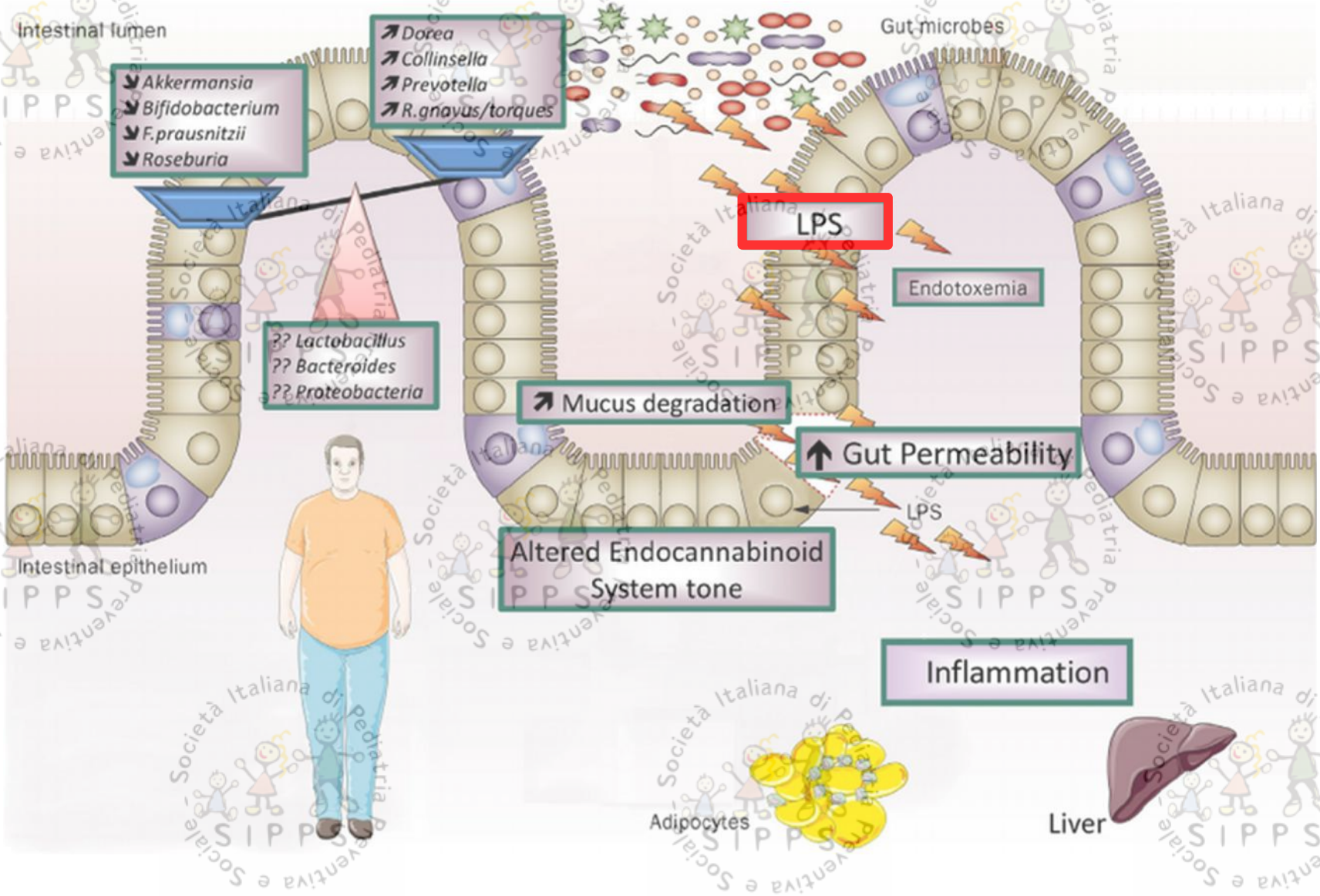
Meri K. Tulic, BSc, PhD,[a] Megan Hodder, BSc,[a] Anna Forsberg, MSc,[b] Suzi McCarthy, BSc,[a] Tara Richman, BSc,[a] Nina D'Vaz, BSc,[a] Anita H. J. van den Biggelaar, BSc, PhD,[c] Catherine A. Thornton, BSc, PhD,[d] and Susan L. Prescott, MD, PhD[a] *Perth, Australia, Linkoping, Sweden, and Swansea, United Kingdom*

J Allergy Clin Immunol 2011;127:470-8.

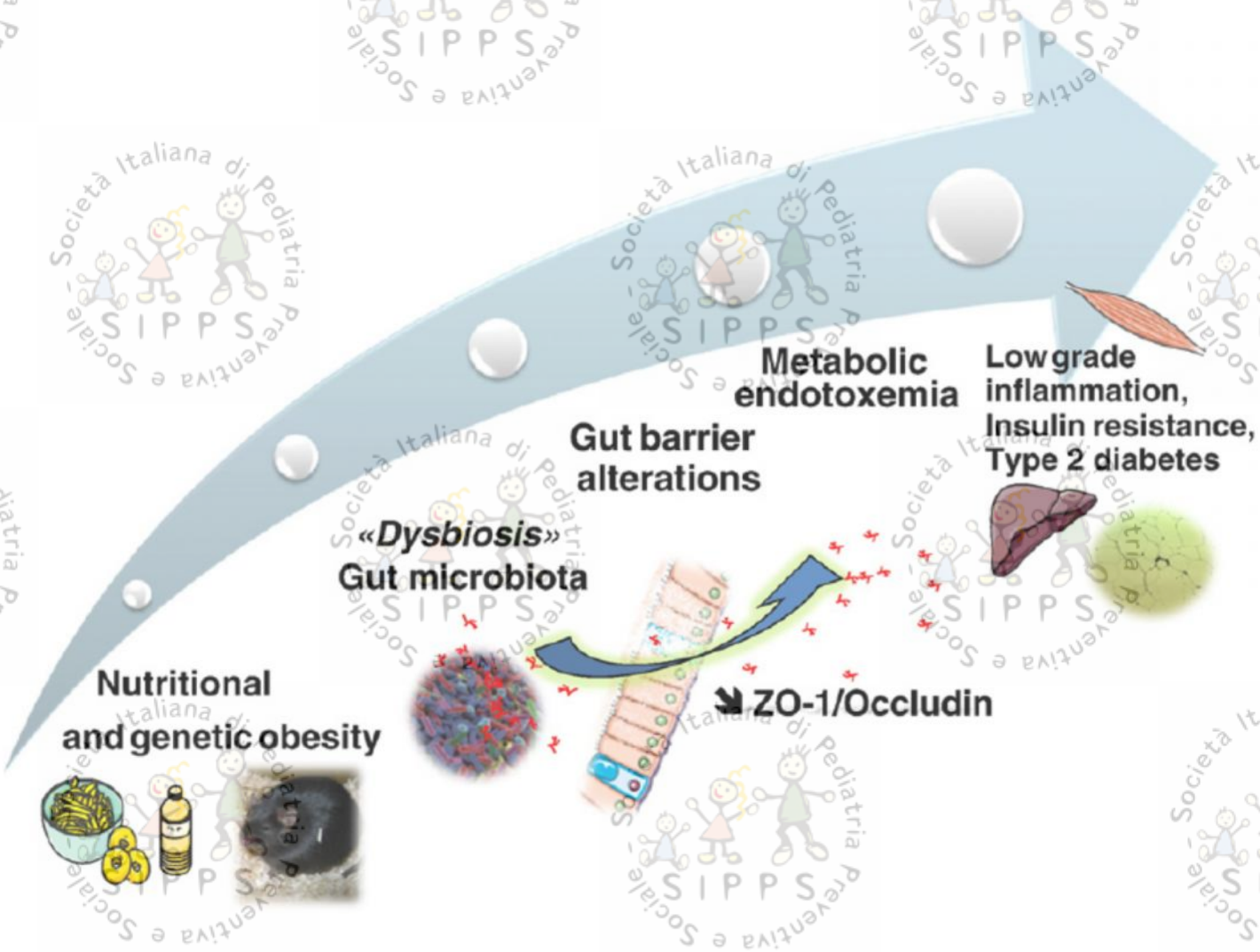
innate immune development



# Alterata Permeabilità Intestinale



P.D. Cani, N.M. Delzenne / Pharmacology & Therapeutics 130 (2011) 202–212



# Sistema Endocannabinoide

Diabetes & Metabolism

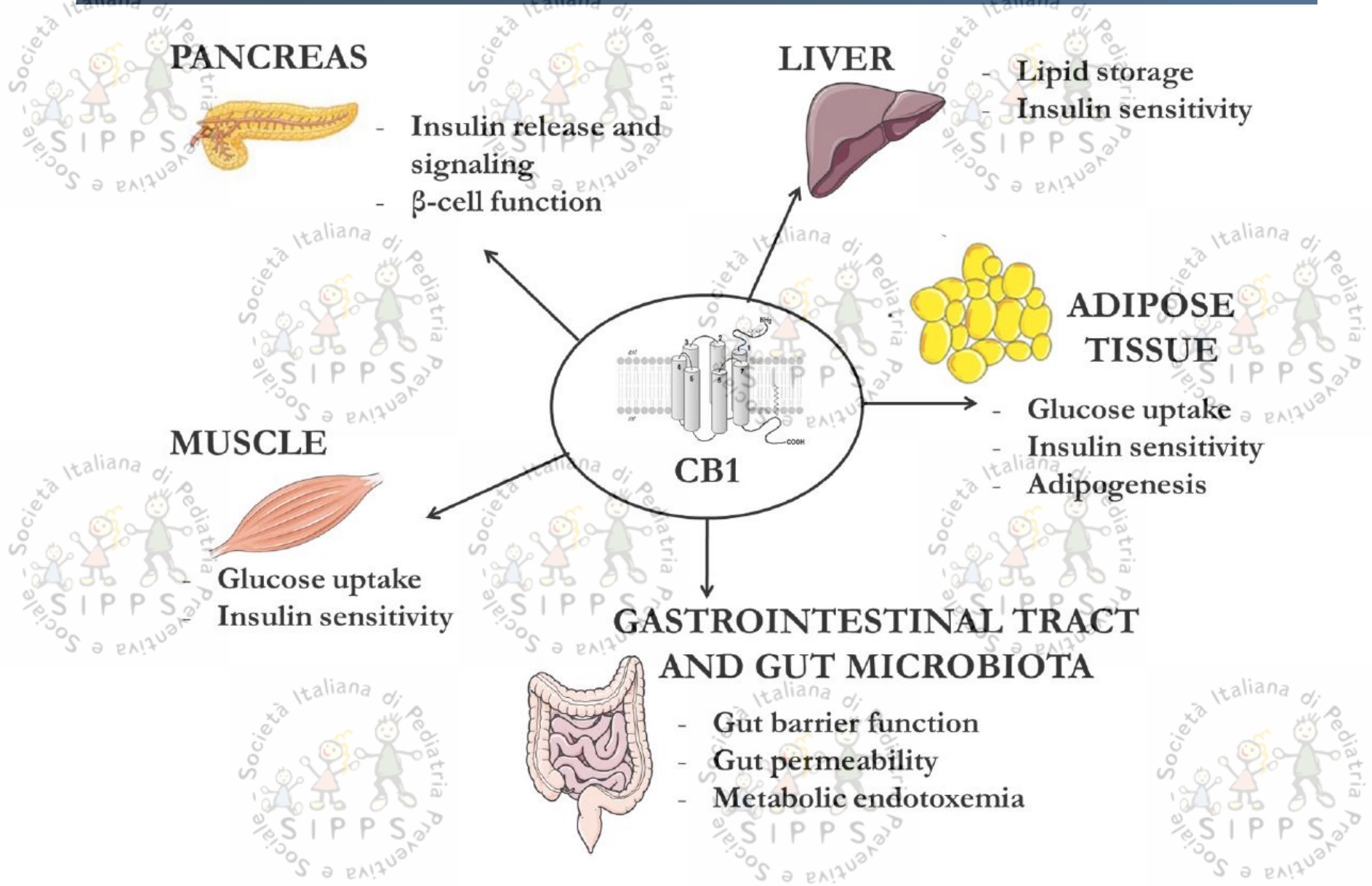
Glucose metabolism: Focus on gut microbiota, the endocannabinoid system and beyond

P.D. Cani

## The endocannabinoid (eCB) system and metabolism

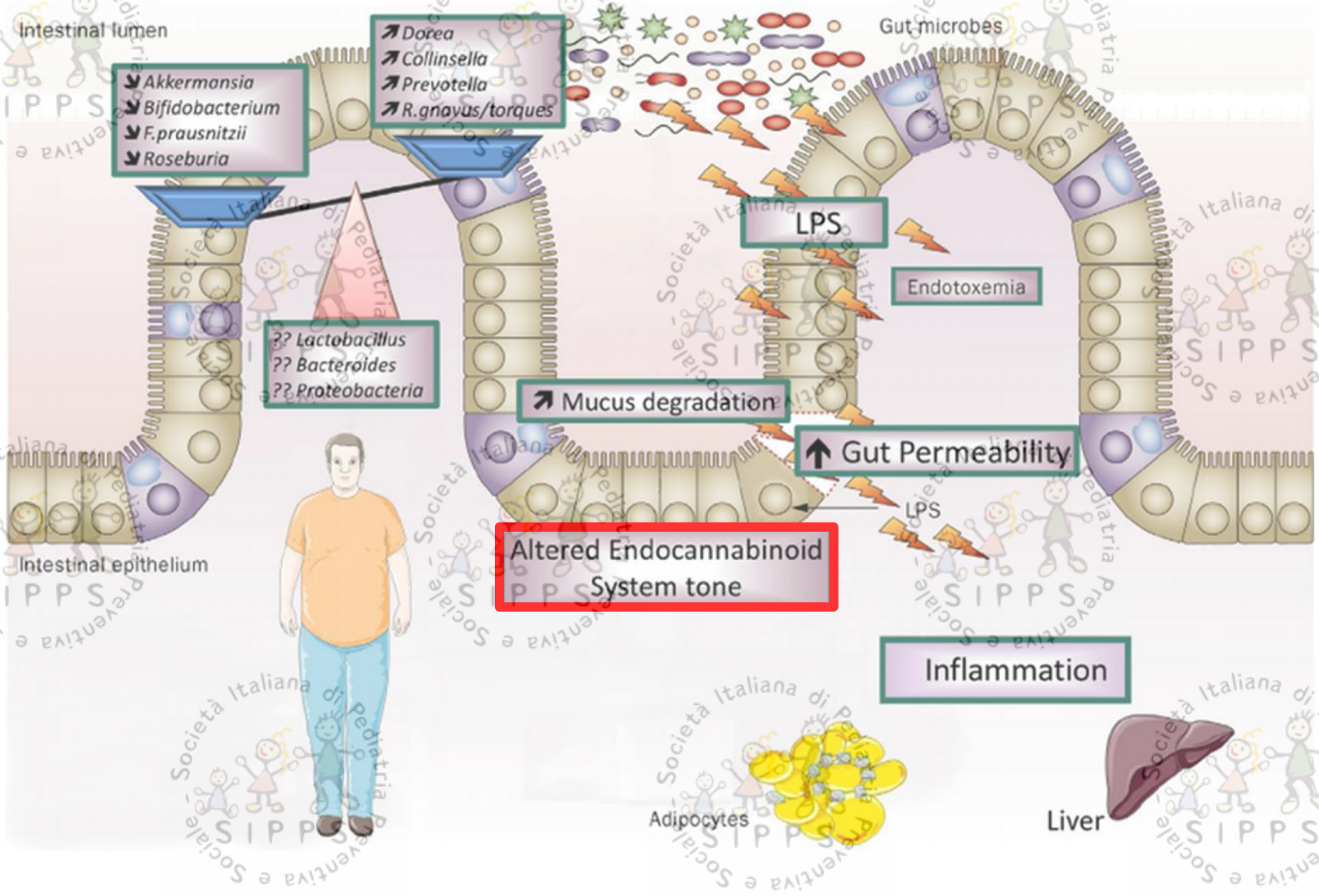
Con il termine cannabinoidi endogeni o endocannabinoidi (eCB) si identifica una classe di composti lipidici accomunati dalla capacità di interagire con specifici recettori presenti a livello centrale o periferico che regolano numerose funzioni fisiologiche e comportamentali. Tutti gli eCB derivano da acidi grassi polinsaturi, strutturalmente differenti dai cannabinoidi di origine vegetale (fitocannabinoidi), come il componente attivo della *Cannabis* (tetraidrocannabinolo), responsabile degli effetti psicoattivi legati all'uso di marijuana.

# Sistema Endocannabinoide




Gut microbiota composition is associated with intestinal eCB content and $CB_1$ receptor

# Sistema Endocannabinoide

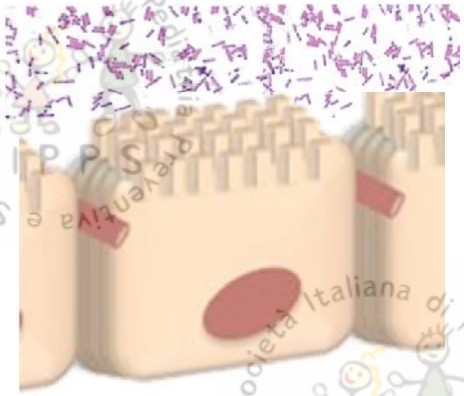


# Dysbiosis

Tight Junctions

↓ ZO-1 (Zonula occludens protein)

↓ Occludin



Gut barrier disruption

**↑ Intestinal Permeability**

⇩

**↑ Metabolic Endotoxemia**
**Gut microbiota-derived lipopolysaccharides ( LPS)**

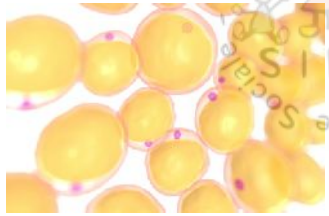
⇨ ë Endocannabinoid System tone

⇩

ë Adipogenesis

**Chronic low-grade inflammation**
**Insulin resistance**

ë Fat storage



# Finestra di vulnerabilità e opportunità

# BIOMODULATORI DEL MICROBIOTA INTESTINALE: tra realtà e futuro

Vito Leonardo Miniello

EDITEAM
GRUPPO EDITORIALE

# biomodulatori del microbiota intestinale

World Health Organization

Food and Agriculture Organization of the United Nations

## probiotici

“Microrganismi vivi che, assunti in quantità adeguata, conferiscono all’organismo ospite un effetto salutare”

## prebiotici

“Costituenti alimentari non vitali che conferiscono un beneficio alla salute, mediante una modulazione del microbiota”

# biomodulatori del microbiota intestinale

## s inbiotic i

Associazione di
Prebiotici e Probiotici”

## p ostbiotic i

Prodotti batterici o derivati
metabolici di microrganismi
probiotici con attività biologica
per l’ospite

# Probiotics

**Initial Intestinal Colonization in the Human Infant and Immune Homeostasis**

W. Allan Walker — Ann Nutr Metab 2013;63(

**Abnormal colonization (dysbiosis) and its accompanying increase in disease expression can be prevented by pre- and probiotics.**

Gut microbiota biomodulators, when the stork comes by the scalpel

Vito Leonardo Miniello — Clinica Chimica Acta 451 (2015)

Under these dysbiosis conditions probiotics can act as surrogate colonizers to prevent immune-mediated diseases.

# Probiotics

## Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort

(J Allergy Clin Immunol 2009;123:335-41.)

Mikael Kuitunen, MD, PhD,[a] Kaarina Kukkonen, MD,[a] Kaisu Juntunen-Backman, MD, PhD,[a] Riitta Korpela, PhD,[c,d]

In a double-blinded, placebo-controlled study we randomized 1223 mothers with infants at high risk for allergy to receive a probiotic mixture (2 lactobacilli, bifidobacteria, and propionibacteria) or placebo during the last month of pregnancy and their infants to receive it from birth until age 6 months. Infants also received a prebiotic galacto-oligosaccharide or placebo.

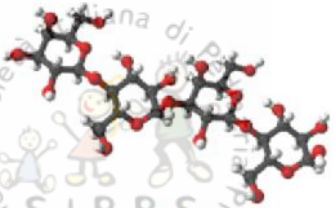
# Probiotics

461

464

PLACEBO

- Lactobacillus rhamnosus GG (ATCC 53103)
- Lactobacillus rhamnosus LC705 (DSM 7061)
- Bifidobacterium breve Bb99 (DSM 13692)
- Propionibacterium freudenreichii (DSM 7076)



GOS

PLACEBO

# Probiotics

At 5 years

Cumulative incidence of allergic diseases (eczema, food allergy,allergic rhinitis, and asthma) and IgE sensitization

"No allergy-preventive effect is extended to age 5 years by perinatal supplementation with probiotics in babies at risk for developing allergies".

Less IgE-associated allergic disease occurred in cesarean-delivered children receiving probiotics.

(24.3% vs 40.5%; odds ratio, 0.47; 95% CI, 0.23% to 0.96%; p=.035).

"Protection is conferred only to C-section babies"

# Probiotics

## Clinical Use of Probiotics in Pediatric Allergy (CUPPA): A World Allergy Organization Position Paper

WAO POSITION PAPER *WAO Journal* 2012; 5:148–167

However, the conclusions of this trial appear relevant for prevention and not treatment purposes, as the main difference was lower rates of allergy among infants born by caesarean section.[80]

# Prebiotici

MINIELLO VL, ARMENIO L.
Infant formula supplemented with prebiotic oligosaccharides: closer to the reference

Agro Food 2004 2: 42-4

MINIELLO VL, MORO GE, ARMENIO L.
Prebiotics in infant milk formulas: new perspectives.

Acta Paediatr 2003; 91: 68-76

MORO GE, MOSCA F, MINIELLO VL et al.
Effects of a new mixture of prebiotics on fecal flora and stools in term infants.

Acta Paediatr 2003; 91: 77-9

# Prebiotici e GLP

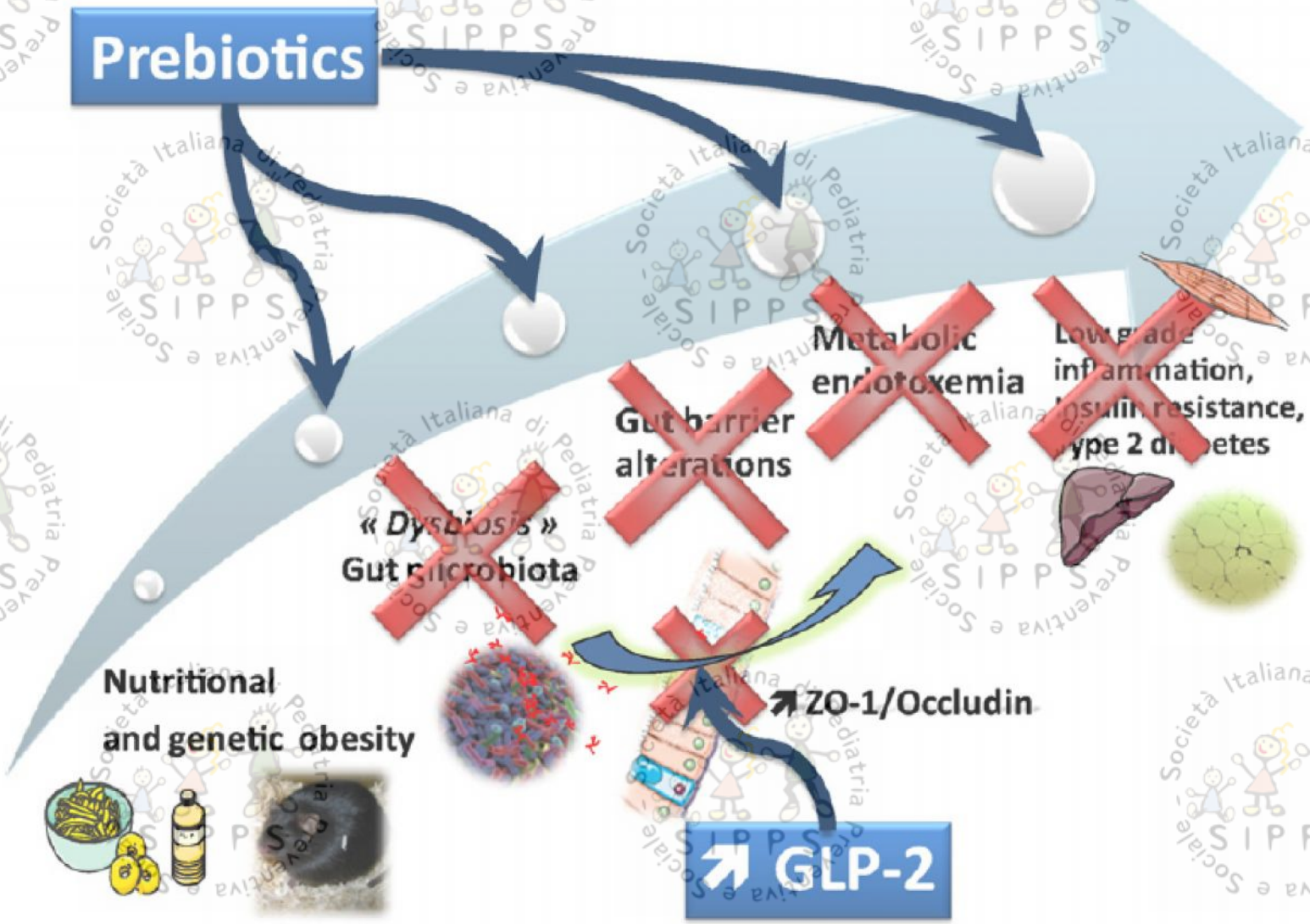
P.D. Cani, N.M. Delzenne / Pharmacology & Therapeutics 130 (2011) 202–212

GLP-1, Glucagon-Like Peptide-1 GLP-2 Glucagon-Like Peptide-2

We investigated this particular peptide because in our previous work, we found that prebiotic-induced changes in the gut microbiota promote GLP-1 synthesis (proglucagon mRNA and GLP-1 peptide) in the proximal colon by a mechanism linked to the differentiation of precursor cells into enteroendocrine cells (Cani et al., 2004, 2005a, b, 2006b; Cani et al., 2007b; Delzenne et al., 2007). Given that both GLPs are produced and secreted by L-cells and that endogenous production of GLP-1 increases upon prebiotic-induced changes in the gut microbiota, we focused our research on GLP-2. We found that increased endogenous GLP-2 production was associated with improved mucosal barrier function via the restoration of tight junction protein expression and distribution

# Prebiotici

P.D. Cani, N.M. Delzenne / Pharmacology & Therapeutics 130 (2011) 202–212




GLP-1, Glucagon-Like Peptide-1

# Prebiotici

**Prebiotics in infants for prevention of allergy**

Osborn DA, Sinn JKH

Cochrane Library

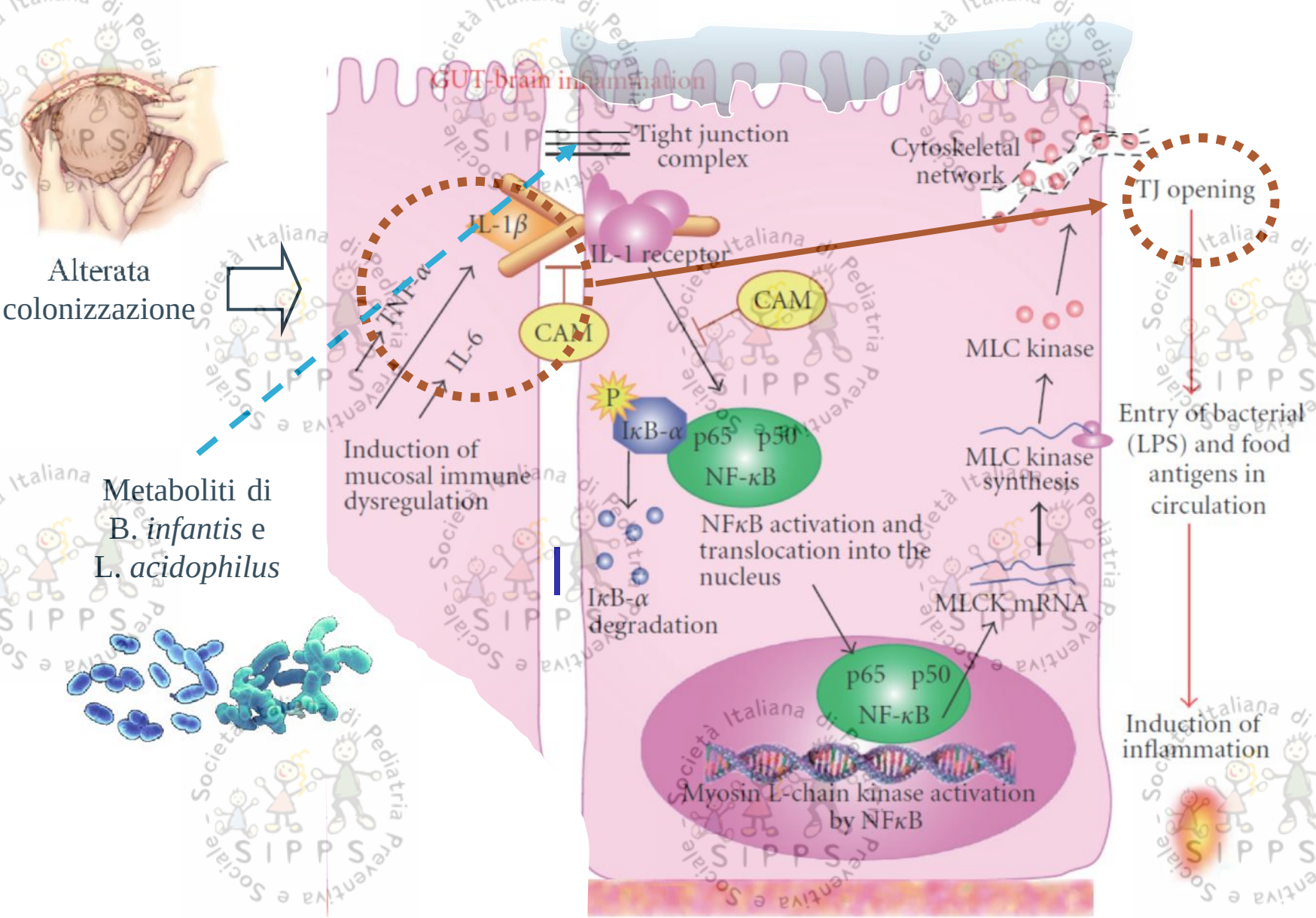
I ricercatori hanno analizzato i dati provenienti da 4 studi condotti su oltre 1.218 bambini, dai quattro mesi ai due anni di età, a cui veniva somministrato latte formula supplementato con prebiotici versus formula standard (controllo).

In base alla revisione, l'eczema e' risultato significativamente meno frequente nei bambini che avevano aassumevano latte con prebiotici.

(GOS/FOS 9:1 ratio)

Meta-analysis of four studies found a significant reduction in eczema

# Postbiotici




Guo SJ , Pediatr Gastroenterol Nutr. 2016

# Postbiotici

Cow's milk and rice fermented with *Lactobacillus paracasei* CBA L74 prevent infectious diseases in children: A randomized controlled trial

Rita Nocerino Clinical Nutrition (2015)

bambini sani (12-48 mesi) assegnati a ricevere giornalmente per tre mesi durante la stagione invernale latte di crescita con matrice fermentata da *Lactobacillus paracasei* CBA L74 o supplementato con placebo (maltodestrine).

L'analisi *intention-to-treat* ha mostrato nel gruppo attivo una minore incidenza (51,8 %) di almeno una delle comuni affezioni infettive, rispetto al gruppo placebo (80,3 %). Analizzando i dati in base al trattamento effettivamente ricevuto dai bambini che hanno portato a termine lo studio prospettico (*per-protocol analysis*), gli Autori hanno riscontrato minore incidenza di infezioni delle alte vie respiratorie (48,2 %) e di gastroenterite acuta (13,1 %), rispetto ai coetanei che assumevano latte con maltodestrine (rispettivamente 70,5 % e 31,1 %).


“Prega per noi peccatori
adesso e nell’ora
della nostra nascita”
Thomas Stearns Eliot

# medieterranea

## 10° Congresso Nazionale di Pediatria

10° ANNIVERSARIO

**21-22 Aprile 2017**

The Nicolaus Hotel - Bari

Vi Aspetto!