Health aspects of probiotics

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PROBIOTIC TARGETS

Intestinal diseases
- ✓Acute diarrhea
- ✓AAD, traveler's diarrhea
- ✓*C. difficile*
- ✓Lactose intolerance
- ✓IBS symptoms
- ✓Inflammatory bowel conditions
- ✓ Colon cancer

Collateral Effects
- ✓ Lowering of cholesterol level
- ✓ Increasing of mineral absorption
- ✓ Lowering of blood pressure

Allergy
- ✓Atopic dermatitis
- ✓Ashma

Oral microbiology
- ✓Dental Caries

Metabolic Syndrome
- ✓Obesity, Diabetes

Urogenital Infections

Inflammation

Colds, respiratory infections

Effect on BRAIN

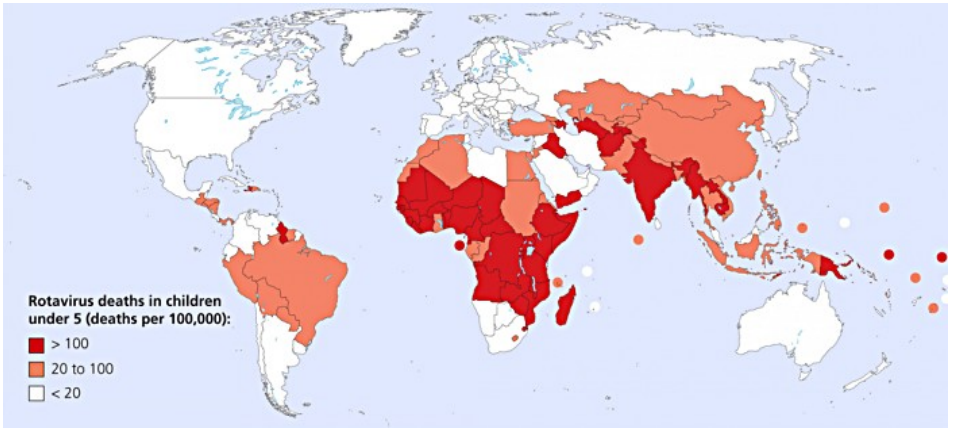
GUT ASSOCIATED DISFUNCTION

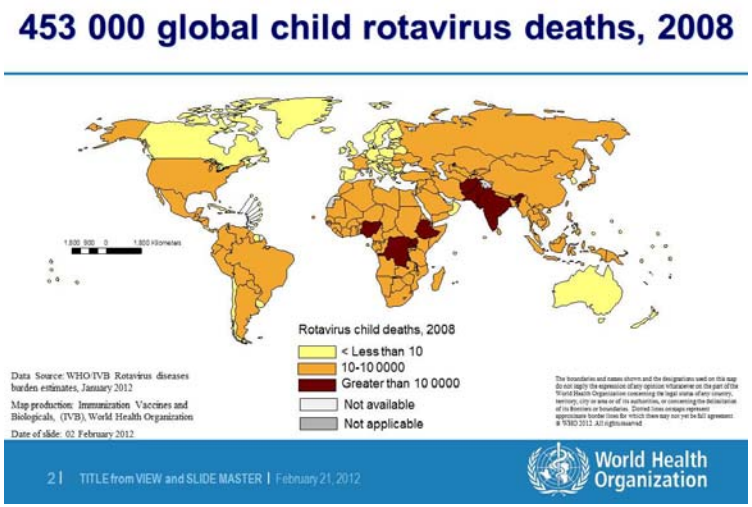
DIARRHEA

• Bacterial agents are responsible for approximately 10% of diarrheal illness in industrialized countries.

• Viruses (especially rotaviruses) are more important in infants but can cause food- and waterborne gastroenteritis in all age-groups.

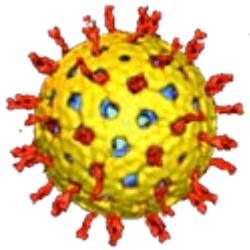
• In developing countries, bacterial enteropathogens, particularly enterotoxigenic *Escherichia coli*, are an important cause of endemic pediatric diarrhea





Rotavirus Diarrhea

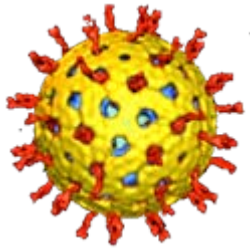
Acute gastroenteritis → first cause of hospitalization in children.
Nearly all children suffer at least one rotavirus infection before reaching 5 years of age, independent of their socioeconomic status



Double-stranded RNA virus in the family Reoviridae. There are five species of this virus (A, B, C, D, E). Rotavirus A, the most common species, causes more than 90% of infections in humans.

Transmitted by the fecal-oral route. It infects and damages the cells covering the small intestine and causes gastroenteritis

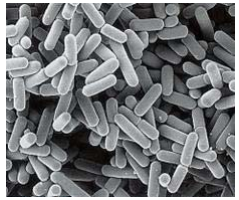
Trials support the use of probiotics in treating rotavirus diarrhea. They would be especially relevant in societies where diarrhea and malnutrition have high prevalence; Effects: decreasing time of diarrhea, of vomiting and of fever will help diminishing (and/or preventing) malnutrition associated to acute diarrhea.



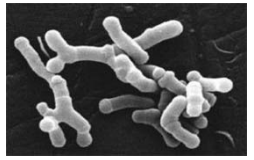
Examples

1. *Saccharomyces boulardii* diminished the time of diarrhoea by 31.4% and shortened time with fever by 73%

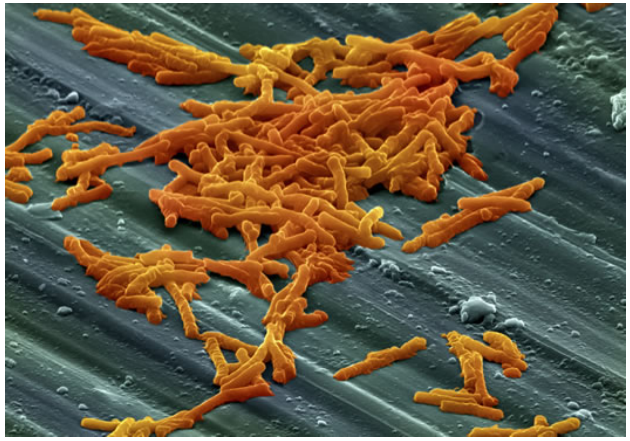
2. A strain *L. rhamnosus* reduced faecal excretion of rotavirus in a dose dependent fashion



3. A novel *Bifidobacterium longum* subsp. *infantis* strain was isolated from infant feces and selected based on its capacity to inhibit *in vitro* rotavirus replication and to protect cells from virus infection



C. difficile Diarrhea



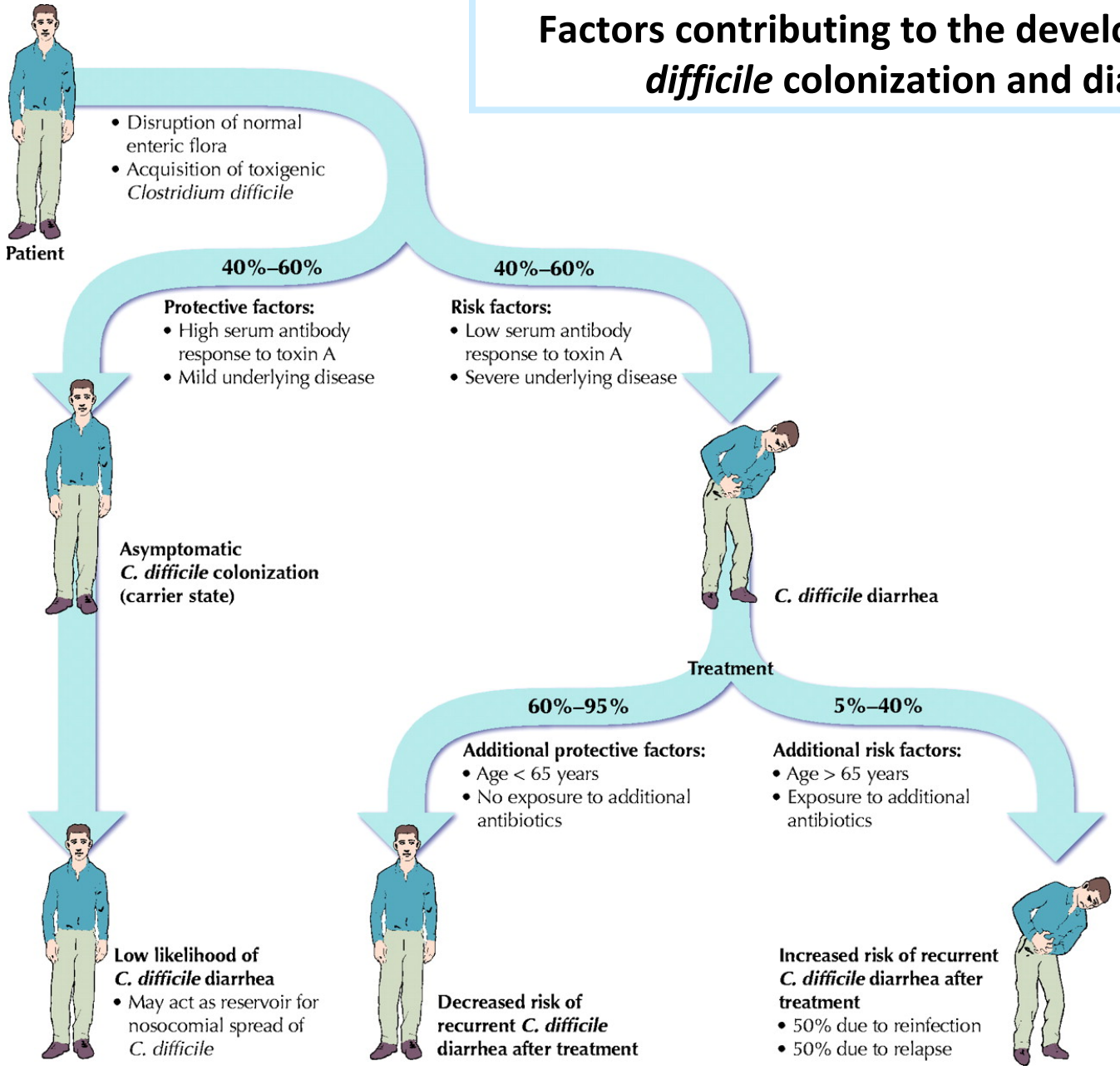
Clostridium difficile is a bacterial species that causes severe diarrhea and other intestinal disease when competing bacteria in the gut microbiota have been reduced by antibiotics.

Clostridia are Gram-positive anaerobic, spore-forming rods. *C. difficile* is the most serious cause of antibiotic-associated diarrhea (AAD) and can lead to a severe inflammation of the colon.

The risk increases with:

1. Antibiotic exposure
2. Gastrointestinal surgery/manipulation
3. Long length of stay in healthcare units
4. Immunocompromising conditions
5. Advanced age

Factors contributing to the development of *C. difficile* colonization and diarrhea



AAD: Antibiotic Associated Diarrhea

Antibiotic-associated diarrhea (AAD) has been suggested to be clinically significant when there are 3 or more episodes per day.

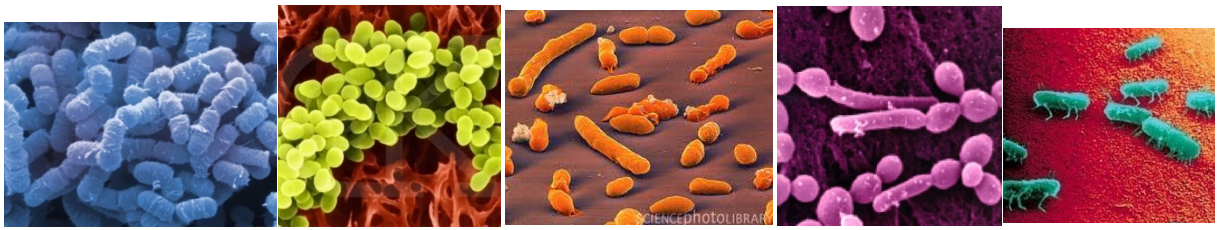
The mechanisms by which antibiotics lead to AAD :

- disturbance of the composition and function of the normal intestinal microbiota,
- overgrowth by pathogenic microorganisms,
- allergic and toxic effects of antibiotics on intestinal mucosa

Clostridium difficile causes nearly **25%** of cases of antibiotic-associated diarrhoea.

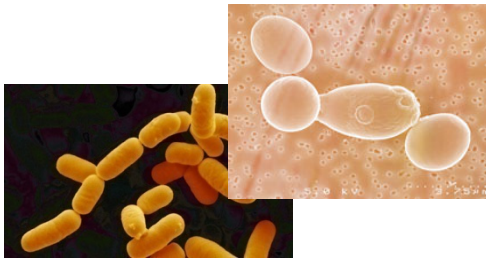
Other bacteria:

· *Clostridium perfringens,*
· *Staphylococcus aureus,*
· *Klebsiella oxytoca,*
· *Candida* spp.
· *Salmonella* spp.



PROBIOTICS VS AAD

A variety of probiotics have been evaluated for their efficacy as a means of treating and preventing antibiotic-associated diarrhea.



EXAMPLES in patients with bacterial caused diarrhea

Caused by *C.difficile*

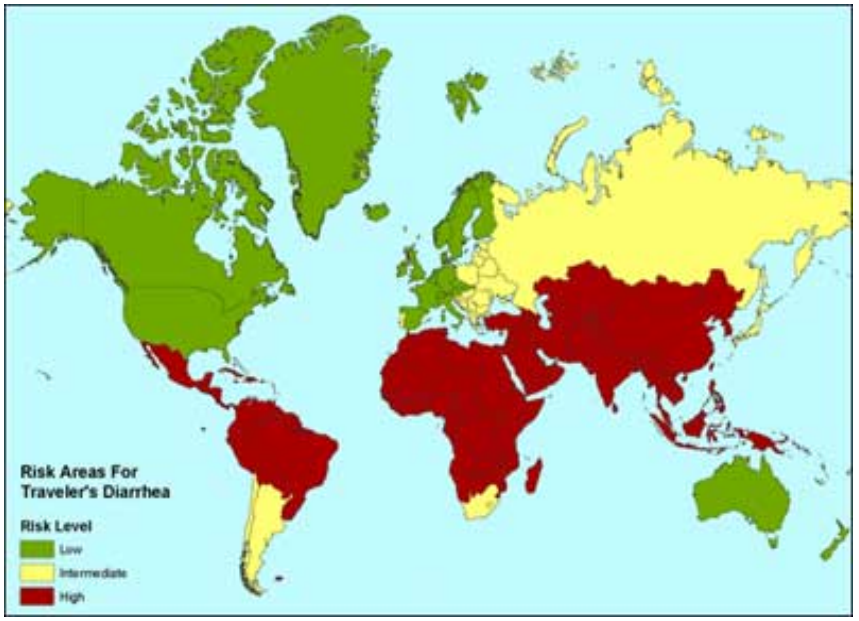
- ✓ Lactic acid – producing *Enterococcus* SF68 was effective in reducing the incidence of AAD
- ✓ *Saccharomyces boulardii*
- ✓ A daily dose of LGG has been shown to be an effective means of controlling diarrhea

Not caused by *C. difficile*

- ✓ In a small study, yogurt containing *Bifidobacterium longum*: reduce erythromycin-induced diarrhea.
- ✓ *Lactobacillus* preparation reduces ampicillin-induced diarrhea

Travellers' diarrhea

- Acute diarrhea occurs in about half of travellers who visit high-risk areas.
- Most cases are mild and self-limiting.
- Antibiotics can be effective means of prophylaxis but are not recommended.
- There is a need for alternative treatments.



An estimated 10 million people—20% to 50% of international travelers—develop it annually. **TD is defined as:** three or more unformed stools in 24 hours, commonly accompanied by abdominal cramps, nausea, and bloating. Its diagnosis does not imply a specific organism, but enterotoxigenic *Escherichia coli* (20-75%) is the most commonly isolated pathogen.

PROBIOTICS MECHANISMS vs DIARRHOEAL DISEASES

The reduction of pathogens load is achieved through **multiple mechanisms**:

- Competitive exclusion (CE): adhesin-mediated attachment of probiotics excludes pathogens,
- Aggregation: probiotics aggregate with pathogens, leading to expulsion from the gut,
- Nutrient Competition: probiotics compete with pathogens for essential nutrients,
- Masking: masking of intestinal receptors for enterotoxins binding by probiotic adhesins,
- Production of Antimicrobial Substances: probiotics produce hydrogen peroxide (H_2O_2), bacteriocins (e.g. lactocins, helveticins, lactacins, curvacins, nisin, bifidocin) and organic acids, the latter, lowering pH, create a forbidding environment for wide range of harmful microorganisms

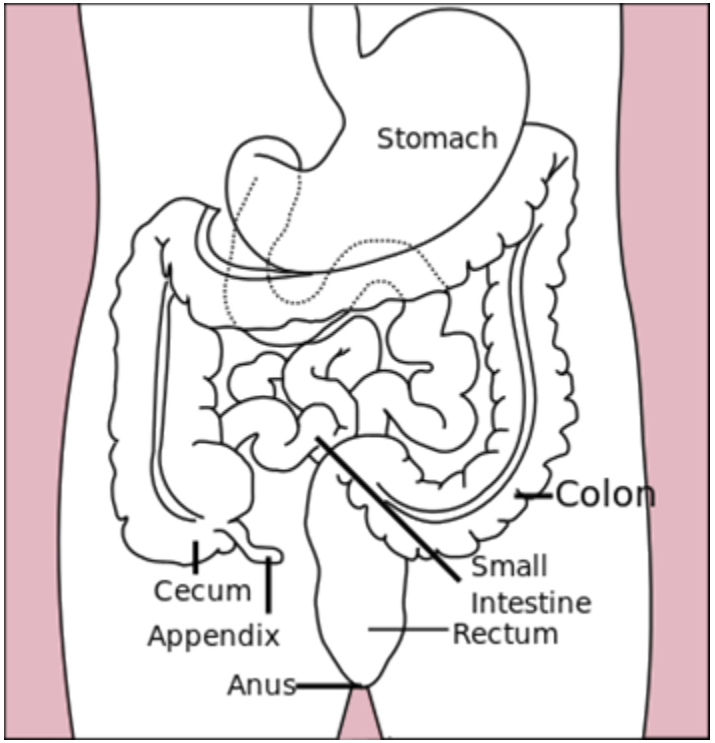
Colon Cancer

Colorectal cancer, commonly known as colon cancer is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine).

Symptoms of colorectal cancer typically include rectal bleeding and anemia which are sometimes associated with weight loss and changes in bowel habits

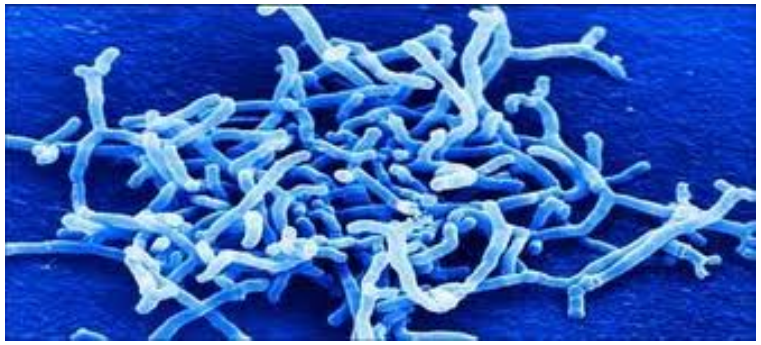
Most colorectal cancer occurs due to **lifestyle** and **increasing age**. Only a minority are associated to genetic origin.

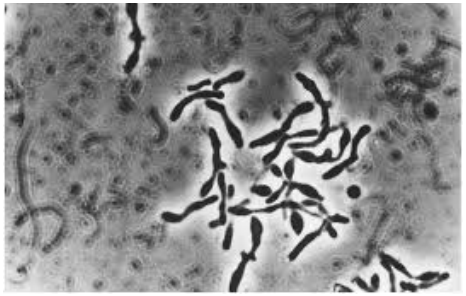
Studies of the intestinal microbiota and its interaction with the host have underlined that **disbiosis in colonic bacterial composition is a risk factor for colon cancer**



PROBIOTIC-PREBIOTIC ANTICARCINOGENIC EFFECT

- Binding/absorption of carcinogens.
- Competition against bacteria producing precarcinogenic metabolites.
- Modulation of intestinal bacteria enzymes
- Production of beneficial compounds (organic acids, SCFA, bacteriocins, vitamins)
- Immune system stimulation



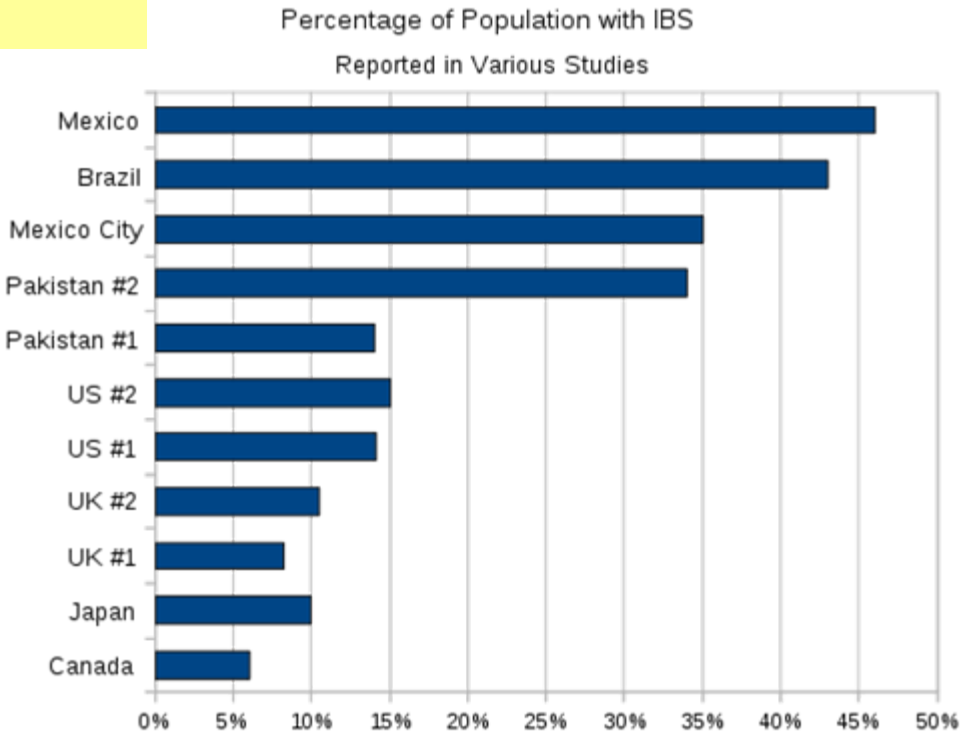


Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS, or spastic colon) is a

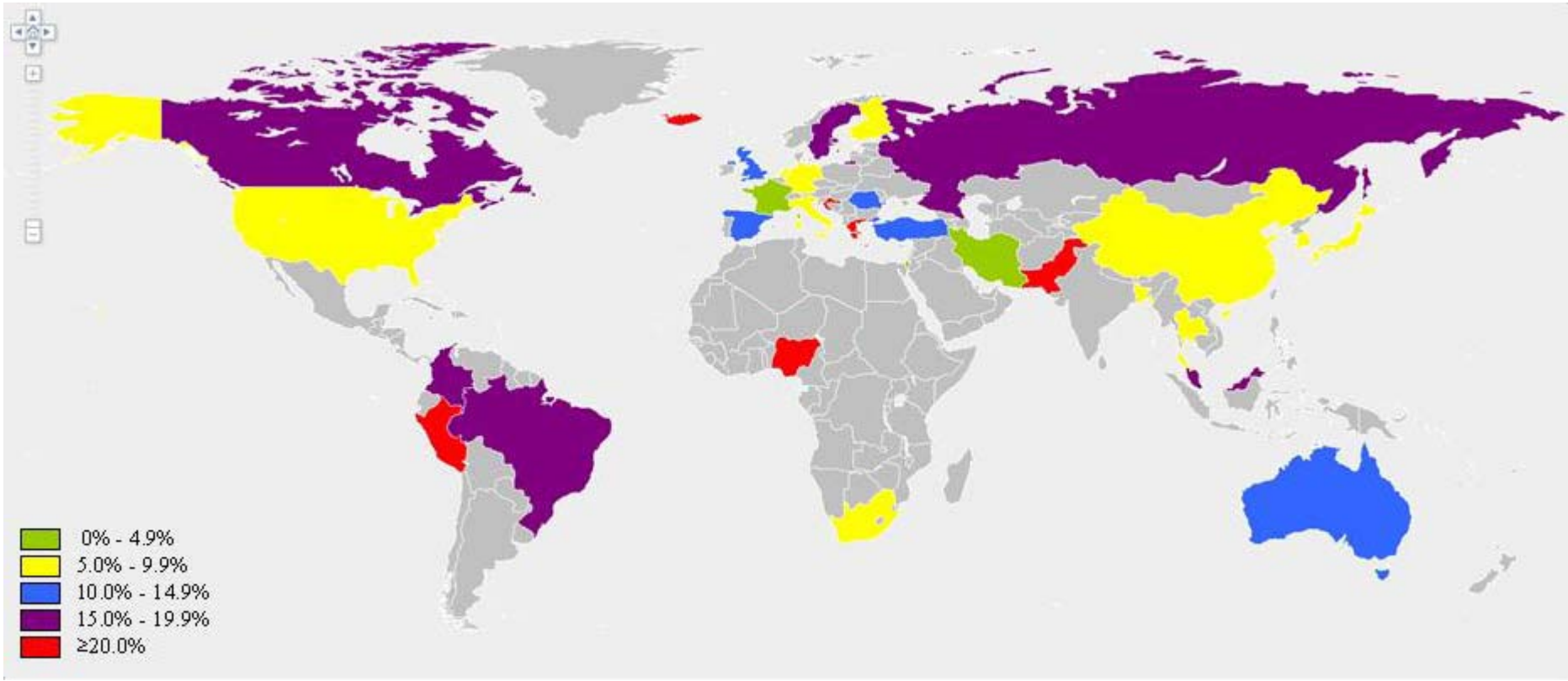
symptom-based diagnosis:

- chronic abdominal pain
- discomfort
- bloating
- alteration of bowel habits.



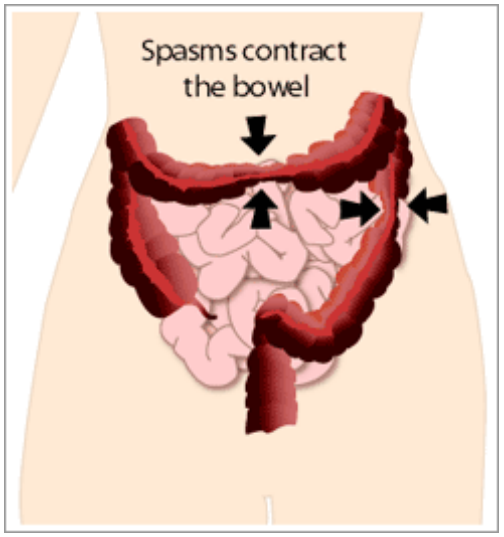
As a functional bowel disorder, the causes of IBS development are still unknown

Diarrhea or **constipation** may predominate, or they may alternate (classified as IBS-D, IBS-C or IBS-A, respectively).



Prevalence map of IBS according to country

IBS has no direct effect on life expectancy but….:

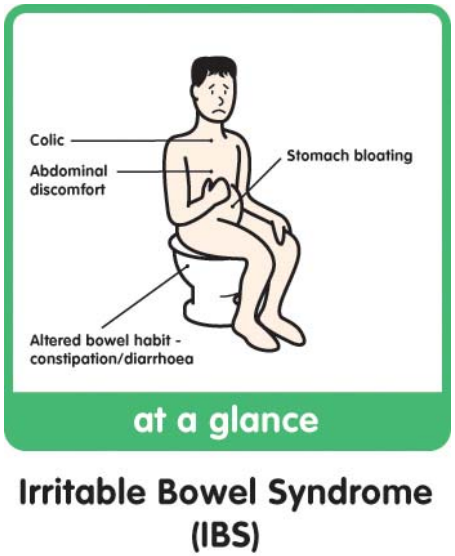


- Chronic pain
- Fatigue
- Diarrhea and/or constipation
- Low life quality

✓ Work absenteeism

✓ High social costs

IBS patients may **develop depression** and are thus more likely to **commit suicide**.



Examples of probiotic applications

The prevailing paradigms of IBS highlight the role of dysmotility and hypersensitivity:

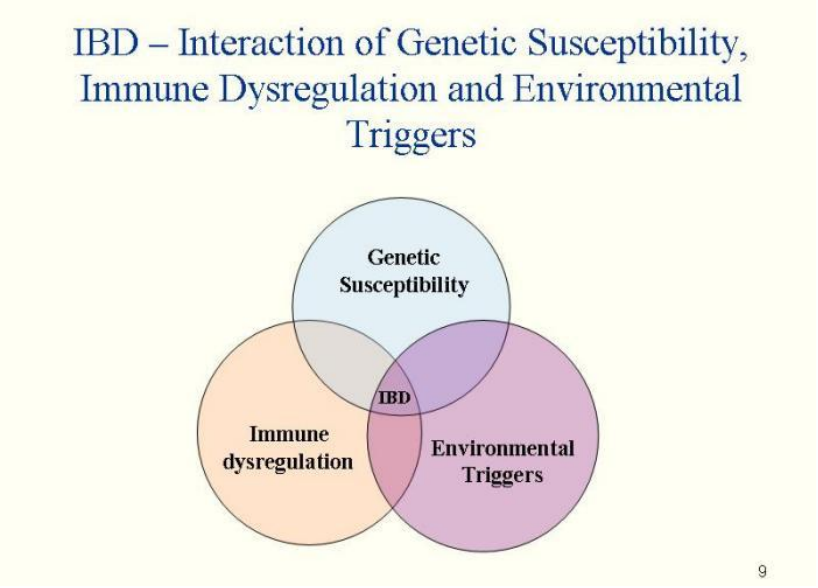
- ✓ *Lactobacillus paracasei* NCC2461 was reported to attenuate post infectious intestinal dysmotility in a mouse model.
- ✓ Animal models have shown that probiotics improve visceral hypersensitivity
- ✓ *Lactobacillus acidophilus* NCFM administration induces the expression of cannabinoid and opioid receptors on intestinal cells → effect on visceral hypersensitivity
- ✓ Administration of *Bifidobacterium infantis* 35624 to IBS patients can effectively reduce abdominal pain, discomfort, distension/bloating, and difficult defecation, the cardinal symptoms of the disease

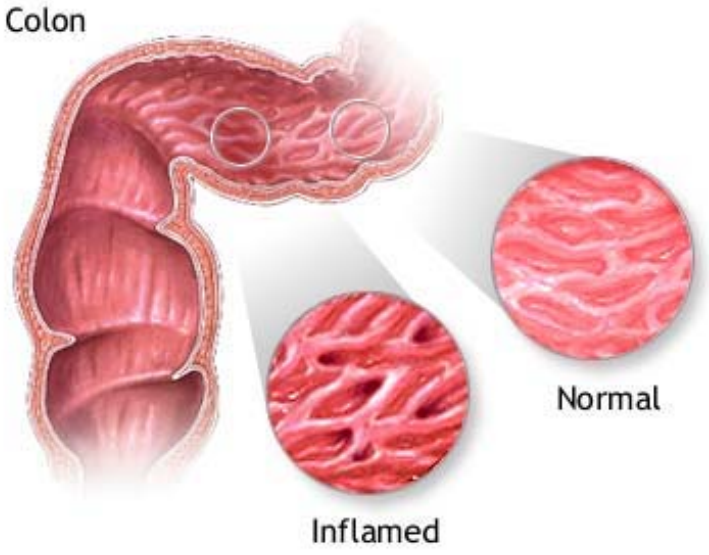
Inflammatory Bowel Disease (IBD)

In medicine, inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine.

The major types of IBD are:

- **Crohn's disease**
- **Ulcerative colitis**





Inflammatory Bowel Diseases are considered **autoimmune diseases**, in which the body's immune system attacks the digestive system

Other forms of IBD (fewer cases) which are not always classified as typical IBD:

- Collagenous colitis
- Lymphocytic colitis
- Ischaemic colitis
- Diversion colitis
- Behçet's disease
- Indeterminate colitis

The main difference between Crohn's disease and UC:

- ***Location*** and

- ***Nature*** of the inflammatory changes

LOCATION:

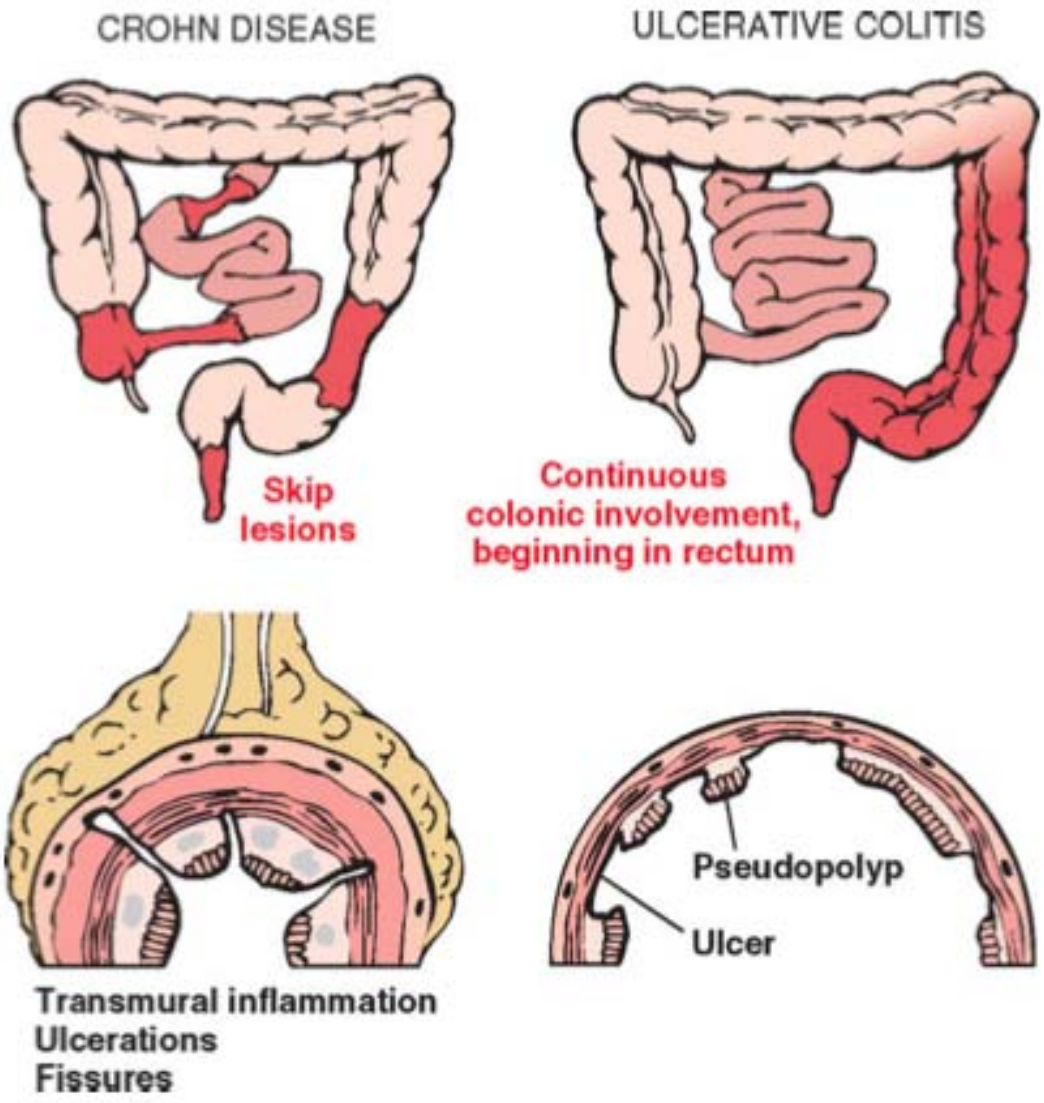
Crohn's can affect any part of the gastrointestinal tract, from mouth to anus, although a majority of the cases start in the terminal ileum.

Microscopically Crohn's disease affects the whole bowel wall ("transmural lesions").

Ulcerative colitis, in contrast, is restricted to the colon and the rectum

Microscopically, ulcerative colitis is restricted to the mucosa (gut epithelial cells)

Crohn's disease VS Ulcerative colitis

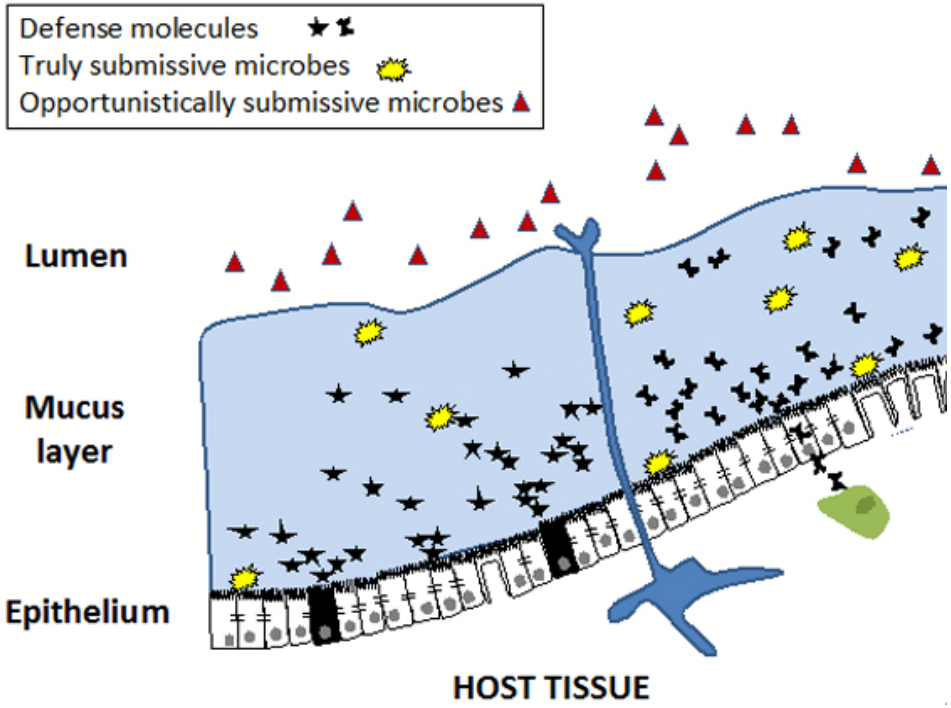


The role of the intestinal microbiota

The intestinal microbiota plays a major role in the development and maintenance of intestinal health

Intestinal epithelial cells (IEC) constitute the interface between the gut lumen and the innate and adaptive immune system.

To maintain intestinal homeostasis, the organized and diffuse compartments of the gut-associated lymphoid tissue have to process the continuously varying information at the interface between the luminal side and the host.

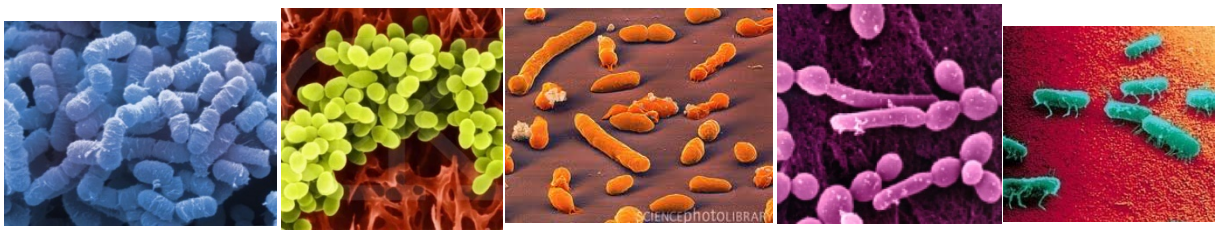


Environment-dependent disturbances of this tightly regulated intestinal balance contribute to the development of inflammatory bowel diseases (IBD) in genetically susceptible hosts.

IBD are characterized by the **loss of tolerance of the intestinal immune system** towards the intestinal microbiota resulting in constant immune activation followed by mucosal tissue damage and chronic inflammation.

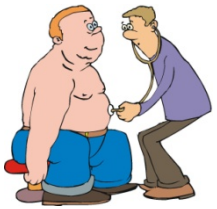
There are only two bacterial preparations, VSL#3 and *E. coli* strain Nissle 1917, that can be referred to as probiotics in the context of IBD

Clinical data indicate that probiotic therapy is more effective in preventing disease onset or recurrence than in the reduction of active inflammation



METABOLIC SYNDROMES

OBESITY: medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health:



-reduced life expectancy and/or

- increased health problems.

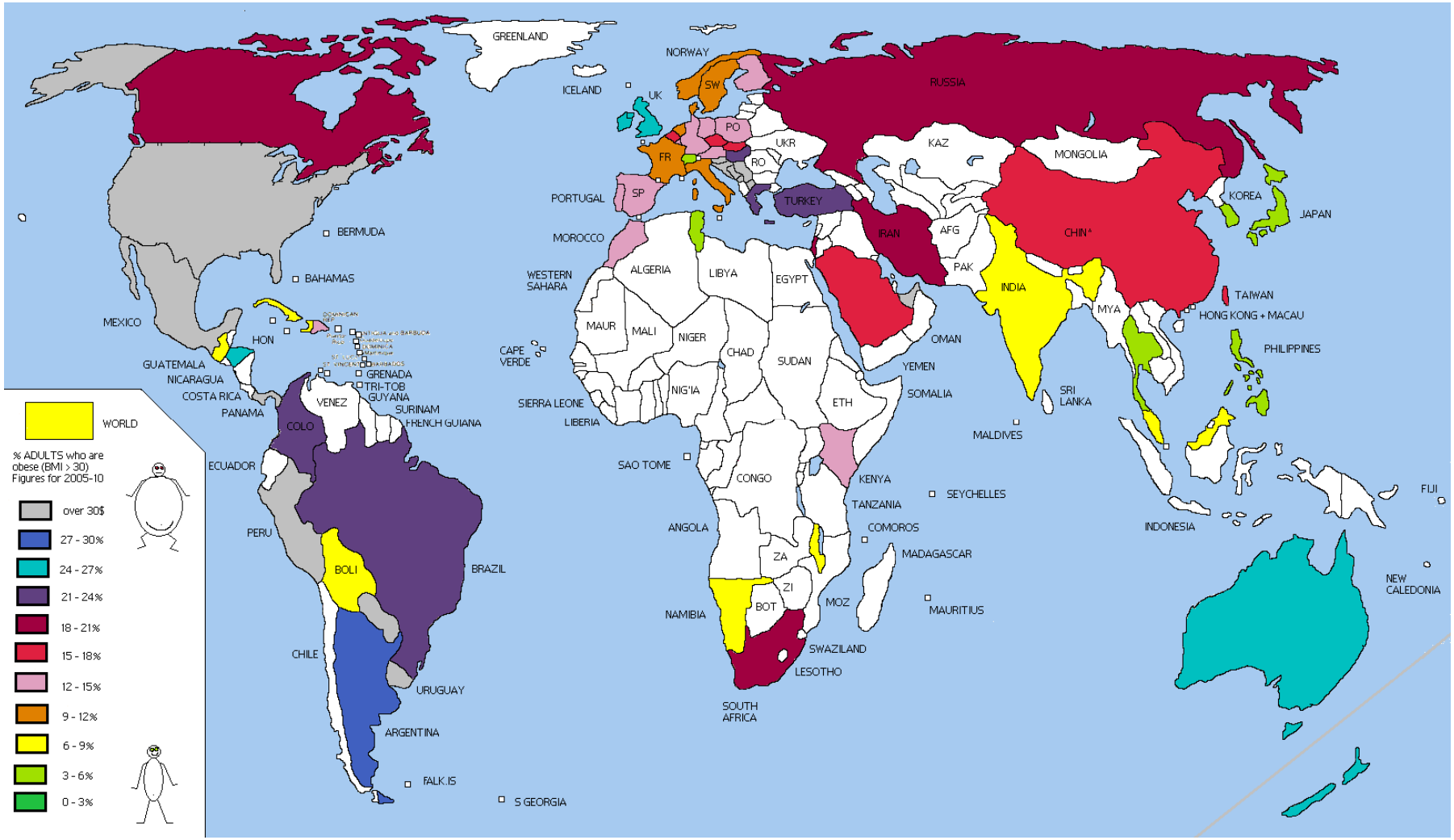
Body mass index (BMI): person's weight (Kg)/the square of the person's height (m) (>30 kg/m^2).

Obesity increases the likelihood of various diseases: **heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis**.

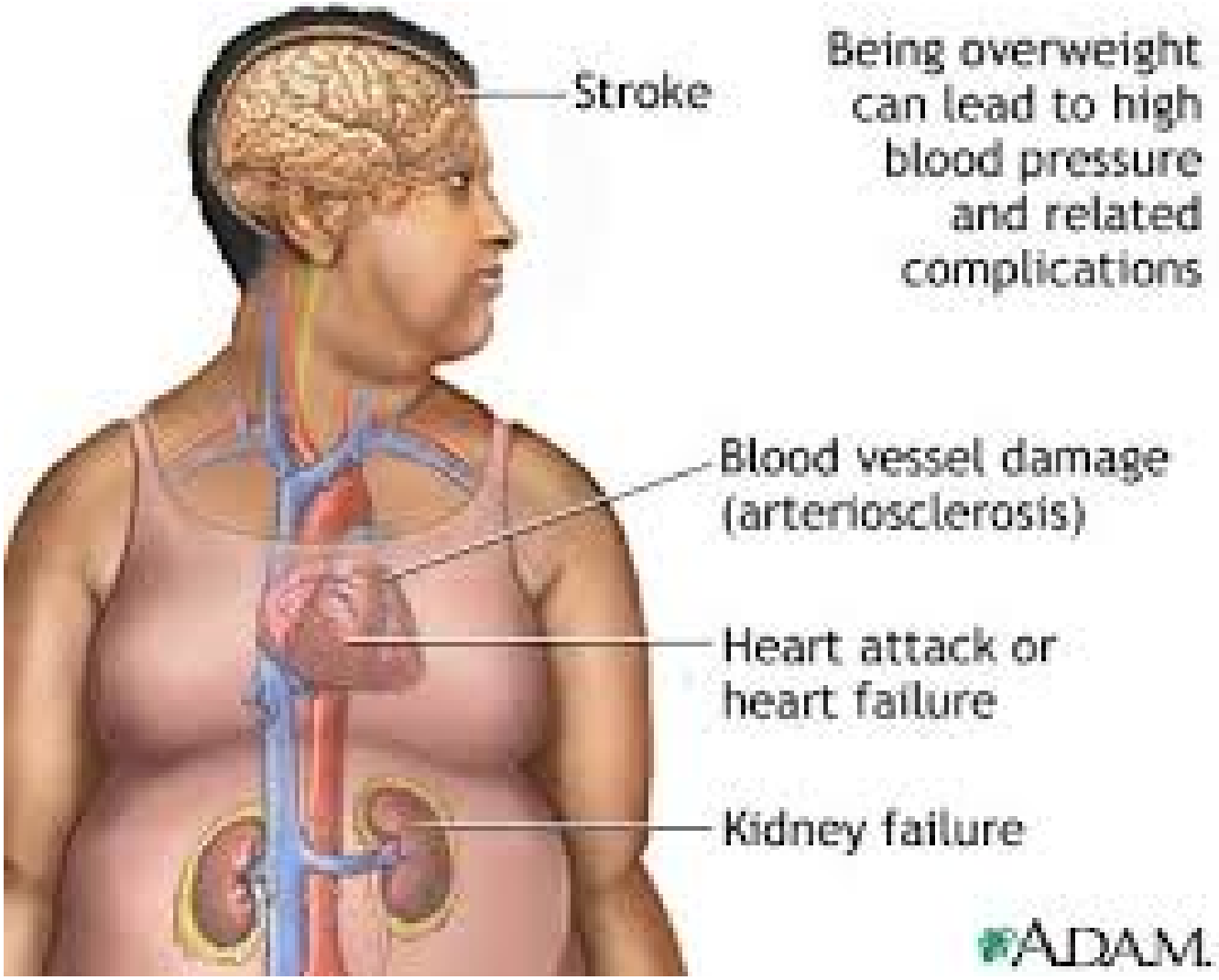
DIABETES: Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because:

- the pancreas does not produce enough insulin (type 1, autoimmune diabetes)
- the cells do not respond to the insulin that is produced (type 2).

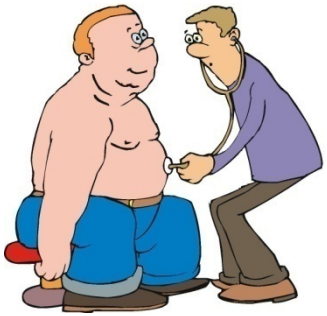
OBESITY 2010



OBESITY


Being overweight can lead to high blood pressure and related complications
Stroke
Blood vessel damage (arteriosclerosis)
Heart attack or heart failure
Kidney failure
A.D.A.M.

MICROBIOTA AND OBESITY

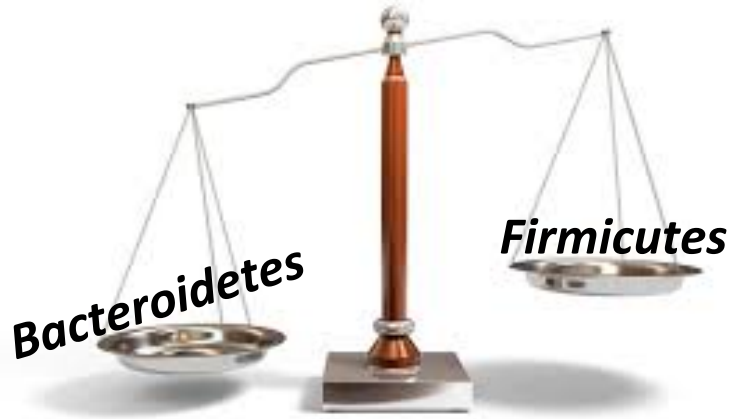


High energy intake and sedentary lifestyle are the main reasons for the worldwide obesity epidemic and related disorders.

<u>The intestinal microbiota plays a role in obesity development</u>

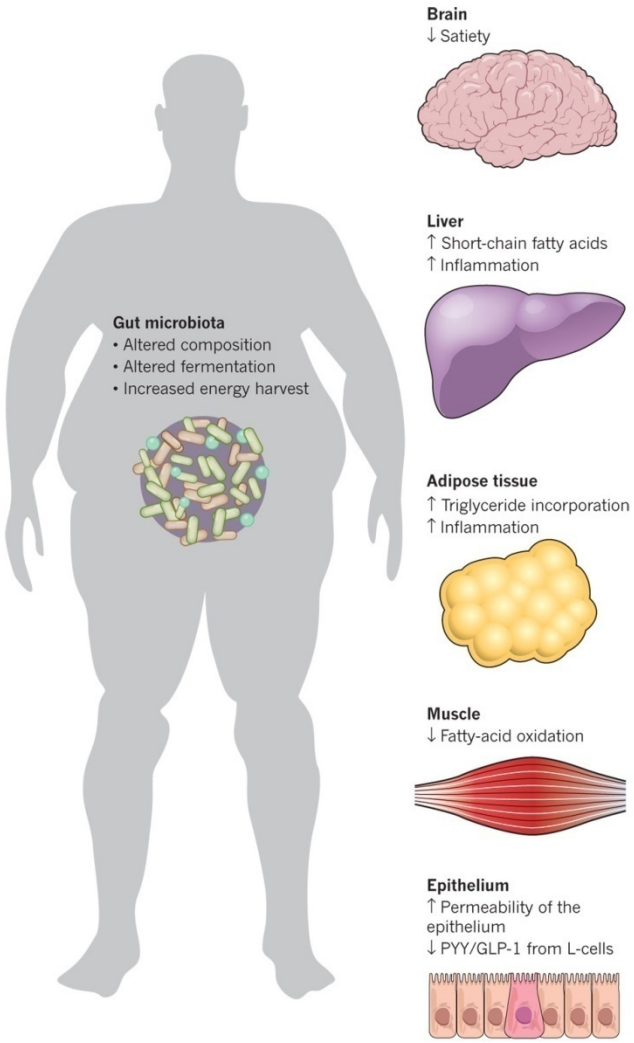
Overweight subjects harbored a higher proportion of *Firmicutes* and a smaller proportion of *Bacteriodetes* than normal-weight subjects

These 2 phyla cover ~ 90% of all bacterial cells in the human intestine.



HOW MICROBIOTA AFFECTS OBESITY ?

- Improved energy harvest from the diet
- Stimulation of fat storage → influencing lipoprotein lipase activity

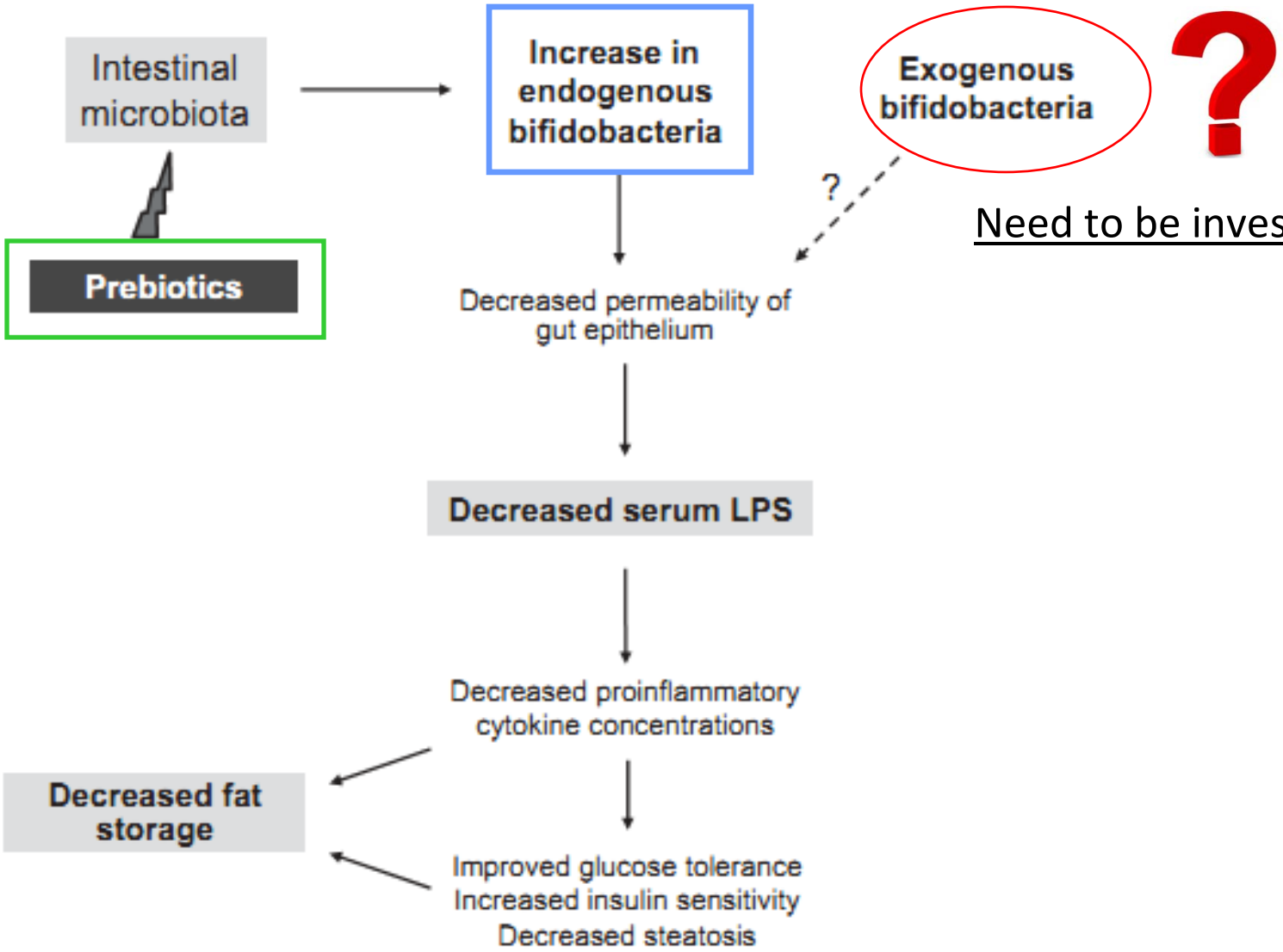


Fat-induced systemic low-grade inflammation

Endotoxemia and insulin resistance

High-fat diet leads to increased levels of lipopolysaccharides (LPS) in the serum. LPS is a component of the cell wall of Gram-negative bacteria causing endotoxemia. Metabolic endotoxemia led to an increase in the concentration of proinflammatory cytokines in various tissues.

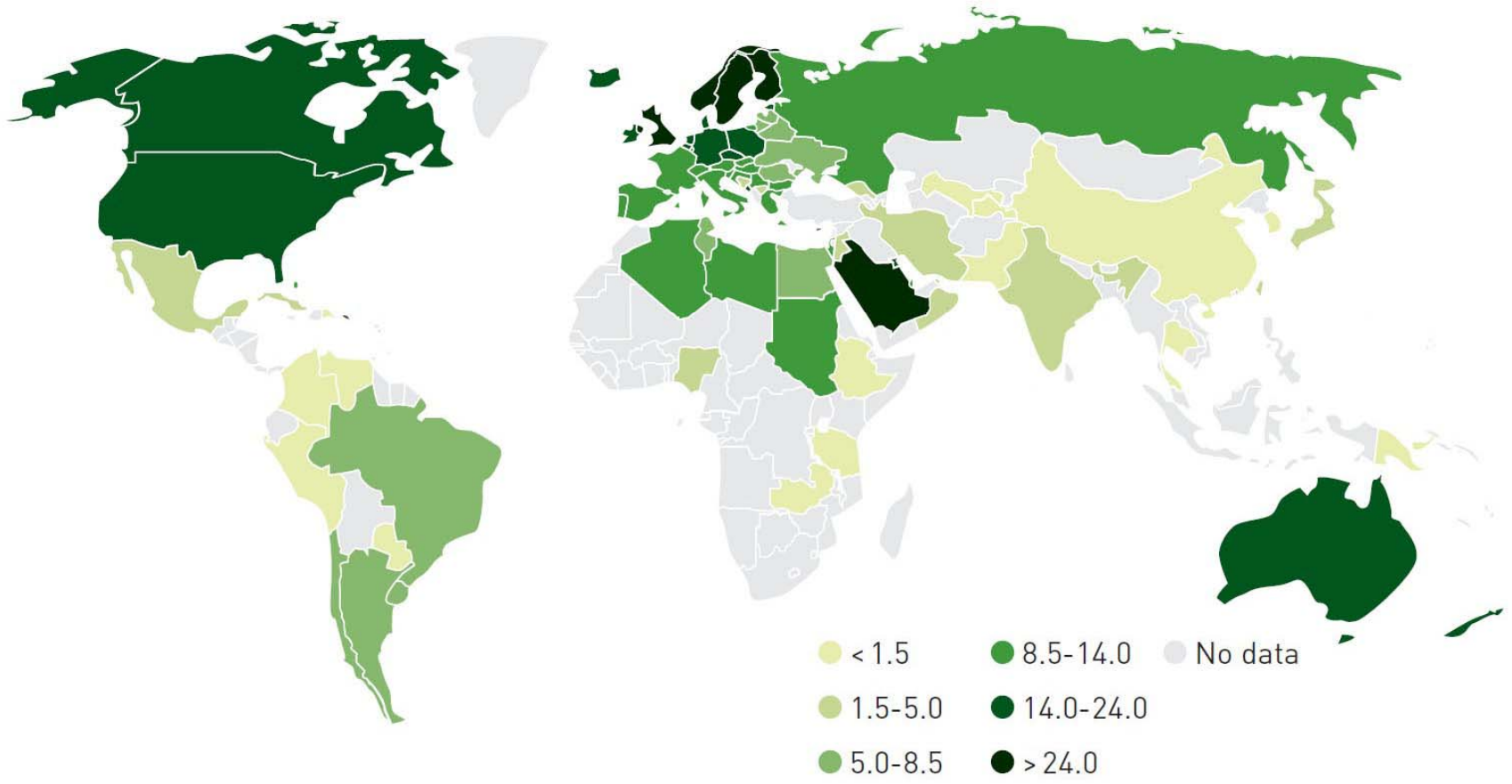
INCREASING ENDOGENOUS BIFIDOBACTERIA



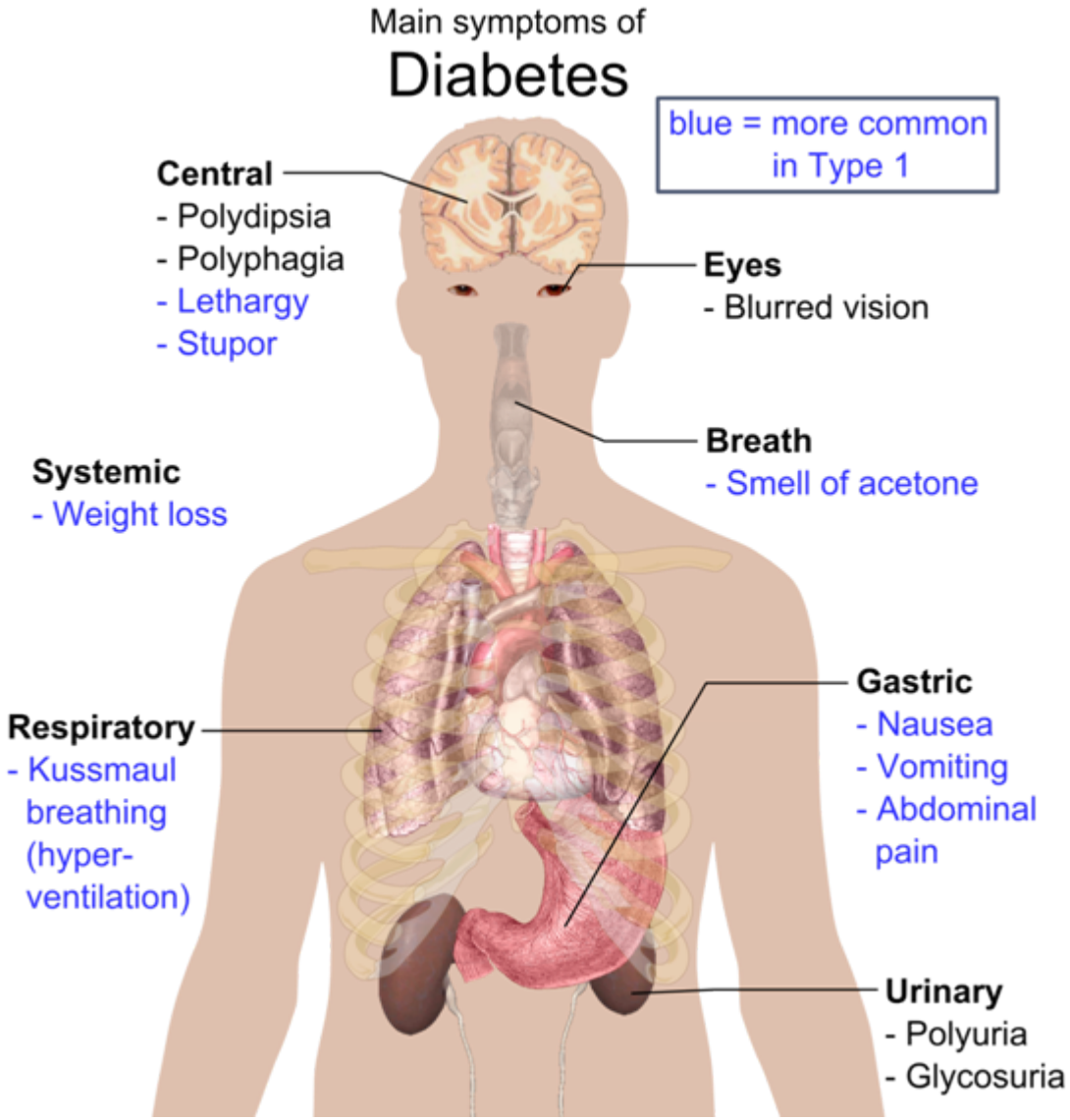
Need to be investigated

DIABETES 2011

Map 2.5. New cases of type 1 diabetes (0-14 years per 100,000 children per year), 2011



DIABETES


Main symptoms of
Diabetes
blue = more common in Type 1
Central
- Polydipsia
- Polyphagia
- Lethargy
- Stupor
Eyes
- Blurred vision
Breath
- Smell of acetone
Systemic
- Weight loss
Respiratory
- Kussmaul breathing (hyper-ventilation)
Gastric
- Nausea
- Vomiting
- Abdominal pain
Urinary
- Polyuria
- Glycosuria

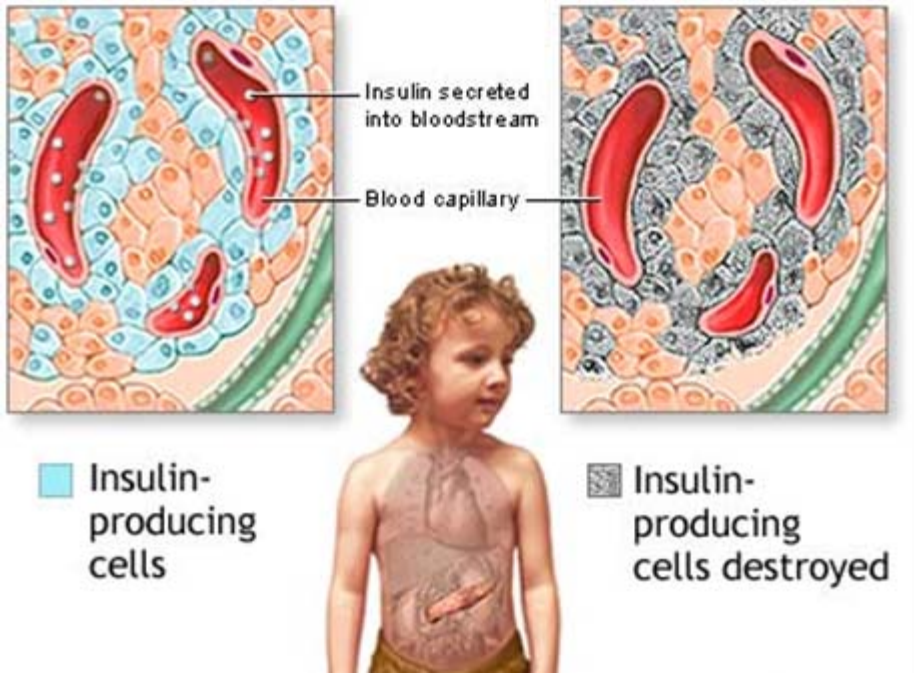
Type 1 diabetes (T1D) is an autoimmune disease

T-cell-mediated destruction of insulin-producing β-cells (pancreas cells)

Its incidence has increased during the past several decades in developed countries, suggesting that changes in the environment (including the human microbial environment) may influence disease pathogenesis.

The microbiota plays a role

Germ-free mice colonized with a defined microbial consortium (representing bacterial phyla normally present in human gut) attenuates T1D



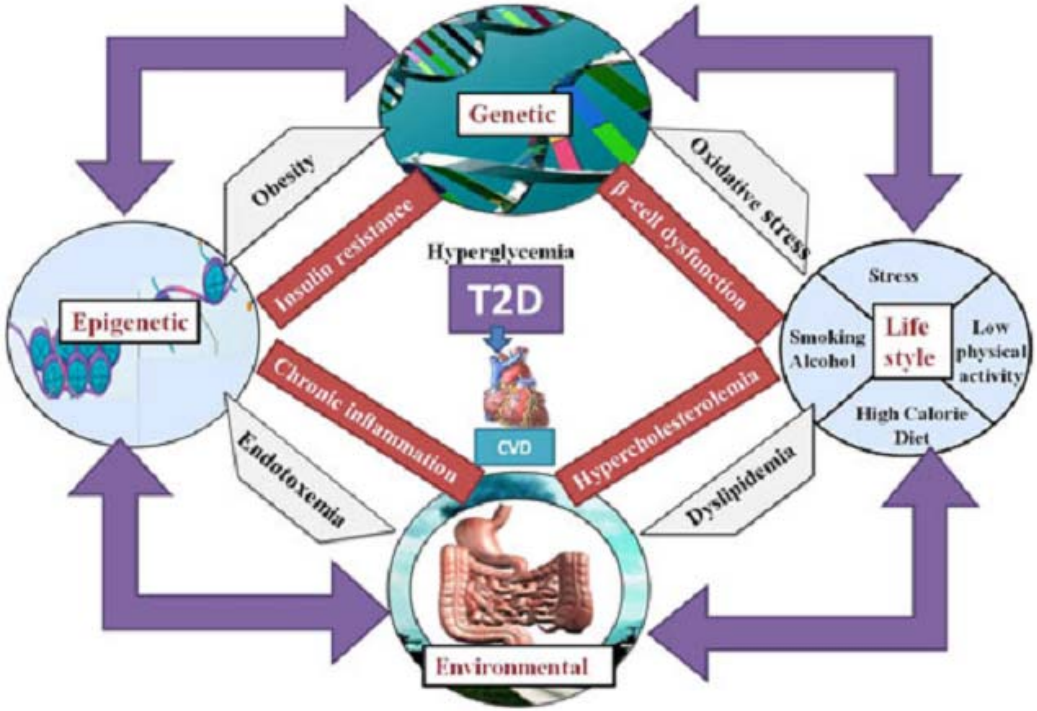
Type 2 diabetes (T2D)

Diabetes mellitus type 2 (noninsulin-dependent diabetes mellitus) is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency.

Type 2 diabetes makes up about 90% of cases of diabetes.

Obesity is thought to be the primary cause of type 2 diabetes in people who are genetically predisposed to the disease.

Gut microbiota has been reported to play a pivotal role in pathogenesis of T2D, obesity and related inflammatory metabolic disorders

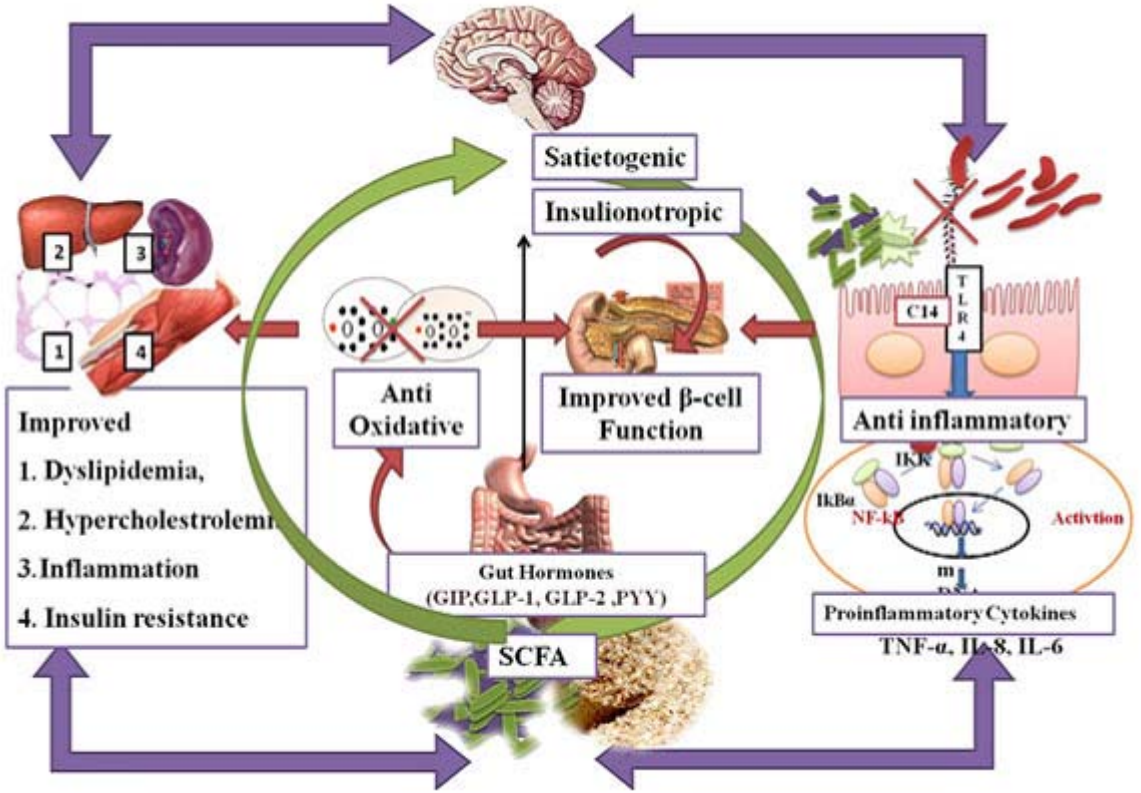


Microbiota shift and probiotics in Diabetes

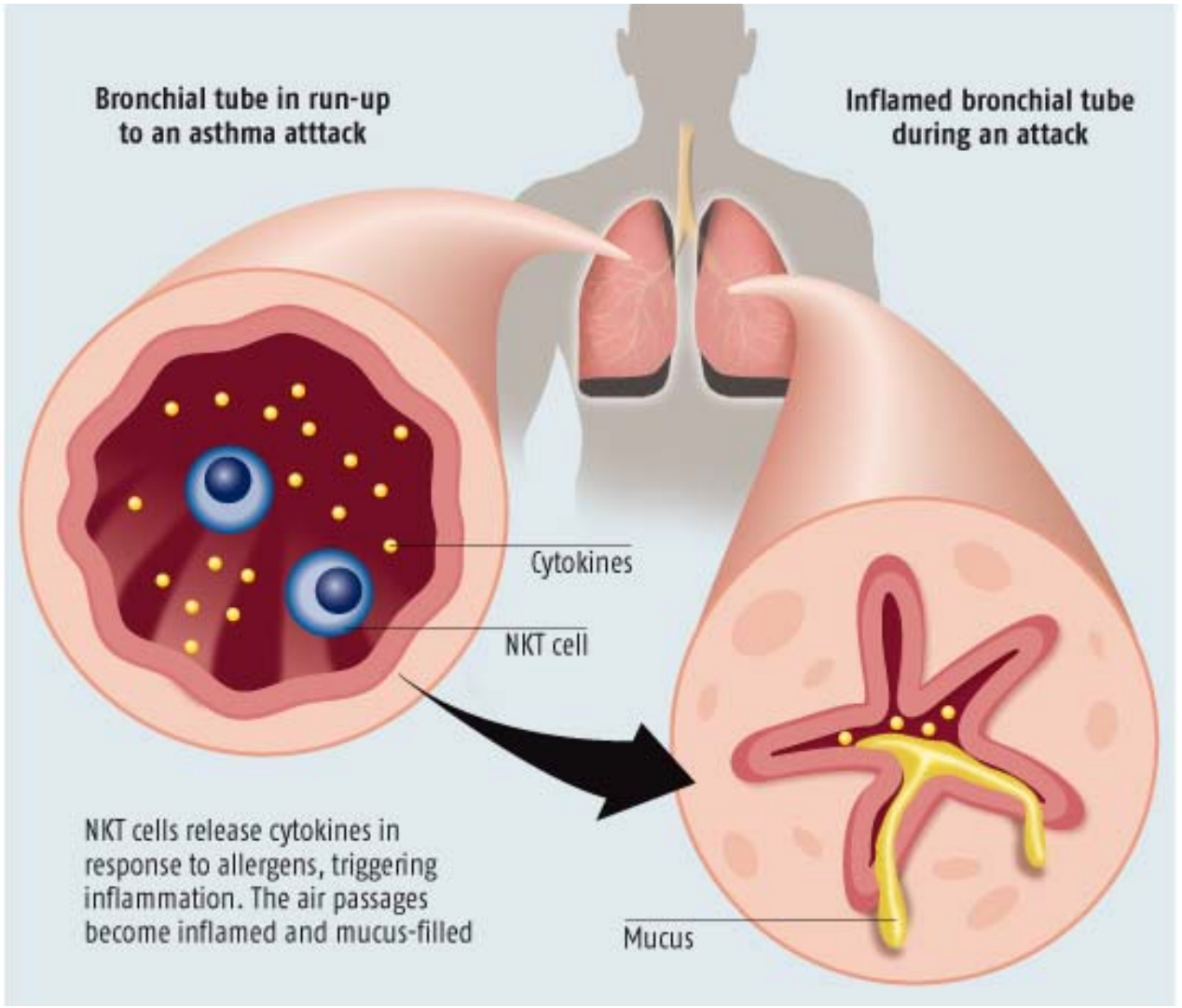
Compositional changes in the group of bifidobacteria and lactobacilli during the onset of insulin resistance in mice. The gut microbial profile of these studies recorded reductions in *Bifidobacterium* spp. and *Lactobacillus* spp. with increased plasma LPS that caused metabolic endotoxemia on the molecular onset of insulin resistance

Probiotics investigated for their effects:

- Hypoglycemic
- Antinflammatory
- Antioxidative
- Insulinotropic
- Satietogenic



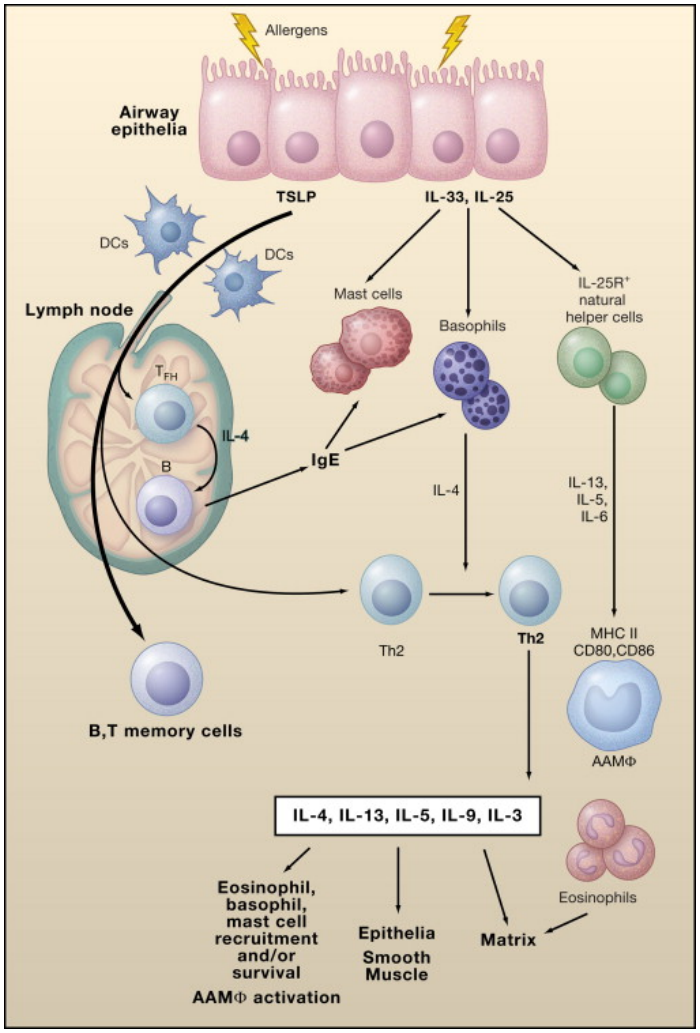
ALLERGY: Atopic Dermatitis and Asthma



ALLERGY

An allergy is a **hypersensitivity disorder of the immune system**.

The immune system reacts to normally harmless substances in the environment.



Immunoglobulin E (IgE) over activate certain white blood cells (mast cells and basophils)

Inflammatory response (from uncomfortable to dangerous)

Atopic dermatitis (AD)

Atopic dermatitis (AD, a type of eczema) is an inflammatory skin disorder

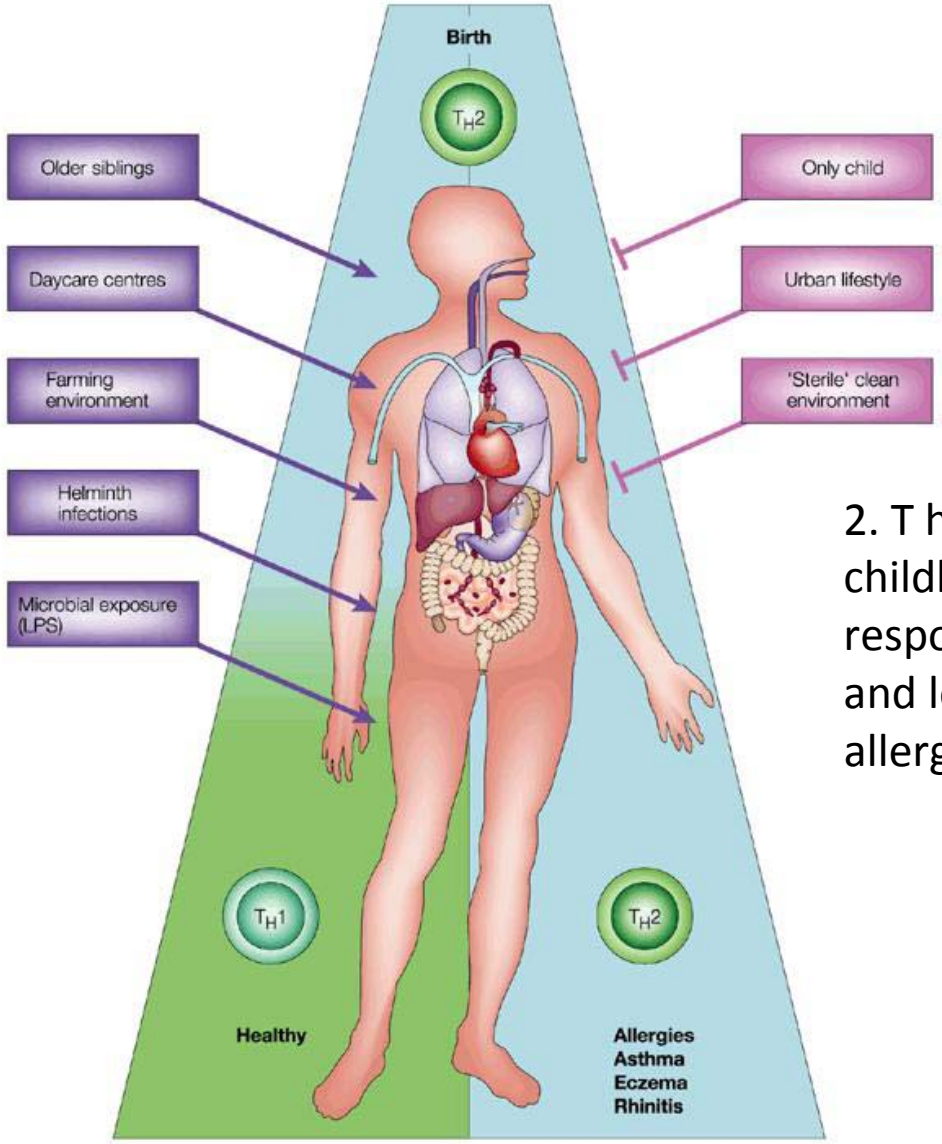
The prevalence of AD has risen over the past decades, especially in western societies where it varies in primary school children between 5% and 20%

AD can be the starting point of the allergic march, the natural progression of allergic disorders such as asthma and allergic rhinitis. Children with AD have a chance of approximately 40% to develop asthma

HYGIENE HYPOTHESIS ??

Several facts are not consistent but still in consideration

HYGIENE HYPOTHESIS



1. General hypothesis that early childhood infections, caused for example by unhygienic contact with older siblings, could prevent the development of allergic disease

2. T helper 1/T helper 2 paradigm: lack of early childhood infections results in a decreased Th1 response, which disturbs the Th1/Th2 balance and leads to an abundant Th2 response, causing allergic diseases

<u>Revised</u> hygiene hypothesis, which considers changes in the intestinal colonization pattern during infancy

INTESTINAL MICROBIOTA

- > 400 SPECIES
- large intestine 10^{11} -10^{12} cells/g luminal content

Factors Influencing colonization

- mode of delivery
- prematurity
- hospitalization
- antibiotic use after birth
- type of feeding
- exposure to older siblings

The relation between the intestinal microbiota and the human host is mostly symbiotic

- Important nutritional functions
- Crucial for the development of the mucosal and systemic immune system

If the revised hygiene hypothesis holds true, then it is feasible that atopic disease can be treated or prevented by manipulating the microbiota:

probiotics, prebiotics, or synbiotics

- Stabilizing of intestinal barrier function
- Stimulation of intestinal IgA-production
- Modulation of cytokine production

Down-regulate Th2 cytokine production:

- Stimulation of Th1 cytokines (IL-12 and IFN-γ)
- Stimulation of regulatory cytokines (IL-10)

PREVENTION and/or TREATMENT OF AD WITH PRO/SYNBIOTICS

POSITIVE OUTCOMES ⟶ but some inconclusive results

- 50% reduction of AD incidence
- Reduction in IgE-associated AD
- Reduction of symptoms

1. Different strains ⟶ Probiotic effects are **strain specific**
2. Probiotic effects are probably **dose-dependent**
3. Differences in **study design**

- Number of participants
- Atopic risk of participants
- Maternal supplementation during pregnancy
- Supplementation directly to infants
- Supplementation period

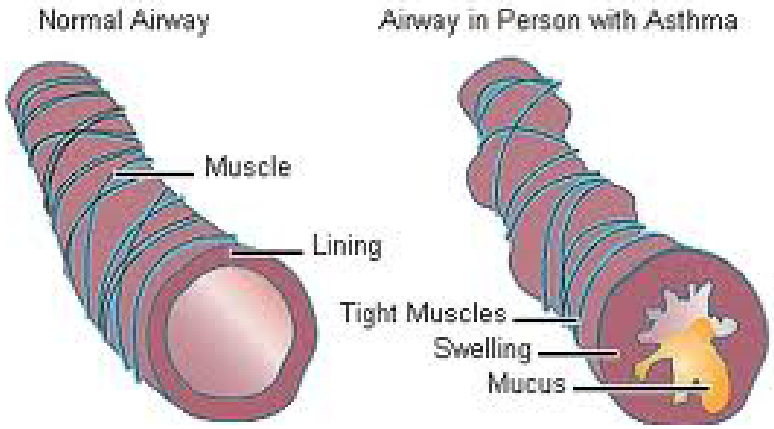
IMPORTANT

Manipulating the intestinal microbiota probably has more effect in early infancy, when immune programming is initiated

Asthma

Asthma is a common **chronic inflammatory disease** of the airways characterized by variable and recurring symptoms, reversible airflow obstruction, and bronchospasm. Common symptoms: wheezing, coughing, chest tightness, and shortness of breath

1. The **hygiene hypothesis** attempts to explain the increased rates of asthma worldwide as a direct and unintended result of reduced exposure, during childhood, to non–pathogenic bacteria and viruses



2. Use of antibiotics in early life has been linked to the development of asthma

3. Delivery via caesarean section is associated with an increased risk (estimated at 20–80%) of asthma

APPLICATION

VSL#3: a commercial combination of probiotic bacteria (*Bifidobacterium, Lactobacillus* and S*treptococcus*)

- Results demonstrated significant protection in the experimental model of allergic asthma

- The protective effect was dose-dependent
- Probiotics function through Toll-like Receptor stimulation

Oral Microbiology

Caries is one of the most common infectious diseases in the world

Dental caries: destruction of the tissues of the tooth by bacterial fermentation of dietary carbohydrates

Some bacteria are considered more caries-promoting than others e.g. *Streptococcus mutans*, a bacterium-producing polysaccharides (dextrans and levans) that causes the dental plaque

Caries-promoting bacteria are acidogenic and aciduric.
The most common bacteria used as probiotics, lactobacilli and bifidobacteria, are, in theory, caries-promoting. At the same time they are able to metabolize the polysaccharides of the dental plaque

They are excellent acid producers, they tolerate low pH-values, and they are frequently found in caries lesions

PROBIOTICS VS CARIES

All studies so far indicate that probiotics have rather beneficial than adverse effects on the caries risk

1. *Lactobacillus rhamnosus* GG, *Lactobacillus reuteri*, and *Bifidobacterium lactis* BB-12 colonize poorly the oral cavity of adults AND showed poor retention to the teeth and oral mucosa of the infants

2. Short-term consumption of LGG, *L. reuteri*, and BB-12 have reduced counts of *S. mutans*

3. The amount of dental plaque has been reduced by some probiotics. Dental plaque is not only a caries risk factor but also associated with periodontal diseases

4. LGG milk reduced caries occurrence in 3–4-years-old children. Milk with *L. rhamnosus* LB21 and fluoride reduced caries occurrence in school children

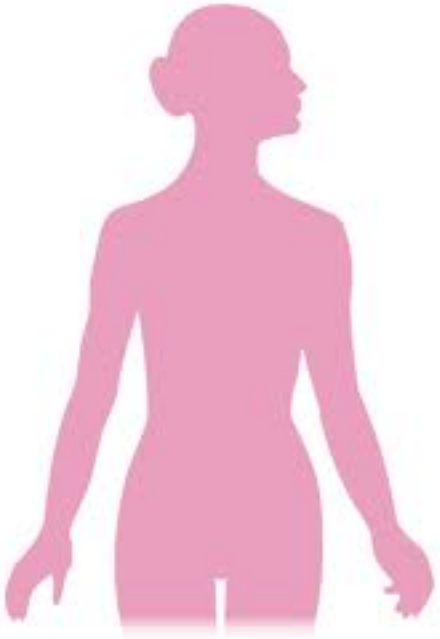
Urogenital Infections

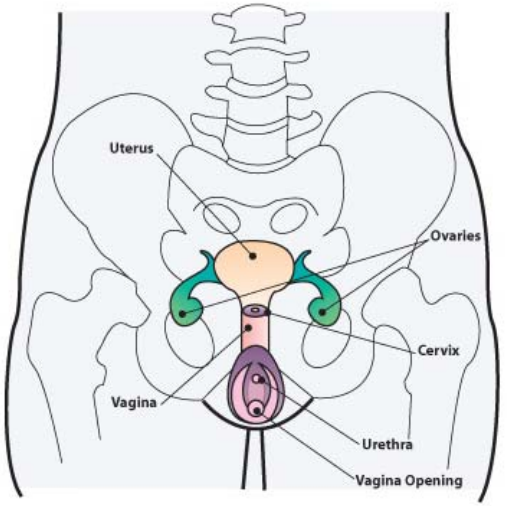
Urogenital infections are a great problem in terms of women infected per year

Annually ~1 billion women around the world suffer from non-sexually transmitted urogenital infections: Bacterial Vaginosis (BV), urinary tract infection (UTI), and yeast vaginitis

Urogenital microbiota, as well as intestinal microbiota, is critical for the health and well-being of humans

The **urogenital microbiota of a healthy woman** comprises about 50 species of organisms, which differ in composition according to reproductive stages and exposure to several factors, including antibiotics and spermicides.



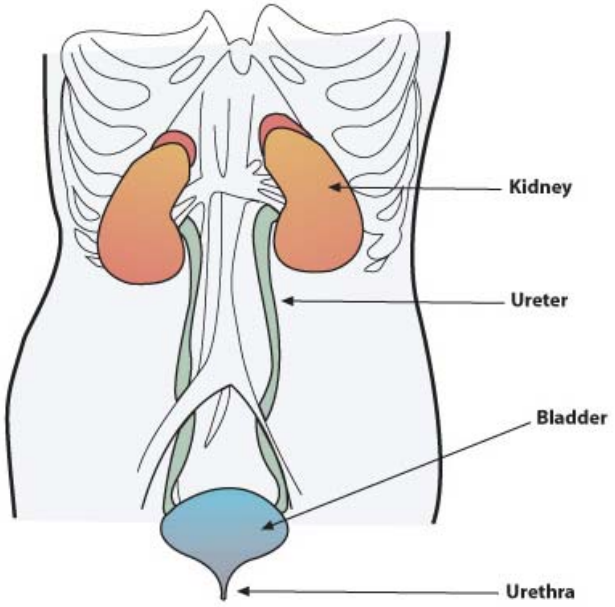


Female Urogenital Tract

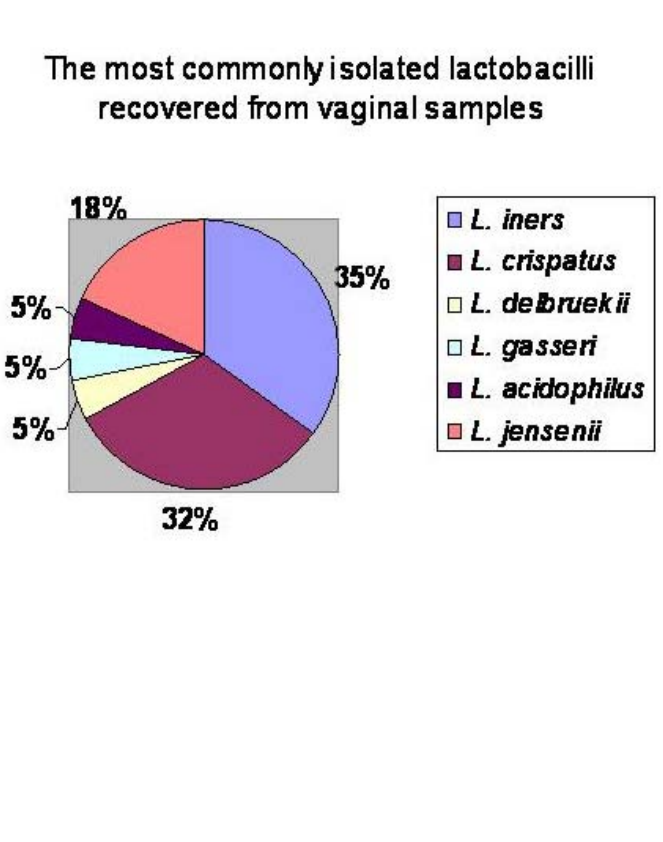
The etiology of bacterial vaginosis is unknown, but we do know that it results from the overgrowth of various anaerobic bacterial species and is associated with the disappearance of hydrogen peroxide-producing lactobacilli, which dominate in the normal vagina

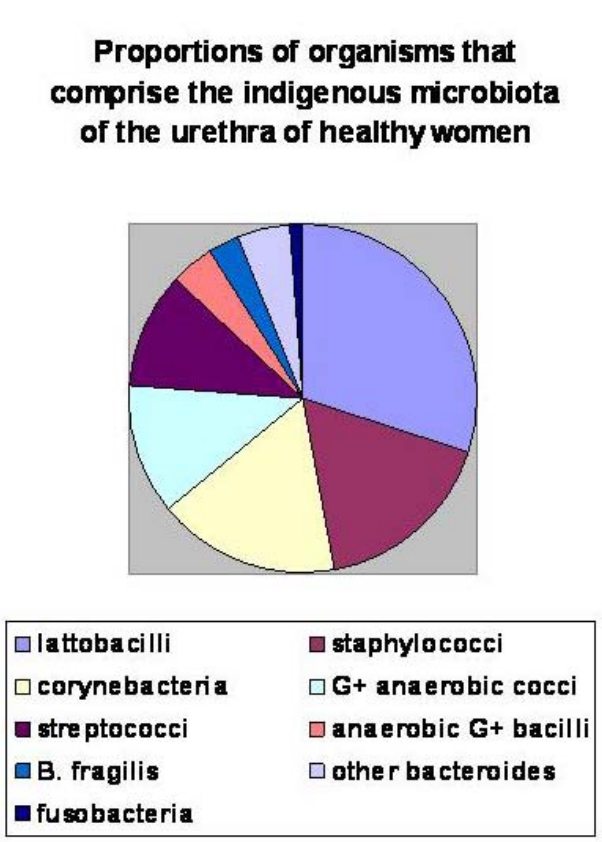
In the urinary system only the urethra is colonised by microbes –the kidneys, ureters and bladder are sterile in healthy individuals.

Urogenital tract infections (UTIs) are very common and annoying diseases. *Escherichia coli* is the responsible agent in most cases (up to 85%), followed by *Staphylococcus saprophyticus* and enterococci.



Female urinary system





The complex vaginal microbiota is dominated by *Lactobacillus* spp. High levels of lactobacilli, particularly hydrogen-peroxide-producing strains, suppress the growth of potentially pathogenic members of the vaginal microbiota, including *Candida albicans* (responsible for vaginal candidiasis or vaginitis) and organisms responsible for bacterial vaginosis (BV) (*Gardnerella vaginalis*, *Prevotella* spp., *Peptostreptococcus* spp., *Mobiluncus* spp.)

PROBIOTICS VS UROGENITAL INFECTIONS

Two strains specifically selected for urogenital applications:

- *L rhamnosus* GR-1 and
- *L fermentum* RC-14.

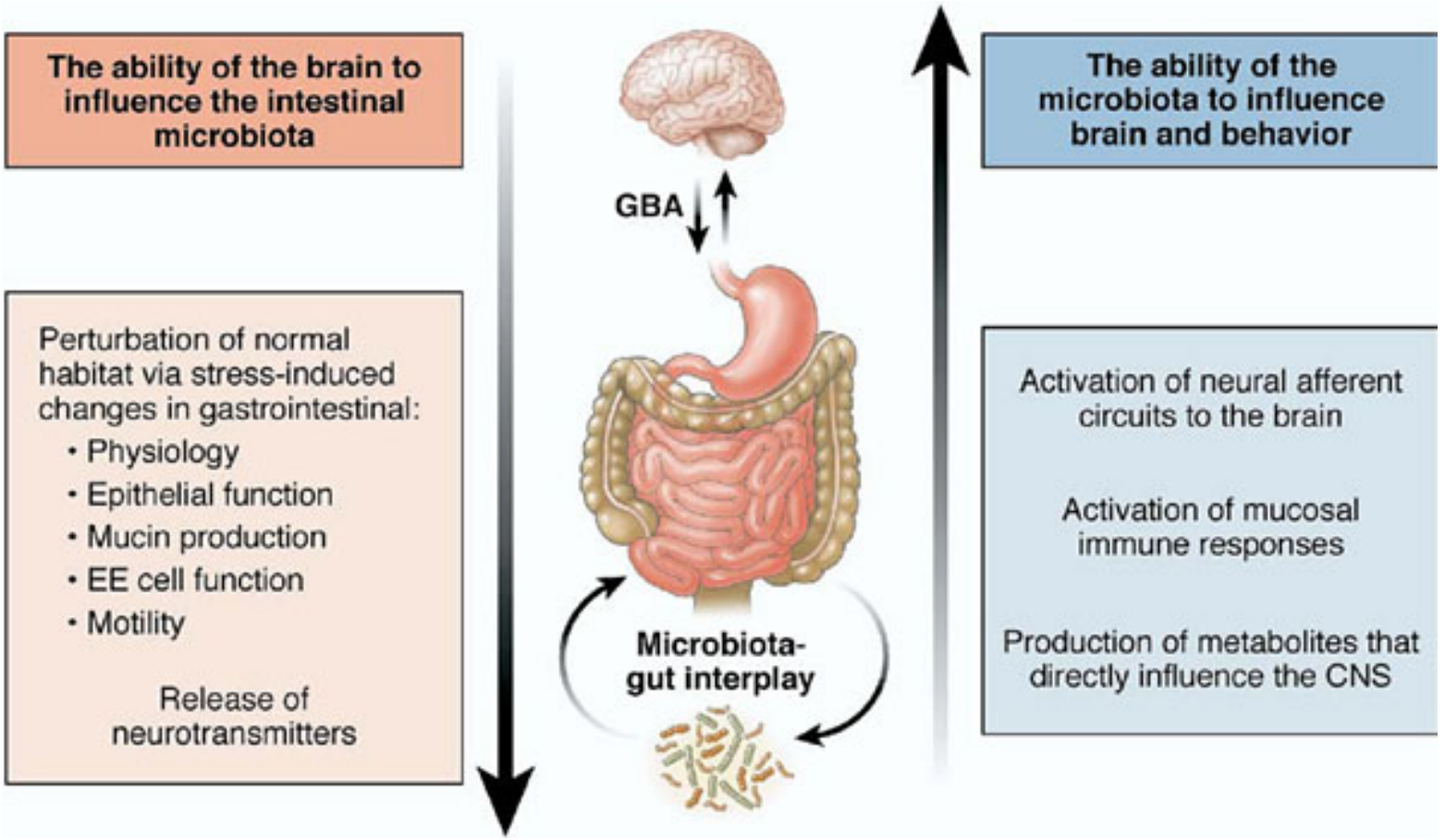
Both *L rhamnosus* GR-1 and *L fermentum* RC-14 or B-54 have shown very encouraging results

The mechanisms include:

- immune modulation,
- pathogen displacement
- creation of a niche less conducive to proliferation of pathogens and their virulence factors
- production of bacteriocins, organic acids and hydrogen peroxide

Effect on Brain Areas

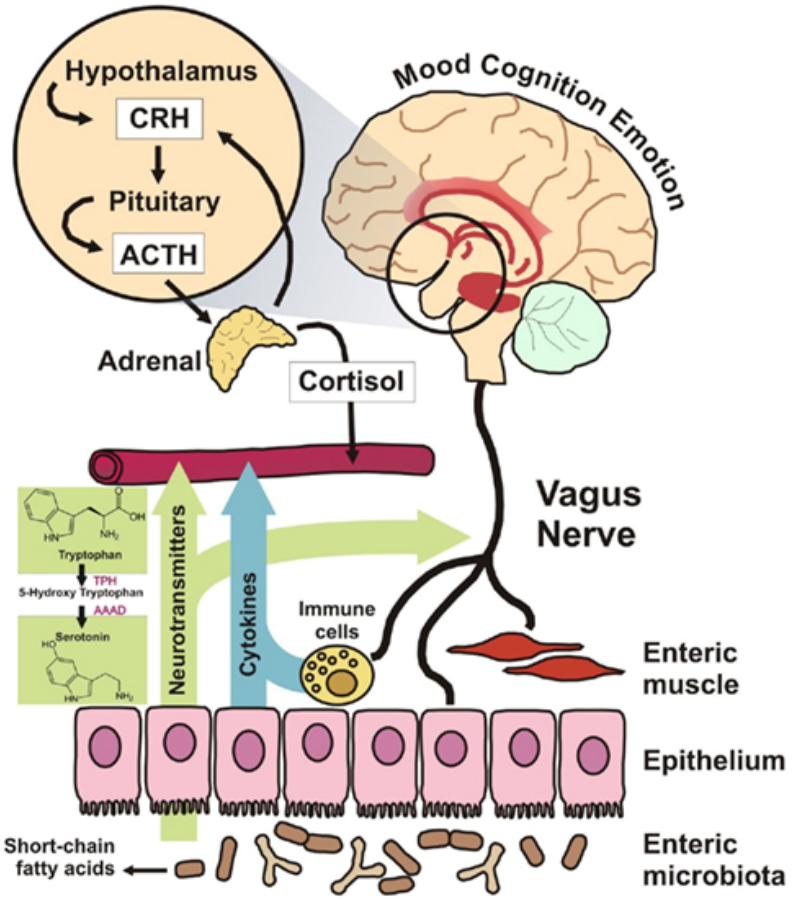
There is a functional bidirectional communication between the gastrointestinal (GI) tract and the central nervous system (CNS)



This communication involves anatomical connections like the vagus nerve and humoral components including the immune system and the hypothalamus-pituitary-adrenal (HPA) axis.

Increasing evidence suggesting a key player in this interaction: **the intestinal microbiota**

MICROBIOTA-GUT-BRAIN AXIS

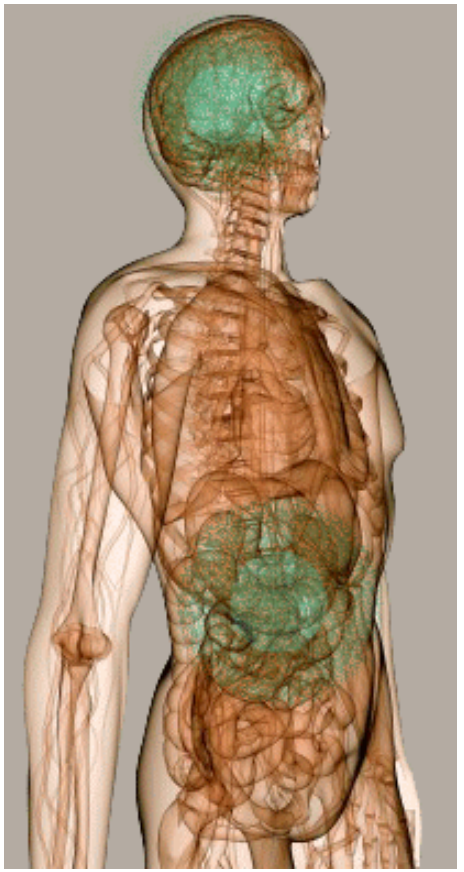


Data suggest that:

1. The presence of intestinal microbiota during early developmental stages is a key component for an adequate brain development → highlighting the complex interaction existing between intestinal microbes, gut and CNS

2. Alterations in the gut microbiota could underlie some of the behavioral traits associated with anxiety and depression

3. Change in diet and the concomitant change in microbiota improve cognitive parameters and also reduce anxiety-like behaviours



PROBIOTICS VS MICROBIOTA-GUT-BRAIN-AXIS

1. Efficacy of probiotics in inducing changes in the CNS (strain-specific effect)
2. Anxiolytic-like properties of some probiotic strains
3. Commensal/probiotic bacteria can signal to sensory neurons and reset their excitability state

Applicability

A) Certain probiotic strains might be used to improve cognitive and emotional aspects of mental health (mood disorders)

B) Probiotic treatment may increase the efficacy of psychopharmacology, as it has been shown that probiotics affect the expression of genes in the brain

C) Selective alteration of the gut microbiota may itself prove to have important beneficial outcomes for many systemic and gut conditions as a result of their actions on the enteric and central nervous systems

A Help from the gut

Thanks to our microbial friends