BY

SILVIA DI MAIO AND FEDERICO MERETA

MICRO BIOTA

THE INVISIBLE FRIEND OF GOOD HEALTH AT ALL AGES

THE ANSWERS OF
7 LEADING
EXPERTS ON

STRESS ALLERGIES
CEREBRAL AGEING
OVERWEIGHT
ANXIETY MENOPAUSE
DIABETES WEAKNESS
CONSTIPATION
SWELLING INFECTIONS

WITH
RECIPES
FOR A
HAPPY
BELLY

GRIBAUDO

Microbiota

Thanks to Salvatore Castiglione and Laura Corridore,
for the innovative idea and determination
without which this project could not have happened.
Thanks to Fondazione Istituto Danone for having always believed (and shown)
that science and information can travel down the same road.

By Silvia Di Maio and Federico Mereta
Translation by Simon Tanner
Photos: Shutterstock Images
Layout: Alessandra Leonardi

Gribaudo editorial office Via Garofoli, 266
37057 San Giovanni Lupatoto (VR)
redazione@gribaudo.it

Special initiatives management: Alias srl
Production manager: Franco Busti
Chief editor: Laura Rapelli
Graphics manager: Meri Salvadori
Progetto grafico: Natascia Adami
Photolith and pre-press: Federico Cavallon, Fabio Compri
Editorial secretary: Emanuela Costantini

FSC
www.fsc.org
MISTO
Carta
da fonti gestite in
maniera responsabile
FSC® C101934

Printing and binding: Grafiche Busti srl, Colognola ai Colli (VR),
azienda certificata FSC®-COC con codice CQ-COC-000104

First edition: 2020 [1(Gh)]
Second edition: 2020 [12(Be)] 978-88-580-2598-7

BY
SILVIA DI MAIO AND FEDERICO MERETA

MICRO BIOTA

THE INVISIBLE FRIEND OF GOOD HEALTH AT ALL AGES

GRIBAUDO

THE INVISIBLE ORGAN THAT REGULATES OUR WELLBEING

Over 500 years ago, Leonardo da Vinci admirably described the human body in his tables of unique drawings. But perhaps, in illustrating the muscles, joints and internal organs, Leonardo forgot a structure which, although lacking the anatomical dignity of the organs we all know, has now been found to be on a par with them, and performs its own precise functions. Some of these have already been discovered, others will emerge with the constant progress of science. But we should certainly not hold this oversight against that genius Leonardo, considering that not even modern manuals of anatomy depict the organ that appears increasingly important in determining our psycho-physical well-being and in regulating the functions of many parts of the body. This organ weighs more or less 800g in an adult, a little less than the heart, which we know well and which we are constantly aware of thanks to its beat, yet it does not appear in any anatomical illustration, despite being composed of a number of cells which far exceeds that of the human body. By studying the invisible, we realize that the human body is basically nothing more than a collection of eukaryotes, bacteria and archaea. And it must be pointed out that there are around ten times more microbial cells, that is bacteria, living in our organism than eukaryotes, those that we more properly consider “ours”. However, bacteria are not distributed throughout the body: most of these prokaryotic cells are found in the human gut. Also in genetic terms, the genomes of these bacteria contain over a hundred times more genes than humans. Although we perceive bacteria as mainly being linked to disease, human life itself is dependent on the presence of “good” bacteria. As we will see, the “world” of microorganisms living inside us is both fascinating and important for our health, both physical and psychological.

WHY A BOOK ON THE MICROBIOTA?

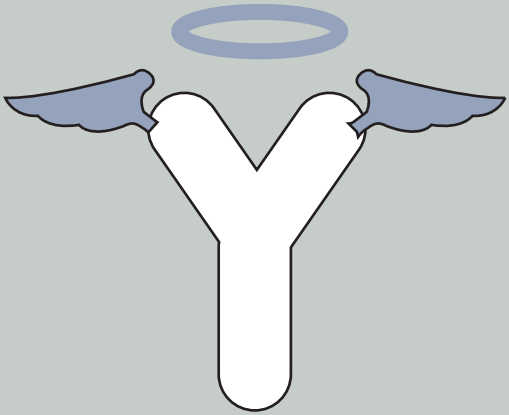
This mix of historical, anthropological and scientific concepts explains why research on gut bacteria, from ancient texts to the most recent scientific evidence, is a sector of great interest for science. But we are talking about Science, with a deliberately capital “s”, the science that goes beyond fake news and is based exclusively on the results of tests that follow strict protocols, both in test tubes and on humans, and which are shared in leading journals, to be further demonstrated in other laboratories. Science has felt the need to shed light on this problem, using – since it is a question of “shedding light” – simple language. With this in mind, the Fondazione Istituto Danone, which aims to disseminate information on food issues, always based on the most recent observations coming directly from research, has set up a multidisciplinary committee to provide clear information on what is known about the microbiota. The analysis carried out by these experts resulted in this book, which recounts clearly, through questions and answers, what the microbiota is, its physiological changes as we age, and the impact it can have on various medical conditions, from allergy to depression. It concludes by offering hope and suggestions on how, both now but above all in the future, we can positively influence the bacteria that coexist with us.

LABORATORIES INSIDE US

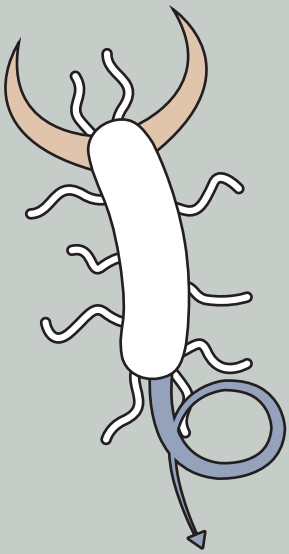
The microbiota is today one of the scientific areas in which research is most concentrated. The bacteria in the digestive tract in fact activate and regulate some fundamental processes, without us even realizing it. When we ingest food, for example, enzymes are set in motion that allow us to transform many of the substances it contains. These enzymes include lipases and proteinases and their activity is, so to speak, "mediated" by bacteria. This is just one of the examples that shows how bacterial cells are like laboratories in the human body: they obviously work primarily for themselves and to achieve replication, but in doing so they release enzymes that act not only on substances in transit (those that come from food digested by the stomach) but also on the cells of the mucous membrane of the digestive system. Would you like some more examples? Thanks to bacteria, vitamin B12 is produced, which is extremely useful, especially for vegetarians, who don't eat meat and therefore can't get it from their food. Furthermore, these invisible "power plants" exploit food proteins to produce a special amino acid, called "beta-alanine". This amino acid, inside muscle, joins with histidine to form carnosine, probably one of the most effective natural antioxidants, which helps protect us from the action of free radicals. As if that were not enough, even the waste products – the junk – of bacteria can be transformed by the bacteria themselves into compounds useful for our well-being: think of short-chain fatty acids (SCFA) produced by germs, such as acetic acid, propionic or butyric acid. These fatty acids, due to their molecular constitution, can be dissolved in water and are therefore easily absorbed by the blood, without the need for special transport systems, which are instead necessary for other types of medium or long chain fatty acids: they thus become one of the main "fuels" for muscles, heart and brain, being a rapidly consumed energy source.

THE GOOD AND THE BAD

Every day, our intestines are the scene of “bloody battles”, and if we are to remain healthy, the “good” microbiota must win. Our “inhabitants” in fact play a fundamental role in defending us from their peers in the “bad tribes”, those that cause diseases. Fortunately, the good bacteria are not interested in martyrdom, but have many defensive weapons at their disposal, such as strengthening the body’s immune system (our immune defences help them to withstand any attacks), thus making it more difficult for the “enemies” to settle on the intestinal cell wall. In any case, the “inhabitants” of the microbiota are not all good and useful for the body. Some of them are pathogenic, that is, they can cause ailments. A case in point are salmonellae, similar to those that cause typhus, or *Clostridium*, which can lead to intestinal infections. Fortunately, eubiotic bacteria, which are mainly from the *Lactobacillus* or *Bifidus* genera, counteract the development of these bad germs. The important thing, in any case, is that the good inhabitants remain far superior in number to the bad ones. Eubiotic bacilli reproduce, exploiting what arrives in the intestine, and therefore subtracting the food required for



the development of pathogenic germs, which consequently cannot replicate en masse. This is the law of the jungle: a species that feeds and grows, while others are destined to remain on the "margins of society", with few inhabitants. By adopting good habits, from a healthy diet to the correct use of antibiotics (to be taken only as an exception, only when prescribed by a doctor, and for the entire course of treatment), we can help the good come out on top against potential invaders. Eubiotic bacteria, for example, need to ferment food fibre to obtain nourishment: this explains the importance of consuming fruit and vegetables, essential for "feeding" them. There are other important variables, such as the intestinal availability of oxygen: the more there is, the lower the risk of developing anaerobic bacteria in large numbers, those that can reproduce even in its absence. What matters, from our point of view, is leading a healthy life. It will then be up to them, our invisible "friends", to work together and promote our well-being.



HOW OUR MICROBIOTA HELPS KEEP US HEALTHY

Many scientific studies show that the bacterial population of the gut plays a fundamental role in human health, and not only for that of the gut itself. Just think of the research evidence available regarding the role of the gut microbiota in stress, mood and the development of widespread pathological conditions, such as obesity, which is nothing short of a modern non-infectious pandemic. An Italian study published in *PLOS ONE*, for example, shows that by acting on the microbiota it might be possible to interfere with the mechanisms that give rise to Alzheimer's disease, thanks to the action of the CNF-1 toxin produced by the bacterium *Escherichia coli*. In mice, this toxin was found able to eliminate the inflammation that accompanies the neuronal damage typical of the disease. The study was carried out in a collaboration between researchers from the Dipartimento del Farmaco dell'Istituto Superiore di Sanità and from the Dipartimento di Farmacia e Biotecnologie and the Dipartimento di Scienze Mediche e Chirurgiche of the University of Bologna. But that's not all: regarding obesity and diabetes, which often complicate the health of overweight subjects, we need merely mention the study by the National Cancer Institute, published in *Nature Communications*, which demonstrates that the action of an antioxidant substance used to treat damage from radiation, which affects the bacterial flora, may have an influence on the onset of obesity. Similarly, research by the University of Gothenburg, also published in *Nature*, demonstrates that the microbiome of diabetics is different to that of healthy people, even in the presence of spikes in blood sugar. Nor should we forget what was discovered in France, by

scientists from the National Institute of Agronomic Research, who had the intuition that the composition of bacteria could affect the way food is metabolized, thus explaining why some people gain weight more easily. Moreover, although it may seem strange, changes in the gut's bacterial population could also be correlated with an increased risk of developing cardiovascular problems, such as a heart attack. How? By modifying the metabolic profile. Gut bacteria in fact have the task of breaking down carnitine, a compound found in red meat. A study published in *Nature Medicine* and conducted at the Cleveland Clinic focused attention on a substance present in carnitine, called trimethylamine-N-oxide (TMAO). This compound has been associated with the possible formation of plaques within blood vessels. Those who regularly consume meat have higher levels of TMAO than vegetarians and vegans. But when steak lovers are treated with antibiotics, TMAO production drops, even after eating foods rich in carnitine.

These are just a few examples. We could give you hundreds more. But what matters is being aware, and this book aims to offer a small – yet scientifically impeccable, thanks to the expertise of leading scholars – contribution to helping us all become aware of what we can do for our well-being. Starting with the invisible world.

Enjoy the read

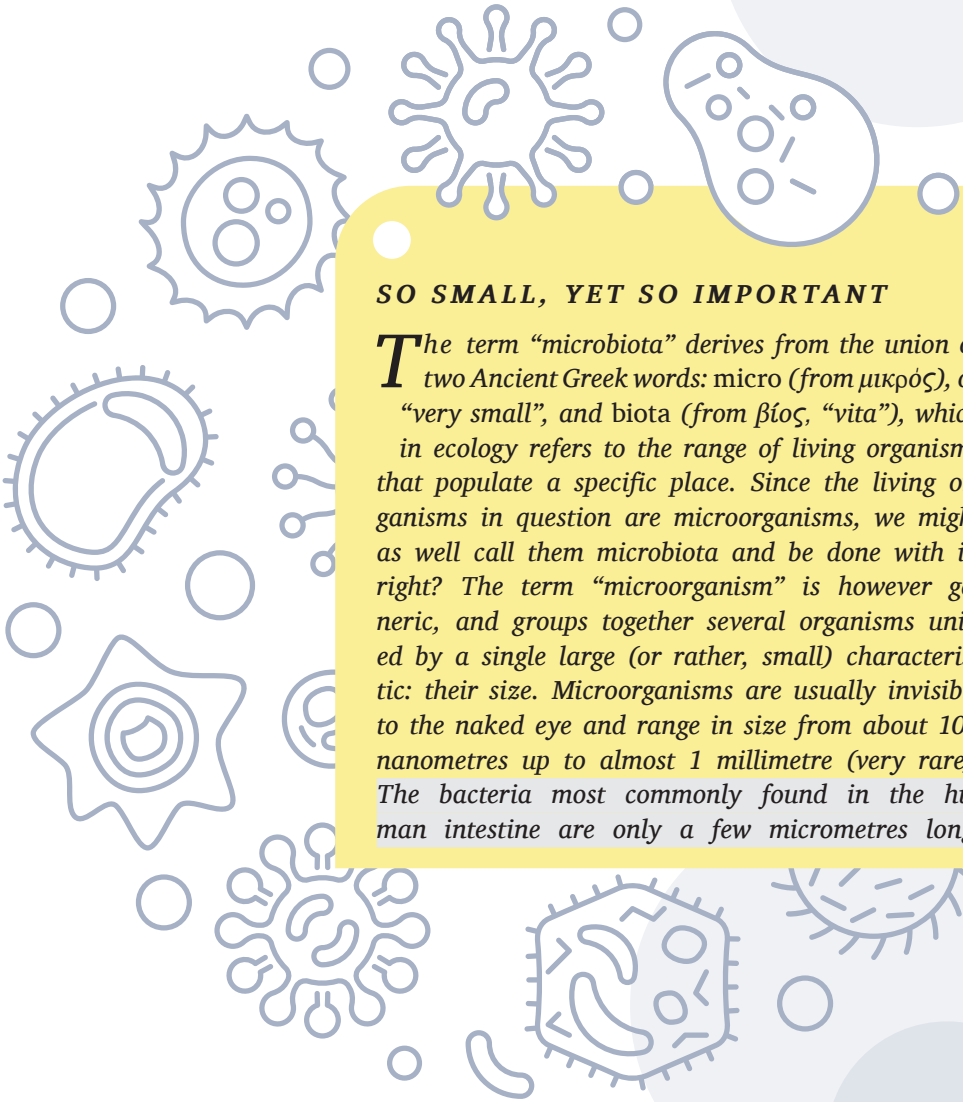
SCIENTIFIC PREFACE: CURRENT AND FUTURE RESEARCH

ARE WE ALONE INSIDE OURSELVES?

Rather than asking the clichéd, as yet unanswered question “Are we alone in the universe?”, we propose a radical change of perspective: “Are we alone inside ourselves?”. No, we are full of life! But what does this mean exactly? We konw that humans are complex structures, made up of numerous bodily ‘districts’. But not everyone knows that some of these districts (e.g. skin, intestine, vagina) are populated by staggering numbers of tiny living microorganisms, which, taken together, constitute the human microbiota.

WHAT IS THE MICROBIOTA?

Let’s get right to the heart of the matter, starting with the scientific definition of the term: ““The human microbiota is the collection of microorganisms situated in the various bodily districts that interact with the outside world”. This is what an expert in the sector would answer today, and it is an answer that summarizes in a single, concise sentence, years of work and study; this apparently straightforward definition embodies decades of research and experimental and *in vivo* observations. In any case, from these few words we may make two principal deductions. Firstly: the human microbiota is the community of microorganisms that live with us. Secondly: bacteria take up residence in those areas of our body that are in contact with the outside world, such as the urogenital system (for example in the vagina), the respiratory system (the air that we breathe in and out is a means of communication with the outside world), the skin (hard to imagine any part of our bodies more exposed than that!) and the digestive system, in which most of the body’s microorganisms reside (about 80% of the total). But what groups of microorganisms make up our microbiota? In terms of scientific classification, some are bacteria, others archaea, still others fungi, viruses or protozoa. Even without going into the taxonomic details of the groups listed above, we are well aware that different names correspond to different characteristics. This means that microorganisms in general (as well as those that make up our microbiota) also differ widely from each other!



SO SMALL, YET SO IMPORTANT

The term "microbiota" derives from the union of two Ancient Greek words: micro *(from μικρός), or "very small", and* biota *(from βίος, "vita"), which in ecology refers to the range of living organisms that populate a specific place. Since the living organisms in question are microorganisms, we might as well call them microbiota and be done with it, right? The term "microorganism" is however generic, and groups together several organisms united by a single large (or rather, small) characteristic: their size. Microorganisms are usually invisible to the naked eye and range in size from about 100 nanometres up to almost 1 millimetre (very rare). The bacteria most commonly found in the human intestine are only a few micrometres long.*

DEAR MICROBIOTA, IF I CHANGE, DO YOU CHANGE TOO?

We must not presume that we all have the same microbiota. Scientists distinguish between "intra-individual" and "inter-individual" variability. Inter-individual variability refers to the fact that the microbiota of every human being is unique, but let's put this issue aside for the moment; we will come back to it in due course. Instead, let's focus on intra-individual variability, which refers to those changes in the microbiota we all undergo in the course of our life. The microbiota is a dynamic entity: as we grow, it changes with us. This means that every human being's gut microbiota is the result of their experiences, and these experiences lead to the formation of what we call the *core microbiota*, which is stable over time and able to respond to external events. During the life of an individual, the microbiota is in fact capable of self-modulation (a phenomenon due to its resistance and resilience) in response to so-called external *stressor events*, in order to ensure bodily equilibrium. It's worth repeating: the microbiota should not be imagined as a set of compartmentalized microorganisms in the gut or other parts of the body, but as a dynamic entity, able to actively interact with us and in particular with our immune, endocrine and nervous systems. Growing in symbiosis with our organism, the microbiota changes in relation to the different life stages of the individual. During the life of each person we can identify above all two critical periods for the acquisition and modification of the gut microbiota: the first three years of life and the passage from adulthood to old age (over 65 -70 years approximately). But why are these in particular the most significant age groups?

It is not the first time that we have heard that the first three years of a child's life are fundamental. For example, it is believed that they are crucial for the development of the main cognitive functions. Well, this also applies to the gut microbiota. In fact, this is when the core microbiota is formed, which will be structurally similar to that of the adult. In the first days of life, it is characterized by "low microbial density and diversity". To put it simply, there are relatively few microbes, and these are more or less all related to each other, belonging to a few different species. In particular, the microbiota of a newborn baby in the first days

of life is mainly composed of bacteria that ferment milk, such as *Lactobacillus*, *Streptococcus* and *Bifidobacterium*. As the days and weeks go by, various factors will contribute to filling the gut with new tenants, until a plateau is reached, in a sense equivalent to a situation of "sold out": bacteria come and go, but the spaces are what they are, and their number will not increase by much. In the first three years of life, any factor capable of altering the early, delicate interaction of the microbiota with the developing immune, endocrine and nervous systems will make the individual more susceptible to disease later in life. Not to mention the particular moment when new foods are introduced: this is when the child comes into contact for the first time with completely new molecules, which are bound to affect the gut microbiota as they pass through the intestine. Therefore, within the first three years of an individual's life, the microbiota reaches its full development, is moulded, and becomes similar to that of an adult.

And what about the second period of great change, between the ages of 65 and 75? This age band roughly corresponds to the transition from adulthood to old age, and marks significant transformation. We must adapt to all these changes, and so must our microbiota! But let's not get ahead of ourselves!

*

HOW THE MICROBIOTA EVOLVE

0-3 years formation and acquisition of the gut microbiota

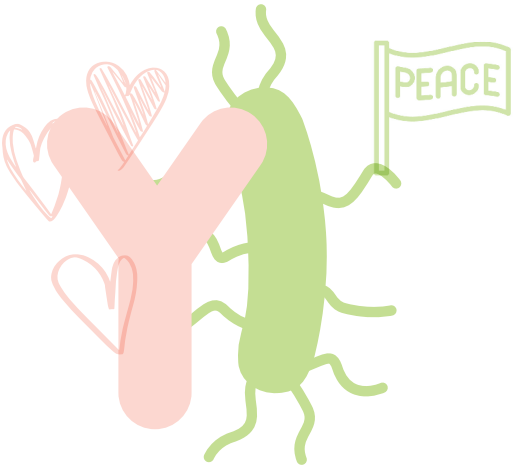
Continuous remodelling of the gut microbiota

> 65/70 years changes in old age to the gut microbiota

THE 1,000+ DAYS OF THE MICROBIOTA: THE FIRST THREE YEARS OF LIFE

LET'S GET ONE THING CLEAR FROM THE START: DOES COHABITATION SUIT EVERYONE?

The microorganisms that live in our gut have found a comfortable habitat suited to their needs. The real gem, however, is this: we need them as much as they need us. To survive in the environment in which it lives, the human body must ensure the functioning of some basic biological functions, such as the absorption of nutrients, breathing, and many others. During the course of evolution, our environmental conditions have gradually changed, and with them the very dynamics according to which our bodies "work": when nature identifies an effective solution, it is prioritized and handed down. Some microorganisms residing in our gut began to prove particularly useful in ensuring the normal functioning of the digestive system (and the immune system, but that's another story, which we will be looking at later!). Scientifically, evolution is said to have occurred in a symbiotic relationship, which means nothing more than a little healthy mutual help, and in a homeostatic one, which for now we will simply define as "in equilibrium", but we will soon return to this definition with a few more details. In biological terms, evolution is the product of the interactions between external environmental factors and our genes. Considering that the many microorganisms living inside us are endowed with their own genes, evolution also takes their presence into account. The population of microorganisms that have ended up colonizing our gut microenvironment have, over the centuries, established a complex, dynamic system of exchange with our organism. So this is the compromise: "If you give us a hand, you can stay".



THE GOLDEN RULE OF BIOLOGY

DNA holds all the instructions that each cell needs to survive and carry out its functions, including that of collaborating with others to build our body (as well as that of any living being). DNA, however, is a single very long structure curled up inside the nucleus of each cell: understanding its commands can sometimes be difficult! To make it easier to read, DNA compartmentalizes its information into shorter segments called genes. Although they may be considered "units" in their own right, genes are physically a continuum with the rest of DNA. The golden rule of biology, which underlies everything, can be described as follows: the instructions present in each single gene are transcribed by special reading machines and "cleaned" of all the accompanying DNA that is not needed, to make sure that the gene commands are received. After this process, the information of the transcribed gene is found in a shorter molecule, called RNA, which continues on its way towards the ribosomes. Ribosomes are those "machines" that translate the language of RNA into a very different language, that of proteins. In the language of RNA (as well as DNA), if we imagine each RNA molecule as a word, the letters of the alphabet are "bases"; in protein language, however, if each protein is a word, each letter is an amino acid. When translating a word "written in RNA" into a word "written in protein", however, we cannot assume that each base corresponds to an amino acid. The bases are in fact organized according to a very precise code, whereby each amino acid will correspond to three bases grouped into a trio. Once the protein is obtained, it will perform the function for which it was created, based on the information contained in the corresponding gene.

✱
FROM DNA TO PROTEINS

ARE ALL MICROORGANISMS WELCOME?

The many microbial species that make up the gut microbiota have a commensal relationship with each other, something that sounds more complicated than it actually is. Two species are defined as commensals when they freely choose to interact with each other, even though both are able to survive independently. One of the two species benefits from interaction with the other, which is not damaged in any way and thus willingly accepts its presence. Having said that, the microorganisms we host belong to many different commensal species, but the pact they have signed with us forces them, as a whole, to guarantee us a certain state of health. The fact that a non-pathogenic microbial species swears that it has no bad intentions certainly does not authorize it to settle and expand until it drives out all the other inhabitants. However, it is also true that we are not too choosy, and even welcome microbial species "with a criminal record", potentially capable of causing human diseases: as long as only a few specimens settle in our intestinal tract, the situation remains under control, with no harmful consequences.

The crux, then, is that in the microbial community there are balances to be respected: the various species live and interact with each other, but each one must, so to speak, stay in its place and not get above itself. The concept of "homeostasis" fits into this context, and refers to the tendency to maintain a situation of equilibrium. Studies have shown that, in healthy conditions, the gut microbiota is characterized by a high level of microbial diversity, i.e. with a vast number of different species coexisting. This is what we mean when we speak of "biodiversity". But nothing is left to chance, not even biodiversity.

NINE MAGIC MONTHS, WHAT HAPPENS TO THE MICROBIOTA?

For years it was believed that at birth, and therefore throughout pregnancy, humans did not host microorganisms, and that colonization by these welcome strangers began only subsequently. Today, however, we can say that this is probably not the case. Probably? Well, the only great truth of science is that there is no absolute truth. Each theory is affirmed as a consequence of painstaking research that leads to the collection of information, of so-called "scientific evidence". But scientific research is based on technology, and just as we have moved from Snake on monochrome mobile phones to Netflix on smartphones, in the same way research laboratories have equipped themselves with increasingly exceptional tools. Technological innovation has made it possible to continue making new discoveries, sometimes conflicting with the evidence obtained previously using tools that are now somewhat outdated. A scientist, therefore, must always be ready to take on board any new information, and prepared to completely change their mind. But before they can scream out loud that none of what they said earlier was true, they must start over with that lengthy, meticulous collection of information that maybe, just maybe, will lead to a new theory. All this means that, contrary to what was previously believed, humans are probably not sterile (in the sense of being free of microorganisms) even at birth. Bacterial communities have in fact been observed that, so to speak, "pitch their tent" on the placenta, in the amniotic fluid, the fetal membranes, the umbilical cord and meconium (substance found inside the fetus's gut). This would suggest that microbes arrive before birth, already during gestation: we could therefore speak of "in utero colonization". However, in order for this theory to replace the idea that the newborn is sterile at birth, we need more evidence: the doubt always remains that the experiments carried out could have been falsified by contamination errors, for example, or that the bacteria identified are not actually alive. Pending the availability of more precise information, it is better to think of the fetus as completely sterile. But, in any case, its microbiota will be formed in a very short time.

THE MYSTERY OF BIRTH: HOW DOES THE MICROBIOME CHANGE?

The first cry is about to arrive. The gynaecologist, together with the mother, decides whether to proceed with natural birth or, if necessary, the surgical alternative of caesarean section. The newborn "forms" their own population of bacteria in the digestive system also depending on how they are born.

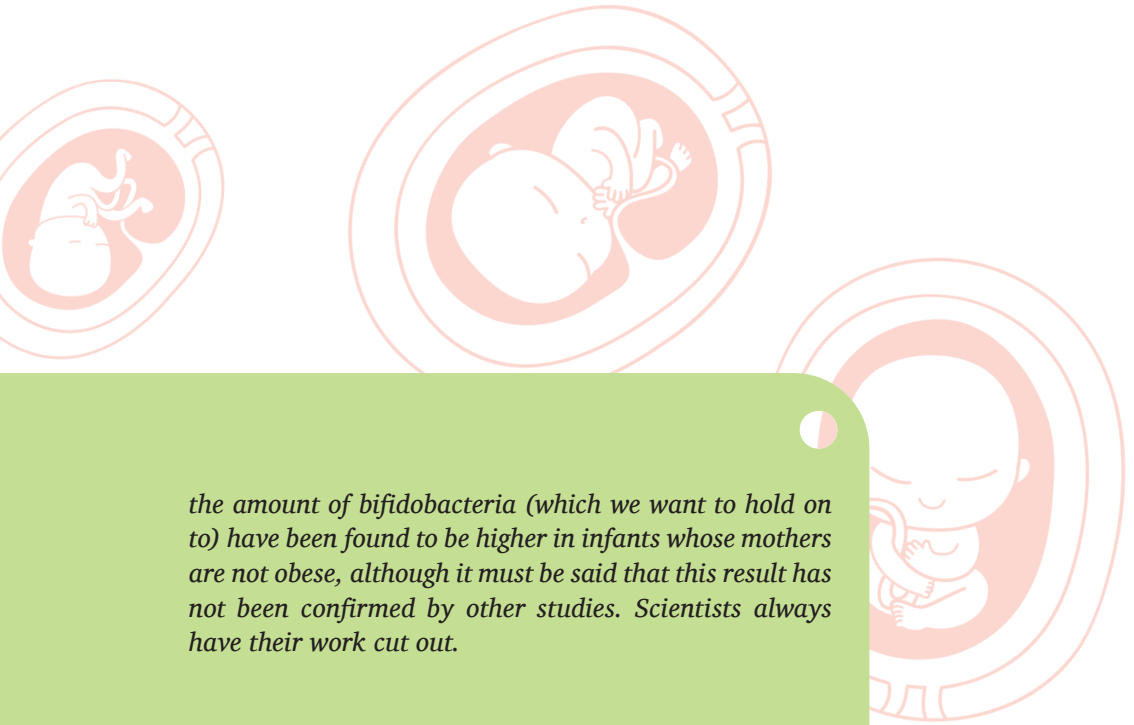
For what reason?

During its passage through the birth canal, the fetus is contaminated by the microorganisms it physically encounters. In short, for the gut microbiota the method of birth is a key factor that can substantially

THE INVISIBLE FACTOR OF MATERNAL NUTRITION

A mother is such not from the moment of childbirth, but from the moment of conception. Right from the start, the little one will need her care and attention and their microbiota can also benefit from this. The microbes that can be found in the baby's faeces are even influenced by the mother's body mass index[1] *and by her weight gain during pregnancy. In general, the concentrations of faecal* Bacteroides *and* Staphylococus *have been found to be significantly higher during the first 6 months of life in infants born to overweight mothers. On the other hand,*

influence its composition. In a natural birth, the newborn comes into contact with the populations of microorganisms found in the mother's vaginal tract and faeces. On the other hand, when the newborn comes into the world by means of caesarean section, they will encounter all the microorganisms that live on the mother's skin and those that are somehow "introduced" by the hospital environment and staff. Some of these microorganisms move, lock, stock and barrel, into the intestine of the unborn child.



the amount of bifidobacteria (which we want to hold on to) have been found to be higher in infants whose mothers are not obese, although it must be said that this result has not been confirmed by other studies. Scientists always have their work cut out.

1 The body mass index is expressed as a number obtained by means of a mathematical calculation that divides a person's weight by the square of their height. Basically, if a person weighs 58 kg and is 1.62 m tall, the calculation will look like this: 58/(1.62)2. Short, quick and painless, it's a handy way to distinguish between underweight, normal weight, overweight and obese. The range of values that identifies a person of normal weight (pre-pregnancy) goes from 18.5 to 24.9.

But where does the difference lie?

The point is that the microorganisms present in the vagina and maternal faeces belong to different species to those present on the skin or in the hospital environment. So Baby A, delivered by natural birth, will have an intestine colonized mainly by microbes called *Lactobacillus* and *Prevotella*. The intestine of Baby B, meanwhile, who was born by caesarean section, will show a less diverse microbiota, and the bacterial community will be more similar to that of the surface of the mother's skin (*Staphylococcus*), and colonization, by Lactobacillus will be delayed. During childbirth, Baby B will mainly be colonized by microbes called *Proteobacteria* and *Firmicutes*, which will then also make room for *Actinobacteria* (which have been observed in faeces a week or two after birth). In both cases, immediately after birth, the intestine begins to accommodate further colonizers, mainly bacteria called facultative anaerobes, which, as we have already mentioned, have an excellent spirit of adaptation and can survive and grow both in the presence and absence of oxygen. The newborn's gut is in fact an environment suitable for the colonization of strictly anaerobic bacteria.

Would you like some examples?

Bacteroides, *Clostridium* and *Bifidobacterium*.

Conclusion: in general, the intestine of those born by caesarean section is more frequently colonized by bacteria of the genus *Clostridium*, including *Clostridium difficile*. In other words, there are numerous differences, including greater heterogeneity in the microbiota of those delivered by natural birth over the first 12 months of life. Interestingly, however, the differences gradually decrease over time.

*

THE DIFFERENCE BETWEEN THE TWO TYPES OF DELIVERY

HOW MUCH DOES THE METHOD OF BIRTH COUNT FOR THE FUTURE OF THE BABY?

The way in which one comes into the world, as the first cry is emitted, amidst the parents' smiles and tears of joy, is also an important factor in "designing" the future microbiota. Obviously it is not the only variable, but it counts. Quite a lot, actually! This is confirmed by the results of important epidemiological research, i.e. studies that exploit statistics to understand the relationship between two or more characteristics (called "variables" by statisticians) of the individuals involved. The number of participants is usually high: in statistics, the more the better! But let's take a step back. A variable could be, for example, the "delivery method" by which an individual is born. Based on the answer, participants in a potential study will be divided into two large groups: those born naturally and those born by caesarean section. Among these individuals, some will develop diabetes, others will suffer from allergies, still others will have, for example, brown eyes or blond hair: all of these are, again, characteristics, or variables. The goal of these studies is to understand whether there is any relationship between the characteristic of "delivery method" and the variables observed in the individuals of the two groups. The observations made indicate the importance of this step and the role of the microbiota. Among individuals born by caesarean section, a sufficiently high number of them go on to be affected by allergies, asthma and type I diabetes, so much so that the type of birth may have a role in determining the development of these conditions. Let's not jump to conclusions, though: at the moment this is just a simple association, therefore a hypothesis, so a study of this type is not enough to tell us with certainty what the causes and consequences are. However, researchers are working to establish whether this association can pave the way for the development of a theory. The question scientists have asked themselves is therefore:

What changes from one delivery method to another?

The microbiota! This led to the hypothesis whereby the differences between the microbiota in the first years of life of those born by caesarean section and those born naturally, even though they smooth out over time,

could affect the state of health throughout adulthood. Even more specifically, the gut microbiota is thought to be so important in the very early stages of life because it may play a role in the maturation and development of the host's immune system, thus influencing it throughout life.

BORN BY CAESAREAN: GREATER RISK OF OBESITY

While caesarean births in Italy are common, also due to the high average age of many women when they give birth, new research shows that this type of childbirth may be correlated with a greater risk of becoming overweight and obesity in adulthood. The study, which took into account data relating to over 10,000 children born between 1991 and 1992, was led by Jan Blustein of the University of New York and appeared in the "International Journal of Obesity". Comparing the development of babies born by caesarean section and that of babies born naturally, the experts observed that the babies in the first group had a greater risk of developing obesity than their peers in the second. This is further proof of the effects that caesarean delivery can have on the development of infants. Already considered among the possible reasons behind the increased risk of obesity and other conditions, such as allergy, scholars are evaluating the influence of caesarean delivery on the microbiota at the time of birth. There would seem to be fewer "good" bacteria in the intestinal bacterial ecosystem of children who come into the world by caesarean section, compared to those born naturally. This is hypothesized in a study conducted at the University of Alberta, in Canada, which appeared in the "Canadian Medical Association Journal". The study confirms the hypothesis whereby exposure to bacteria present in the vaginal tract could play a positive role in the development of the microbiome in infants..

WHAT HAPPENS IN BABIES BORN PREMATURELY?

A newborn who comes into the world before 37 weeks of gestation have elapsed is classified as a "preterm birth". But parents are not the only ones who are not yet ready for this early arrival: newborns, most of all, may be somewhat unprepared. They are fragile, their immune system is still immature and cannot defend them to its full potential. They often suffer from respiratory and neurological diseases, and theirs are therefore births that require a great deal of attention on the part of the doctors who have them in their care. But, above all, the intestine is not yet able to face the challenges of the outside world, as it is functionally immature and, perhaps, not yet perfectly formed. Fortunately, modern medicine makes it possible to facilitate the transition to the "real" world despite this short notice: according to how premature the birth is and the baby's state of health, infants may be hospitalized in an intensive care environment and benefit from the help of artificial respiration systems. Furthermore, since the intestine is not yet able to carry out its work independently, nutrients can be administered directly, not through the mouth but using other means. To complete the picture, drugs and especially antibiotics are also included.

Science tells us that "all of these factors can interfere with the natural pattern of acquisition and development of the microbiota, with consequent deviations in the composition of the gut microbiota". Basically, when comparing full-term and premature babies, there is some difference in the microbiota itself. Pharmaceuticals, antibiotics, intensive care and all the other factors listed above may induce the confusion, delays and inconveniences we associate with a serious public transport strike in the forming microbiota. To begin with, anaerobic commensal microorganisms arrive later in the gut of premature babies than they do in the gut of those born at term. This is the case, for example, with *Bifidobacterium* or *Bacteroides*. On the contrary, higher numbers of *Enterobacteriaceae*, *Enterococcus* and other opportunistic pathogenic microorganisms have been observed in the faeces of preterm infants, which in some way "exploit" the abnormal situation to multiply. Of course, it is not possible to

generalize in this case either, because substantial differences are often found between one newborn and another. However, a minimum common denominator has been identified among preterm infants: their intestines are colonized by anaerobes (which live also or only in the absence of oxygen) and, in particular, by germs of the genus *Clostridium*. Unfortunately, these peculiar characteristics of the microbiota in preterm babies make it more unstable than that of those born at term. In other words, the microbiota itself needs more time to achieve the typical composition of the adult microbiota, with possible implications for the child's well-being. Maybe even in the short term.

Let's look at some examples. When the immune system interacts with an altered microbiota, infections are more likely to appear, with a higher risk of potentially serious (albeit rare!) consequences such as necrotizing enterocolitis (severe intestinal disease of the newborn) or sepsis (spread of germs in the blood and therefore throughout the body). Furthermore, the gut microbiota of the premature baby differs not only in composition but also in function. In particular, there appear to be differences in lipid metabolism. To try and better explain what has been observed, we should first of all note that scientists are able to trace these changes simply by analysing the faeces of infants. Hard to believe, isn't it? In fact, in premature babies, smaller quantities of short-chain fatty acids have been found, but greater quantities of bile acid derivatives than in those born at term. What are they, and why should these characteristics imply an altered lipid metabolism? First of all, fatty acids are fundamental components of lipids and we can think of them as a kind of pearl necklace. Each pearl is represented by a carbon atom. Based on the number of carbon atoms present, the chain varies in length and, based on this, it is possible to classify fatty acids into short, medium or long chain fatty acids. Those with short chains will therefore be like chains of pearls which would not even be long enough for a bracelet.

*

THE STRUCTURE OF FATTY ACIDS

carbon atoms

"necklace":
long-chain fatty acids

"complete bracelet"
medium-chain fatty acids

"bracelet that doesn't close":
short-chain fatty acids

This, however, makes them anything but useless. Indeed, if their quantity in the faeces of preterm babies is lower than found in full term births, it is not a good sign: short-chain fatty acids are in fact known to exert a beneficial effect on the body! And what about the derivatives of bile acids we talked about earlier? First of all, bile acids are not fatty acids, but substances produced by the liver that facilitate digestion and the absorption of fats. The microbiota is able to transform fats into new substances called “derivatives of bile acids”, and the increased presence of the latter in the faeces of preterm infants is a symptom of an altered gut microbiota.

IS BREASTFEEDING IMPORTANT?

The infant's nutrition is based on feeding with milk, but not all babies are fed in the same way. Some are exclusively breastfed by the mother, some receive formula milk, and some are fed both ways. The mother's milk obviously offers a unique cocktail of nutrients and particular substances that can also have an effect on the gut microbiota. Some ssubstances, called "antimicrobials", are capable of killing microbes or of counteracting their growth; other substances, on the other hand, benefit them. Mother's milk provides an ideal blend of all these components, and that's not all. It has been shown that this blend can send to the newborn's gut an impressive team of microorganisms, which work together to optimally exploit the mother's milk for the child's necessary metabolic processes, for their growth and for the promotion of an efficient immune system. In particular, in order to be efficient, the immune system must be able to modulate its responses to stimuli in the best possible way, without exaggerating, so that it can tolerate everything that is not dangerous.

But that's not all. Apparently, breast milk also contains bacteria. However, it is not yet known precisely how they get there. May the very mammary gland that produces milk be involved? If so, the origin of these bacteria would be in the mother's intestine, where special "mononuclear cells" would carefully choose the bacteria to be captured and transferred to the breast, from where they would then enter the milk. An alternative hypothesis suggests that the bacteria present in breast milk instead come from the infant itself. In this case, bacteria present in the child's oral cavity, or on the skin, are thought to sneak into the milk ducts and rise up, so to speak, in the opposite direction. Eventually, although coming from the outside, they would still be able to find their way into breast milk. Despite this, bacteria have also been found in the breast tissue of women who have never breastfed in their life, which clearly goes against the idea that the bacteria come from the newborn. However, as said, we are still not certain about this.

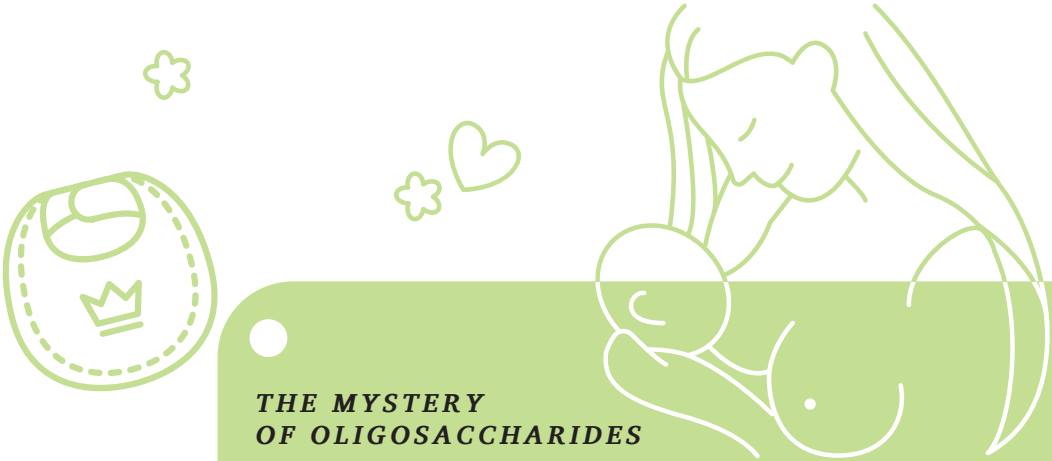
Setting theories on bacteria aside, it has been proved that other components in breast milk can affect the infant's microbiota. We are

referring, for example, to oligosaccharides, which are the third most abundant component in breast milk after lactose and fats, and can affect the growth and function of the microbiota. One litre of human milk ready for drinking contains 5 to 20 grams of these complex sugars, and even greater quantities are found in colostrum.

But what is colostrum?

Due to its different composition, the liquid produced by the mammary gland in the first days of breastfeeding is not called milk, but colostrum.

If the mother does not breastfeed in this period, or if feeding is mixed, something necessarily changes. The different composition of formula milk compared to human milk promotes different quantities and compositions of nutrients and bacteria, and therefore a different microbial colonization of the infant's gut. In the faeces of breastfed animals we find more bifidobacteria and lactobacilli, microorganisms capable of providing benefits for humans. In addition, the inhabitants of the gut of breastfed animals are more varied, and are dominated by staphylococci, bacteroids, clostridia, enterococci, enterobacteria and the genus *Atopobium*. The short-chain fatty acids mentioned above, for their part, being produced by intestinal bacteria, display different concentrations in the faeces of breastfed infants and of those fed with formula milk. Specifically, propionate and butyrate (stars in the world of short-chain fatty acids) are present at higher levels in infants fed with formula milk. Finally, the microbiota of the latter soon becomes similar to that of adults, faster than occurs in breastfed babies.



THE MYSTERY OF OLIGOSACCHARIDES

Oligosaccharides are molecules that belong to the group of carbohydrates, and are made up of few (the Greek word ὀλίγος *means "little") repeated units. These repeated units are called monosaccharides (*μόνος *means "only", therefore "one unit only"). Which monosaccharides combine to form the oligosaccharides found in breast milk? Glucose, galactose, N-acetylglucosamine, fucose and sialic acid. Combined in various ways, these five monosaccharides can give rise to more than a hundred different oligosaccharides. Oligosaccharides are able to reach the distal small intestine and colon intact, resisting the acidic environment of the newborn's stomach (low pH), enzyme digestion in the pancreas and the intestinal brush border. But why do they interest us so much? Because once the danger has been avoided, the oligosaccharides in breast milk can exert their prebiotic action undisturbed, actively stimulating the growth of some specific members of the infant's gut microbiota. Not anyone's, then. The oligosaccharides mainly, although not exclusively, encourage the growth of bifidobacteria, whose presence is more than welcome. It has even been observed that the abundance of microorganisms in the gut of breastfed animals, in particular of biofidobacteria, changes according to the oligosaccharides found in breast milk. This means that mother's milk is able to pilot, albeit indirectly and only partially, the*

development of the infant's microbiota by exploiting the substances it contains. Science tells us that there are links between the microbiota of breast milk (i.e. the microorganisms it contains), the composition of oligosaccharides and the gut microbiota of the infant. Furthermore, it seems that the effect of breast milk depends on its quantity! But that's not all: the microbiota of breast milk changes over time during breastfeeding, and also differs between mothers who breastfeed exclusively and those who breastfeed, but not exclusively. The gut microbiota of breastfed infants exhibits less variability than that of formula-fed infants. Breastfed infants in fact tend to have a more stable microbiota. Finally, scientists tell us that "the infant's feeding method also influences the gene expression of the host's intestinal epithelial cells". In practice, everything starts with the intestinal epithelial cells which densely line the entire internal wall of the intestine. Like all the other cells, they are alive and well. As in all cells which are alive and well, DNA commands and proteins follow orders. DNA provides cells with the information they need to correctly create proteins, whose job will be to perform the specific function that DNA already had in mind for them. Through these mechanisms, breastfeeding boosts metabolic functions and those related to the immune defences of the newborn.

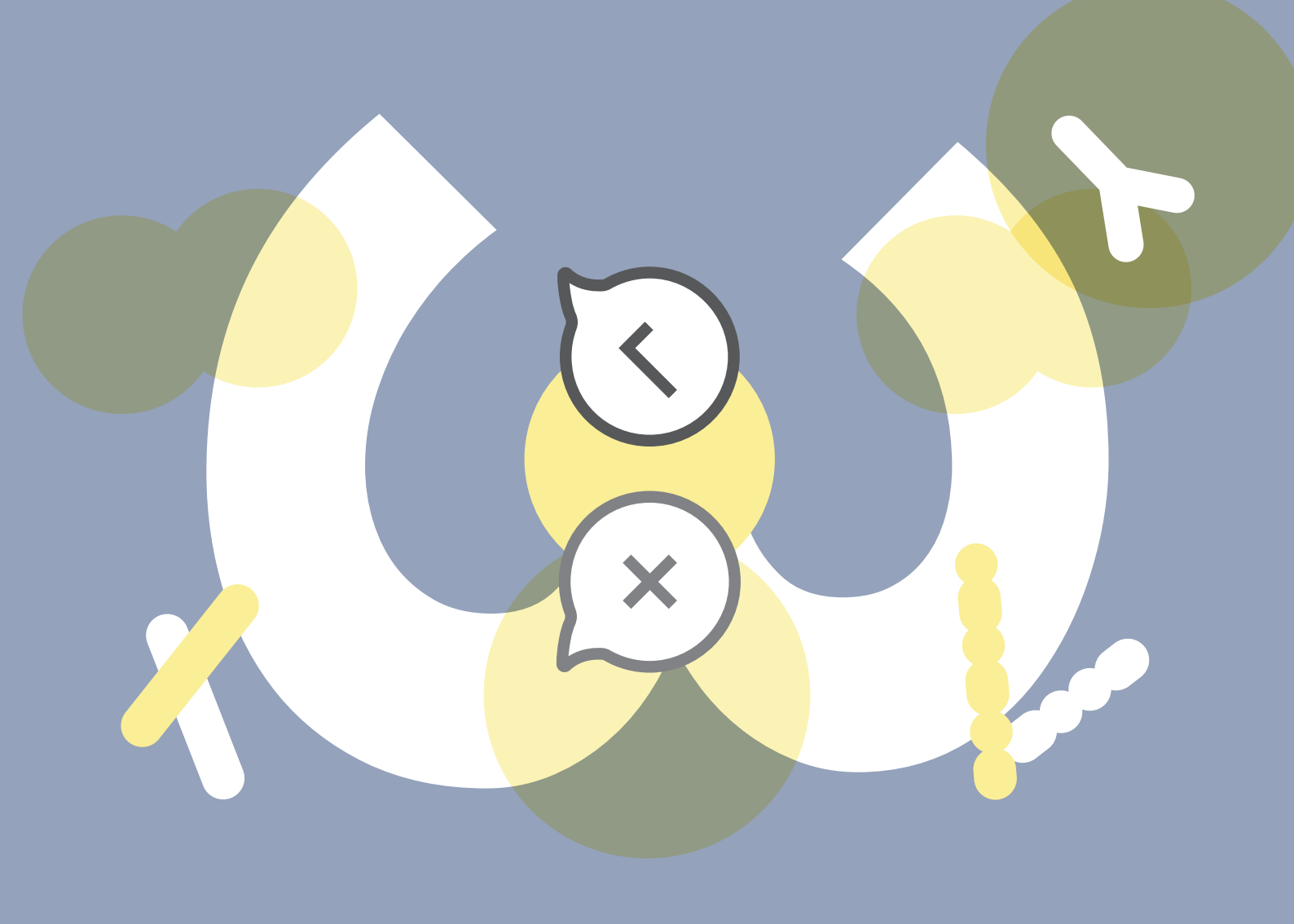
MUM, I'M BIG NOW, DO BACTERIA GROW UP TOO?

As long as the infant has an exclusively milk-based diet, the composition of their microbiota varies over time, becoming increasingly diverse until the baby is weaned, when it becomes more stable and complex, similar to that of the adult. During weaning, so-called microbial diversity, i.e. the number of species that populate the intestine, increases in the gut microbiota. *Proteobacteria* and *Actinobacteria* are replaced by *Firmicutes* and *Bacteroidetes*, which become the new dominant members of the microbiota. Comparing the microbiota to a company, we would therefore say that in the period between 9 and 18 months there are staff cuts and important new hires. Even when we say that a species is particularly abundant in our intestine, we are always speaking in relative terms, and mean that quantities are higher than those of other species. Therefore, the relative abundance of important bacterial families such as *Lachnospiraceae*, *Ruminococcaceae*, *Eubacteriaceae*, *Rikinellaceae*, *Sutterellaceae* and others becomes more significant in this period. On the contrary, we see a drop in the relative abundance of *Bifidobacteriaceae*, *Actinomycetaceae*, *Veillonellaceae*, *Enterobacteriaceae*, *Lactobacilaceae*, *Enterococcaceae*, *Carnobacteriaceae*, *Fusobacteriaceae* and *Clostridiales incertae sedis XI* (which sounds like the name of a pope, but isn't). In short, Latin names aside, what we must remember is that some microorganisms come and other microorganisms go.

But why precisely at this stage?

Well, everyone has their own preferences in terms of food! And why shouldn't this also hold true for the microorganisms that live inside us? During the complementary feeding phase, the newborn begins to take in more proteins with food, which could attract bacteria from the *Lachnospiraceae* and *Bacteroidetes* families, and ward off bacteria that eat sugars (saccharolytic bacteria), such as *Bifidobacteriaceae*. At this stage, food fibre is also a new factor, and is associated with higher levels of Prevotellaceae and Firmicutes. Taken together, these new molecules result in a rapid increase in microbial diversity.

It is precisely in this phase of the child's life that the microbiota, previously acquired from the mother, is shaped and made similar to that of an adult. Interestingly, two other species also begin to increase, *Faecalibacterium prausnitzii* and *Akkermansia municiphila*, which are usually absent, or only present at very low levels, during early infancy. At 12 and 24 months respectively, the relative abundance of these two species rises to typical adult levels. Another substantial change that characterizes weaning is the transition from human milk to cow's milk. Already a few days after the cessation of human milk intake, there is an increase in the relative quantities of *Bacteroides*, *Blautia* and *Ruminococcus*, among others, and a decrease in the relative quantities of *Bifidobacterium*, *Lactobacillus* and *Enterobacteria*. Microbial diversity (the number of species residing in the gut) and faecal pH also increase. And to refresh the memory of those who may have heard of it in school, remember that a higher pH simply means a less acidic stool. In short: in this phase microbial diversity grows. This is also followed by changes in terms of functions: we see an increase in the total levels of short-chain fatty acids, the degradation of complex carbohydrates, starches and xenobiotics (as all substances foreign to our organism are classified as either natural or synthetic), and increased vitamin production. The microbiota, previously acquired from the mother, is then shaped and made similar to that of the adult, to the point that we can identify the enterotype to which it "belongs". What is it? Scientists classify the gut microbiota of adult individuals into three groups (*Prevotella*, *Bacteroides*, *Ruminococcaceae*), called enterotypes, which depend on the species present in the microbiota, and which we will examine later. Furthermore, a microbiota similar to that of the adult is functionally more complex, and structured to metabolize polysaccharides derived from plants, providing mutual benefits for the host and the bacterium. Polysaccharides are molecules which, like oligosaccharides and monosaccharides, belong to the group of carbohydrates, but which are characterized by their larger size and considerable structural complexity.



GOOD AND BAD: HOW THE MICROBIOTA CHANGES IN THE COURSE OF OUR LIFE

IS THE MICROBIOTA THE PHILOSOPHER'S STONE OF OUR TIMES?

The transition from adulthood to old age (approximately from 65 to 70 years) is the second phase with a significant impact on the gut microbiota, which has to adapt to profound changes in the organism caused by ageing. In this context of great change, the microbiota struggles to remain stable and it becomes essential to try and maintain its biodiversity. In the elderly, the gut microbiota is likely to change in terms of quality and quantity (a phenomenon called "dysbiosis"). This may be primarily due to dietary changes, which can easily occur at this delicate time in our life. We often witness a reduction in the consumption of proteins and fibre, which may also be accompanied by dehydration. The elderly also find it hard to chew, which does nothing to aid their nutrition, and their gut becomes less efficient in absorbing nutrients. These conditions have a cumulative effect and are sometimes so serious that they lead to malnutrition! Furthermore, many elderly people no longer live at home, but are forced to move to a retirement home, or to spend long periods in hospital. Moreover, the elderly, for reasons we will not go into, often suffer from an underlying proinflammatory state. All these factors lead to alterations in the gut microbiota which, in turn, expose them to greater risk of disease. In particular, there is a relative reduction of protective and anti-inflammatory bacteria in the gut (for example *Akkermansia municiphila*, *Faecalibacterium prausnitzii* and *Bacteroides fragilis*) and an increase in aggressive pathobionts (*Clostridium*, *Actinobacteria* and *Proteobacteria*). Last, but not least, is the effect that the gut microbiota has at a cognitive level. In a later chapter we will discuss this issue at length, but for now let's just say that gut dysbiosis seems to potentially cause the cognitive changes characteristic of the neurodegenerative diseases of the elderly. Numerous studies have in fact shown a link between gut dysbiosis and psychiatric conditions such as autism and schizophrenia, or neurodegenerative diseases, such as Parkinson's and Alzheimer's. But in light of these data showing the damage that

dysbiosis can cause, may we suppose that maintaining normal homeostasis can help us age healthily?

*** FACTORS THAT DESTABILIZE THE MICROBIOTA IN THE ELDERLY**

– dietary changes
– dfficulty in chewing
– intestine less efficient in absorption
– life in a care home/long-term hospital stay
– basic proinflammatory state

WHAT IS AGEING?

We always heard our grandmother complain of wrinkles and pain in her hands that prevented her from knitting. We have all seen an elderly man struggling to get on the bus, although as a young man he too, like all his peers, “could jump over ditches”. But why is he no longer able to do so? A doctor’s answer would be that “ageing is characterized by a physiological and progressive reduction of the functional reserves of organs and systems”. In other words, human beings, like everything else for that matter, are affected by the passing of time. The kidneys begin to lose some efficiency, the heart and blood vessels undergo alterations that worsen their functioning. The nervous system is no longer at its best and, as they say, people begin to have the occasional “senior moment”. In addition to all of this, our immune systems also age. The elderly are more exposed to disease, certainly more than a young man of twenty, who comes out of the nightclub in a T-shirt in the middle of winter and at worst catches a cold. In the elderly, on the other hand, we see a situation known as “immunosenescence” which is scientifically described as “reduced activity of the innate and adaptive immune system”.

HOW IS THE PHENOMENON OF IMMUNOSENESCENCE EXPLAINED?

When we talk about the immune response, we can distinguish between innate and adaptive response. Their names provide a clue as to their function.

Basically, innate immunity is part of the baggage we carry with us from birth, and does not need to learn how to behave.

Adaptive immunity meanwhile, is acquired over time. How? One possible way is known as natural and active: the immune system tends to have a good memory, which sometimes prevents it from getting fooled twice by the same disease. Another way of acquiring adaptive immunity is also natural, but passive: the mother passes to the fetus some weapons (to be scientifically precise, some antibodies), pre-packaged and ready to strike the target for which they were created, despite the fact that the child has never encountered it before. The third and final method of acquisition is instead the result of human intervention and is therefore called artificial. Let's talk about vaccines, for example, which in a certain sense "show us an identikit of the enemy" and warn us against it. Various factors are involved in innate and adaptive immunity, and each performs its function with a view to cooperation and collaboration. In the elderly, however, both types of immune response display the reduced function that we call immunosenescence. While in conditions of normal efficiency the immune system reacts to certain factors, called "mitogens", with a proliferative response, in the case of immunosenescence this response is reduced. Similarly, the activity of cells such as Natural Killer and T cells, which participate in innate and adaptive immunity respectively, is also reduced. There is also a relative increase in proinflammatory compared to anti-inflammatory cytokines. The term "cytokines" groups proteins that may differ significantly from each other in terms of function and origin. Among those produced by the cells of the immune system, some play a major role in regulating inflammation. Under physiological conditions, regulation is also guaranteed by the correct balance of anti- and proinflammatory cytokines. Under conditions of immunose-

nescence, the imbalance in favour of proinflammatory cytokines (for example TNF-α, IL-6 and IL-8) causes a state of chronic inflammation. In short, the elderly organism does not fare as well as that of a young person. Still, some worsening of conditions is physiological. "Physiological" is the scientific term used to indicate that a slight decline is also normal and ultimately inevitable. All in all, if we manage to stay in the physiological range we have nothing to complain about. The important thing is to try and avoid "pathological" events. It is in this context that the concept of "frail elderly person" comes in, which in medical terms means a clinically unstable individual of advanced age suffering from multiple chronic diseases, who often also has to live with socio-economic problems such as loneliness and poverty. Faced with an ageing population and an increasing average life span, scientific research has responded by broadening its interests to include the promotion of healthy ageing! And this explains why research has turned its gaze towards the microbiota, trying to understand its potential role in this phase.

*

VARIOUS TYPES OF IMMUNITY

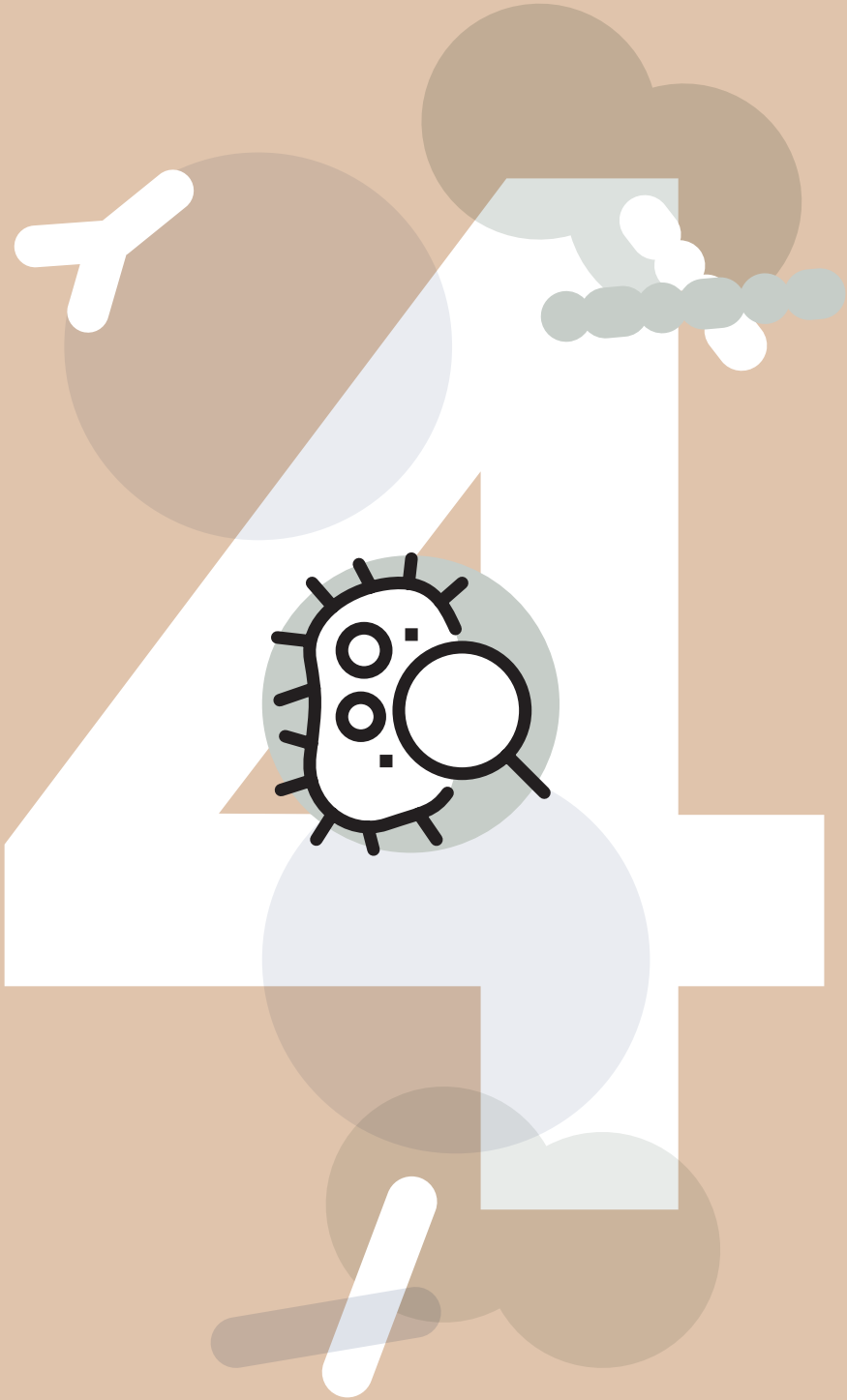
– Innate immunity: *we have this since birth, and it doesn't need to be learned*

– Adaptive immunity: *this is acquired over time and may be of 3 types.*

1. Natural active: *the immune system remembers what has already happened in the past and doesn't repeat the same mistakes.*

2. Natural passive: *based on antibodies transmitted directly from the mother to the fetus.*

3. Artificial: *resulting from human intervention (as in the case of vaccines).*



UNIQUE, IRREPLACEABLE AND FUNDAMENTAL: IDENTIKIT OF THE MICROBIOTA

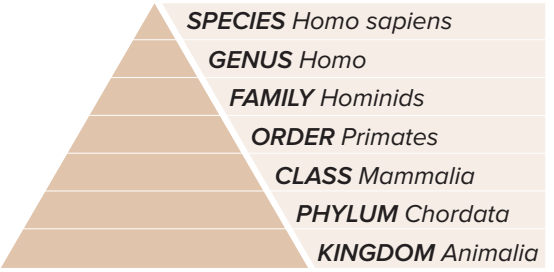
HOW MANY MICROORGANISMS REALLY LIVE IN THE HUMAN BODY?

Science tells us that the gastrointestinal tract is the "most densely populated" district, with about 10^{11}-10^{12} microorganisms per millimetre! The total is a difficult figure to imagine: 100,000 billion microorganisms – that's how many "microscopic hosts" can inhabit the intestines of each one of us! To get an idea of what this figure means, just consider that the global human population is under 8 billion! But let's get back to the invisible level. Of these, 100,000 billion microorganisms, about 70-80% belong to one of only two groups (or to put it in scientific language, *phyla*): *Firmicutes* and *Bacteroidetes*.

But what does "phyla" mean?

Science uses a highly hierarchical classification system for organisms. At the bottom, the groups are fewer and less selective than those that one encounters continuing through the hierarchy, as the criteria become gradually more restrictive. *Phyla* are nothing more than a very precise hierarchical level of classification. *Firmicutes* and *Bacteroidetes* are certainly not the only phyla whose members contribute to form the gut microbiota, but they are undoubtedly numerically predominant. The other *phyla* present include *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria* and *Fusobacteria*. But bear in mind that these are just a few examples...

✱ THE HIERARCHY OF ORGANISMS



HOW IS THE WORK OF MICRO-ORGANISMS ORGANIZED?

"No one can whistle a symphony. It takes an orchestra to play it."
H.E. Luccock

Every microorganism found in our gut performs the functions typical of its species in order to survive. However, we can imagine each species as part of a team, the gut microbiota, where they each contribute and collaborate with the others. As a result, the gut microbiota will be able to perform tasks that none of the species comprising it would be able to carry out independently. This is a symphony of functions, the result of a well-orchestrated team. If we think about it, a similar situation occurs in each of our organs. The heart, for example, pumps blood throughout the body, but for this to happen, muscles, valves and many other structures need to coordinate precisely and effectively. In the same way, the gut microbiota can, indeed must, be imagined as a real organ which, although not native to the human body, is able to perform specific functions important for our well-being, as a result of the effective cooperation of various species. "Species-specific" functions depend on differences in the DNA between one species and another. If we add up all the genes of the different species present in our gut, we have a total of about 3.3 million. Taken together, these genes make up the genetic pool of the gut microbiota, so special that it has a name of its own: microbiome. This genetic richness is bound to result in an equally rich pool of metabolites (products of the various metabolic pathways), which interact with our body's complex metabolic networks.

BUT WHAT DO MICROORGANISMS DO EXACTLY?

We said that the microbiota also works to our advantage. But can we state clearly what it does? Let's look briefly at some of its roles; later we will have a chance to examine each function individually.

It helps bones grow and become stronger. The microbiota is in fact comfortable where it is and has no intention of retreating to make room for new invading microorganisms, with which it is in fierce competition. It thus also counteracts the invasion and/or spread of pathogenic species, which can potentially cause disease in humans. Furthermore, scientific studies have shown that the gut microbiota is also able to modulate and activate the immune system during its development. By also establishing a delicate balance with the intestinal epithelial cells themselves, the microbiota plays a protective role, capable of limiting the production of toxic substances associated with an imbalance at the immune (and neurovegetative) level. In the gastrointestinal tract, the microbiota also affects intestinal motility and digestion. Our bodies can supply the bacterial microflora with nutrients for its sustenance. In turn, the microorganisms deal with breaking down and processing some food residues for us that we would otherwise be unable to process. By fermenting fibres and breaking down protein compounds, our gut microbiota produces short-chain fatty acids, a source of nourishment for the colon cells. These processes, moreover, produce vitamins and other compounds, which can process drugs, counteract the proliferation of pathogens and, as we have already mentioned, contribute to the development and growth of the cells in our body's immune system. Furthermore, our gut microbiota provides an important contribution to metabolising the fats we ingest, and is even able to stimulate angiogenesis (i.e. the formation of blood vessels). We should also consider that there are continuous exchanges between the intestinal lumen and the area "behind" the intestinal wall, and that the microbiota also influences them affecting both the secretion of substances in the intestinal lumen, and the permeability of the intestinal wall.

As if that were not enough, the microbiota may also be able to influence communication between the intestine and the brain by intervening in the mechanisms of so-called "visceral sensitivity", which is responsible for receiving and transmitting stimuli from the internal organs, as we will discuss in detail later. In short: our bacteria could affect mood and metabolism.

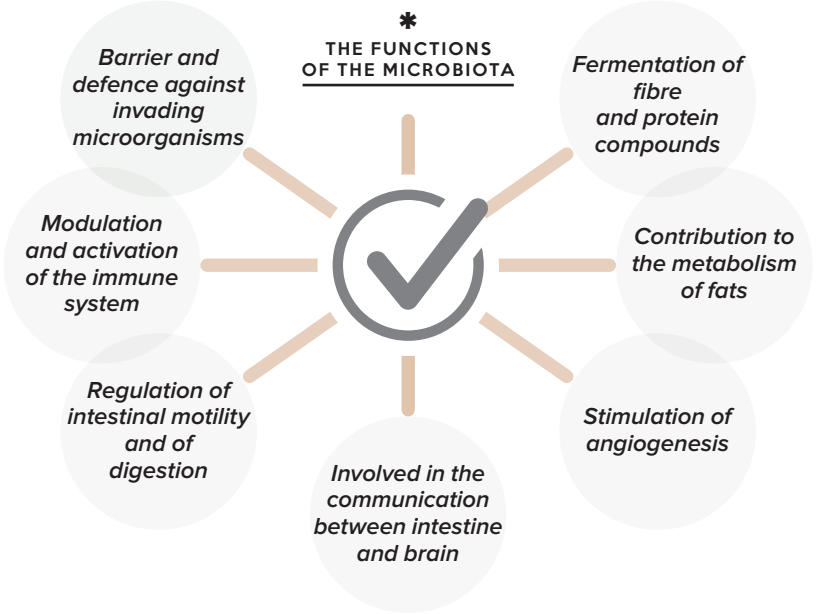
THE SUBSTANCES PRODUCED BY THE MICROBIOTA

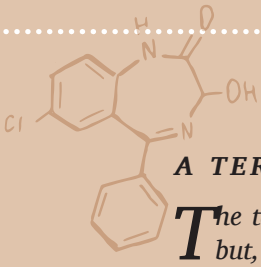
So, when it is in a state of equilibrium (called "eubiosis", as we will explain in greater detail below), our microbial gut flora contributes to our well-being in a multitude of ways. First of all, it is able to produce a wide variety of molecules that perform an equally wide range of different functions:

- *growth factors, which act at the level of the intestinal epithelium (a layer of cells that lines the intestine, as we will see in detail later);*
- *antibacterial substances, which protect the wall of the intestinal canal and the structures attached to it (collectively called the "intestinal barrier") and maintain its integrity (we will discuss this in detail too);*
- *neurotransmitters and hormones, that is substances the nature of which we will examine below. For the moment, we will merely say that, by producing these substances, the gut microbiota influences the perception of pain, motility and intestinal secretion (every day, in fact, about 1.8 litres of intestinal juice flow into the intestinal canal).*

EUBIOSIS AND DYSBIOSIS: WHAT ARE WE TALKING ABOUT?

Eubiosis is defined as "a situation of qualitative and quantitative equilibrium of the species present in the microbiota". In order to achieve eubiosis, the various species that make up the gut microbiota must interact with each other and with the host organism, maintaining the latter's homeostasis. The Greek word eu means "good", and eubiosis benefits the health of our entire body. On the contrary, when the intestinal microbial community undergoes qualitative and/or quantitative changes, the associated functional mechanisms also change. In this case we speak of dysbiosis, where the prefix "dys" expresses distortion of the normal balance. This condition is often associated with human diseases such as obesity, diabetes mellitus, asthma, chronic inflammatory intestinal diseases, and neurodegenerative and psychiatric diseases. But what came first: the chicken or the egg? That is, is it dysbiosis that contributes to the onset of some diseases, or is it these diseases that lead to imbalances in the intestinal microbial flora (thus causing dysbiosis)? This is an extremely complex issue, and we still have some way to go before we can provide an answer!

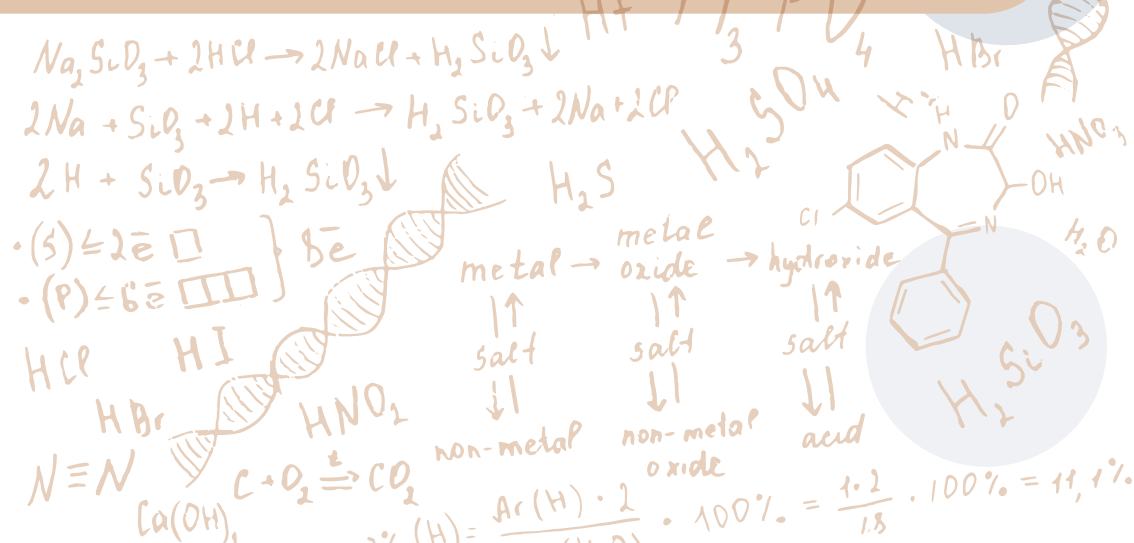




A TERM IN SEARCH OF AUTHORS

The term "dysbiosis" was first used by a German researcher in 1959, but, as often happens in these cases, it took some time to become popular, and only began to be more widely used in 2010. How can we date the popularity of a scientific term? Research is based on extensive collaboration between various laboratories, and therefore between various scientists. When a discovery is made, it is explained to the rest of the (scientific) world through the publication of articles in specialist journals. The goal of these articles, known as 'papers', is for researchers to share with their colleagues, even those on the other side of the world, what has been discovered, and how, so that everyone can take advantage of it in their work. To facilitate dissemination of these papers, various easy-to-use web portals hold huge archives of scientific literature. An example is the PubMed database, which allows users to subdivide articles by topic, or carry out searches by keywords.

So, let's go back to the story of our term. When looking for articles dealing with the concept of dysbiosis, we see that most of them date back to after 2010. The term "dysbiosis" is now widely used: the more authors talk about it, the more the issue under discussion is considered a hot topic, of increasing interest and relevance in the eyes of science (and the wider public).



HOW DOES THE MICROBIOTA RESPOND TO EXTERNAL FACTORS?

In the present context, “resistance” is scientifically defined as “the gut microbiota’s ability to remain stable in the face of disturbances from the outside world”. This ability is based on the collaboration established among the many microbial species present in our gut. The microbiota wants to resist, and must therefore remain united and compact in the face of disturbances. What the microbiota seeks to avoid is not only the proliferation of unwanted pathogenic microorganisms, but also the excessive multiplication of microorganisms of some species, whether pathogenic or non-pathogenic, which may already be present in our gut, but which must not proliferate further. Biologically, this resistance is due to competition between different species. Basically, we may imagine that bacteria live peacefully in the intestinal loops, according to the concept of “we’re already here, and there’s no room for the rest of you”. However, this competition helps maintain the stability of the gut microbiota, even if the defence system does not always work, as happens in everyday life, at work or in the family. The important thing is knowing how to bounce back and react. Here resilience comes into play, which “defines how quickly and to what extent the microbiota will recover its composition following a disturbance”. In other words, resilience is the ability to deal with a challenge and restore the previous state, both in terms of the body’s inhabitants and of functions performed. In the course of our life (and obviously also that of our body’s invisible inhabitants), the microbiota is in fact continuously adapting, as we will discuss later, and dynamically responds to external challenges to ensure its host’s homeostasis. For example, studies have shown that the guts of people suffering from ailments such as inflammatory or autoimmune diseases – diseases in which, for various reasons, our own immune system attacks us, as if we were its enemies – display less variety of microbial species. For modern medicine it is therefore a priority to understand “the mechanisms through which the ‘healthy’ microbiota promotes a condition of stability and resilience”. If the microbiota is able to help achieve this condition, perhaps we can take a cue from it, implementing strategies

with the same aims, including precisely that of increasing our resilience to disease.

DOES THE MICROBIOTA HAVE "INFLUENCERS"?

Just like social media, albeit without smartphones and PCs, the microbiota too is subject to factors that "guide" its composition. The "influencers" in this case are those external factors capable of altering the quantitative and/or qualitative composition of the microbiota. The first that comes to everyone's mind, also because we all eat, is our daily diet. The human diet has changed over the centuries, depending on the historical period and the food and economic resources available. Since the gut microbiota is in direct contact with the food we ingest, it has been forced to adapt to changes in diet. Several studies have shown that food is able to modulate the microbiota even in the short term, for example as a result of weight loss diets. The microbiota responds rapidly to food-induced alterations, but in most cases is able to return to baseline once the diet is interrupted. As we will examine in greater depth in the chapter dedicated to food, the Western diet is rich in fats and low in fibre, and contrasts with the Mediterranean diet, which is instead rich in protective foods and beneficial substances with an anti-inflammatory effect on the body. Among the quality components of the Mediterranean diet we find various nutritional substances such as polyphenolic compounds, antioxidants, fibre, prebiotics and omega-3 fatty acids, which are increasingly arousing interest in the context of possible nutritional strategies for the future (so-called "functional food").

* THE INFLUENCERS OF THE MICROBIOTA

01 *Diet*

02 *Delivery method*

03 *Breastfeeding*

04 *Use of antibiotics*

05 *Female hormones*

DO THE METHODS OF BIRTH AND FEEDING AFFECT THE MICROBIOTA?

Science has no doubt (and at this point neither do we). As we have already seen, the type of birth and feeding will affect the baby in its first months of life and in the future, during its growth. Childbirth and feeding can therefore be seen as influencers! Through delivery, the newborn baby comes into contact for the first time not only with the outside world, but also with the maternal microbiota, which will be different depending on the type of birth (natural or caesarean). Infants delivered by caesarean section acquire the bacteria present on the mother's skin (for example *Staphylococcus*, *Corynebacterium* and *Propionibacterium spp.*), and the composition of their newly formed gut microbiota will be negatively affected in terms of both quality and quantity. In natural birth, on the other hand, the newborn's microbiota is characterized by the bacteria of the maternal vaginal flora (*Lactobacillus* and *Prevotella*). All of this will subsequently affect the child's life and their response to stress. For example, the development and maturation of the immune system may be affected by the relative abundance of *Bifidobacterium*, making it less susceptible to chronic diseases such as asthma and inflammatory bowel diseases.

Once born, how is the baby fed?

Many studies have underlined the importance of breastfeeding both from a nutritional point of view and in terms of the psycho-physical well-being of the mother and fetus. Breastfeeding allows optimal interaction between mother and child, laying the foundations for a solid bond that will accompany the child until adult life. As we have already said, breast milk has many properties, which we will analyse in more detail later. Babies who are not or cannot be breastfed must be fed with formula milk. Numerous improvements have been introduced over the last few years to ensure correct balance in formula milk, although studies show that the guts of non-breastfed infants contain different bacteria to those who are breastfed. For example, *Escherichia coli*, *Bacteroides* and *Clostridioides* difficile are present in particularly high numbers. These bacteria, normally also present in healthy

microbiota, are prevalent in children fed on formula milk, and these differences will accompany the child throughout their life.

WHAT DO ANTIBIOTICS DO?

"Use antibiotics well, considering the environment, the animal and obviously the human being within a single vision." This is the modern approach, which aims to counteract resistance to antibiotics by pathogenic bacteria. In general terms, of course, this is of great concern, especially for people who need targeted treatments capable of defeating infection. Pathogenic bacteria, those that cause diseases, in fact learn to defend themselves over time. Sometimes they produce enzymes capable of destroying the drug taken to do the same to them. In other cases, they act more subtly, preventing the antibiotic from reaching its target, by modifying it or creating "traffic disruption" inside the body. As if that were not enough, they are able to exploit metabolic pathways that allow them to bypass the effect of the drug. These bacteria, then, are shrewd, chameleonic and also mutually supportive – since they pass these strategies on to their descendants and also to the bacteria living around the resistant germ –, and thus sometimes manage to get the better of antibiotics. So far, however, we have been talking about general health. The situation changes when it comes to the gut microbiota. First of all, antibiotics are molecules that are often eliminated through the digestive tract and which are therefore also able to affect the gut microbiota. First of all, antibiotics are molecules that are often eliminated through the digestive tract and which are therefore also able to affect the gut microbiota. For example, antibiotic therapy can lead to intestinal dysbiosis and diarrhoea. In some cases it can also fail to be sufficiently selective, affecting "good bacteria" and compromising the intestinal balance. In these situations, pathogenic microorganisms such as Clostridioides difficile, normally present but kept under control, can take over, and cause infections. The problem of antibiotics affects people of all ages, from the elderly to children. But let's try to look at things from the perspective of the microbiota! The administration of antibiotics to children (often for infections such as those of the ear)

causes the premature destruction of their intestinal microbiota, consequently damaging some of the mechanisms fundamental for proper growth. Furthermore, in the child, the microbiota is developing jointly with the immune, nervous and endocrine systems: any alteration of the interaction between these systems will lead to changes in the normal development of the gut microbiota, with a predisposition in adulthood to the development of various diseases. Let's be clear: antibiotics are really important drugs that can save lives. They should not be demonized, but must be used appropriately. Some of them, in particular Rifaximin, have even been found to exert a positive effect on microbiota balance. Rifaximin is an antibiotic that is not absorbed in the intestine, but which can interact with and modulate the composition of the gut microbiota.

DO FEMALE HORMONES AFFECT THE POPULATION OF THE DIGESTIVE SYSTEM?

The answer is definitely “yes”. There are phases in a woman’s life in which the body undergoes significant hormonal remodulation. Let’s look at an example. Think about what happens during menopause, characterized by the ovaries’ loss of their reproductive function. There are obviously changes in hormone levels, with a reduction in circulating oestradiol and an increase in follicle stimulating hormone (FSH). Correlated symptoms can vary greatly from woman to woman in both intensity and type. However, they generally consist of sleep disturbances, hot flushes (vasomotor symptoms), urogenital atrophy (inflammation), headache, breast pain (mastodynia), mood changes (anxiety and/or depression) etc. During menopause, in addition to metabolic, cardiovascular and immunological alterations, the reduction in oestrogen levels causes intestinal dysbiosis. More generally, oral, intestinal and vaginal microbial flora in women are strongly affected by hormonal changes, and in particular by reduced circulating oestrogen levels. It is in fact known that oestrogens can affect the gut-immune axis, altering the interaction of the microbiota with the immune system. We should also consider the possible role that the microbiota could play in the onset of some conditions to which women are particularly prone, especially those linked to autoimmunity, where the body’s defensive system is erroneously unleashed on its own cells or tissue, considering them “foreign”. Women are in fact more predisposed than men to the development of systemic lupus erythematosus (SLE), rheumatoid arthritis and Sjogren’s syndrome, but also to chronic inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease. In all these conditions, alterations of the intestinal microbiota have also been found. Not surprisingly, if autoimmune diseases appear, they often do so at two important stages in a woman’s life: around the onset of menstrual cycles, or at any rate at a young age, and with the approach of menopause.

Intestinal dysbiosis also predisposes to the development of vaginal infections such as bacterial vaginosis and candida vulvovaginitis. The use of probiotics has also proved useful in these cases: the administration of *Lactobacillus* restores the vaginal microbial balance and prevents the onset of genital tract infections during menopause.

But how do Lactobacilli have a protective effect?

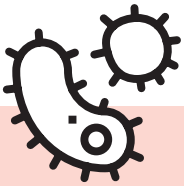
The vaginal microbial flora, under the influence of oestrogens, is usually colonized by *Lactobacilli*, which, by breaking down glycogen and producing lactic acid, maintain a low vaginal pH and protect against the development of infections. In menopause, the decrease in the level of circulating oestrogens also reduces the level of *Lactobacilli*, leading to increased colonization by pathogens (*Enterobacter*, *Escherichia coli*, *Candida*, *Gardnerella*). Furthermore, during menopause, due to the lack of oestrogens in circulation, women are more prone to osteoporosis, resulting in the increased risk of fractures observed over the age of 50. Some studies have shown that the administration of probiotics containing *Lactobacillus helveticus* and *L. reuteri* restores microbial balance and thus reduces the risk of fractures. The microbiota, therefore, plays a crucial role during menopause, a crucial phase in a woman's life, also in relation to the changes that the body undergoes as a result of decreased oestrogen levels. Obviously, in terms of prevention, careful nutrition, the introduction of prebiotics and probiotics in the diet and physical exercise can aid the recovery of normal microbiota composition if this should prove necessary, promoting well-being.


PASS

DOCUMENTS, PLEASE. MEET THE BACTERIA THAT LIVE INSIDE US

WHAT ARE WE DOING TO GET TO KNOW "OUR" BACTERIA?

To date, it is estimated that every human being can host over 1,000 different species in their gut! In the past, the only possible techniques for counting species necessarily required the cultivation of bacteria through so-called "cell cultures". Cell cultures? Yes, that's right. Growing bacteria basically means choosing the right "soil", providing the necessary nutrients, "planting" a few bacteria and waiting for more to grow. However, we don't go and plant the bacteria in the fields of the Po Valley: the growing medium is not actual soil and, moreover, much less space is needed. We are talking about culture medium, which contains a mixture of ingredients specifically chosen for the bacteria we are interested in, of both solid and liquid consistency, as appropriate. The important thing is to get the right mix of ingredients to allow the growth of the bacterium we want to see proliferate. Are you wondering if there is a universal formula that can make any bacterium grow? No, of course not? Just like plants, different bacteria need different nutrients, different temperatures and growing conditions. The problem is that we don't always know the best growing conditions



QUICK "GARDENING" COURSE

Where do we plant bacteria? If the culture medium is of solid consistency, we usually use plastic or glass plates, called "Petri dishes", which are sterile and resealable with a lid. In the case of liquid media, on the other hand, we can use special containers, usually made of glass, called "flasks", whose size varies according to needs.

for each bacterium! Sometimes, we lack the secret ingredient that allows the proliferation of a certain type of bacterium, but no one has yet managed to identify it. Consequently, even by planting everything we find in the intestine, not all the bacteria actually present will be able to grow in artificial cultures, because the researcher will have unknowingly failed to provide some necessary element. This is why, as long as we relied on technologies that inevitably required the use of cultures of microorganisms, there were "only" a few hundred species.

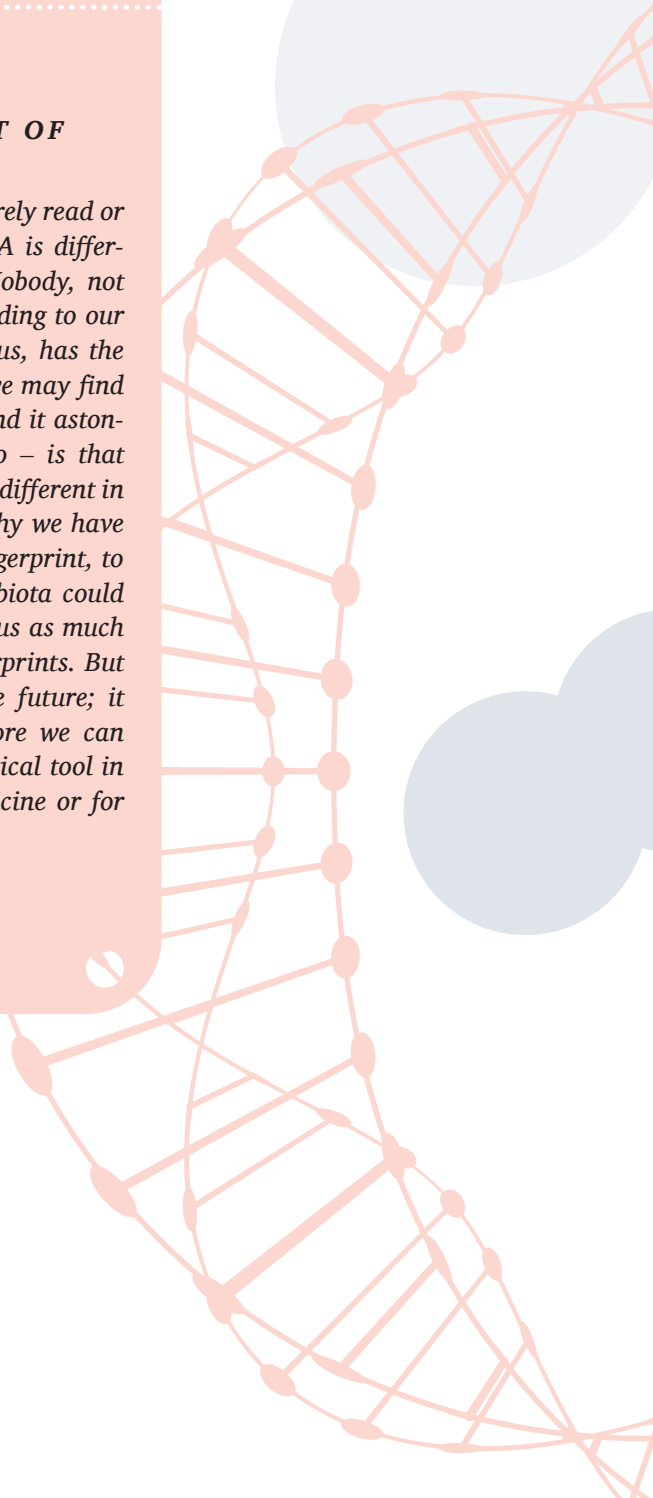
How are bacteria planted? Let's imagine we have some bags, with identical contents, full of unidentified seeds that we are unable to count, for some strange reason, for example a bet (not being able to count the seeds helps us to reproduce the situation in which scientists find themselves when they decide to grow the bacteria present in the intestine, but can neither see them nor count them). Let's then imagine preparing a field suitable for growing tomatoes, a soil suitable for growing lemon trees and many other soils, as many as possible, hoping to identify the nature of each seed found in the bags. We plant the seeds from each bag in each type of field. If lemons grow, it means that among our seeds we had one of lemon; if tomatoes don't grow, it means that we didn't have any tomato seeds! Yet, since we have planted all our seeds and we don't have any more in the bags, and since we didn't count them at the beginning, we can't know if all the seeds in the bags found a place to grow. In fact, there may be some seeds that fail to grow, because none of the soils are suited to their needs. The same happens to a scientist who tries to count the species present in the intestine by trying to make each of them grow in a specific medium.

HOW HAS TECHNOLOGY CONTRIBUTED?

As is now the case in any sector, recent innovative technologies have revolutionized the old system of counting species and have made it possible to identify over 1,000: although we are not yet able to grow them in our artificial media, we now know that they are part of the gut microbiota. In other words, you no longer reap only what you sow! It is also curious to note that the analysis of large numbers of human gut microbiota samples has led to the discovery of a wide range of new species which had never been identified in any other ecosystem. In most cases these newly identified species are strictly anaerobic, i.e. they do not survive in the presence of oxygen. They are also numerically much more abundant than facultative anaerobic species (present in their hundreds or thousands), which can, on the other hand, survive in both the presence and absence of oxygen.

THE FINGERPRINT OF THE MICROBIOTA

Many people have surely read or heard that the DNA is different in each one of us. Nobody, not even a sibling, who according to our aunt looks so much like us, has the same DNA as us. What we may find even more surprising – and it astonished many scientists too – is that the gut microbiota is also different in each one of us! This is why we have the term gut bacterial fingerprint, to underline that our microbiota could contribute to identifying us as much as DNA and actual fingerprints. But we are talking about the future; it will take some time before we can imagine using this analytical tool in the field of forensic medicine or for other applications.



SO CAN WE SAY "EACH TO HIS OWN MICROBIOTA"?

It seems that each of us has a unique microbiota, different from that of anyone else. But what if groupings could be created? Some scientists have proposed and shown the existence of "enterotypes" (similar to blood groups): all of humankind can be divided into groups, called enterotypes, precisely on the basis of the characteristics of their gut microbiota. In other words, we can classify humans into different groups, based on their bacteria! Who would have ever imagined it? To date, three enterotypes have been identified. But what characteristics of microbiota led to their classification? First of all, its composition. The three enterotypes identified in humans are therefore established on the basis of genus (a hierarchical level higher than species and lower than class) to which the majority of the bacteria present in each individual belong. In particular, it seems that in each one of us, one of the following is a predominant type, and therefore enterotype:

- *Bacteroides*
- *Prevotella*
- *Ruminococcus*

Predominant, of course, does not mean sole! What, then, of the other bacterial groups? There are hundreds of them, and they play an important role: all these groups become part of the microbiota in numbers such as to maintain a more or less constant numerical ratio with the genus characterizing the enterotype. Keep the enterotype in mind, because it could be of great help in the future in revealing some unresolved aspects of cardiovascular diseases, diabetes, or cancer, serving as a basis for targeted preventive strategies. Just think of heart failure, a condition in which the heart progressively loses its ability to pump blood round the body. A study recently conducted in Norway suggests lower biodiversity in the microbiota in subjects with heart failure compared to the healthy population, with a reduced ratio between *Firmicutes* and *Bacteroides*. This difference was found to be particularly significant when the imbalance was not caused by ischemia. When this result was correlated with nutrition, it was seen

that in the case of a high fibre diet, the biodiversity of the bacterial population increased, with increased levels of Firmicutes and a microbiota profile similar to that of healthy subjects. It's just an example, but it serves to show that scientific research now also focuses on the composition of the gut microbiota and its relation to the host's state of health or predisposition to certain diseases. At the moment, even leading microbiologists tell us that we are still a long way from obtaining a scientifically robust view of microbiota composition in health and disease. We are, then, currently unable to explain in detail the role of the microbiota in the health of its host or possible differences related to the enterotype to which it belongs.

WHAT LAWS APPLY IN THIS "JUNGLE"?

"For the strength of the pack is the wolf,
and the strength of the wolf is the pack."
R. Kipling

Rudyard Kipling, narrating the story of Mowgli, with the expression "Law of the Jungle" offers us a strict code of ethics and solidarity, the only way to maintain peace and balance in the ecosystem. In the ecosystem of the gut microbiota, the desirable condition of equilibrium is called, as we have already said, eubiosis. In order for this to be maintained, *eubiosis* is regulated and controlled by numerous relationships, including interrelationships between different bacteria, but also between bacteria and the intestinal epithelium, and between bacteria and the immune system. This interaction and cooperation between different members of the ecosystem allows the individual success of a single species, because, as we said, strength lies in the pack. Consequently, the resilience of one species may even depend on the "choices" of the others! For this reason, the scientific community agrees in saying that:

- in healthy conditions, the gut microbiota ecosystem is in a state of equilibrium (homeostasis);
- when it is in equilibrium, members of a limited number of species will be present in far greater numbers than those of other species, which may be more numerous, but are represented by fewer members. As we will see in the next section, however, this does not necessarily mean the latter are less important.

All this suggests that homeostasis in the bacterial composition of the gut microbiota is of utmost importance for maintaining the good health of this particular organ of our body and, as a consequence, of the entire human organism that hosts it.

But we are also used to associating the so-called “Law of the Jungle” with the infamous “law of the strongest”, and we certainly don’t want to play down this aspect. On the other hand, we have already touched on the concept of resistance to colonization by new microorganisms: bacteria that already reside in the intestine can, in fact, limit the entry of new tenants, and retain their own place by means of competitive mechanisms. Isn’t this the law of the strongest at work?

DO YOU REALLY NEED TO BE NUMEROUS TO MAKE A DIFFERENCE?

We have said that the enterotype is defined on the basis of the genus which predominates and is thus considered to be characterizing. This, however, does not mean that the most abundant groups are necessarily also the only important ones: we should never lose sight of the less numerous groups, which could prove to play a significant role in supporting the well-being of the host organism. Scientists are well aware of this, and the researchers who identified the existence of enterotypes themselves stressed that species or genera with high numbers of specimens alone cannot explain the functional complexity of the gut microbiota. Some “minor” species, where minor means “numerically less represented”, perform essential, beneficial functions for the body: those who make less noise do not necessarily have less to say. Some important functions may therefore be performed exclusively by poorly represented species. To give an example, the methanogenic bacteria (capable of producing methane) present in the gut microbiota are among the less abundant species, but their functions are precious for our well-being.

THE PIONEERS OF THE MICROBIOTA

"Whatever concerns health is of real public interest. I take advantage of this to make my address less arduous for you. I shall moreover use the opportunity to show you the practical value of pure research."
E. Metchnikoff, 11 December 1908

Science is made up of women and men, is made up of stages and is made up of names. In 1908, Elie Metchnikoff was awarded the Nobel Prize for medicine, for having discovered how immune system cells known as macrophages operate. During his Nobel lecture, he explained that if we take a group of subjects exposed to the same danger, it may happen that only some of them contract a given disease. His observations also led him to suggest the use of the bacterium Lactobacillus bulgaricum *as an agent capable of providing resistance against infectious bacteria. It was an extremely innovative and revolutionary idea, which for the first time suggested how the composition of the gut microbiota could significantly influence the susceptibility of different individuals to diseases.*

STRESS, ANXIETY AND DEPRESSION: LOVE, PASSIONS, PSYCHE AND MICROBIOTA

DO WE (ALSO) THINK WITH OUR BELLIES?

The intestinal barrier can be described in various ways. It is clearly an extensive interface with the external environment, involved in complex interactions with the gut microbiota. But that's not all. The intestinal wall can in fact also be considered, to all intents, as a second brain. Being a crossroads of elaboration and integration of various types of neural information, this takes the name of "enteric nervous system".

A LITTLE HISTORY

William Beaumont was an American surgeon known for his studies on digestive processes conducted on a patient (Alexis St. Martin) who, following a gunshot wound that never completely healed, had a fistula in his stomach, i.e. a direct link between this organ and the outside world. This "window on the stomach", gave a unique opportunity to directly observe digestive processes and gastric mechanisms. It was 1825, and for the first time it was possible to identify the effect of certain negative emotions directly on the functions of the stomach. About 100 years later, Stewart Wolf and Harold Wolff systematically studied a man (a certain Tom) in whom the presence of a gastric fistula made it possible to observe correlations between behaviour, emotions and gastric function.

"MENS SANA IN CORPORE SANO": IS IT TRUE?

The relationship between mind and body has been the subject of debate since Plato's time, and two main theories have been supported over the centuries: the monist theory, which denies any distinction between mind and matter, and the dualist theory, which on the contrary, supports the ontological difference between mind and body and the impossibility of dialogue between them. However, René Descartes himself (1596-1650), one of the main exponents of dualism, when addressing the mind-body dichotomy, admitted and envisaged the possibility of integration between the two. It is now widely known that our central nervous system can have complex effects on gut activity. Conversely, we know less about the opposite direction of communication, from the gut back to the brain, and it is on this intricate axis that we need to focus. Far from being a narrow one-way street, the gut-brain axis is more akin to a motorway, and supports two-way communication: not only can everything that occurs at the level of the central nervous system (for example stress, anxiety symptoms and depression) have consequences on the normal functioning of the intestine, but the balance of the central nervous system itself can be influenced by intestinal function, and by whether this is healthy or pathological. Furthermore, as is usual, in a relationship there are always two parties, and one makes up for the other's shortcomings! Experts tell us that "both the intestinal autonomic nervous system and the central nervous system provide a significant contribution. Indeed, if gut control by the central nervous system is reduced, a form of visceral control may come into play, in turn able to alternately modulate gut activity". The continuous exchange of information between the parties is carried out thanks to a dense network of mediators of various origins (typical of the nervous, immune or endocrine systems), which act as messengers to make communication possible even over long distances. Serotonin, for example, is a molecule known for its role in the central nervous system, but scientific evidence also shows that over 95% of serotonin is produced in the intestine, particularly in the "enterochromaffin cells" located along the gut mucosa.

DOES THE MICROBIOTA PLAY A CRUCIAL ROLE?

When we talk about the intestine and its mechanisms, we cannot ignore the gut microbiota. We don't wish to boost its "ego", but if gut function is able to influence the balance of the central nervous system, we expect that the microorganisms living in the digestive tract inevitably also play a role in this flurry of interactions. Research tells us that "the microbiota is able to influence complex higher functions such as mood and neurocognitive functions"! How can we be so sure that this is the case? First of all, it has been seen that the microbiota is altered in various pathological conditions, including irritable bowel syndrome and neuropsychiatric disorders. Moreover, some neuropsychiatric disorders could even be treated using two intervention methods that seek to modify the gut microbiota: psychobiotics and transplantation of the microbiota itself. The term "psychobiotics" refers to a therapeutic intervention based on the use of specific intestinal microorganisms, called "probiotics". Probiotics are by definition "living and vital organisms, which, when administered in adequate quantities, are able to bring a real benefit to the health of the host", potentially influencing their behaviour as well. The fact that psychobiotics can promote and restore mental health means that they are becoming increasingly important for therapeutic purposes.

DEAR MICROBIOTA, WHAT ARE YOU UP TO?

"Sorry, you know, I would never wish to disturb you…"
Tiziano Ferro

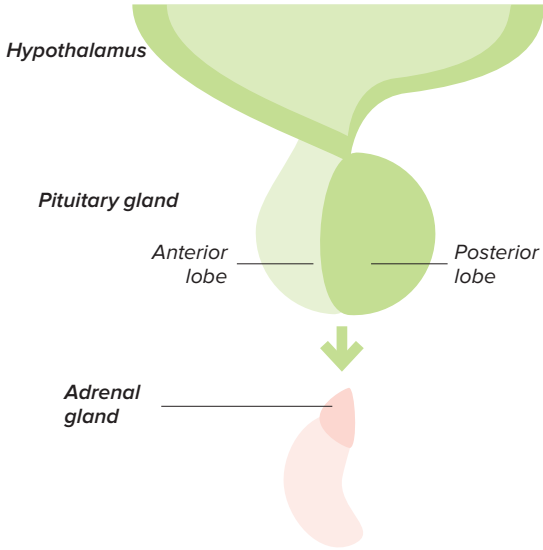
If it could, our microbiota would sing this song. Its intentions would undoubtedly be not to cause damage and, on the contrary, to help us as much as possible. The problem is that when something disturbs its equilibrium, it is thrown into confusion and, like it or not, risks making mistakes. When the biodiversity of the intestinal microbial ecosystem is altered, the overall genetic makeup of the microbiota, or the "microbiome", is also inevitably changed. Chronic stress, for example, can induce significant changes to the microbiome and, more generally, these alterations may depend on dysfunctional lifestyles, such as high-fat diets or the abuse of antibiotics. These alterations can contribute to the onset of problems of various kinds, from metabolic to neurodegenerative and neuropsychiatric disorders. The neuropsychiatric disorders for which an alteration of the microbiome has been described include Alzheimer's disease, Parkinson's disease, multiple sclerosis, major depression and schizophrenia. Experts tell us that "the analysis of the interactions between the microbiome and the central nervous system, is in fact beginning to provide a valuable contribution in the determinism of complex disorders that influence cognitive functions such as cognition, personality, affectivity and the emotional-behavioural sphere".

WHAT IS HIDING BEHIND MAJOR AFFECTIVE DISORDERS?

The “major affective disorders” are a large class of psychiatric disorders mainly concerning mood alterations and anxiety, distinct from the other large category, which includes “psychotic disorders”. While studies relating to the relationship between alterations in the microbiome and psychotic disorders are still in their infancy, there is instead greater evidence that the microbiome plays a decisive role in anxiety disorders and major depression (the most serious of the mood disorders).

No microbiota, no party

Have you ever heard that you don’t realize the true value of something until you lose it? This saying holds true in the world of science too! One of the approaches that researchers use to understand the functionality of a gene or molecule for example, is to create a model devoid of it, in order to observe what happens in its absence (speaking of cause-effect always requires some caution!). If I can’t explain the role of the ingredients in a recipe, I can try removing them one at a time: if I remove yeast from pizza, for example, the dough will be sub-standard, and the role of yeast will become clear! In a similar way, researchers may conduct their studies on rodents without a gut microbiota, in order to assess the possible consequences of its absence on our physical and mental health. In 2004, the *Journal of Physiology* published one of the first studies concerning the possible relationship between microbiota and mood disorders. In their work, the authors (Sudo and collaborators) highlighted that rats without a gut microbiota responded to stress abnormally, compared to animals with a normal microbiota. The authors analysed the activation of the hypothalamic–pituitary–adrenal axis, one of the pathways in our body that guarantees the transmission of information between points that may be distant from each other.



✱
HYPOTHALAMIC -PITUITARY -ADRENAL AXYS

In this case, as the name suggests, "at the top end" we have the hypothalamus, part of the central nervous system, which, mediated by the pituitary gland, coordinates the action of the adrenal gland (in particular its external portion, known as the "cortical zone"). The response of the cortical zone of the adrenal gland is expressed through the production of hormones, called "corticosteroids", each sent to perform a specific function. In brief, we need merely mention that the activity of the hypothalamic–pituitary–adrenal axis was found to be abnormal in rats without gut microbiota: the excessive activation of the hypothalamus at the top of the axis resulted in excessive hormone production lower down. The authors also noted that hyperreactivity of the axis was further accentuated by the administration of pathogenic microorganisms (for example *Escherichia coli*), while it normalized following the administration of non-pathogenic microorganisms (*Bifidobacterium infantis*). The researchers came to the following conclusions:

- whereas in rodents the activity of the hypothalamus-pituitary-adrenal axis depends on levels of stress, and considering that in the absence of gut microbiota the activity of this axis was abnormal, it is possible to deduce that the gut microbiota is essential for eliciting a normal response to stress;

- some pathogenic microorganisms accentuate an altered response, while others (non-pathogenic) are able to normalize it;
- moreover, there is a precise time interval after birth beyond which manipulation of the microbiota does not seem to produce effects in terms of reaction to stress.

A second study conducted by O'Mahony and colleagues confirmed the association between gut microbiota and the level of activity of the hypothalamus-pituitary-adrenal axis. The study showed that, in rats, early separation from the mother (considered a stressful event in the early stages of life) resulted in an increase in corticosterone production by the adrenal cortex. Given that the production of this hormone is the ultimate consequence of the passage of information along the axis, its increased production is an indication of increased activity of the hypothalamus-pituitary-adrenal axis itself. Among the consequences of increased corticosteroid levels was an altered immune response and – this is important – an alteration of the faecal microbiota. The study also highlighted the involvement of a series of mediating molecules which, just like in an assembly line, played their part once corticosterone had "pressed the start button". Among these, the authors found vasoactive intestinal peptide, serotonin, melatonin, gamma-aminobutyric acid, histamine and acetylcholine. Although these apparently complicated names have long been known to doctors and researchers, their role and the ways they interact in this cascading process have not yet been fully clarified. Finally, by studying rats with no gut microbiota, in 2013, Dinan and Cryan showed that some symptoms of anxiety and depression could be attenuated by restoring the regular composition of the microbiota. In particular, following the administration of *Lactobacillus sp.*, *Bifidobacteria sp.*, *L. helveticus*, *B. longum*, *L. rhamnosus* and *Lactobacillus farciminis*, there was a marked improvement in symptoms of depression and anxiety in rats.

Should alterations in the microbiome be a cause for concern?

As explained in the previous section, scientific research sometimes tries to explain the roles and functions of something (genes, molecules, microbiota, etc.) by analysing its absence. But a second possible approach is to study how the alterations of a certain element (a mutated gene, for example) may be associated with particular situations. Step by step, this helps to reconstruct its causes and consequences. As for the gut microbiota, studying its alterations means studying dysbiosis! Various studies have focused on the association between dysbiosis and mood disorders. An example is the case of lipopolysaccharide, a component of the outer membrane of some bacteria that is a powerful toxin for humans (known as an "endotoxin", since it acts only once it has entered our cells). In conditions of equilibrium of the microbial flora, the quantity of lipopolysaccharide of bacterial origin that enters the bloodstream of our body is kept at acceptable levels. In conditions of dysbiosis, plasma lipopolysaccharide release increases, also altering gut permeability and stimulating an inflammatory and immune response. As we have already seen, these two reactions are defence mechanisms that our organism activates to defend itself from any damage (physical, chemical or biological). In summary, the inflammatory response is non-specific: regardless of who the enemy is, it behaves in roughly the same way. The immune response, meanwhile, is prepared and directed specifically against the enemy at hand. One of the hypotheses regarding lipopolysaccharide suggests that among the consequences of increased lipopolysaccharide in conditions of dysbiosis is disproportionate, excessive inflammatory and immune responses, implemented through the release of substances such as proinflammatory cytokines and quinolinic acid, which are able to cause inflammation of the nervous system ("neuroinflammation"). The information that can be gleaned from the scientific research therefore shows that:

- lipopolysaccharide is able to influence the functioning of the central nervous system by increasing, for example, the activation

of specific areas of the brain responsible for emotional control. Among these is the amygdala (a complex almond-shaped structure);

- it has been confirmed that neuroinflammation plays a role in psychiatric disorders, particularly in major affective disorders. In patients suffering from psychiatric disorders, the normal parameters of inflammation are in fact altered in both the central nervous system and the peripheral nervous system; these alterations are correlated both structurally (alteration of white and grey matter) and functionally, with the disorder itself and its symptoms.

Furthermore, it has been seen that in conditions of dysbiosis, certain stimuli from the intestinal lumen are able to generate signals in some cells of the intestinal epithelium (the aforementioned enterochromaffin cells). When these signals reach the central nervous system, they affect functions such as the perception of pain, the immune response, and a person's emotional and behavioural reactions. In order to assess dysbiosis, we can look directly at the composition of the species of the microbiota. Recently there has been an increase of some species, such as *Alistipes* (from the *Bacteroidetes* strain), in the microbiota of subjects suffering from major depressive disorder, as well as in those suffering from chronic fatigue syndrome and chronic inflammatory bowel disease. These observations led to the hypothesis that the *Alistipes* species can alter the permeability of the intestinal barrier and induce an abnormal release of some "messengers" into the blood, such as proinflammatory cytokines, capable of influencing mood. Furthermore, it has been seen that the *Faecalibacterium* species is also associated with major depression: symptoms of depression seem to be more severe in those patients in whom *Faecalibacterium* specimens are less numerous. This and other clinical studies, therefore, demonstrate the existence of alterations in the composition of the gut microbial flora in patients with major depression.

Do the brain and drugs for the central nervous system affect the microbiota?

Research not only aims to understand how the microbiota can affect the central nervous system, but also the other way around. Emotional stress can in fact trigger changes in the composition of the microorganisms of the gut microbiota. The same also seems to apply to the alteration of circadian rhythms, that is our internal clock that regulates, for example, the alternation between sleep and wakefulness. This means that intense emotional states, psychological distress and chronic stress levels can induce hyperreactivity of the hypothalamic-pituitary-adrenal axis, damaging the gut-brain axis, abnormally increasing the permeability of the intestinal mucosa and activating an immune and inflammatory response. Numerous studies conducted on animal models, in particular on rodents, suggest a relationship between alterations in the gut microbiota, anxiety disorders and major affective disorders. Recent studies are increasingly demonstrating the relationship between alterations in the levels of some intestinal microorganisms, anxiety disorders, emotional dysregulation and altered mood regulation.

On the therapeutical front, let's assume, for example, that we are dealing with a drug, or a compound of a different nature, effective against a specific medical condition. If we see that that drug also acts at the microbiota level, isn't this perhaps an indication that the microbiota could be counted as one of the causes of this condition? In short, tell me what cures you and I'll try to tell you who you are! For example, melatonin is known for its ability to offer relief from stress, in both humans and animals. Studies have shown that the molecule modulates the composition of the gut microbiota, increasing, for example, levels of *Lactobacillus johnsonii* and *Lactobacillus reuteri*, while reducing those of *Prevotellaceae*. This raises the question: could melatonin reduce stress levels by acting on the microbiota? Let's take another example: ketamine is an antidepressant molecule that is able to counteract inflammation. Interestingly, it has been observed that its effect can also be mediated by the action performed on the gut microbial

flora. In particular, ketamine has been shown to increase the concentration of *Lactobacillus* (by 3.3 times), *Turicibacter* (by 26 times) and *Sarcina* (by 42 times) in rats. In the same study, a reduction was found in the concentration of opportunistic germs such as *Mucispirillum* and *Ruminococcus* (2.6 and 26 times, respectively). Furthermore, by administering some species of *Lactobacillus*, there was a significant improvement in some symptoms of depression; on the contrary, levels of species of the *Mucispirillum* strain were associated with greater intestinal inflammation (which obviously we don't want!).

So could probiotics be useful, then?

When we talk about "psychobiotics", we are referring to probiotics that can affect our mental health. Probiotics undoubtedly have a direct effect on the composition of the gut microbiota, and in addition, are also able to modulate the integrity of the gastrointestinal barrier. First of all, probiotics induce an increase in the production of mucin by the goblet cells (which we will learn more about below): if the mucus layer thickens, the barrier is less permeable and also more protected. In addition, probiotics make the barrier more closely-knit by strengthening the tight junctions and increasing apical adhesion between the cells. Finally, they are also involved in the modulation of immune and inflammatory responses. And in psychiatric disorders, the main focus of this chapter, what effects can they have? Some studies have analysed the effect of probiotics as modulating agents in anxiety and major depressive disorders. Pre-clinical studies (conducted on animal models) and clinical studies (on humans) help to form the scientific literature that associates probiotics and prebiotics with these medical conditions. Let's have a look at some of them together. Some studies in rodents have shown the potential of psychobiotics to reduce symptoms associated with major depression and anxiety. In combination with n-3 polyunsaturated fatty acids (known for example for their cardiovascular protective effect), it appears that psychobiotics are capable, among other things, of reducing the symptoms of post-heart attack depression (as a highly traumatic experience, a heart attack can also have repercussions on the patient's mental health). It was also

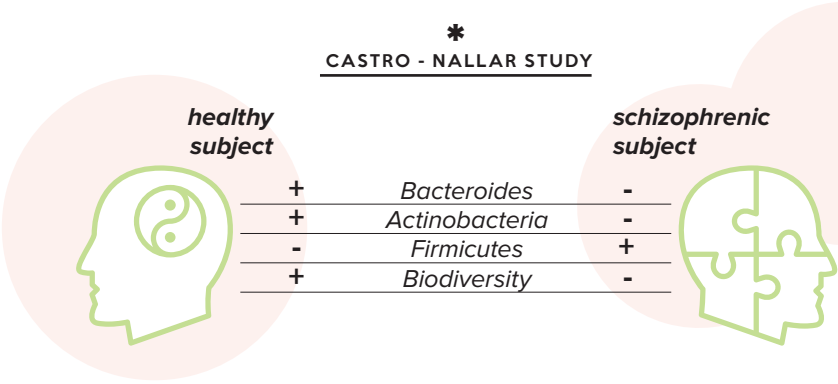
observed that the presence of some probiotics (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*) is associated with significant changes in cognitive symptoms and in the emotional sphere: the stress response is improved and anxiety is reduced. And what about human studies? There are still only few, and with modest results. In general, we can say that the scientific literature is accumulating data that correlate an improvement in mood or symptoms related to psychological stress to the administration of psychobiotics.

IS FAECAL TRANSPLANTATION A HOPE?

This is an extremely interesting area of research. Let's make this clear: we are not referring to the kind of operation – often life-saving – that makes it possible to replace an organ (or tissue) with another from the same individual or from a donor. If we consider the microbiota to effectively be an organ, why shouldn't we be able to transplant it? Well, we can! We are talking about a "faecal microbiota transplant", which is a therapeutic strategy used in the treatment of some disorders, including those of a neuropsychiatric nature. What does it involve? Balanced microbiota is taken from the faeces of a healthy subject, or from the same individual for whom the transplant is intended, who has previously "donated" their faeces. The goal is to restore the microbial ecosystem and homeostasis of the colon. One of the first faecal microbiota transplants dates back to 1958, by Ben Eiseman, who infused faecal material in four patients with pseudomembranous colitis, an inflammatory condition of the colon, often associated with infection with *Clostridium difficile*, a microorganism that is pathogenic for humans. Some therapies similar to a faecal microbiota transplant were already sporadically used in ancient times, but only after the pioneering experience of 1958 were attempts to treat infections with *Clostridium difficile* reported. By analysing the scientific literature available today, we see that it supports the hypothesis whereby microbiota transplant is effective in the treatment of recurrent *Clostridium difficile* infections. But what do we currently know about faecal microbiota transplantation as a therapy for neuropsychiatric disorders or, more generally, about the association between gut microbiota transplantation and psychiatric symptoms? Although still only adopted rarely, there are cases of autistic subjects in whom faecal microbiota transplantation has been associated with an improvement in specific symptoms. In addition, an association was observed between faecal microbiota transplantation and improved sexual functions and mood in patients suffering from Crohn's disease, a chronic inflammatory disease of the intestine. These data lay the foundations for further studies, with the aim of considering microbiota transplants a valid therapeutic tool for patients with major affective disorders.

IN THE MOST COMPLEX CASES, SUCH AS PSYCHOTIC DISORDERS, HAVE WE ANYTHING SIGNIFICANT TO SAY?

Psychotic disorders are a broad category of severe psychiatric conditions, including, for example, schizophrenia. Why are we interested in them here? Because associations with the microbiota have been seen here too! For example, according to some evidence, there is a correlation between psychotic symptoms, an abnormal immune response from the intestinal epithelium and altered composition of the gut microbiota. Significant differences have been found in the composition of the gut microbiota of schizophrenic subjects compared to healthy subjects, already at the onset of the disease. In particular, the differences included a reduced prevalence of *Lactobacillus* and *Bifidobacteria* species. Differences in the microbiota were also correlated with the severity of negative symptoms and reduced remission levels after 12 months, but were not correlated with the duration of treatment with antipsychotic drugs. In 2015, a study by Castro-Nallar and colleagues highlighted a higher presence of the *phylum Firmicutes* in schizophrenic subjects compared to healthy subjects, through analysis of the mouth and pharynx microbiome. Furthermore, in healthy subjects, the relative concentration of strains such as *Bacteroidetes* and *Actinobacteria* was higher than that found in schizophrenic subjects. Last but not least, the same study found differences in the biodiversity of the species present: healthy subjects were decidedly richer in the number of strains, but showed a decidedly less uniform distribution than subjects with schizophrenia. This and other studies therefore suggest alterations in the gut microbiota (and metabolic pathways) associated with schizophrenia.



In the midst of so many studies focused on bacteria, Yolken and collaborators, studying the oropharyngeal microbiome (located in the mouth and throat), focused attention on particular viruses capable of infecting bacteria, called "bacteriophages". These viruses are capable of altering the metabolism of bacteria and their ability to replicate. Among these, levels of the bacteriophage *Lactobacillus phage phiad* were found to be significantly higher in schizophrenic subjects than in healthy subjects. In cases of schizophrenia, levels of this bacteriophage appeared to be associated with metabolic disturbances and diabetes. Furthermore, these levels were even higher in schizophrenic subjects treated with valproic acid, a drug used in clinical practice, typically to stabilize mood. This therefore suggests that valproic acid is able to modify microbiota composition, which represents a good starting point for the hypothesis that some molecules, including sodium valproate, are able to modulate schizophrenic disease by acting directly on the composition of the gut microbiota. However, further studies are needed to confirm the existence of other compounds with the same properties, or others potentially even more suitable, for the treatment of schizophrenia.

Finally, in terms of research, as in the case of major affective disorders, also with regard to psychotic disorders some preliminary data support the use of probiotic compounds capable of modulating the gut microbiota and the body's immune response, attenuating some symptoms of these conditions. Most of the studies that analyse the relationship between psychotic disorders and probiotic administration have however been carried out on animal models. In order to establish in greater detail the role of the gut microbiota in complex conditions such as schizophrenia and other psychotic disorders, further studies with therapeutic agents capable of altering the microbiota, or modulating the levels of gastrointestinal inflammation, are therefore necessary.

A LAST QUESTION: COULD THERE BE A RELATIONSHIP BETWEEN MICROBIOTA AND AUTISM?

Among the topics that have more recently been the subject of psychiatric research are autistic disorders. Why do we mention them? Because, also here, alterations in the composition of the gut microbial flora seem to play an important role, albeit by means of mechanisms which are still unclear. Two recent studies from 2019 examined this relationship. Wang and collaborators noted in specific autistic subjects alterations both in terms of compounds (altered levels of glutamate and drastic reduction of 2-keto-glutaramic acid, a typical marker indicating the presence of autism spectrum disorders) and in terms of species composition in the microbiota. In autistic subjects there was also a potentially harmful increase in the strains of the species *Bacteroides vulgaris* and higher levels of *Eggerthella* lenta and *Clostrium botulinum* compared to healthy subjects. Liu and colleagues, on the other hand, showed that in subjects with autism spectrum disorders, in addition to alterations in the composition of the gut microbiota, there were also alterations in the short-chain fatty acids in the stool. Specifically, acetic acid and butyric acid levels were lower, and faecal valeric acid levels higher. In line with this, levels of species such as *Fusobacterium*, *Barnesiella*, *Coprobacter* and bacteria associated with valeric acid increased, while on the contrary, those of species such as *Rumenococcaceae*, *Eubacterium*, *Lachnospiraceae* and *Erysipelotrichaceae* decreased. After this list of names, all we can do is try to sum up the gist of the story! Modifying the composition of the intestinal microbiota, and in particular acting on those microbes capable of producing butyric acid, is currently one of the most promising alternative treatment strategies for autism spectrum disorders.

WE ALL LOVE SPORT... BUT WHAT ABOUT OUR BACTERIA?

HOW DOES PHYSICAL ACTIVITY HELP KEEP US HEALTHY?

The World Health Organization offers us nine simple but essential tips for healthy living. Here they are in no particular order.

1. *Eat healthily.*
2. *Don't smoke.*
3. *Don't drink alcohol.*
4. *Get vaccinated.*
5. *Practice safe sex.*
6. *Avoid stress.*
7. *Respect road safety rules.*
8. *Take care of your daily hygiene.*
9. *Engage in regular physical activity.*

In this chapter, we focus attention on the importance of physical activity in our life and on how the microbiota can also play a role in this area. Before examining specifically "mechanical" issues, let's listen for a moment to what the main organizations dealing with public health and prevention have to say. They tell us that a sedentary lifestyle is one of the top ten causes of death globally! Furthermore, a sedentary lifestyle also increases risk factors for "non-communicable diseases" such as cancer, diabetes and cardiovascular diseases. Minimizing sedentary activities and keeping as active as possible, on a daily basis, is a real "elixir for long life", and it's simple, fun, has no side effects, and is free. Physical activity helps us prevent and cure – sometimes better than medicines – a wide range of diseases. These include the

chronic-degenerative conditions paradoxically associated with human progress, such as type-2 diabetes, obesity, atherosclerosis and many others. The advice is therefore as follows: regularly practice any form of physical exercise, which is an effective way to benefit our organism and promote a state of health that guarantees real protection against many diseases.

WHAT HAPPENS WHEN WE ARE ACTIVE?

The muscles we train when we are physically active are called "skeletal muscles". One of the characteristics that distinguishes them from other types of muscles, such as the heart (yes, the heart is a muscle, but you already knew that), is that our skeletal muscles respond to our will! We are the ones who decide how, when and whether to put them to work! The heart, however, is a law to itself. Similarly, the walls of our digestive tract and of our blood vessels are equipped with their own muscles. But have you ever found a way to tell your blood vessels to dilate or contract? If this were the case, even the shyest amongst us would be able to order their cheeks not to blush, since this phenomenon is merely the result of dilated capillaries. But to get back to the main point, we have clarified that "skeletal muscle" refers to the muscles we use when we go for a run, or those in the fingers of my hands as I strike the letters on my keyboard at this very moment. Every movement we make is caused by the contraction and relaxation of our skeletal muscles.

During physical activity, the contraction of skeletal muscle, however it takes place, produces a large number of substances that have a positive effect on our health, albeit in different ways. These substances (cytokines, myokines and growth factors) are produced by the muscles, but they are able to act "remotely" also on other tissues, organs and systems. Bearing this in mind, we can already begin to imagine that the beneficial effects of physical activity can be extremely varied and sometimes unexpected. Many scientific studies include in the list of positive effects of regular physical activity not only a "simple" increase in energy expenditure, but also, for example, an anti-inflammatory and

antioxidant action. It is thought that the anti-inflammatory effect is mediated by the substances produced by skeletal muscles during contraction, and explains the reduced susceptibility (or improved symptoms) to those diseases commonly associated with low-grade chronic inflammation (known as *metabolic inflammation* or *meta-inflammation*). What are these diseases? Once again, we are referring to diseases associated with human progress.

WHAT BENEFITS ARE ASSOCIATED WITH REGULAR PHYSICAL ACTIVITY?

One of the first benefits that comes to mind when we think about physical activity is that using our muscles is of great help in reducing excess body fat (making it essential for fighting excess weight and obesity). For some time, however, we have also been aware of its benefits for the cardiovascular system in preventing arterial hypertension, heart attacks and many other cardiovascular diseases. Furthermore, using muscles also helps in the event of impaired ability to metabolize both sugars (which leads to excessive blood glucose levels, insulin resistance or diabetes), and fats (which cause high levels of cholesterol or triglycerides in the blood, and even atherosclerosis). Those suffering from metabolic syndrome can also benefit from physical activity. Metabolic syndrome – which would take an entire chapter to discuss in depth – is characterized by the coexistence of predisposing factors that increase the risk of developing brain and cardiovascular diseases, and diabetes. Similarly, those suffering from other diseases such as certain diseases of the liver (non-alcoholic fatty liver or fatty liver) or gut (such as irritable bowel syndrome) benefit from it. Some forms of cancer, but also states of anxiety and mood disorders, including depression, can benefit from a commitment to keeping ourselves physically active. Even the risk of thrombosis and inflammatory phenomena, which are increasingly considered the causes of many diseases, could be reduced by regular physical activity!

---

1 Metabolic syndrome corresponds to a clinical condition in which the patient displays at least three out of five symptoms: abdominal obesity, high blood sugar, high triglycerides, low HDL cholesterol and blood pressure ≥130/85 mmHg

*

CRITERIA USED TO DIAGNOSE METABOLIC SYNDROME

Criteria	*Value*
Waist circumference (cm)	*> 102 for men*
	> 88 for women
Fasting blood glucose (mg/dL [mmol/L])	*≥ 110 [≥ 6,11]*
Blood pressure (mmHg)	*≥ 130/85*
Triglycerides, fasting (mg/dL [mmol / L])	*≥ 150 [≥ 1,7]*
HDL cholesterol (mg/dL [mmol/L])	*< 40 [< 1,04] for men*
	< 50 [< 1,29] for women

WHAT ROLE CAN IT PLAY IN PROMOTING HEALTHY AGEING?

As it ages, our body feels the effects of time. This is inevitable, but can we reduce the damage? Happily, yes: science is increasingly finding ways to help us age optimally! The benefits attributed to physical activity also concern ageing-related pathologies, such as sarcopenia (loss of quantity and quality of muscles), which leads to more complex situations such as dynapenia (loss of muscle strength), osteoporosis and, in the worst cases, loss of self-sufficiency. Furthermore, keeping active and exercising regularly helps, for example, to prevent and slow down cognitive impairment, as well as to reduce the risk of dementia (a broad term, which also includes Alzheimer's disease).

IS PHYSICAL ACTIVITY ALWAYS A GOOD IDEA?

You can have too much of a good thing: even physical activity, if engaged in too frequently and intensely, or incorrectly, can be a stress factor for the body! This can happen, for example, when the physiological limits of recovery and adaptation, specific for each individual, are exceeded. In many sports, when athletes overtrain, they risk performing below their best, and may suffer from fatigue, injuries, mood alterations and even gastrointestinal problems! You may have heard of "overtraining syndrome", a condition that can affect athletes subjected to prolonged and intense physical workloads and psychological stress, during both periods of training and competition. This particular disorder is characterized by difficulty in recovering quickly, with the onset of fatigue and mood swings, in addition to respiratory and gastrointestinal problems. The physical and psychological demands that arise during physical exercise can be a source of stress for athletes. The cause of stress is thought to be our old friend, the hypothalamic–pituitary–adrenal axis, which, when activated, releases so-called stress hormones.

PHYSICAL ACTIVITY, EXERCISE OR TRAINING?

PHYSICAL ACTIVITY *any movement of the body obtained by contracting the skeletal muscles with a consequent increase in energy expenditure.*

EXERCISE *Planned, structured, repetitive sequence of movements with the aim of improving or maintaining physical components or abilities.*

TRAINING *Dynamic process whose purpose is to improve physical and sporting performance, characterized by alternating between workload (progressively increased) and rest phases.*

IS ALL TRAINING THE SAME?

Overall, it is estimated that 20-60% of athletes suffer from stress caused by excessive intensity training without adequate recovery. Yet not all types of training and physical exercise are the same, or have the same effect. For example, it is believed that sport-associated stress is more common in the case of what are known as “endurance sports”, such as swimming, rowing, cycling, triathlon and, to some extent, even long-distance running. In these disciplines, professional athletes train for 4 to 6 hours a day, 6 days a week! This may continue for several weeks, often without an opportunity for the athlete to devote time to other activities.

AEROBIC EXERCISE *In this type of exercise the main muscle groups contract cyclically and rhythmically, for prolonged periods of time.*

RESISTANCE TRAINING *This type of exercise promotes increased muscle mass, power, strength and resistance to the work of the skeletal muscle system. It is called “resistance training” because the muscles work against a force or weight that resists their movement.*

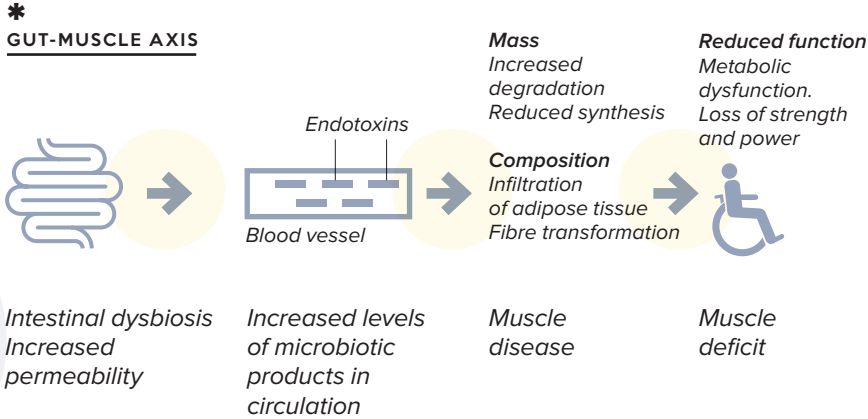
AND AT THE INTESTINAL LEVEL?

An active lifestyle and correct physical activity programmes have been shown to also have beneficial effects on gut function. Among the benefits identified, it has been observed that, for example, the time taken for faeces to pass through the gut is reduced. And why should this be in some way beneficial? The contact of possible pathogens with the mucus layer lining the intestinal walls is de facto reduced, and we observe a lower incidence of chronic inflammatory bowel diseases and colon cancer. Even in this case, however, excess can have unpleasant consequences. About 30-50% of athletes suffer from abdominal symptoms, both in the upper and lower abdomen, of various kinds, including bloating, nausea, stomach pains and cramps, but also flatulence and diarrhoea. Consequently, the hardest-working athletes often complain of impaired intestinal function. But what factors may be involved? These include increased internal body temperature, dehydration, excessive use of supplements, antibiotics or anti-inflammatory NSAIDs (a classification that depends on their chemical structure and identifies them as “not belonging to the category of steroid drugs”). Similarly, the contribution of anxiety, emotional stress, diets high in simple carbohydrates (such as fructose and glucose) and in proteins has also been suggested. All these factors could, for example, damage the tight junctions between the cells of the intestine wall, thus leading to an alteration of the intestinal barrier and causing a proinflammatory response. Furthermore, the barrier could be more permeable to bacteria and toxic substances. Once absorbed and introduced into the blood, these toxic substances could negatively affect the immune response of the whole organism! There is also clearly a difference between a healthy population and patients suffering from chronic gastrointestinal diseases and disorders. In the healthy population, physical exercise certainly has a significant impact, albeit reversible, on gastrointestinal integrity and function. In patients suffering from chronic gastrointestinal diseases, on the other hand, further investigations are needed to try and clarify the consequences, probably worrying, that intense and prolonged exercise may have on their state of health, and how “safe” it is for them to practise sports.

The fact that physical exercise is a stressor suggests that there may be a significant association between it and alterations in the composition of the gut microbiota.

IS THERE A GUT-MUSCLE AXIS?

The skeletal muscle and the gut also engage in fruitful dialogue. In short, the gut is a great talker. Is there a bidirectional relationship between gut microbiota and skeletal muscle? Absolutely! We attribute important effects on health and on our body's physical-athletic capacities to this axis, and the ability of the microbiota to remotely regulate skeletal muscle tissue is well known. A recent study conducted by Grosicki and colleagues shows that the alteration of the intestinal microbiota due to ageing can in turn, by means of various steps and mechanisms, alter muscle function. Greater gut permeability due to microbial alterations is one of the mechanisms proposed as a bridge between the two structures. This would result in an increased flow of microbial products into the blood, in turn triggering proinflammatory signals. One of the consequences of these signals could be structural alterations in the muscles, with subsequent loss of their functional capacity. Inevitably, these conditions lead, especially in the elderly, to reduced independence and lower quality of life.



IS PHYSICAL EXERCISE ONE OF THE FACTORS THAT CAN CHANGE OUR GUT MICROBIOTA?

It has thus become clear that the gut microbiota can affect muscle function. But can physical activity affect the microbiota? The results that scientists have obtained in their search for an answer to this question are extremely interesting! It's nothing new to say that physical activity practiced in a gradual, regular manner, suited to one's state of physical efficiency and health, brings numerous benefits. What's new is that positive changes are also found in the gut microbiota and, more generally, in terms of gastrointestinal health. Recently, an increasing number of scientific publications have highlighted that physical exercise can affect the diversity, composition and metabolic activity of the the by now well-known short-chain fatty acids. In other words, physical exercise is thought to be able to influence the diversity and relative quantities of bacterial species, even in different nutritional contexts, and modulate the balance between the interaction of the host and the microbiota. Some studies have also shown that gut health may even depend on the amount and intensity of the exercise.

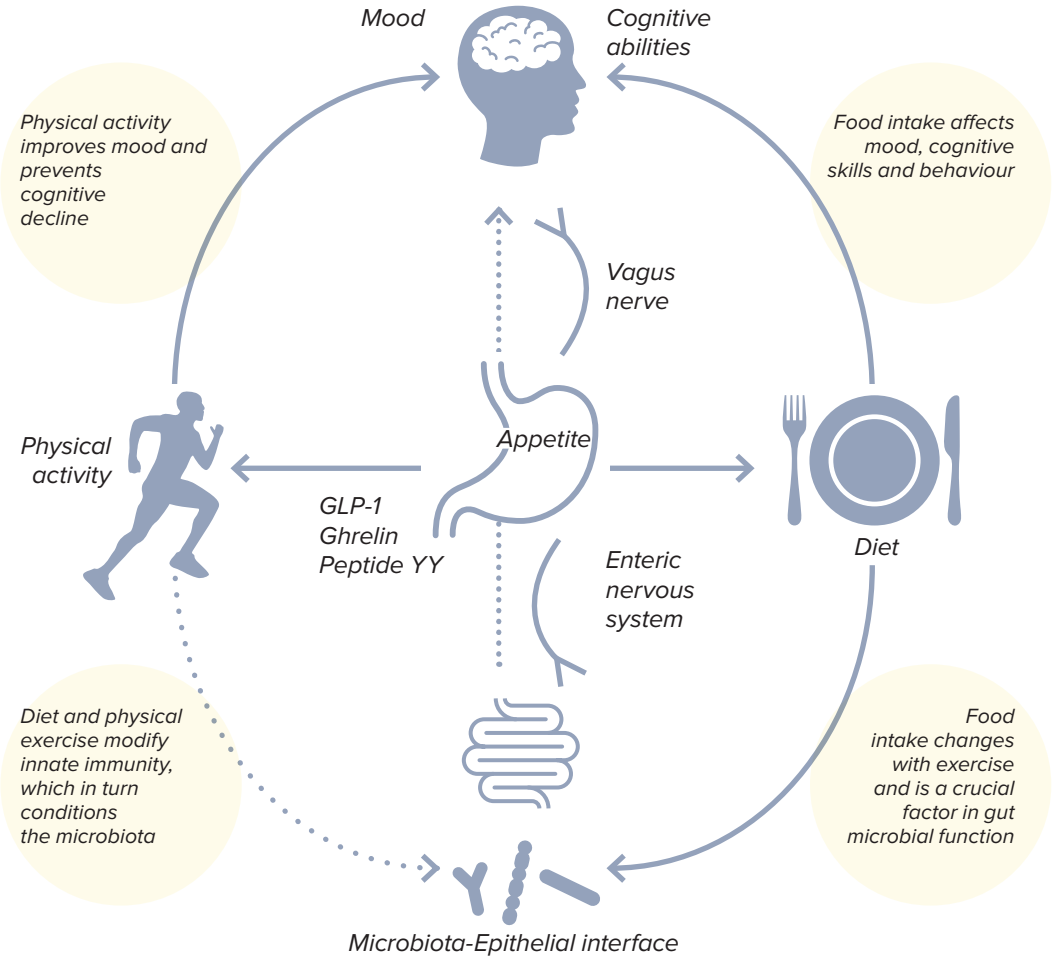
Furthermore, we know that when our microbiota is qualitatively and quantitatively in good shape, this has a positive impact on the entire human organism. This shows that the favourable influence of physical activity on our microbiota could improve not only gut health, but many other bodily functions, as happens in the case of nutrition and its effect on the microbiota.

The potential sites of interaction between biological adaptations to physical exercise and the microbiota are numerous. Physical activity involves a wide variety of biological responses, including influences on the brain-gut-microbiota axis, diet-microbiota-host metabolic interactions, neuroendocrine and neuroimmunological interactions. As we know, exercise increases the tone of the vagus nerve and this is, in turn, closely linked to gut health, thanks to its anti-inflammatory and immunomodulatory activity. The positive effect of physical exercise on the composition of the gut microbiota could even be the indirect result of its ability to improve vagus nerve tone.

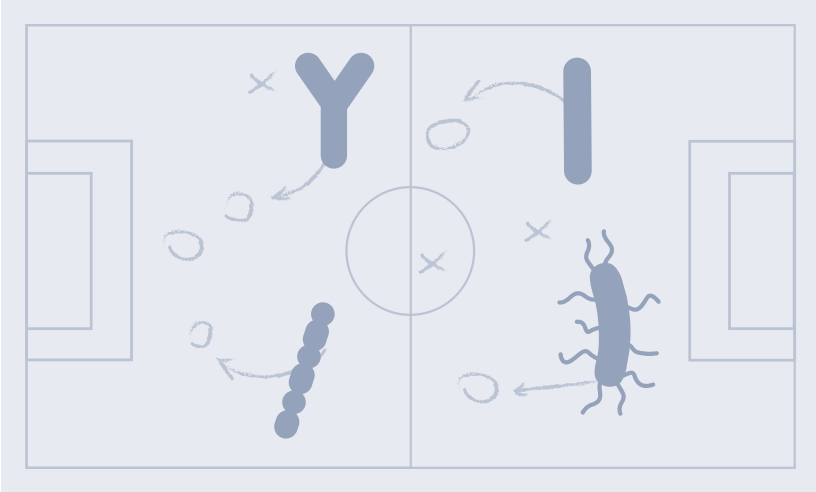
A study carried out by Clarke and colleagues in 2014 can be considered in some ways pioneering. In their work, they examined a group of athletes from the Irish rugby team at a pre-World Cup training camp.

✱

POTENTIAL SITES OF INTERACTION BETWEEN BIOLOGICAL ADAPTATION TO PHYSICAL EXERCISE AND MICROBIOTA



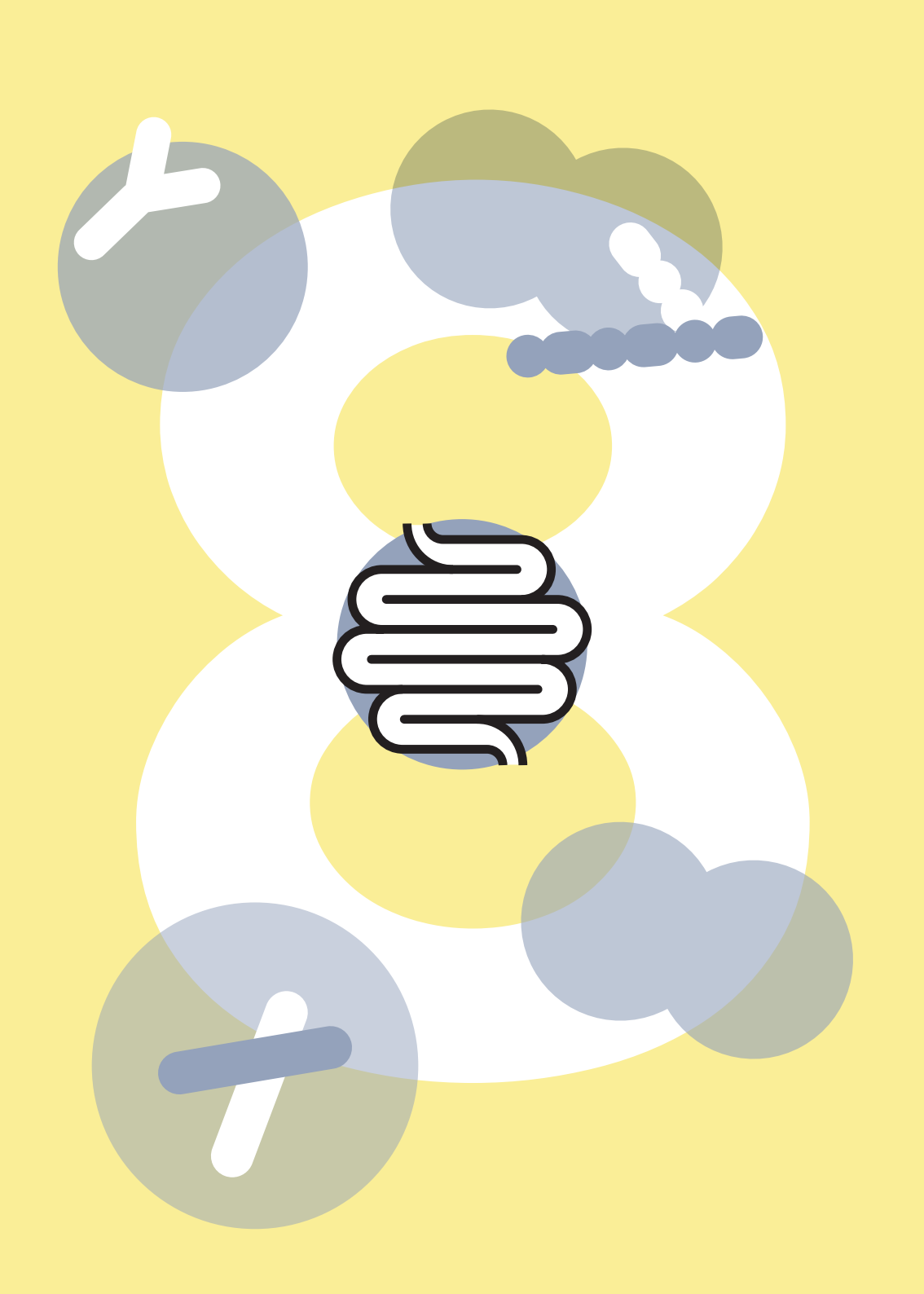
All the athletes were obviously subjected to very intense training, and were compared with two control groups of non-professional athletes, in good health and with different levels of physical activity and body mass index (BMI). On the basis of the results obtained, the authors of the study believe that physical exercise is able to promote greater diversity of the gut microbiota (22 different phyla in professional athletes, compared to 11 in the control groups), in turn associated with a lower incidence of high BMI and lower risk of metabolic and degenerative diseases. The greater diversity of intestinal microorganisms in athletes correlated positively with protein consumption. Furthermore, in athletes with a low BMI, the levels of a particular bacterial species, *Akkermansia muciniphila*, are significantly higher than in the group with a high BMI. Last but not least: inflammatory cytokine levels were lower in athletes than in control groups. It is true that caution is needed in interpreting studies that reveal associations, but not cause-and-effect relationships. Despite this, the authors believe they have provided strong evidence of how strenuous exercise and food intake can significantly affect the gut microbiota.



WHAT ROLE DO PREBIOTICS AND PROBIOTICS PLAY?

Various studies on prebiotics and probiotics have also looked at the close relationship between gut microbiota and physical exercise. Scientific evidence suggests that the consumption of prebiotics and probiotics can help preserve and promote optimal health in athletes and sportsmen. How? By improving metabolism, antioxidant and immune defences and barrier function. To this end, a 2016 study highlighted that daily probiotic supplementation could limit the exercise-induced reduction in levels of an amino acid called tryptophan, and decrease the incidence of infections in the upper respiratory tract (nasal cavity, oral cavity, etc.). In the same year, another study conducted on sixteen male athletes (who had undergone endurance sport training) analysed the effect of the daily intake of two strains of probiotics (*Bifidobacterium breve BR03* and *Streptococcus thermophilus FP4*). The results of the study showed that the probiotic preparation appeared to have an anti-inflammatory effect. Furthermore, the expected decline in performance following traumatic muscle work was attenuated in subjects taking the probiotic supplement.

In conclusion, we may argue that the habit of regularly consuming prebiotics or probiotics as part of our diet could be an effective and safe way to improve our metabolism, immune system and barrier function. How is this possible? It is because they encourage the proliferation of specific microbial genera, such as *Bifidobacteria* and *Lactobacillus*, and the production of metabolites, such as short-chain fatty acids. Further studies are certainly necessary to clarify the potential mechanisms underlying the observations made so far, and to correctly assess the dose of prebiotics and probiotics necessary to obtain the expected functional response. Probiotics, however, can certainly exert a positive effect and be effective in reducing the incidence of respiratory and gastrointestinal diseases in those who regularly practise various kinds of sports. In particular, they help athletes engaged in intense, prolonged training programmes, or in phases of greatest stress, such as during competitive events.



THE “CUSTOMS OFFICE” IN OUR GUT: THE ROLE OF NATURAL BORDERS AND THE MICROBIOTA

IS THE INTESTINE JUST A DIGESTION FACTORY?

There is a crucial, but little known, activity of the intestine, or rather of the entire gastrointestinal tract: its immunological function. Ladies and gentlemen, we are pleased to confer the title of our body's largest immune organ on the gastrointestinal tract! Immune organ? Immune function? What are we talking about? Let's take a step back: in the human body there is a complex system, the "immune system", which coordinates our defence against damage of various kinds, from invasion by viruses or bacteria to wounds. Furthermore, the immune system is sometimes unleashed against enemies but "hits the wrong target", as happens in the case of allergies.

In any case, the immune system coordinates a large number of cells and structures, which are separated by barriers. These are distributed in different areas of our body, including the undisputed star of our story: the gut. Despite their common protective function, these barriers take on different forms and act in different ways. Think, for example, of the skin, or of chemical or even biological barriers. At the gut level, these three types of barriers work together – unity is strength! – and what we get is the largest, most important interface between our internal world and the external environment. But why must the gastrointestinal tract also take care of our defence? Isn't it challenging enough to manage everything we eat and drink on a daily basis (especially considering that we sometimes treat our gut without mercy)? The reason is very simple. The intestine is in direct contact with the external environment: at each end there is a cavity in communication with the outside world, and it processes food and drinks that can be contaminated by unpleasant, unwanted microorganisms. It goes without saying, therefore, that the gastrointestinal tract requires particular protection. For this reason, the intestine is a line of defence of primary importance in our organism, and also plays a central role in maintaining our immunological balance ("immunological homeostasis"), by allowing the survival of the entire crew of symbiotic and commensal microbes we host, while eliminating those that expose us to risks ("pathogenic microorganisms"). Epithelial cells, cells of the immune system and stromal cells (those that in practice organize the

"structure" and support it) work together to counter pathogenic micro-organisms, limiting their direct contact with the intestinal epithelium. The gut microbiota itself helps our cells in this mission; although it is not native to the human body, it is in effect an important, functional and active component of our intestinal barrier.

LET'S GET TO KNOW THE BARRIER: WHO'S FIRST?

The intestinal barrier is not a simple, static, passive wall. Just think how in *Game of Thrones*, merely building a barrier of ice and snow was not enough to protect Winterfell – the Night's Watch was also sent to guard it, just as soldiers eager for battle and glory are stationed at the Bastiani Fortress in the novel *The Tartare Steppe*. If George R. R. Martin and Dino Buzzati placed barriers at the far north of their famous fictional worlds, perhaps the time has come to try and make our much more real and equally fascinating intestinal barrier famous too. But let's not get ahead of ourselves!

Let's imagine that we are inside our digestive tract: looking around us we are not able to clearly see the walls of the tube, which are hidden and protected by a blanket of mucus.

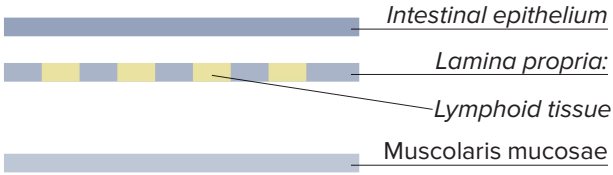
Wait! We have just encountered the first element of the intestinal barrier: mucus. But let's proceed.

Hidden and protected by the mucus, the actual intestinal wall comprises four layers. The layer closest to the intestinal lumen, therefore closest to the hollow intestine tract, is the "mucosa", and is in turn made up of two layers:

- the "intestinal epithelium", formed by a single layer of cylindrical cells in close contact with each other, turned towards the lumen. These cells, peculiar to the intestinal epithelium, are called "enterocytes";
- the "lamina propria", i.e. the connective tissue underlying the epithelium.

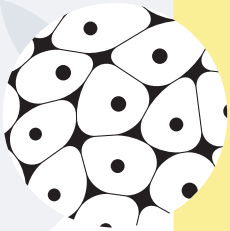
So, this is the second component of the intestinal barrier we have so far encountered: the intestinal epithelium.

✱
THE MUCOSA



We have said that the epithelium is the most superficial layer of the mucosa. Under the epithelium, but still in the mucosa, we find scattered agglomerates of lymphoid tissue that take the name of "gut-associated lymphoid tissue", which seems clear enough. Gut-Associated Lymphoid Tissue (or GALT) can therefore be considered the third element of the intestinal barrier.

Lymphoid tissue is mainly made up of cells called lymphocytes, which belong to the immune system. Observing the mucosa of the intestinal wall (and not only) we find aggregates of lymphoid tissue, scattered here and there along the gastrointestinal tract, like assistants sent by the immune system to support the intestinal barrier. But immune system cells are not the only active component. As we have already mentioned, the microbiota can also be considered an important ally: it stays outside the barrier, and is not allowed to enter, but in order to stay close to it, it helps us to fight the White Walkers (any reference to things, people and the Undead is purely coincidental). We can thus see that the barrier is extremely active and dynamic. The layer of cells forming the epithelium controls the gates and, after careful selection, allows nutrients, water and electrolytes, i.e. mineral salts, to pass through. However, it takes steps to block any microorganisms, toxins and antigens, which for no reason must pass through the intestinal wall.



WE ARE MADE OF CELLS

In biology, cells similar in structure, function and origin that aggregate give rise to "tissue". If we continue through the hierarchy of our organism, we discover that different tissues are organized together to form "organs". Various organs, joining forces for a common purpose, can form a "system" or "apparatus", as appropriate.

HOW CAN WE SUMMARIZE THE BARRIER IN A FEW LINES?

We have repeatedly said that the intestinal barrier is far from being "just" a wall, although we do have a wall that performs a "simple" physical blocking action. Hidden and protected by the first layer of mucus, we find a layer consisting of cells, physically joined together by complexes of proteins specializing in this role, which simultaneously seal the spaces between one cell and another. But let's take a closer look at everything.

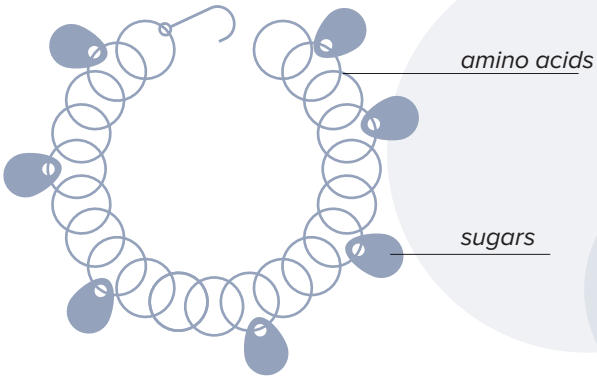
HOW DOES MUCUS WORK?

The outermost portion of the intestinal barrier is made up of mucus, which actually has two layers: the innermost is denser, while the outermost (nearer to the intestinal lumen) is looser, i.e. with a greater liquid component. Along the gastrointestinal tract, the mucus barrier is not constant in form, but thickens as it continues towards the intestine, reaching its maximum thickness in the colon. In each case and at every point, the mucus constitutes the first, highly efficient, line of defence, as it is able to prevent the penetration of most bacteria, especially thanks to the high density of its innermost layer. Although welcome, even the "friendly" microorganisms that settle in our intestine must not be allowed to reach the epithelium whenever they feel like it! Intact bacteria, and oversized particles, must be stopped first. The mucus not only blocks penetration of the latter, preventing the infiltration of most microorganisms, but is also able to choose who it lets adhere to it. How? The proteins that make up the mucus have been selected for their particular structure, which allows only some bacteria to adhere, the ones that we actually want to hold onto.

LET'S HAVE A LOOK, AS IF WE HAD THE SCRUTINIZING EYE OF A MICROSCOPE, AND SEE WHAT HAPPENS

Firstly, we're all familiar with mucus, but what exactly is it? From a chemical point of view it's a hydrated gel. From a biological point of view, what interests us is the fact that it is rich in particular proteins called "mucins", which are what give it the viscosity typical of gels. Mucins are produced by special cells, called "goblet cells", cylindrical in shape (despite the misleading name), located in the intestinal epithelium. Therefore, observing the expanse of cells that form the intestinal epithelium, we see that, among the most numerous cells (the enterocytes), here and there are some particular cells capable of producing mucus, as if they were daisies in a spring meadow. The goblet mucus cells produce mucins, whose particular structure is able to explain two important elements: the viscosity of the mucus and its ability to choose which bacteria may adhere. They are in fact "highly glycosylated" proteins. If we think of all proteins as chains, each link in the chain is an amino acid. Chains fold in various ways, forming proteins of different shapes and sizes. A glycosylated protein has an additional feature: it is equipped with "pendants" which, chemically speaking, are sugars. Mucins, in fact, can be accessorized with more than 100 different oligosaccharides.

✱ **MUCINS**



The mucins present in each of us are very similar to each other: in biological terms they are said to be "conserved". This means that during evolution, at a certain point, functional mucins in particular were formed, and owing to natural selection, from that moment on they changed as little as possible: they work well just the way they are. But in what sense do they work well? In the selection of intestinal commensal bacteria, for example. To put it in other words, nature realized that certain mucins were suitable for letting bacteria that were basically good for us adhere, and so decided to "keep" them as they were, so that, being handed down from generation to generation, they could always facilitate selection of the right bacteria. In fact, only some of them (for example *Clostridium*, *Lactobacillus* or *Enterococcus*) have the structures necessary to anchor themselves to the mucus (almost exclusively to its outermost layer), are able to use it as a source of nutrients, and are able to obtain access to the epithelial surface. This depends on whether or not these bacteria have proteins called "lectins", capable of binding and anchoring to the mucus. Among all the sugars decorating the mucins there will be one, or more than one, with a conformation that allows bonding with the lectins present on the bacteria, like two pieces of a puzzle. Some lectins have been isolated by beneficial bacteria, our friends (for example *Lactobacillus reuteri*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus*). Unfortunately, some pathogenic microorganisms (for example *Helicobacter pylori*, *Clostridium jejuni* and *Norovirus*) also have lectins which are able to bind to the sugars present on our mucins.

CELLULAR JUNCTIONS: HOW ARE THEY ORGANIZED?

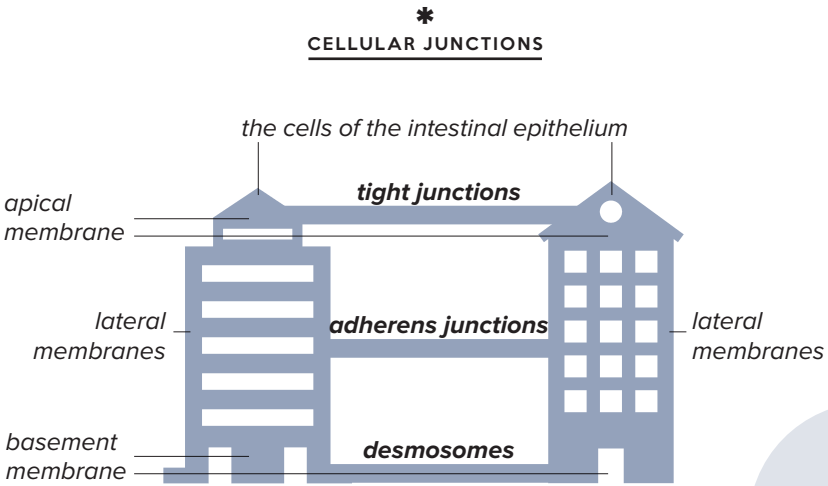
If we imagine the intestinal barrier as an active, dynamic, complex machine, cellular junctions are a further important component of it. What are they? These are particular structures whose main tasks are to seal adjacent cells together and prevent the entry of pathogens, while also regulating the entry of water, nutrients and ions.

Let's imagine we are looking at two intestinal epithelium cells, adjacent to each other. Being cylindrical cells, we see that they are taller than they are wide, and we can therefore envisage them as two adjacent vertical buildings. We see that these buildings (cells) are held close to each other by cell junctions.

But where exactly are they located and how are they structured?

Still remaining within the cell-building metaphor, we can say that the roof corresponds to the apical membrane, the ground floor to the basement membrane and the walls to the lateral membranes. Since the adhesion complexes aim to keep adjacent cells united to each other, we also find them on the lateral membranes. From a structural point of view, the junctions of epithelial cells are made up of "transmembrane proteins", i.e. proteins that are positioned inside the cell membrane and that cross it entirely (each cell is, in fact, enclosed within a "cell membrane"). The transmembrane proteins of two adjacent cells come into contact with each other through the protein portion that emerges from the two cells (extracellular domain) and in doing so keeps them together. Inside the cell, however, the intracellular domain of the proteins makes contact with the "cytoskeleton", the framework that keeps the cell structure intact. This time, a connecting bridge between the cytoskeleton and the transmembrane proteins of the adhesion complexes is provided by so-called "scaffold proteins".

But that's not all. As proof of the complexity of our organism and the precision with which all this takes place, we can also add another detail: the junctions are located on the lateral membrane, but the lateral membrane of a cylindrical cell is relatively high. Depending on their position, the structure and name of the junctions change! So let's imagine running our gaze down the wall from top to bottom. In the upper region, the "apical part", the junctions are called "Tight Junctions" (TJs). Continuing downwards we meet the "Adherens Junctions" (AJs) and finally in the lower region ("basal part") we find the "desmosomes". Taken together, these three types of junctions form the apical junction complex. As we can expect, different structures also correspond to different functions! Desmosomes and adherens junctions are considered to be the most important components in the actual mechanical junction. The tight junctions, for their part, are instead more involved in sealing the intercellular spaces and in regulating the selective transport of substances (ionic solutes) through the pathway between one cell and another (the "paracellular pathway").



TRANSMEMBRANE PROTEINS

Transmembrane proteins are equipped with:
• a part ("domain", to put it in scientific terms) outside the cell membrane;
• a part which is effectively trapped in the membrane;
• a part that also protrudes from the membrane, but this time into the cell.

- **Adherens junctions**

Adherens junctions are therefore located higher than the desmosomes and lower than tight junctions. Among the transmembrane proteins that make up the adherens junctions, the best known are undoubtedly the so-called "cadherins". Cadherins are able to recognize each other and interact in a way that science calls "homotypic interaction" (*homos* means "same"), showing how interaction occurs between proteins of the same type. Therefore, cadherins interact with other cadherins and in particular, the interaction occurs in their extracellular domain. Inside the cell, however, the intracellular domain of cadherins attracts other proteins, called "catenins". To be honest, not just any catenin will do; particular cadherins are required, such as beta-catenin, gamma-catenin and p120-catenin. Details aside, the function of catenins is to create a bridge between the intracellular domain of the cadherin transmembrane protein and the cytoskeleton. The bond can take place directly between catenins and actin, a typical cellular scaffold protein, or can exploit the mediation of adaptor proteins such as afadin.

The link between the catenins and cadherins is not only important for the junctions between adjacent cells, but also for the role they play in maintaining cell polarity. Cell polarity? This term refers to the fact that cells have a precise direction in which they must be positioned. We can't just take a cell and turn it upside down. It goes without saying that cells do not have a head, but the structures that are in their

"top" part are there for a reason and play their role there, and that is where they have to stay. The adherens junctions are also involved in the maintenance of this polarity, and even intervene in the assembly of the tight junctions, ensuring that it takes place efficiently. How did this become clear to us? Scientists' work often involves the use of "models" capable of reproducing the interactions between different proteins or different cells. We then have in vitro experiments. Through studies of this kind, the intervention of proteins called E-cadherin and α-catenin in contributing to the assembly efficiency of tight junctions was checked.

- **Tight junctions**

The tight junctions are the cellular junctions found in the highest part of the lateral membrane of epithelial cells in mammals. These are organized in such a way as to form a ring, a sort of intermittent belt around the cells, almost at the meeting point of the lateral and apical membranes (walls and roof, to follow the analogy): the membranes of the two adjacent cells do not fuse together in long stretches, but at these junctions, intermittently, the cell membranes are so close to each other that they eliminate the intercellular spaces, at places known as *kissing points*. As for the adherens junctions, the transmembrane proteins that form the tight junctions also exploit the intracellular domain to interact with various other cytoplasmic proteins and to regulate attachment to the cytoskeleton, cell polarity, cell signalling and vesicle trafficking. Note: the tight junctions are the main controllers of the permeability between one cell and another. We can imagine them as gates capable of selecting the molecules to let through at the entrance, both on the basis of their size and on the basis of their electric charge, if present. Some molecules called "ions", in fact, have a positive or negative electric charge (like the + and - on the batteries of our TV remote control) and depending on this may or not be allowed to pass through tight junctions.

THE DARK MYSTERY OF TIGHT JUNCTIONS

Let's consider an intact monolayer epithelium (which has only one layer of cells): passing through the tight junctions, we can identify two different paths that allow the epithelium to be crossed, called the pore pathway and the leak pathway. The pore pathway is the most selective pathway as regards both the charge and size of the molecules it allows to pass (in and out). The leak pathway, on the other hand, has limited selectivity, is more tolerant and gives the green light to more molecules. Let's now focus our attention on the structure of the tight junctions. We have already explained that they are made up of transmembrane proteins which, as the name suggests, cross the cell membrane and emerge on both the cell interior and exterior. If we wish to provide more biological details, we can add that transmembrane proteins belonging to five very specific families contribute to forming the tight junctions. Which ones? Occludin, claudin, JAMs, tricellulins and scaffold proteins. Let's provide a quick identikit.

- *Occludin*

It helps regulate the permeability between cells and mediate cell adhesion. Where is it found? Mainly at the tight junctions of epithelial cells (for example epidermis, or intestinal epithelium) or endothelial cells (such as those lining the innermost part of the blood vessel wall), but also in astrocytes and neurones (cells of the nervous system) and dendritic cells (cells of the immune system).

- *Claudins*

Among the transmembrane proteins of the tight junctions, they are considered the most important and the most critical in protein-protein interactions, both between identical ("homophilic interactions") and different ("heterophylic interactions") proteins. They regulate selectivity, helping decide which ions to let through. In addition, they regulate the function of the intestinal

barrier, the invasion of epithelial cells and their motility. Claudins can in turn be divided into two subgroups: proteins whose role is to seal (sealing proteins: claudins 1, 3, 4, 5 and 8) and proteins that have the task of forming pores (claudins 2, 7, 20 and 12). The former reduce permeability, the latter increase it.

- Junctional adhesion molecules (JAMs), or "junctional adhesion proteins"

These transmembrane proteins belong to the immunoglobulin superfamily, a large family whose membership criterion is, at a structural level, possession of a domain with a constant form, known as the "immunoglobulin domain". JAMs regulate the formation of functional tight junctions, as well as the formation of cell-cell boundaries and also play a role in the adhesion between leukocytes (immune system cells) and epithelial cells.

- *Tricellulin*

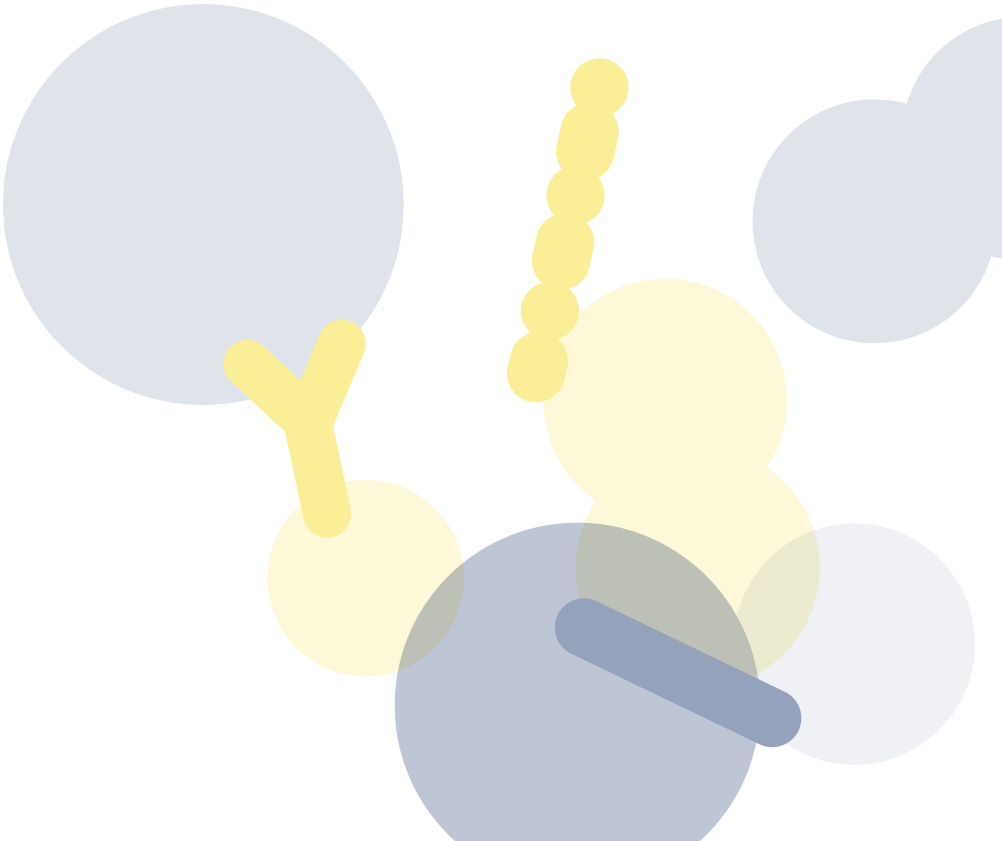
Appears to be able to keep the structure of the tight junctions stable and to regulate the passage of macromolecules, such as carbohydrates, lipids and proteins, through the tight junctions.

- Scaffold proteins – *zonula occludens (ZO-1, ZO-2, ZO-3)*

These are proteins composed of multiple domains ("multi-domain") which form a bridge to mediate the anchoring of some proteins of the tight junctions to the actin of the cytoskeleton.

IT'S (NOT) MOVING, YET IT LIVES

We have repeatedly said that, contrary to what was believed to be true in the past, the intestinal barrier actively interacts with the gut microbiota and with the cells of the immune system, in particular playing a role in innate immunity. In the light of these interactions, in which the barrier plays an integral, leading role, science speaks of a biotic surface, with a clear reference to the life (*bios* in fact means "life") of the barrier. The intense communication between epithelial cells, immune system cells and microbiota results in a tailor-made immune response aimed at specific targets. This helps to balance out the margin of tolerance previously granted, and the immune function of the effectors, those factors that react to nerve impulses, which are part of the real workforce of our immune system.



HOW DOES THE INTESTINAL BARRIER CONTRIBUTE TO OUR IMMUNE DEFENCES?

To clarify this point, we need to make a brief premise. We have seen that the intestinal epithelium is made up of special cells called "enterocytes". What we haven't yet seen is that enterocytes are capable of producing antimicrobial substances. Chemically, the antimicrobial substances produced by our epithelium are peptides, i.e. chains of amino acids so short that they are not considered proteins. To be honest, they are not only produced by enterocytes, but also by "Paneth cells". These particular cells are found at the bottom of the so-called "Lieber-kühn crypts", glands located in the intestinal mucosa beneath the epithelium, in the layer known as the "lamina propria". The antimicrobials produced by enterocytes and Paneth cells include alpha-defensin, lysozyme C, phospholipase, type C lectin and RegIIIgamma. As we have often said, different names, different functions, but the ultimate goal is the same: in this case, to keep potential enemies, that is, pathogenic bacteria, under control.

But enterocytes are tireless, and this is not all they do for our immune defences! Thanks to receptors called PRR (pattern recognition receptors), such as the so-called Toll-like or Nod-like receptors, enterocytes are able to identify and recognize the bacteria with which they come into contact. The same role is also assigned to dendritic cells, which are also equipped with PRR. These receptors are in fact able to bind microorganisms and activate the immune response, attracting the "staff" needed to fight it to the site of infection.

ANTI-INFECTION TEAM: WHO'S PART OF IT?

An impressive collection of immune system components, both innate and adaptive! These include, for example, T cells, plasma cells, macrophages and dendritic cells, but also chemokines and cytokines, which are molecules involved in the innate response.

Finally, Goblet cells (which, as we have said, produce mucins) also take part, as cells presenting antigens. What does this mean? Antigens can be imagined as identity tags that each microorganism carries. This is true for both unwanted and welcome microorganisms. In the case of unwanted microorganisms, their intention is certainly not to announce themselves: if they could, they would gladly hide their identification tags, but our immune system has learned to recognize them so as not to be caught unprepared. On the other hand, the antigen of a microorganism that we are willing to host works almost like a white flag, as if it were telling us: "It's me, I come in peace". The antigens found in the intestinal lumen must somehow manage to reach the dendritic cells, which are located at the lamina propria and have the job of taking them to specialized organs. In particular, it seems that the dendritic cells called CD103+ present in the mucosa receive the antigens of the intestinal lumen following a passage mediated by Goblet cells. CD103+ dendritic cells therefore take on the welcome antigens, taking them into the presence of T cells, located in organs which may be distant from the intestine, to which they will say something like: "Did you get a good look at them? They can stay, let's not attack them!". In scientific jargon, we thus say that dendritic cells promote the development of regulatory T cells, which will learn to be tolerant of antigens belonging to microorganisms that we don't want to expel.

In other words, one of the crucial points for our well-being is being able to distinguish friends from foes. We have compared antigens to identification tags. From a biological point of view, antigens are sub-

stances capable of provoking an immune response. Logically, molecules produced by our cells are also antigens, but since they are "self-produced", science defines them as "self antigens". If our immune system waged a war against our organism's own molecules, we would find ourselves in a constant state of civil war, which would be dangerous for our health. The immune system is thus educated to recognize self antigens, and also to recognize innocuous antigens such as those typically found in food, or, to stay on the subject, the antigens of symbiotic microbes. As we have already mentioned, a certain amount of "tolerance" is needed, and this is the very term that science uses to explain the ability of our immune system to distinguish between friend and foe, without indiscriminately attacking everyone.

But who are the enemies?

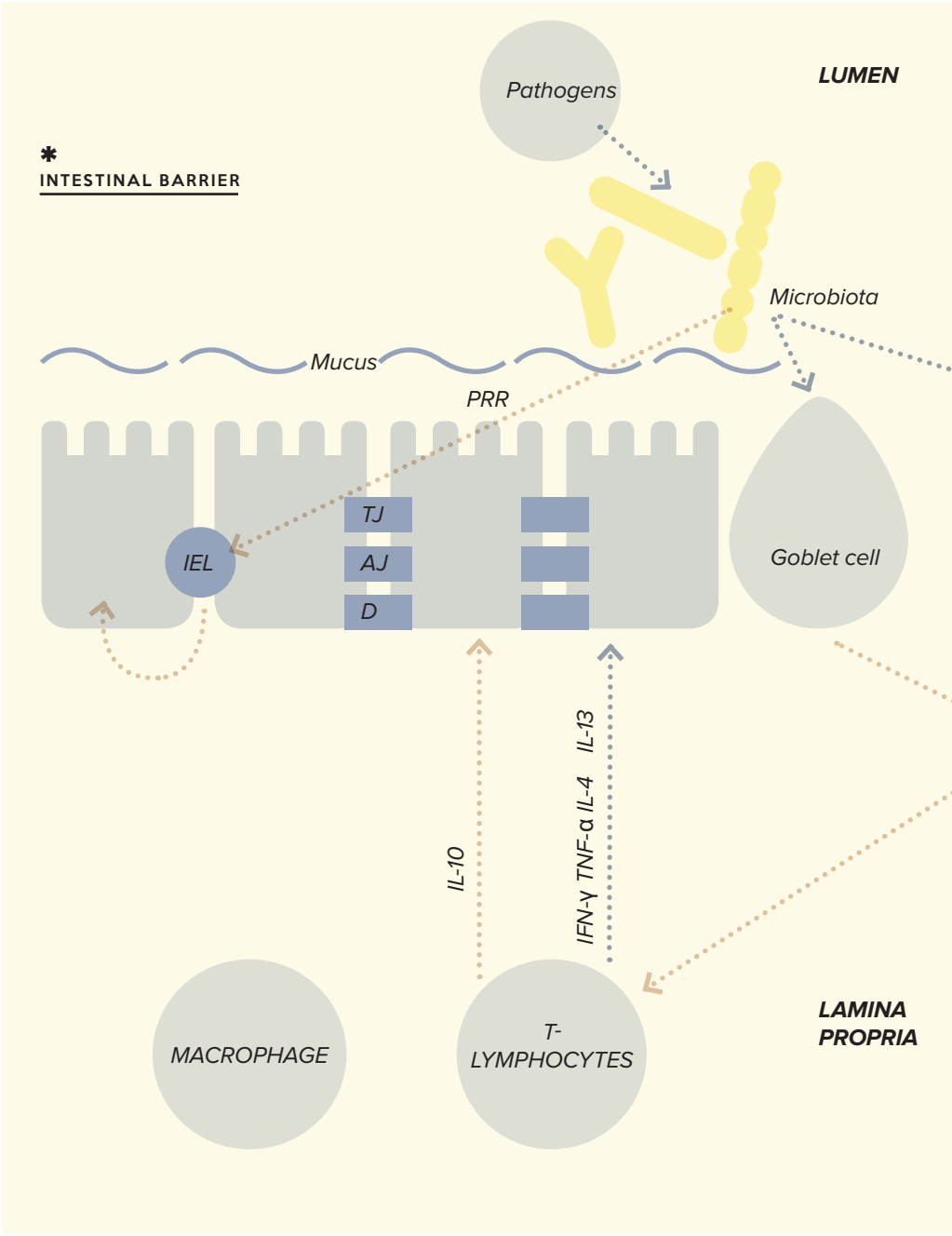
For example, some pathogenic microorganisms, or toxins. In fact, when our immune system is able to recognize its antigens, we are happy to see it react: defence is the best form of attack!

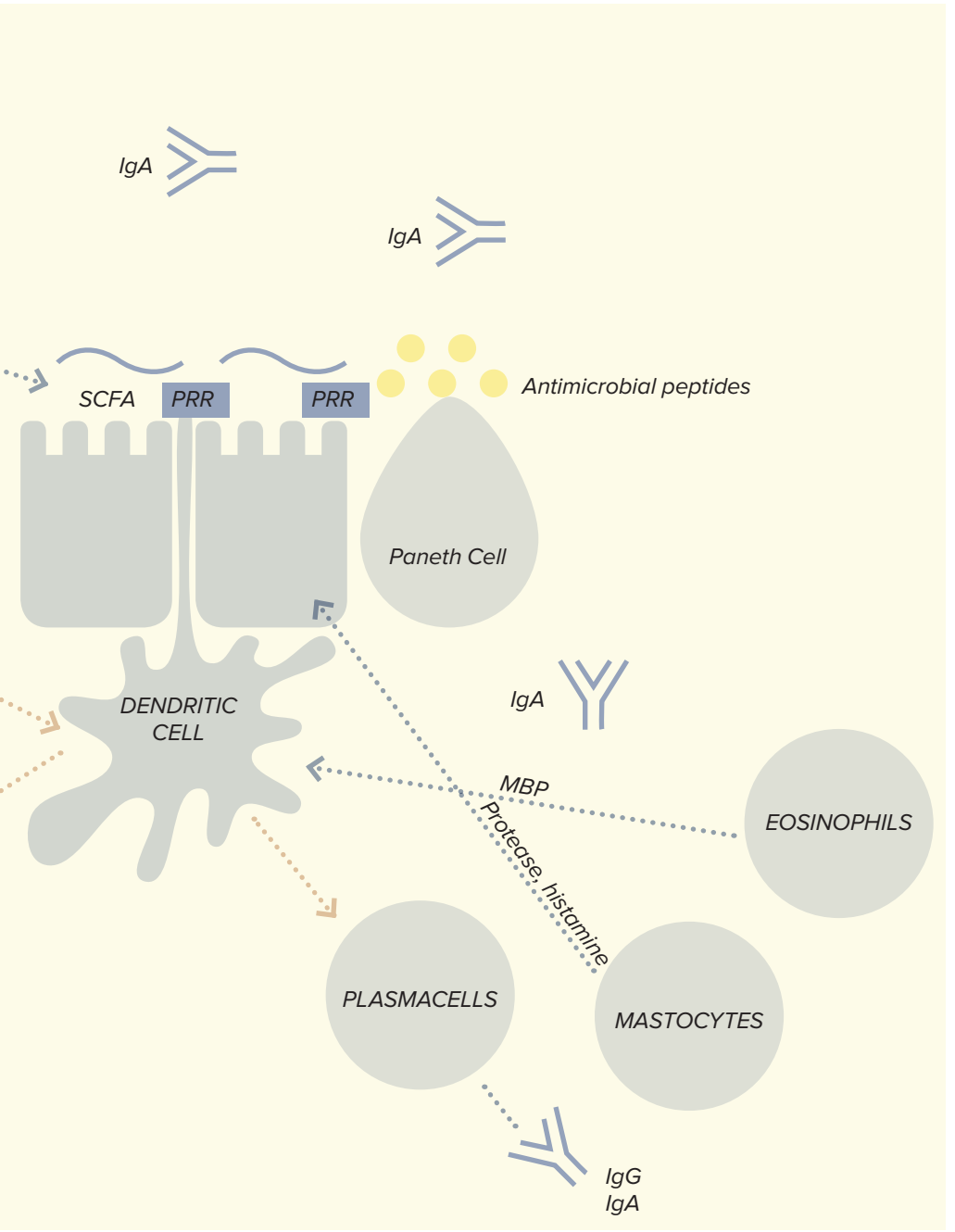
WHO IS IN CHARGE OF IMMUNITY IN THE GUT?

The immune system of the gastrointestinal tract is constantly stimulated by antigens from the lumen. In general, it is estimated that the gastrointestinal tract can house up to 70% of the lymphocytes present in our body, making it our largest immune organ. In these pages we will try and provide an identikit of the components of the immune system found in the gastrointestinal tract.

Beneath the epithelium, the lamina propria hosts dendritic cells and gut-associated lymphoid tissue (GALT), which includes "Peyer's plaques", lamina propria lymphocytes (LP-lymphocytes) and intraepithelial lymphocytes (IELs). Let's have a closer look at these components.

- Dendritic cells are, by definition, specialized in the presentation of antigens. In fact, they capture and process the antigens with which they come into contact, display them in plain sight on their surface and transport them to the lymphoid organs of the immune system. Here they present the antigens to T lymphocytes (cells of the adaptive immune system), inducing either a specific immune response (attack) or tolerance. In particular, saying that a specific immune response is induced implies that specific antibodies are produced for the microbe in question. Without going into the details of the classification of antibodies, we may mention immunoglobulin A (IgA), each time specific to the identified microorganism, and secreted to inhibit its growth and penetration.
- Peyer's plaques are GALT structures scattered along the digestive tract but mostly concentrated in the ileum (a section of the intestine). The plaques host a large number of immune system cells, which are necessary to induce a targeted, specific response. At the site of these plaques we find a specialized epithelium consisting of M cells, capable of capturing antigens and transporting them from the lumen to beneath the epithelium, to present them to the "immune machinery" located here.


LUMEN
Pathogens
*
INTESTINAL BARRIER
Microbiota
Mucus
PRR
TJ
AJ
D
IEL
Goblet cell
IL-10
IFN-γ TNF-α IL-4 IL-13
MACROPHAGE
T-
LYMPHOCYTES
LAMINA
PROPRIA


IgA
IgA
SCFA
PRR
PRR
Antimicrobial peptides
Paneth Cell
DENDRITIC CELL
IgA
MBP
Protease, histamine
EOSINOPHILS
PLASMACELLS
MASTOCYTES
IgG
IgA

M cells can be considered a double-edged sword. On the one hand, they in fact mediate the passage of antigens towards the lymphoid tissue found in the intestinal mucosa, allowing initiation of the immune response where necessary. On the other hand, however, they can also serve as an entry point for various microorganisms, including pathogenic ones that have learned how to deceive our defences.

- Lymphocytes of the lamina propria and intraepithelial lymphocytes: lymphocytes are considered cells of the adaptive immune system and are also named according to where they reside, as in this case. The adaptive immune system, which secretes factors called "effectors", contributes to the defence of the intestinal barrier.

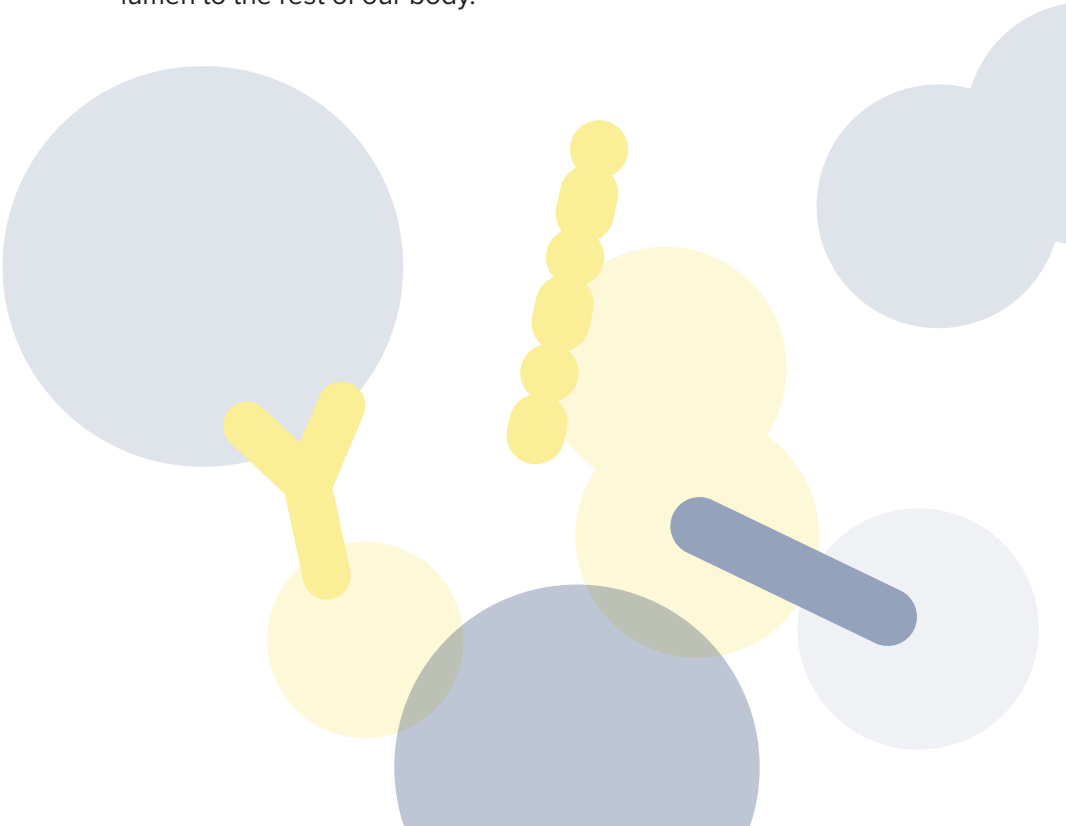
WHAT DOES THE MICROBIOTA DO?

The further we proceed, the more we realize that the gut microbiota is vital for our health, which is obviously also protected by the immune system. We are thus now going to discuss the role that the microbiota plays in this context, in particular in the defence against pathogens. How does it help us?

A real competitive battle is waged in the gut between welcome and unwelcome microorganisms: space and nutrients are in fact limited. Because of this, the microbiota also produces antimicrobial substances with the aim of fighting invaders. A stable microbiota, supported by the mucus layers of the barrier, is essential to prevent pathogenic bacteria from causing infections in the host.

But this is not the only way in which the microbiota contributes to the physiological well-being of its host (understood as the human organism in which it resides). Among other things, the microbiota is able to produce beneficial substances for our organism during its metabolic processes, in particular during the anaerobic fermentation (i.e. in the absence of oxygen) of food residues found in the digestive tract. The main products of bacterial fermentation in the colon are short-chain

fatty acids, which we have already discussed. These include acetic acid, butyric acid and propionic acid. By managing to insinuate themselves through the intestinal epithelium, these short-chain fatty acids are able to interact with our cells, influencing the immune response and the risk of disease. Short-chain fatty acids work hard for us. While on the one hand they are, for example, an important source of energy for the intestinal epithelium cells, on the other they intervene in our physiology and immunity through a variety of regulatory functions, generally being considered beneficial and endowed with anti-inflammatory properties. In addition, short-chain fatty acids boost defence mechanisms by fortifying the barrier function of intestinal epithelium cells. Some scientists have observed that these fatty acids are also able to exert an effect on the Goblet cells, precisely by increasing mucus production. Furthermore, it seems that short-chain fatty acids facilitate the assembly of tight junctions in intestinal epithelial cells and inhibit the passage of toxins, including lethal ones, from the intestinal lumen to the rest of our body.



WHAT ELSE INFLUENCES THE INTESTINAL BARRIER?

Introducing all the members of the Immune System Team one by one would require an entire book. However, we can begin to get to know those characters who also dedicate some of their time to regulating the intestinal barrier, sometimes in the first person, sometimes by sending substances they have produced to the front line. We'll start then with a list of characters, but be patient: more details will not be long in coming! There are T cells, mast cells, eosinophils, also cytokines produced by subpopulations of T cells (cells (T helper 1 and 2 cells), and external factors such as alcohol and intestinal microorganisms. So let's start by talking about T cells and the substances they produce. These are interesting characters, at times a little aggressive, with murderous instincts, at others good-natured, tolerant and patient. The activation of T cells impacts the intestinal barrier. In general, we can summarize their effect by saying that they promote an increase in the permeability of the intestinal epithelium. This occurs both for the paracellular pathway, i.e. through the spaces between one cell and another, and for the transcellular pathway, that is, through the cells themselves. Some compounds can also breach the epithelium by entering from one side of a cell and exiting the other, thus crossing it internally. T cells increase traffic through both possible routes (para- and transcellular), both inbound and outbound, sometimes even creating a little confusion. Let's have a look how.

- **Transcellular pathway**

  It seems that T cells are able to increase the passage of molecules through the transcellular pathway by directly affecting the rhythm of action of the transporters present at the plasma membrane level. Which transporters? What are we talking about? The cell membrane is not to be imagined simply as a protective envelope, but also as an interface between the external and internal environment of each cell. Scattered in the membrane we can thus find proteins whose role is to transport molecules across the cell membrane. Why? Because it is true that molecules such as

oxygen can cross the membrane at any point, without the need for any help, but there are other molecules that require well-structured, specific passageways to pass from the outside to the inside of a cell, and vice versa. T cells are able to alter the permeability of the membrane by modifying the normal rhythm of action of these transporters, allowing the flow of molecules such as glucose, sodium, potassium and chlorine.

- **Paracellular pathway**

  As for the passages between one cell and another of the epithelium, the activated T cells enhance the flow, sending small molecules to act for them. In particular, permeability is increased by the action of two molecules, belonging to the family of cytokines, which take the name of "interferon-gamma" (IFN-γ), produced by Th1 cells (type 1 T Helper), and "tumour necrosis factor alpha" (TNF-α), produced by Th2 cells (type 2 T Helper). In what sense? Some scientists have noted that the intervention of these molecules at the epithelial level results in the destruction of the tight junctions present between one cell and another in the epithelium. Since the tight junctions seal the cells together and regulate the permeability of the epithelium, their destruction is bound to increase the flow that the junctions previously limited. A similar effect has been found, for example, to be caused by another human protein, called zonulin, which induces the dismantling of tight junctions, thereby increasing permeability between cells. Cytokines produced by type 2 T Helper cells also increase permeability. Among these, one cytokine in particular, called interleukin 13 (IL-13), increases permeability through the tight junctions and in particular through the channel of the very junctions formed by the claudins (pore pathway). It also seems that by stimulating the epithelial cells in the colon, not only IL-13, but also IL-4 (also produced by Th2 cells) can increase permeability at the epithelial level. In addition, IL-10, once again produced by Th2 cells, is a cytokine with anti-inflammatory properties, able to exert a protective function on the intestinal barrier.

Among all the lymphocytes that populate our body, there are some that take the name of intraepithelial lymphocytes precisely because they are closely associated with the intestinal epithelium, in particular with the basolateral portion of the membrane of the intestinal epithelial cells. Given the presence of particular receptors called γδ, these lymphocytes are called "γδ positive", and are involved in the maintenance of the intestinal barrier.

Among the other immune system "actors", mast cells can also be found in the gastrointestinal tract. Once activated, these release a large array of inflammatory mediators, including histamine, neutral proteases (tryptase, chymase, carboxyl peptidase A), prostaglandins, leukotrienes, platelet activating factors and various cytokines, including TNF-α, IL-3, IL-4, IL-5, IL-6 and GM-CSF.

And what role do external factors play?

Chronic alcohol consumption is associated with increased intestinal permeability, inhibition of the transport of vitamins and nutrients and a reduction in the absorption of sodium and water. In fact, the action of alcohol on the intestinal barrier is caused by two products that derive from the metabolism of alcohol itself, namely acetaldehyde and nitric oxide. Scientific evidence suggests that inflammatory cells and various mediators, including cytokines, reactive oxygen species, leukotrienes and histamine also contribute to barrier dysfunction. In some cases, drugs can also perform this activity, especially if used in excess, such as when we take too many non-steroidal anti-inflammatory drugs (or NSAIDs) to combat inflammation and pain. Finally, some pathogenic bacteria also seem to be able to regulate the barrier function of tight junctions. This happens, for example, when the enterotoxin of *C. perfringens* interacts with specific claudins. But, as in many cases we have mentioned, this is just an example.

IF THE BARRIER DOESN'T WORK, DO WE GET ILL?

In the last decade, increasing evidence has been found of associations between damage to the intestinal epithelial barrier and predisposition to the development of an increasing variety of diseases, both intestinal and systemic. On the one hand, altered barrier function, with increased intestinal permeability, can be a consequence of various diseases. On the other hand, however, increasing evidence shows how it could also be a predisposing factor! Unfortunately, most of the studies are limited to finding correlations, and not to clarifying cause and effect. However, coeliac disease and inflammatory bowel diseases are exceptions, for example. Furthermore, dysbiosis could promote damage to the intestinal barrier and be linked to a greater susceptibility to some diseases. The importance of the mucus layer, for example, is highlighted by observations that show an altered mucin structure in conditions such as active enterocolitis. Similarly, it has been observed that spontaneous colitis develops in rats lacking the main mucus protein.

Allergies! We've all heard of them! At the biological-molecular level, an allergy is an exaggerated reaction of our immune system against the antigens of particular foods. For an allergy to develop, the antigens in question must be presented to the components of the immune system located in the intestinal mucosa. This leads to so-called "antigen sensitization", resulting in the production of cells and antibodies directed against the antigen itself. When dealing with food, there should be no need for this reaction, but sometimes it happens! It has been hypothesized that, in situations of impaired intestinal barrier functionality, the transport of antigens through the intestinal barrier increases and, as a consequence, the immune system of the mucosa is more exposed to antigens. This could then contribute to the development of a response directed against the excess of food antigens. Increased intestinal permeability has consistently been noted in infants with food allergies, when compared to healthy babies, even after 6 months of an allergen-free diet.

When we talk about "chronic inflammatory bowel diseases", such as Crohn's disease and ulcerative colitis, we are talking about diseases whose course is characterized by "remissions" and "relapses", that is a prolonged coming and going over time, with periods in which we feel fine alternating with times when the symptoms come back with a vengeance. Recently, a new model is emerging to explain how these diseases develop, and it suggests that three essential factors are involved:

a. the functionality of the intestinal barrier fails;

b. the immune cells of the lamina propria are exposed to the contents of the intestinal lumen;

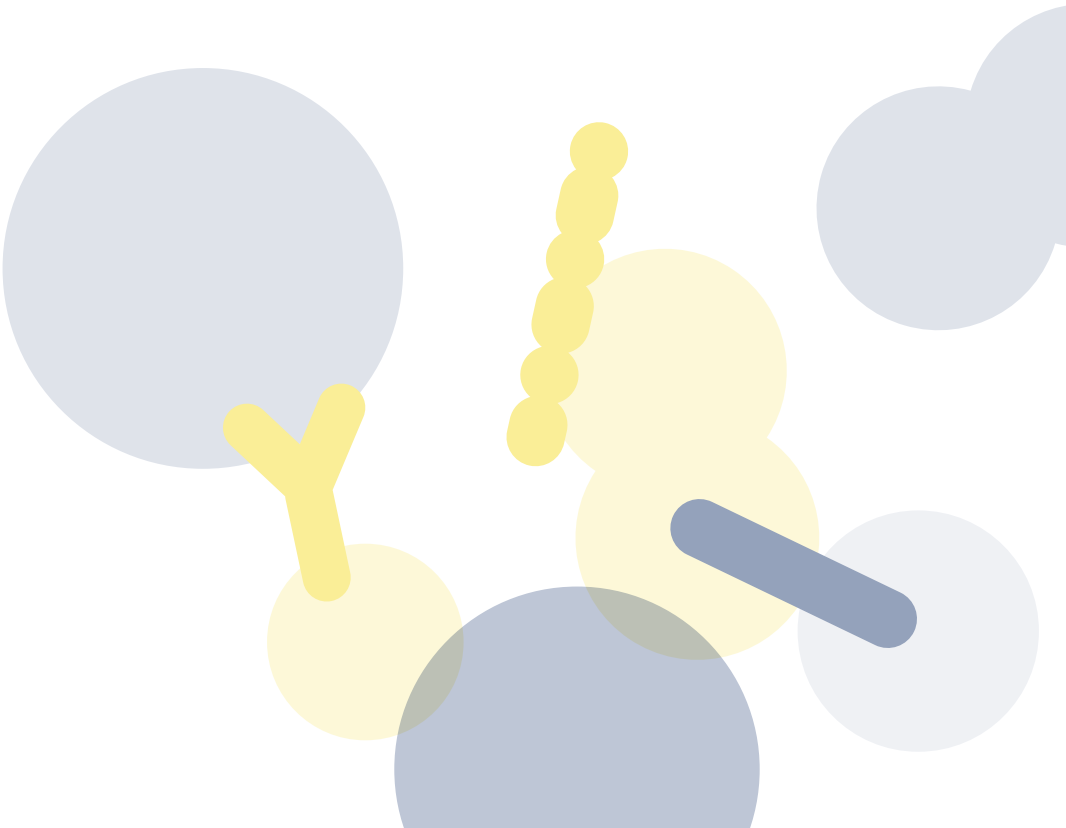
c. an excessive immune response is activated.

But what factor is at the origin of this self-perpetuating cycle? This is still unclear. However, scientific evidence is increasingly beginning to support the hypothesis that increased intestinal permeability is a primary causal factor in the development of these conditions. Although it is a key element, however, it is not enough to determine predisposition to the development of chronic intestinal diseases. It is interesting to note that in the mucus of "damaged" sites of patients with Crohn's disease an alteration of the gut microbiota has been observed, with higher numbers of *Escherichia* (*phylum Proteobacteria*) and lower proportions of *Lachnospira*, *Faecalibacterium*, *Blautia* (all *Firmicutes*) compared to what was found at undamaged sites in the same patient. What does this tell us? That dysbiosis could promote or maintain lesions associated with Crohn's disease.

Coeliac disease, which you have probably heard of on various occasions in recent years, is caused by a malfunction of the immune system. What happens is that T cells react inappropriately to the ingestion of gluten and in particular of a component called "gliadin". Some studies suggest that an alteration in the function of the intestinal barrier can also play a role in the development of coeliac disease, allowing gliadin to cross the intestinal barrier and activate the immune system. It has been observed that coeliac patients show increased intestinal permeability and altered tight junction morphol-

ogy. These distortions, however, have been seen to persist even in patients following a gluten-free diet who no longer show the typical symptoms of coeliac disease.

Reduced intestinal barrier function was also noted in patients with type 1 diabetes before onset of the disease. Recently, dysbiosis and bacterial invasions have been observed in patients with ankylosing spondylitis. If biopsies are carried out in areas where the ileum is inflamed, increased zonulin levels are found. Increased blood levels of zonulin alter protein levels at the tight junctions (lower occludin levels) and correlate with greater intestinal permeability.



THE MICROBIOTA DIET: WHAT AND HOW TO EAT

DOES FOOD INFLUENCE THE MICROBIOTA?

Age, genetics, method of birth. But what we eat every day also affects the composition of our microbiota. It's the easiest factor to act on, while respecting personal tastes, and can truly become an effective way to improve the well-being of our microbiota. Why? Because, as the experts say, "it's the simplest factor to modify and lends itself to becoming a concrete method of prevention and cure". This relationship has been known for some time: already in 1919 one of the first scientific articles dedicated to the relationship between diet and microbiota was published, back when the microbiota was still called "bacterial flora" and only a few far-sighted scientists had realized its importance.

ARE WE WHAT WE EAT?

It doesn't take much for food to affect the microbiota, which reacts very quickly to dietary changes. It can make a significant difference to switch from an animal-based diet to a plant-based one, or, for example, to add 25 grams of specific fibres to one's daily diet. Or to switch from a low-fibre, high-fat diet to one that, conversely, is low-fat and high-fibre. By adopting one of these eating habits, after only 1-2 days there would already be changes in the composition of the microbiota! This in itself shows that it is possible to influence the microbiota through diet. But take note: long-term eating habits are the most important variable in determining the composition of one's individual microbiota! For example, diets with a high protein and animal fat content lead to an increase in *Enterobacteriaceae* (*Shighella* and *Escherichia*), *Bacteroides* and *Firmicutes*. A diet rich in carbohydrates and fibre, on the other hand, is associated with an increase in *Bacteroidetes* such as *Prevotella*. You may remember that we have already encountered *Prevotella* and *Bacteroides* above all as the dominant groups of two enterotypes. This suggests, then, that enterotypes could also be associated with dietary characteristics in the long term: constant eating habits over time make the difference! But let's have a look together, step by step, at the effect that macro- and micronutrients have on our microbiota.

I LIKE CARBS! WHAT INFLUENCE MIGHT THEY HAVE ON MY MICROBIOTA?

Carbohydrates are a class of macronutrients and also include a wide variety of different compounds, ranging from very simple molecules to long, complex chains. Let's have a look.

What are the positive and negative aspects of fructose?

*

LIPOPOLYSACCHARIDES AND INSULIN RESISTANCE

Excessive consumption of sugars, in particular fructose (e.g. Western Diet) → *Increase in* Enterobacteriaceae *and* Escherichia coli → *Increased quantity of lipopolysaccharides* → *Inflammation and Insulin resistance* → *Altered blood sugar levels*

Many will have heard of the Western Diet, also known as the "Standard American Diet" or SAD. The foods mainly consumed in this diet include red and processed meats, and pre-packaged foods. In light of studies in the literature, the Western Diet is hardly a panacea to be recommended for daily life. Its characteristics include huge amounts of sugars and in particular an excessive intake of fructose. Fructose is associated with an increased presence of *Enterobacteriaceae* and *Escherichia coli*, which belong to a group of bacteria characterized, among other things, by the presence of a molecule called lipopolysaccharide on their membrane. If levels of *Escherichia coli* and *Enterobacteriaceae* increase, levels of lipopolysaccharides increase accordingly.

These molecules enter particular particles called chylomicrons, which, like specialized shuttles, transport them to the liver. In addition to inducing a mild state of inflammation, lipopolysaccharides can promote insulin resistance. What does this mean? Under physiological conditions, insulin (which is in fact a hormone) regulates blood sugar levels. Glucose is a monosaccharide, therefore a simple sugar, very widespread and extremely precious as the main source of energy for cells (and living organisms): consider that some cells, such as those of the nervous system, are so fussy that they only want (and are able to feed on) glucose! Some cells in our body are insulin-sensitive, since their ability to detect glucose varies in response to the presence and action of this hormone. Such cells include muscle and fat cells, which have specific receptors, that is, particular proteins that can recognize insulin and receive its message. The response to insulin will be that of taking glucose from the outside to the inside of the cellular environment. In the case of insulin resistance, normally reactive cells progressively lose the ability to carry out insulin commands. Insulin resistance is one of the most common metabolic problems in societies where overweight and obesity are increasingly common.

As regards, then, the complex relationship that binds starches and the microbiota, let's start by saying that starch is one of the main components of our diet, as it is commonly contained in pasta, rice, bread, etc. Some of the starch we consume, however, is known as "resistant starch" (RS), existing in 4 different forms (called RS1-4) and, as the name itself suggests, these forms of starch resist digestion. Supplementation of diets with resistant starch has been shown to be associated with an increase in *Ruminococcus bromii* and *Eubacterium rectale* in the faeces. Furthermore, their presence is related to the production of short-chain fatty acids. The response to resistant starch supplementation, however, can vary from individual to individual. There will be some who, for example, despite supplementation with resistant starch, will not show increased levels of *Ruminococcus bromii*. The faeces of such subjects have been found to contain undigested starch. What conclusion springs to mind? If *Ruminoccous bromii* levels do not increase, nor does "our" ability to digest resistant starch. This suggests that the very composition of the microbiota can make some nutrients usable or not. Furthermore, the very production of short-chain fatty acids can be influenced by microbial differentiation.

What foods contain resistant starch?

Legumes, extruded durum wheat pasta (extrusion is one of the main ways of producing industrial pasta), bread made with partially whole grains, and cooked and chilled foods, such as parboiled rice (which is not a particular variety of rice, but a method of processing). However, not all forms of resistant starch are fermented equally. Further studies are needed to better understand how the gut microbiota can be influenced by the structure of the nutrients it comes into contact with.

WHAT HAPPENS WHEN WE EAT PROTEIN-RICH FOODS?

It is known that excessive meat consumption, also typical of the Western Diet, is related to an increase in the incidence of intestinal cancer. But what if this increased incidence were also mediated by activity in the gut microbiota? Have you ever thought about that? Maybe not, but luckily doctors and researchers have! And what have they discovered? That, as far as the microbiota is concerned, the consumption of animal proteins is associated with an increased presence of *Bacteroides*. Some of the nutrients found in red meat, a low-calcium diet, emulsifiers and choline can modulate the microbiota, promoting the growth of species such as *Fusobacterium nucleatum*, *Escherichia coli* and *Bacteroides fragilis*. And it is precisely the levels of these species in the microbiota that seem to be associated with the incidence of colon cancer. But that's not all. The increased risk of cancer associated with excessive consumption of red meat could also be attributed to the presence of the "haem group" (just be patient for a minute, and we'll tell you more about it) and to the way in which the microbiota metabolizes it for us. Haem is a molecule, a pigment, which gives its typical colour to red meat and whose biological function is mainly linked to the production of energy in the muscles. In fact, haem can cause damage to the intestinal epithelium, and the microbiota can actually facilitate this damage, as it is able to breach and damage the barrier. How? Through the action of bacteria that degrade mucin, thus thinning the protective mucus layer of the membrane. Furthermore, the microbiota is able to metabolize L-carnitine, a nutrient found in red meat. The product of this metabolization is a compound called trimethylamine (TMA) which, if it reaches the liver, undergoes a chemical transformation (called "oxidation") and becomes TMA N-oxide. Some studies suggest that this compound may even be associated with the acceleration of atherosclerosis and, therefore, with a greater risk of developing cardiovascular disease.

DO YOU WANT TO UNRAVEL THE MYSTERY OF FIBRE?

Vegetable fibre is made up of complex molecules called polysaccharides (complex carbohydrates). To ensure that the fibres can be broken down and metabolized, special enzymes are needed that the human organism, per se, does not possess. Once again, the gut microbiota comes to our rescue! Some species, such as *Bacteroides thetaiotaomicron* and *Bacteroides ovatus*, not only have these enzymes, but have them in significant quantities. Enzymes, being proteins, are the final result of the well-known golden rule of biology:

gene ▶ mRNA ▶ protein

Well, it seems that these species possess a large number of genes that lead to the production of enzymes capable of metabolizing fibre, called lyases and glycosidases. How large a number? More than twice as many as man possesses! But let's get back to the microbiota. When we ingest non-digestible carbohydrates, mainly in the form of fibre, the microbiota ferments them. The main product of fermentation is short-chain fatty acids, such as acetate, propionate or butyrate. These have a significant role at the metabolic level: they provide about 10% of our energy needs, but, above all, constitute between 60 and 70% of the energy source for the cells of the colon. Furthermore, great attention is paid to their possible role as "signalling molecules" between microbiota and host. Examples of processes that can be mediated by short-chain fatty acids are regulation of the metabolism, appetite, body composition, and modulation of the immune system. Here is an interesting fact provided by studies comparing rural and urban populations: the diversity of microbiota species is greater in rural populations, whose diet shows a higher fibre content. The high levels of microbial differentiation correspond to a greater production of short-chain fatty acids. Furthermore, the relationship between the species present in the microbiota also changes between rural and urban populations. If the man on the fourth floor of a building in central Milan decides to leave the stress of city life behind him and move to a developing country, among the countless changes he will encounter, one will be in the ratio of *Bacteroidetes* to *Firmicutes*, which

will increase twofold! What could this be due to? Well, for now we can say that it is related to the different quantities and types of fibre consumed.

✱
THE MICROBIOTA OF RURAL POPULATIONS

Diet richer in fibre → *Better ratio of* Bacteroidetes *to* Firmicutes

↓

Greater microbial differentiation

↓

Increased production of short-chain fatty acids

↓

Better gut health

WHAT ARE PREBIOTICS?

In 2017, an authoritative International Consensus Document drawn up by a team of experts defined a prebiotic as a "substrate that is selectively used by host microorganisms, providing a health benefit". To date, these compounds have been the subject of numerous studies. They include, for example, fatty acids such as conjugated linoleic acid or omega-3, but also various types of polyphenols, as well as oligosaccharides such as fructo-oligosaccharides (FOS), inulin, lacto-oligosaccharides (GOS), mammano-oligosaccharides, xylo-oligosaccharides (XOS) and the oligosaccharides contained in breast milk (HMOS).

MORE FIBRE FOR EVERYONE! WHY?

Some types of fibre are particularly welcome to the microbiota, which uses them selectively: these are called "prebiotic fibres". Undigested fibre and polysaccharides are the main modulators of the composition and functionality of the microbiota. How does this affect our well-being? If the microbiota get its favourite (prebiotic) fibres, we ourselves benefit: let's just say that if the microbiota has everything it needs to survive, it leaves us alone! In the case of a low-fibre diet, meanwhile, the microbiota looks for nutrients elsewhere and even uses the glycoproteins in the mucous membrane of our intestinal wall! To say that the microbiota turns against us would perhaps be excessive: "The hunger did what sorrow could not do!". By eroding the intestinal wall, this inevitably reduces its effectiveness as a barrier and, consequently, exposes us to risks! Some species endowed with the ability to erode the intestinal mucosa thus lead to greater vulnerability of the enterocytes and sometimes can even cause lethal colitis (for example in the event of an attack by *Citrobacter rodentium*). Considering the safety, availability and cost of undigested fibre and polysaccharides, increasing the quantity and carefully choosing the type of fibre to consume are simple strategies that can be easily implemented to improve health in many different situations, through the microbiota.

CONJUGATED LINOLEIC ACID: WHY IS IT A PREBIOTIC?

Linoleic acid is an essential fatty acid. When we talk about conjugated linoleic acid (CLA), we are referring to a group of substances that correspond to different forms of linoleic acid. The main source of conjugated linoleic acid for humans is milk and its derivatives. Its production in fact requires the action of various bacteria and enzymes that ruminants are endowed with. Furthermore, if we analyse the products obtained following the fermentation of milk, we find higher levels of CLA than those found in unfermented milk. *Lactic bacteria* and *Bifidobacteria* therefore make it possible to produce fermented milks with higher levels of CLA. Among the products that exploit this process, the most commonly used

is yogurt. But why are we interested in consuming conjugated linoleic acid? It seems that one of its properties is the ability to favourably influence body composition and some cardiovascular risk factors. In addition, the 2017 International Consensus Document listed it as a prebiotic. But conjugated linoleic acid is not the only precious substance contained in yogurt: we must not forget "bioactive peptides". "Peptide" is a term that refers to a chain of amino acids that is not long enough to be called a protein; "bioactive", meanwhile, refers to their ability to act on a biological level. In our case, the bioactive peptides present in yogurt are able to stimulate the production of mucin in the intestine (and therefore of mucus), which is essential for protecting mucosa integrity.

AND AS FOR POLYPHENOLS, IS THERE ANYTHING INTERESTING TO ADD?

Polyphenols are also considered prebiotics. So why ignore them? It is estimated that about 90-95% of the polyphenols present in food are not absorbed in the small intestine, but manage to reach the colon. It is here that the polyphenols are metabolized by the microbiota. Normally, the metabolism of bacteria reduces the activity of nutrients such as polyphenols, but some specific products of the transformations carried out by the microbiota on the latter have shown useful properties for humans. The benefits for our health associated with the consumption of polyphenols could be partly explained precisely by the use the microbiota makes of them: rather than the original compounds, in fact, we need the metabolites produced by microorganisms. For example (and brace yourselves for a strange name), the ellagitannins contained in pomegranates are able to stimulate the growth of *Akkermansia muciniphila*. It is just an example, but an example that reveals the impact that these substances can have on the lives of others (and not just microorganisms!).

AND WHAT ABOUT FATS, WHAT EFFECT DO THEY HAVE?

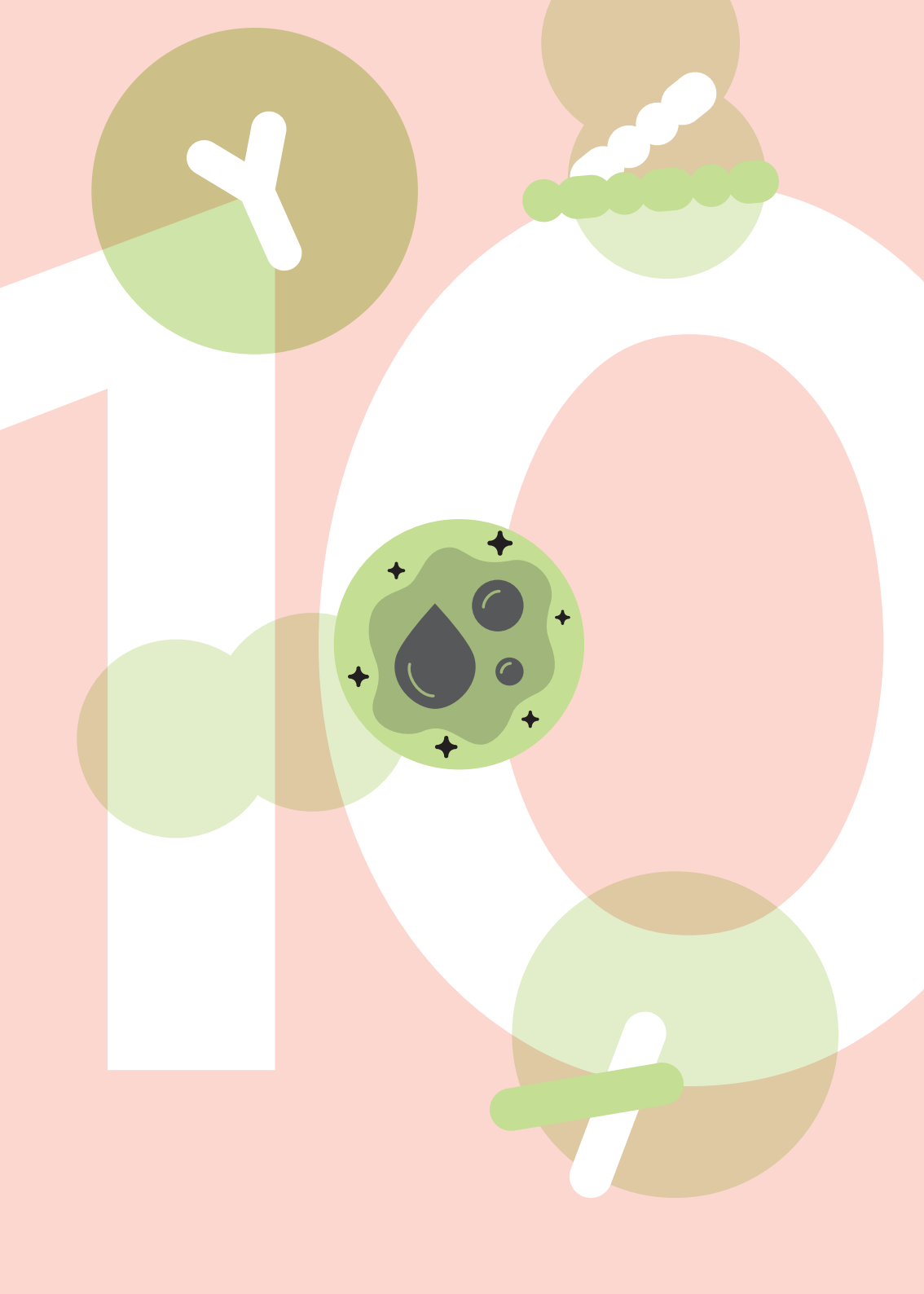
We're leaving the best until last. High-fat diets also modulate the composition of the microbiota. In this case, levels of *Firmicutes* and *Proteobacteria* increase, to the detriment of *Bacteroidetes*, which instead witness a reduction. All this happens extremely quickly: studies on mouse models have shown that the change occurs within 24 hours (shipping at no additional cost!) following the intake of fats. But how do the different types of fatty acids manage to modulate the microbiota? The mechanism is still unclear, but you must not be disappointed when you read in a science book that something "is not yet known". For every "not yet known" you encounter, there is at least one scientist who, full of enthusiasm, is racking their brain trying to interpret the results of their experiments: this curiosity is precisely what drives them. The theories regarding the mechanisms by which fatty acids can modulate the microbiota involve, for example, their ability to act at the cell membrane level, or to interfere with energy production. On the other hand, they can inhibit the activity of some enzymes, i.e. proteins whose role is to intervene in specific biochemical reactions – to each enzyme its own reaction – to speed them up. Inhibiting the activity of enzymes therefore means counteracting some biochemical reactions necessary for cells. Fatty acids can also inhibit the growth of some types of bacteria. Furthermore, the increase in fat intake corresponds to an increase in lipopolysaccharides. As to why, there are two possibilities. On the one hand, it may be that lipopolysaccharides are captured by chylomicrons together with other fats. On the other hand, there may be increased permeability of the intestinal wall, such as to allow lipopolysaccharides to reach blood vessels and enter circulation "without needing to hop on a bus". In support of the second hypothesis, it has been observed that a diet rich in saturated fats leads to alterations in the microbiota, with the secretion of a molecule called chemokine C (CCL5), thus increasing intestinal permeability to microbes such as lipopolysaccharides. Once in the liver, the lipopolysaccharides would be able to exert effects related to the development of liver diseases, such as non-alcoholic fatty liver disease and non-al-

coholic steato-hepatitis (NAFLD and NASH). At the molecular level, lipopolysaccharide would be able to activate two receptors present in the liver cells (Toll-like 4 and 9), which would increase the secretion of a molecule called Tumour Necrosis Factor (TNF), whose presence is connected to the liver diseases mentioned above.

As often happens, however, not only the quantity of fats consumed in a diet is important, but also the quality of the source. A diet rich in extra virgin olive oil has a very different effect to a diet containing lots of butter! First of all, the blood insulin levels associated with a diet rich in extra virgin olive oil are lower, as are the levels of leptin (a hormone involved in controlling food intake). Furthermore, these parameters also correlate with the composition of the microbiota: the higher the insulin levels in the blood, the lower the levels of the *Desulfovibrio* genus, the higher the levels of leptin, and the lower the levels of *Sutterellaceae*, *Marispirillum* and *Mucilaginibacter*. Rats fed with extra virgin olive oil showed an increase in microbial differentiation, which translates into beneficial effects for health. And do you remember omega-3 polyunsaturated fatty acids? Supplementation with omega-3 fatty acids is associated with an increase in *Bacteroidetes* and bacteria capable of producing butyrate, part of the *Lachnospiraceae* family, as well as an increase in *Bifidobacterium*, *Roseburia* and *Lactobacillus*.

Finally, it has been shown that high-fat diets can cause alterations not only in the gut microbiota, but also in the stomach ("gastric microbiota"). In these cases, there is a reduction in species differentiation and a considerable reduction in *Akkermansia muciniphila*, observed first in the stomach and then in the intestine. This means that alterations of the microbiota as a consequence of high-fat diets might first manifest themselves at the gastric level and only subsequently in the intestine.

Years of studies and research, therefore, confirm that diet also has a hand in the balance of our gut microbiota!



THE PRECISION MICROBIOTA: THE FUNCTIONAL FOODS OF THE FUTURE

DIET AND MICROBIOTA: WHAT DOES THE FUTURE HOLD FOR US?

In recent centuries, modern society has had to face profound socio-economic and cultural changes: urbanization has drastically altered our lifestyle, and in particular our dietary habits. Increased productivity, and the consequent increase in the number of hours worked per day, leading us to increasingly optimize our time, has resulted in a clear reduction in the time dedicated to food preparation. This results in greater consumption of processed foods and ready meals, which contain high levels of food additives such as preservatives, sweeteners and emulsifiers, i.e. substances that allow two immiscible elements, such as oil and water, to create a stable mixture. Faced with such a scenario in which, among other things, the use of food additives increases, the microbiota cannot remain indifferent: it is affected, altered, and this predisposes the individual to the development of various chronic and precancerous conditions. Among the substances that have been shown in the laboratory to alter our microbial composition, for example, are carboxymethylcellulose and polysorbate, classified as emulsifiers. Rats administered these substances showed important changes in the diversity of their gut microbiota: increased levels of *Proteobacteria* and *Escherichia* coli and lower levels of *Bacteroides* and *Clostridia*. Other studies have shown that artificial sweeteners (sucralose, aspartame and saccharin), if used consistently over time, might lead to insulin resistance in predisposed individuals. Insulin resistance is the main characteristic of type II diabetes, in which the cells that normally respond to insulin commands begin to resist and stop obeying promptly. According to the data, sweeteners exert this effect due to an increase in the level of *Bacteroides*, *Clostridia* and *Enterobacteriaceae*. In addition, they are thought to cause increased levels of some bacterial genes in the liver, genes that, once present, lead to the formation of proinflammatory proteins.

WILL WE HAVE FOODS "TAILOR-MADE" FOR THE MICROBIOTA ON THE TABLE?

Without any doubt. There is now great interest in *functional food*. In recent years, several experts in the sector have focused their energies on the study of functional foods, and research also aims to understand how these can be used in clinical practice for the maintenance of our health. Let's have a closer look at what this means. FUFOSE (the European Commission Concerted Action on Functional Food Science in Europe) tells us that functional foods are "foods or dietary components consumption of which may have associated health benefits beyond the basic nutritional properties that the foods possess". Functional food must therefore be understood as an integral part of a normal diet and must not be taken in the form of a pill, capsule or dietary supplement. Let's look at an example. Polyunsaturated fatty acids can be divided into omega-3 and omega-6, depending on their chemical structure. Let's focus our attention on omega-3s: where can we find them? They are contained in various types of fish (cod, herring, mackerel, sardines, salmon) and some microalgae. Omega-3 fatty acids, unlike omega-6, have anti-inflammatory properties. This function is performed not only at the cellular level, but also through direct interaction with the gut microbiota. In particular, they help restore the relationship between *Firmicutes* and *Bacteroidetes* in individuals with dysbiosis. The administration of adequate doses of omega-3 would seem able to cause an increase in bacteria whose metabolism produces short-chain fatty acids (SCFA), with consequent anti-inflammatory potential for the intestinal mucosa. Other studies, conducted mainly in the elderly, have shown that the regular intake of polyunsaturated fatty acids could lead to a reduction in the level of proinflammatory molecules.

*
FUNCTIONAL FOOD

Functional food	*Substances contained*	*Properties*
Fish (cod, herring, mackerel, sardines, sardines, salmon etc.) and microalgae	*Polyunsaturated fatty acids Omega-3*	*Anti-inflammatory properties Restoration of the ratio of* Firmicutes *to* Bacteroidetes
Fruits, vegetables, cocoa, tea, coffee, cereals, seeds, wine	*Polyphenols*	*Antimicrobial, antioxidant and anti-inflammatory properties Reduction of the growth of pathogenic bacteria*
Oats	*ß-glucan Saponins*	*Reduction of total and LDL cholesterol ("bad cholesterol")*
Soy (also in the form of edamame, tofu, tempeh, miso)	*Phytochemicals (isoflavones, genistein, etc.)*	*Reduction of total and LDL cholesterol ("bad cholesterol")*
Tomato	*Lycopen*	*Anti-inflammatory properties Reduction of cardiovascular risk*
Walnuts, almonds, cashews, pistachios	*Vitamin E Monounsaturated fatty acids*	*Antioxidant properties Reduction of the risk of coronary heart disease*
Grape juice, red wine	*Resveratrol*	*Cardioprotective*
Green leafy vegetables larga (spinach, kale, broccoli etc.)	*Phytochemicals*	*Reduction of access of carcinogens in the cells*

POLYPHENOLS ARE A HOT TOPIC: HOW COULD THEY HELP US AND OUR MICROBIOTA?

We have already had an opportunity to explain this in part in the previous pages. Polyphenols are molecules contained in numerous foods, such as fruit, vegetables, cocoa, tea, coffee, grains, seeds and wine. These are natural organic substances, generally classified as flavonoids, tannins, lignins, anthraquinones and melanins. They are known for their antimicrobial, antioxidant and anti-inflammatory properties. Their beneficial effect on the body depends, however, on bioavailability, that is, how effectively our body is able to absorb them. Many of these compounds are in fact unable to cross the intestinal barrier and are therefore not absorbed. In the colon, however, polyphenols interact bidirectionally with the gut microbiota, which allows their biotransformation into active metabolites. These, in turn, are able to modulate the microbiota itself, determining its structural and functional changes. Several studies have evaluated how administration of the active biocomponents of tea reduces the growth of numerous unwanted bacteria, such as *Helicobacter pylori*, *Staphylococcus aureus*, *Escherichia coli O157:H7*, *Salmonella*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*. In another study, quercetin supplementation was found to potentially alter the ratio of *Firmicutes* to *Bacteroidetes*, causing dysbiosis

.

FORECASTS FOR THE FUTURE?

The use of *functional foods* can be put into practice at different levels; some of the foods that are normally found on our tables can be modified by human intervention in order to "enhance" their functionality. For example, by adding foods rich in omega-3s (linseed, fish oil or seaweed) to chickenfeed, eggs are obtained which are rich in polyunsaturated fatty acids in spite of the saturated fatty acids normally present (with levels of up to 350mg of omega-3 per egg, compared to the 60mg contained in a normal egg). By definition, however, functional foods also include some natural foods which, if consumed in adequate quantities, perform all their beneficial actions on the body. To name but a few, oats contain ß-glucan and saponins, which reduce total cholesterol and LDL (known as "bad cholesterol"), thus helping to lower blood pressure. Soy (in the form of edamame, tofu, tempeh, miso) is rich in phytochemicals (naturally present in the soy plant) such as isoflavones and genistein. Although we may not have heard of them, these substances are extensively studied because, as we have said, they contribute to the reduction of total cholesterol and LDL levels. Furthermore, lycopene, a substance contained in tomatoes, reduces inflammation and cardiovascular risk. Walnuts, almonds, cashews and pistachios contain vitamin E, known for its antioxidant properties, and monounsaturated fatty acids, which help reduce the risk of coronary heart disease. Grape juice and red wine contain resveratrol, known for its cardioprotective effects. Finally, the phytochemicals contained in green leafy vegetables (spinach, cabbage, broccoli, etc.) have the ability to interfere with the entry of carcinogens into the cells, thus providing protection for the body. In any case, the *sine qua non* for the food of the future is that it is not only functional but also eco-sustainable. Microalgae, for example, are precious natural sources of bioactive compounds such as vitamins, essential amino acids, polyunsaturated fatty acids, minerals, carotenoids, enzymes and fibre, making them perfect! Their added value lies in the fact that they are highly eco-sustainable and therefore perfectly combine the dual need that the future poses for us. It is thus clear that they could be used as functional ingredients to improve the nutritional value of foods!

IN SHORT, WILL WE CURE OURSELVES BY EATING?

Hippocrates, father of medicine, already 2,500 years ago famously said: “Let food be your medicine and medicine your food”. So saying, he emphasized the importance of proper nutrition in human health. The target for the future is the development of microbiota-directed foods (MDF). Acting on the microbiota through food and thus promoting its balance could be a possible way to treat chronic inflammatory diseases linked to intestinal dysbiosis.

In conclusion, it is clear that the medicine of the future will have to be personalized and modulated on the basis of the dynamic modifications of the gut microbiota. However, we must always keep in mind that various factors often contribute to the origin of a disease (we speak of “multifactorial aetiology”, to end with a final technical term): the treatment of gut dysbiosis through “food that targets the microbiota” must be framed within a much broader vision of the management of the individual’s health.

OLD FRIENDS: LACTOBACILLUS, BIFIDOBACTERIUM AND STREPTOCOCCUS THERMOPHILUS

STREPTOCOCCUS THERMOPHILUS

Streptococcus thermophilus *is an old friend of ours, proposed as a bacterial species in 1919 by Orla-Jensen. Its physiological, biological and technological characteristics make it particularly suitable for development in milk. A single* Streptococcus thermophilus *cell measures approximately 0.50.6 µm and is spherical in shape.* Streptococcus thermophilus *cells, however, do not like to be alone, which is why they arrange themselves in chains of various lengths, some as short as just a couple of cells.*

BIFIDOBACTERIUM

Bifidobacterium *is a genus of bacteria of great interest as probiotics; they are normally part of our gut microbiota, where they can reach very high concentrations. These bacteria resemble rods, but have the ability to change their form, sometimes assuming particular X or Y shapes. In fermented milks,* bifidobacteria *play a role in the fermentation of lactose.*

LACTOBACILLUS

A genus of bacteria which includes, for example, L. delbrueckii, L. lactis, L. leichmannii. *These bacteria are called "bacilli" due to their shape which recalls that of a rod. They are quite long and can be found either isolated or in short filaments.*

NEW FRIENDS: AKKERMANSIA MUCINIPHILA, FAECALIBACTERIUM PRAUSNITZII AND ARCHEA

AKKERMANSIA MUCINIPHILA

This is a bacterial species which has only recently "gained popularity". The cells are immobile, oval in shape, about 0.6-1 μm long. They may be arranged singly, in pairs, in short chains or even in aggregates. The optimal growth temperature is 37 °C, which corresponds approximately to the internal temperature of our body. Furthermore, we know that they are able to grow on gastric mucins.

FAECALIBACTERIUM PRAUSNITZII

This is one of the most abundant species in our gastrointestinal tract. In addition to being particularly abundant, it is also known for its potential role in promoting intestinal health: studies have shown that in the presence of some intestinal disorders, Feacalibacterium prausnitzii *is less abundant. Furthermore,* F. prausnitzii *is considered one of the major producers of butyrate, a short-chain fatty acid with benefits and effects for our health.*

ARCHEA

When we talk about archaea we are talking about one of the three domains existing in nature into which life can be grouped, together with eukaryotes and prokaryotes. The probiotics currently used belong to the groups of eukaryotes (for example yeast) and bacteria (for example lactobacilli). And archaea? No probiotics currently belong to this domain; yet there are no known pathogens for humans among the Archaea, and many of them live in close contact with us. These characteristics mean that some archaea have good credentials as candidates for consideration as probiotics. On the other hand, some of them are already naturally present in our gastrointestinal tract!

WEEKLY MENU
FOR THE HAPPY BELLY

MONDAY

BREAKFAST *1 cup of coffee or 1 cup of tea / Whole milk yogurt (1 pot) / 1 teaspoon honey / 6 dry biscuits / Fresh fruit (1 small piece of fruit)*
SNACK *Fresh fruit (1 medium piece of fruit)*
LUNCH *Stewed lentils (mp) / *Mixed salad (mp) / 1 cup of coffee / 1 small square of dark chocolate*
SNACK **Yogurt with rhubarb, fox grapes, pomegranate and redcurrants (1 small bowl)*
DINNER *Grilled chicken or turkey breast (mp) / Sautéed chicory (mp) / Wholemeal bread (1 slice)*

TUESDAY

BREAKFAST *1 cup of coffee or 1 cup of tea / Semi-skimmed milk (1 cup) / Sliced wholemeal bread (1 slice) / 2 teaspoons of jam or 1 teaspoon of honey / Fresh fruit (1 small piece of fruit)*
SNACK *Fresh fruit (1 medium piece of fruit)*
LUNCH **Legume and barley soup (1 bowl) / Raw leafy vegetables, seasoned (mp) / 1 cup of coffee / 1 small square of dark chocolate*
SNACK *1 crispbread: biscuit with spelt, flax and pumpkin seeds*
DINNER **Cabbage and meat rolls (mp) / Baked cubed potatoes with moderate addition of extra virgin olive oil and spices (mp) / Mixed sautéed mushrooms (mp)*

WEDSDAY

BREAKFAST *1 cup of coffee or 1 cup of tea / Plant-based product, such as yogurt, with only fruit and no added sugar (1 pot) / 3 biscuits with legume flour / Fresh fruit (1 small piece of fruit)*
SNACK *Fresh fruit (1 medium piece of fruit)*
LUNCH *Spaghetti with anchovy sauce, garlic and parsley (mp) / Mediterranean vegetables (sautéed or baked) with aubergines, courgettes, peppers and onions (mp)*
SNACK *Whole milk yogurt (1 pot), 2 teaspoons honey / 2 walnuts*
DINNER *Cuttlefish alla livornese (with spinach) (mp) / *Artichoke and potatoes (mp) / *Green radicchio salad, radishes, spring onion, pumpkin and flax seeds (mp)*

mp: medium portion

** recipe on the following pages*

Download the whole day-by-day diet with portions

THURSDAY

BREAKFAST *1 cup of coffee or 1 cup of tea / Whole milk yogurt (1 pot) / Low-fat plain yogurt (1 pot) / 3 tablespoons granola / 2 tablespoons rolled oats / Fresh fruit (1 small piece of fruit)*
SNACK *Fresh fruit (1 medium piece of fruit)*
LUNCH **Pasta and chickpeas (1 bowl) / Baked onions with capers and almonds (mp)*
SNACK *1 cup of tea / 5 wholemeal shortbread biscuits with inulin*
DINNER **Creamy sweet potato and bitter root soup (1 bowl), Parmesan cheese (mp) / 2 slices crispy wholemeal rye bread / Boiled spinach dressed with extra virgin olive oil (mp)*

FRIDAY

BREAKFAST *1 cup of coffee or 1 cup of tea / Whole milk yoghurt (1 pot) / Bruschetta: toasted bread with extra virgin olive oil (1 small slice) / Fresh fruit (1 small piece of fruit)*
SNACK *Fresh fruit (1 medium piece of fruit)*
LUNCH *Bean puree with chicory (mp) / Bruschetta: toasted bread with extra virgin olive oil (mp) / Fresh fruit (1 medium piece of fruit) / 1 cup of coffee / 1 square of dark chocolate*
SNACK *2 small squares of dark chocolate / 2 wholemeal crackers with beta-glucans*
DINNER *Winter vegetable gratin with artichokes or Jerusalem artichokes, turnips, cauliflower and saffron (mp) / Boiled unrefined black Venere rice dressed with extra virgin olive oil (1/2 mp) / Swordfish with cherry tomatoes and olives (mp)*

SATURDAY

BREAKFAST *1 cup of coffee or 1 cup of tea / Soy drink (1 cup) / Wholemeal bread (1 slice) / 3 teaspoons of jam / Fresh fruit (1 small piece of fruit)*
SNACK *Fresh fruit (1 medium piece of fruit)*
LUNCH **Tuscan ribollita (1 bowl) / Homemade toasted bread (1 slice) / Raw leafy vegetables, dressed (mp) / Fresh fruit (1 medium piece of fruit)*
SNACK *Small fruit salad (mp) / 8 almonds*
DINNER **1 prebiotic pizza*

SUNDAY

BREAKFAST *Semi-skimmed milk (1 cup) / Bran flakes or high-fibre bran balls (2 tablespoons) / Multigrain breakfast cereals (2 tablespoons) / Rolled oats (1 tablespoon) / 1 teaspoon honey / Fresh fruit (1 small piece of fruit)*
SNACK *Fresh fruit (1 medium piece of fruit)*
LUNCH **Pasta with sardines (mp) / Raw leafy vegetables, dressed (mp)*
SNACK **Fruit salad of plums, peaches, elderberry, green banana and blueberries (mp)*
DINNER *Creamy leek soup, broad beans and broccoli (1 bowl) / 2 scrambled eggs, omelette-style / Rye bread (1 slice) / Finely chopped green radicchio, dressed (mp)*

MIXED SALAD

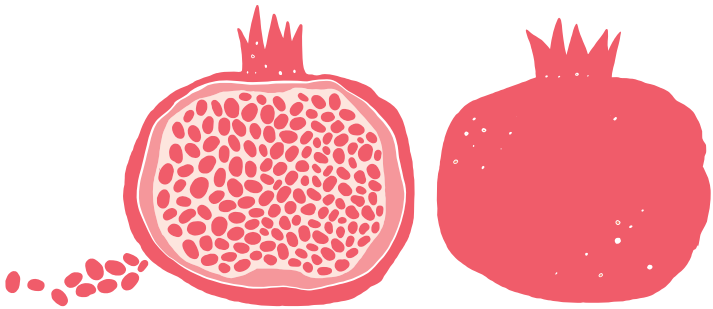
WITH RED CABBAGE, FENNEL, CARROTS, RADICCHIO AND POMEGRANATE

INGREDIENTS

SERVES 4

- 2 LARGE CARROTS (140G)
- 1 SMALL FENNEL BULB (130G)
- 1/4 RED CABBAGE (120G)
- 2 HEADS OF RADICCHIO (200G)
- 1 POMEGRANATE
- 1 BUNCH PARSLEY
- 1 SPRIG TARRAGON
- 1 SPRIG DILL
- 1 FRESH OR DRY CHILLI PEPPER, JUICE OF 1 LEMON (50ML)
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, WHITE PEPPER

1. Wash and peel the vegetables, cut the carrots into julienne strips, the fennel into 3mm thick slices, the cabbage into thin slices and the radicchio into thick slices. Peel the pomegranate and separate the arils; combine everything in a salad bowl. **2.** Add the chopped fresh aromatic herbs (parsley, dill, tarragon) and the chopped chilli **3.** Mix and dress well with oil and lemon, season lightly to taste.



MIXED SALAD AND THE MICROBIOTA

The vegetables for this salad were chosen as being particularly good for the microbiota: fennel contains prebiotic fibre, and if you wish to get as much as possible, you should also eat the heart of the bulb (thinly sliced). Cabbage is a member of the Brassicaceae, a family of vegetables rich in fibre but also in useful substances such as glucosinolates, used by the microbiota and then transformed into isothiocyanates, which are attributed with important health benefits (some studies demonstrate their antitumoral properties). Radicchio, both red and green, has a particular characteristic: its bitter taste, which indicates the presence of polyphenols, substances which perform a prebiotic action. And radicchio, in addition to its precious fibre, when analysed, has also been shown to contain a good quantity of polyphenols. Known polyphenols number hundreds: assuming a wide variety is useful for microbial diversity, which is why pomegranate has also been included in this salad, since it contains large amounts.

SALAD
OF GREEN RADICCHIO, RADISHES, AND SPRING ONION, WITH PUMPKIN AND FLAX SEEDS

INGREDIENTS
SERVES 4

- 1 HEAD GREEN RADICCHIO (200G)
- 12 RADISHES (180G)
- 1 MEDIUM SPRING ONION OR 1 SHALLOT OR 1 LEEK (70G)
- 1 TABLESPOON PUMPKIN SEEDS (15G)
- 1 TEASPOON FLAX SEEDS (10G)
- 16 LAMPASCIONI BULBS IN OIL (120 G)
- 2 TABLESPOONS APPLE CIDER VINEGAR OR LEMON JUICE
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, PEPPER

1. Wash and squeeze the green radicchio, cut it into thin strips using a sharp knife or a mandolin; wash and cut the radishes into slices or julienne; then wash and cut the spring onion into very thin slices. **2.** Combine all the vegetables in a bowl, add the toasted pumpkin seeds, the flax seeds and the lampascioni in oil. **3.** Dress the vegetables with extra virgin olive oil, vinegar or lemon juice, a little salt and pepper to taste."

SALAD OF RADICCHIO AND SPRING ONION AND THE MICROBIOTA

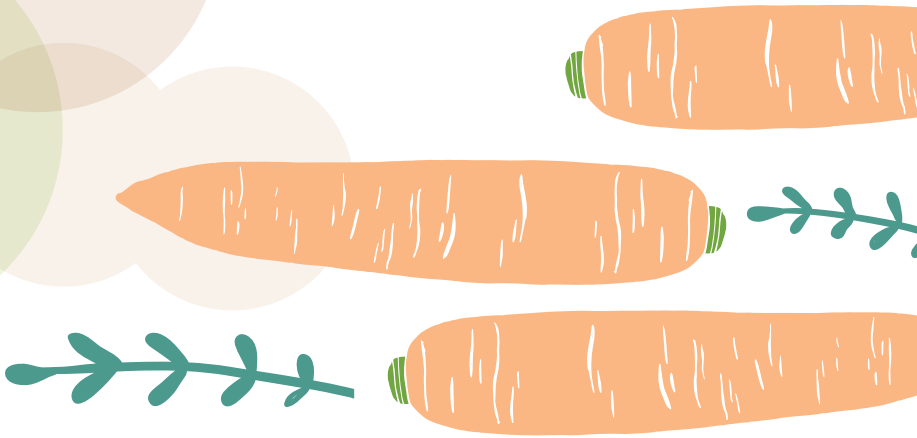
The his salad, a popular dish in Italy, combines the polyphenols contained in radicchio and the prebiotic substances found in large quantities in onions, spring onions, shallots and leeks, including inulin, one of the pre-biotic substances most studied in literature. In addition, there are the glucosinolates contained in radishes, a cruciferous vegetable, and some polyphenols called lignans found in flax seeds which, through the action of the microbiota, can provide useful substances for our body. Also interesting is the presence of particular types of polyphenols in lampascioni, a typically Mediterranean vegetable similar to garlic, which however does not lead to the digestive problems sometimes caused by the latter.

RIBOLLITA
TOSCANA

INGREDIENTS
SERVES 4

- 2 CLOVES GARLIC
- 1 SMALL ONION (70G)
- 2 LARGE CARROTS (140G)
- 1 SMALL STICK CELERY (40G)
- CANNELLINI BEANS – FROZEN OR TINNED (300G), OR DRY (80G)
- 1 SMALL HEAD CHARD (250G)
- 1/2 SMALL SAVOY CABBAGE
- 1 MEDIUM POTATO (200G)
- 1 BUNCH CAVOLO NERO (150G))
- 3 MEDIUM RIPE TOMATOES (180G) OR PEELED TINNED TOMATOES (200G)
- 4 SLICES ARTISAN BREAD (50G EACH)
- A FEW SPRIGS OF THYME
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, PEPPER

1. If you are using dried beans, first soak them overnight. Dried legumes take a long time to prepare, and to reduce the time you can use frozen legumes which, unlike tinned versions, do not contain added salt. If you prefer tinned beans, rinse them well under running water to remove the residues of salty brine. Then cook the cannellini beans (fresh, frozen, or dried and soaked) in water. Set aside about 1/3 of the beans and sieve the rest with their water. If using tinned beans, add 1 glass of water to the sieved mixture **2.** Begin to fry the onion and 1 clove of chopped garlic over a low heat for about 10 minutes. Meanwhile, wash and chop all the other vegetables. Add ripe or peeled tomatoes to the garlic and onion and cook. After a few minutes, add the bean purée and the whole beans kept aside (if you use tinned beans, they must be added only 10 minutes before the end), mix well and finally add all the other vegetables, chopped. Lightly salt, and add pepper and thyme to taste. **3.** Once everything has been mixed well, add about 6 ladles of water and bring to a boil. Simmer for 2 hours, stirring occasionally. **4.** After cooking, toast the slices of bread and rub them with fresh garlic. Serve the ribollita with the crispy bread.



RIBOLLITA AND MICROBIOTA

This is one of the traditional regional dishes which offers most metabolic substrates to the microbiota through the synergy between different brassicas, kale and savoy cabbage, and also helps reduce the presence of sulphur-reducing bacteria. Legumes are an important source of a particular type of fibre, called galacto-oligo-saccharides (GOS), which have a documented beneficial action for our microbiota. The dish also contains onion and garlic, which are important sources of pre-biotic fibre. During the summer, ribollita is always eaten cold, and traditionally fresh spring onion and extra virgin olive oil are added, providing additional useful nutrients.

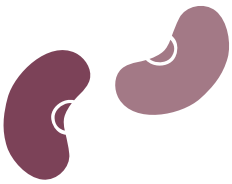
SOUP
OF LEGUMES AND BARLEY (OR ROLLED OATS)

INGREDIENTS
SERVES 4

- LENTILS: DRIED (25G) OR TINNED (90G)
- BEANS: DRIED (25G) OR FRESH, FROZEN OR TINNED (90G)
- CANNELLINI BEANS: DRIED (25G), OR FROZEN OR TINNED (90G)
- CHICKPEAS: DRIED (25G), OR FROZEN OR TINNED (90G)
- PEARL BARLEY (120G) OR ROLLED OATS (100G)
- PARMIGIANO REGGIANO (20 G)
- 1 SMALL ONION (70G)
- 2 LARGE CARROTS (140G)
- 1 SMALL STICK CELERY (40G)
- VEGETABLE STOCK (1/2L)
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, PEPPER

As an alternative to chickpeas, you can use a mix of legumes for soups (100g). If you use dried legumes: the day before cooking, soak the beans and chickpeas for about 12 hours. The various legumes should then be cooked separately and in accordance with their individual cooking times. If you use fresh or frozen legumes, they should simply be cooked separately, respecting their cooking times, after having been thoroughly rinsed.

1. Wash, peel and chop the carrots, celery and onion. Fry the vegetables and add the previously prepared legumes, barley, vegetable broth and water. Simmer for at least 30 minutes until the barley is well cooked. As an alternative to barley, you can use rolled oats, preferably coarse, but they should be added after cooking. **2.** Sprinkle with Parmigiano Reggiano. Serve, and if desired add extra virgin olive oil and a few twists of the pepper mill. **3.** If you like, you can complete the dish with a touch of heat: season with a drizzle of chilli-infused extra virgin olive oil, or with chilli flakes.



SOUP OF LEGUMES AND CEREALS AND THE MICROBIOTA

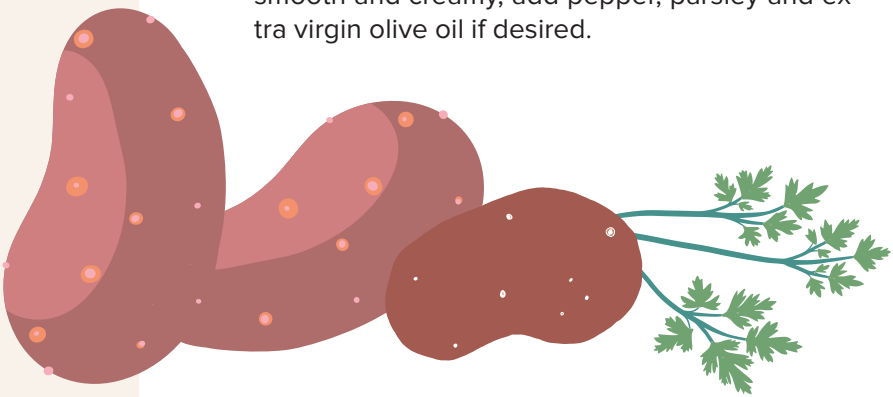
The consumption of legumes is of fundamental importance for the health of the microbiota: they are rich in fermentable fibres such as GOS, but also in a particular form of starch, called resistant starch type 1 (RS-1), which is not absorbed in the intestine but fermented by the microbiota. In order to optimize the prebiotics contained in the soup, it is a good idea to associate barley or oats with them, since they contain a particular type of fibre, called beta-glucans, which have been shown in randomized clinical trials to have prebiotic properties.

CREAMY
SWEET POTATO SOUP WITH BITTER ROOTS

INGREDIENTS
SERVES 4

- 3 SWEET POTATOES, PREFERABLY PURPLE (400G)
- 1 BITTER ROOT (200G)
- 1 SHALLOT (50G)
- A FEW CLOVES OF GARLIC
- 1/2 LITRE VEGETABLE STOCK
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- PARSLEY
- PEPPER

1. Wash, peel and cut the sweet potatoes and bitter roots into large cubes, but keep in mind that the cooking of the bitter roots takes a little longer if they are more woody. **2.** Prepare a soffritto with the garlic and shallot, add half a litre of vegetable stock and if necessary water, and boil for 20 minutes, then blend everything to make the soup smooth and creamy, add pepper, parsley and extra virgin olive oil if desired.



CREAMY SWEET POTATO SOUP AND THE MICROBIOTA

This is a creamy soup with a marked prebiotic action, to be consumed in moderation, since the microbiota must be progressively trained to metabolize prebiotic nutrients. Sweet potatoes contain starch, fibre and polyphenols, which have a known prebiotic action; the purple variant of sweet potatoes contains particular types of starches capable of increasing the population of Bacteroidetes, Lachnospiraceae and Rumenococcaceae, and activates the production of short-chain fatty acids. Bitter roots are the food with the highest content of inulin, which is also extracted from them to produce nutraceuticals. If you are not used to eating them, they can cause excessive bloating and should therefore be consumed in moderation, which is why this recipe uses a modest quantity. If well tolerated, you can progressively increase the amount the next time, decreasing that of sweet potatoes, eventually using equal quantities of each.

PASTA
WITH SARDINES

INGREDIENTS
SERVES 4

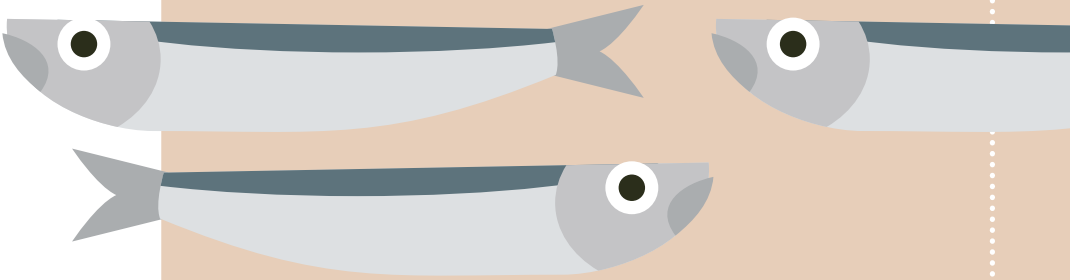
- MACCHERONCINI (320 G)
- FRESH SARDINES (500G)
- 6 SPRIGS WILD FENNEL
- RAISINS (40G)
- PINE NUTS (25G)
- 4 ANCHOVY FILLETS IN OIL
- 1 SMALL ONION (70G)
- 1 CLOVE GARLIC
- 1 BUNCH PARSLEY
- 1 SACHET SAFFRON OR 1/2 TEASPOON SAFFRON PISTILS
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, PEPPER

1. Cook the wild fennel in plenty of salted water, counting 15 minutes from the start of boiling. Drain, set aside and reserve the cooking water. **2.** Prepare the sardines: remove the head, butterfly from the belly side and debone them. **3.** In a pan, heat 1 tablespoon of extra virgin olive oil (15g) with garlic and parsley, add the sardines, pour in half a glass of water and cook for 10-15 minutes, then add salt. Add the saffron dissolved in a little water with salt and pepper; stir so that the sardines break up, cook for 10 minutes, remove from the heat and set aside. **4.** In another pan, stew the chopped onion with half a glass of water and a tablespoon of extra virgin olive oil (15g), add the finely chopped wild fennel, the soaked and squeezed raisins, the pine nuts, and the anchovies, and cook for 10 minutes. **5.** Cook the pasta in the reserved fennel cooking water, drain, dress with the sardine and saffron sauce, and mix. **6.** Sprinkle with parsley and serve.

The original Sicilian recipe involves frying some of the floured sardines in extra virgin olive oil. Once the pasta has been cooked, layer pasta and fried sardines in an oven dish, and bake for 15 minutes.

PASTA WITH SARDINES AND THE MICROBIOTA

This pasta dish combines the resistant starch type 1 of the pasta with the omega-3 contained in the sardines; in particular, it reduces Faecalibacterium and increases Bacteroidetes, thus increasing the production of butyrate, a metabolite essential for gut health. In addition to these properties are those of saffron, rich in healthy substances such as the carotenoid crocetin, which the microbiota transforms into trans-crocetin, making it bioavailable to our body.



PASTA
AND CHICKPEAS

INGREDIENTS
SERVES 4

- CHICKPEAS: FROZEN OR TINNED (400G), OR DRIED (110G)
- REGINETTE PASTA (120G)
- 1 SMALL ONION (70G)
- 2 FRESH MEDIUM TOMATOES (180G)
- 2 CLOVES GARLIC
- 2 SALTED ANCHOVIES
- 2 SPRIGS OF ROSEMARY
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, PEPPER

1. If you are using dried chickpeas, soak them for about 12 hours, then cook them in water for 2 hours with the rosemary and a clove of garlic tied together to form a bouquet garni. Halfway through cooking, salt lightly. **2.** Once cooked, purée a third of the chickpeas and add them to the rest with the cooking liquid. **3.** Cook for about 30 minutes and add more water if necessary. If you are using frozen chickpeas, you can follow the same procedure as for soaked chickpeas. If you prefer tinned chickpeas, you will need to wash them well, then divide them: a third should be puréed and then added to the remainder with 1 litre of water, with a bouquet garni of rosemary and garlic. Place in a saucepan and bring to a boil. **4.** Meanwhile, gently fry the sliced onion, then add the fresh tomatoes, peeled and coarsely chopped. To peel the tomatoes, cut a cross into the base using a sharp knife and dip them for a few seconds in boiling water: they will then peel easily. **5.** Once cooked for about ten minutes, when it has lost excess water add the chickpea sauce and cook for a few more minutes so that all the ingredients mingle. At this point, bring back to the boil and add the

PASTA WITH CHICKPEAS AND THE MICROBIOTA

A traditional dish from Southern Italy that combines the resistant starch type 1 found in pasta, an important prebiotic capable of increasing the production of short-chain fatty acids by the microbiota, with the fibre contained in legumes. In particular, chickpeas are rich in an oligosaccharide called raffinose; even if tinned, they have been shown to increase the production of butyrate by the microbiota and to reduce the quantity of putrefactive bacteria

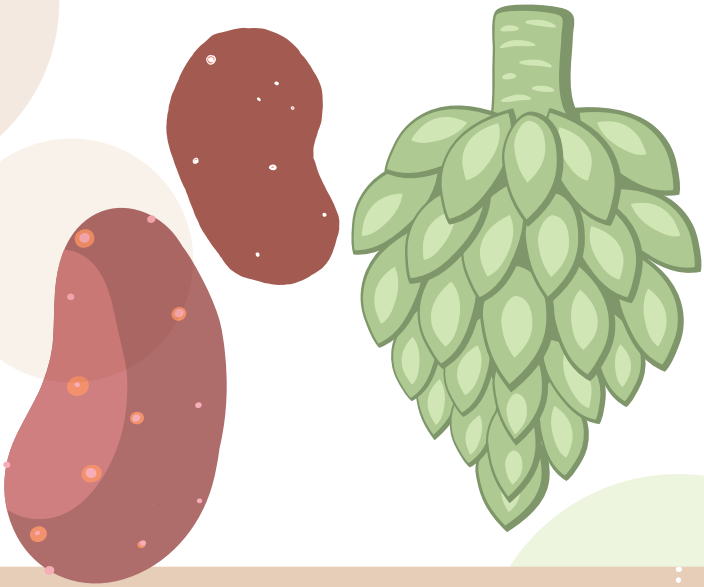
reginette pasta, which you have snapped into short sections, into the chickpea soup; stir until the pasta is completely immersed. **6.** Meanwhile, desalt the anchovies by washing them under running water, chop finely with a clove of garlic and loosen with 2 tablespoons of the chickpea broth to form a paste. A couple of minutes before removing the pasta and chickpeas from the heat, add this anchovy paste and mix well. **7.** Before serving, sprinkle with pepper and if desired drizzle over some extra virgin olive oil.

ARTICHOKES
AND POTATOES

INGREDIENTS
SERVES 4

- 8 ARTICHOKES (900G)
- 3 MEDIUM POTATOES (600G)
- 1 CLOVE GARLIC
- 1 BUNCH PARSLEY
- JUICE OF 1 LEMON
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, PEPPER

1. Clean the artichokes and stems, cutting away the outer leaves and removing the inner petals and choke. Set aside some of the outer leaves to add during cooking to flavour (or to make a vegetable stock). Cut the artichokes in half lengthways and the stems into 2-3 parts. Leave them to soak in water and lemon juice to prevent them from blackening. **2.** Peel and cut the potatoes into rounds. Fry a clove of garlic with extra virgin olive oil in a large deep saucepan over a moderate heat to prevent it from burning. Drain the artichokes and brown them for a minute over high heat in the pan with the garlic. **3.** Then add the sliced potatoes and sauté with the other ingredients. Pour half a glass of water or stock prepared with the outer leaves of the artichoke over the vegetables, season with salt and pepper, and cover the pan. **4.** Continue cooking in a covered pan for 30-40 minutes over a low heat, stirring occasionally, adding water or stock if necessary. **5.** When cooked, sprinkle with chopped parsley and serve the vegetables lukewarm.

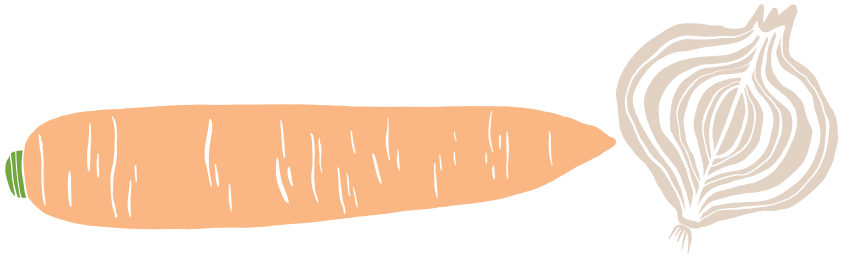


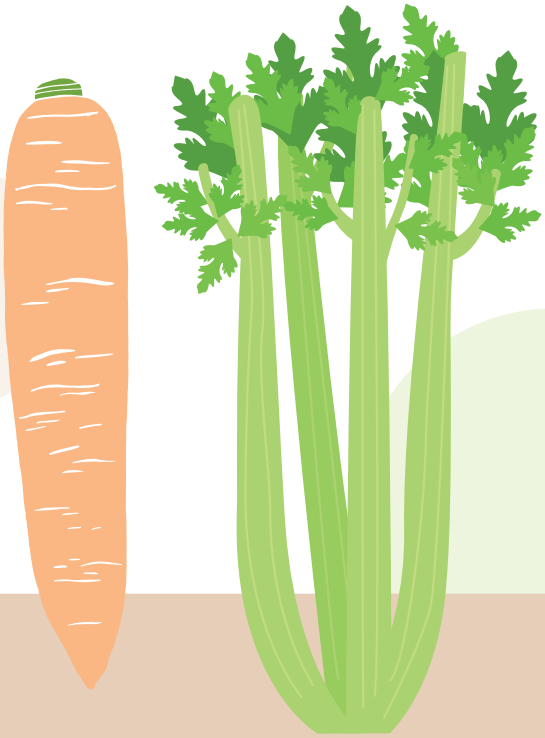
ARTICHOKES AND POTATOES AND THE MICROBIOTA

Artichokes contain large amounts of inulin and oligofructose, and have traditionally been considered good for the liver. This is probably due to their beneficial action on the microbiota rather than to the presence of a specific hepatoprotective nutrient. Some fibre beneficial for the microbiota is also found in the stem of the artichoke, which is usually discarded but edible, and is used in traditional recipes in central and southern Italy. When eating this dish, it is worth noting that if left to cool, the starch in the potatoes undergoes a process called retrogradation in which resistant starch type 3 is formed. On the one hand, this becomes a substrate with a prebiotic action, and on the other it lowers the glycaemic response to potatoes.

SOFFRITTO

The *soffritto* is a distinctive feature of Mediterranean cuisine, and has the function of flavouring dishes. If done well, it contributes to the success of more complex recipes. But, like all simple preparations, achieving the best results requires attention to detail. To prepare a soffritto, you must first carefully cut the onion into slices about 5 mm thick – for some more richly-flavoured dishes you can also add carrot and celery – and fry in extra virgin olive oil over a medium heat. The onion will start to turn transparent in a few minutes. In order to enhance the properties of the ingredients used, it is important to check the soffritto and stir it often, to prevent the vegetables from coming into direct contact with the hot pan. If you are cooking a small amount (with, for example, 1/2 onion, 30g of extra virgin olive oil, and perhaps 1/2 carrot and 1/2 stalk of celery) it may help to tilt the pan a little to combine oil and vegetables in a single point, where they can fry correctly. This method also allows you to use less oil. Once the onion is golden, you must be careful not to burn the vegetables: the cooking time may be increased a little by deglazing with water or white wine. Water or wine must be added with parsimony, otherwise the temperature will drop excessively and we will no longer have a soffritto. A good soffritto needs to cook for 12-15 minutes.





SOFFRITTO AND THE MICROBIOTA

There are various reasons why a soffritto may benefit the health of the microbiota, and contribute to making Mediterranean cuisine one of the healthiest in the world. In fact, onions are rich in fructans, and in particular in fructo-oligosaccharides (FOS), substances with documented prebiotic activity; moreover, the consumption of extra virgin olive oil is associated with positive modulation of the microbiota. As the soffritto cooks, the bioavailability of some polyphenols and carotenoids contained in the vegetables increases; we now know that most polyphenols are not absorbed but are used as prebiotics by the microbiota. We should also add that the soffritto makes dishes containing large amounts of vegetables more appetizing, so that the intake of prebiotic substances essential to stimulate so-called microbial diversity, crucial for a healthy microbiota, becomes more enjoyable.

BAKED ONIONS
WITH CAPERS AND ALMONDS

INGREDIENTS
SERVES 4

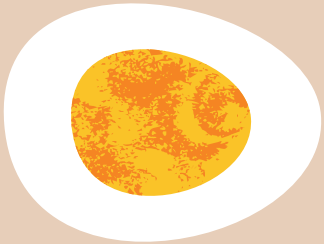
- 4 LARGE RED ONIONS (800G)
- 2 TABLESPOONS CAPERS, DESALTED OR IN OIL (20G) OR 8 CAPER BERRIES
- FLAKED ALMONDS (20G)
- BREADCRUMBS (50G)
- 1 EGG
- 4 SAGE LEAVES
- 40G EXTRA VIRGIN OLIVE OIL
- SALT, PEPPER

1. Wash and peel the onions (in order to stop your eyes from watering, fill a bowl with water and peel the onions while keeping them immersed). Bring a saucepan of salted water to the boil. Add the onions and let them cook for about 10 minutes. Drain and let cool. **2.** Now for the capers: wash in warm water, dry well and chop. Chop the sage. Mix the capers and sage in a bowl. Add the breadcrumbs, whole egg, half the oil, a pinch of salt and a generous twist of pepper; if the mixture is too viscous, add 1 tablespoon of the onion cooking water.

3. Cut the onions in half, spread the remaining oil on the bottom of an oven dish, preferably in terracotta, arrange the onions close together so that, touching at the sides, they stay upright. **4.** Sprinkle over the chopped capers, sage and breadcrumbs, then the almond flakes, and bake in a preheated oven at 200 °C for about 20 minutes. **5.** Remove from the oven and leave to cool for a few minutes before serving.

BAKED ONIONS AND THE MICROBIOTA

Onions are one of the foods richest in fruit oligosaccharides (FOS) and also contain polyphenols, such as quercetin: these two substances perform a prebiotic action. When cooked properly they do not cause digestive problems: a recipe like this allows you to consume larger quantities than those consumed when the onion is used in a soffritto, and the prebiotic effect of FOS on the microbiota also depends on the quantity eaten. We also have the polyphenols typical of capers, such as kaempferol, which has a documented beneficial action on the microbiota.



SAVOY CABBAGE
AND MEAT ROLLS

INGREDIENTS
SERVES 4

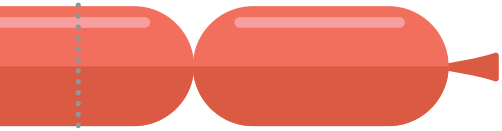
- LEAN VEAL MINCE (220G)
- FRESH PORK SAUSAGE (100G)
- 1/2 SMALL SAVOY CABBAGE (200G)
- BREADCRUMBS (20G)
- PARMIGIANO REGGIANO (20G)
- 1 EGG
- 1 SMALL ONION (70G)
- 1 CLOVE GARLIC
- 1 BUNCH FRESH PARSLEY
- NUTMEG
- 2 TABLESPOONS EXTRA VIRGIN OLIVE OIL (30G)
- SALT, PEPPER

In this traditional recipe, found in various pre-alpine areas of Lombardy, meat is combined with brassicas.

1. Prepare the filling by mixing the minced meat with the sausage and egg, add chopped parsley and garlic, breadcrumbs, Parmigiano Reggiano, salt, pepper and nutmeg, and mix well.

2. While the filling rests, cook the cabbage leaves in boiling water for 2-3 minutes, then let them dry on a tea towel. Prepare the soffritto with extra virgin olive oil and onion. Use the filling to form meatballs about 6 cm in diameter and place each meatball on a cabbage leaf; wrap the leaf around the filling and secure with kitchen twine to obtain a spherical or egg shaped parcel.

3. Place these in the soffritto and cook for about 20 minutes in a large, thick-bottomed pan, covered, over medium-low heat. If they start to dry out during cooking, add a little water or stock



SAVOY CABBAGE ROLLS AND THE MICROBIOTA

Eating less meat is one of the suggested ways to benefit the health of the microbiota, and those who consume too much meat display excessive levels of Bacteroides; moreover, the carnitine present in red meat is converted by the microbiota into trimethylamine, a compound associated with cardiovascular diseases. However, cutting out meat entirely does not lead to greater advantages than reducing its intake, so we chose a recipe in which the amount of meat can be limited in favour of other ingredients, such as cabbage. Cooking cabbage has its own benefits: by inactivating the enzyme myrosinase it makes the glucosinolates available to the microbiota, thus carrying out a prebiotic action.

“PREBIOTIC” PIZZA

WITH DOUBLE TOMATO SAUCE, TROPEA ONIONS, OLIVES, GARLIC AND CAPERS

INGREDIENTS

SERVES 4

- 700G PIZZA DOUGH
- 800G TOMATO SAUCE
- 24 CHERRY TOMATOES
- 1 CLOVE GARLIC
- 2 SMALL TROPEA ONIONS (140G)
- 24 OLIVES IN OIL
- 16 MARINATED GARLIC CLOVES
- 2 TABLESPOONS CAPERS, DESALTED OR IN OIL
- OREGANO
- 40G EXTRA VIRGIN OLIVE
- SALT

If you decide to make homemade pizza, use a classic dough recipe, with 300g of “00” flour and 10g of brewer’s yeast. After it has rested and has risen, roll it out with a rolling pin and give it a round shape. If you are using ready-made pizza dough, all you have to do is roll it out, let it rest for about 20 minutes and add the toppings. **1.** Preheat the oven to 250 °C. Chop the garlic, add it to the tomato sauce and season with two tablespoons of extra virgin olive oil and salt; if it is too liquid, cook to reduce. Thinly slice the Tropea onions, halve the cherry tomatoes and the marinated garlic cloves and desalt the capers well. For those unfamiliar with them, marinated garlic cloves do not cause digestive problems, unlike fresh garlic, and are sold in jars similar to those used for olives. **2.** Once the tomato sauce has reduced and intensified in flavour, spread it over the dough. **3.** Then add the cherry tomatoes, the sliced Tropea onions, the olives, the capers and the marinated garlic cloves and bake for about 6 minutes. Add basil and the remaining olive oil.

"PREBIOTIC" PIZZA AND THE MICROBIOTA

Pizza itself is not particularly good for the health of the microbiota, however if the ingredients are carefully chosen, its properties can be optimized. First of all, a good quantity of tomato puree and cherry tomatoes are a source of lycopene, to which we should add the fructo-oligosaccharides (FOS) contained in the onion, the inulin contained in the garlic, the kaempferol in the capers, and the polyphenols in the olives and extra virgin olive oil; all of these are very beneficial for the health of the microbiota.

FRUIT SALAD

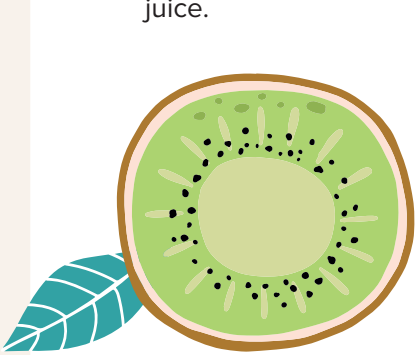
OF DAMSONS, PEACHES, KIWIS, ELDERBERRIES, GREEN BANANA AND BLUEBERRIES

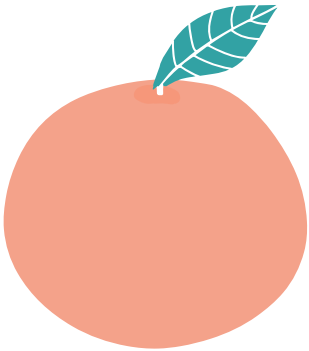
INGREDIENTS

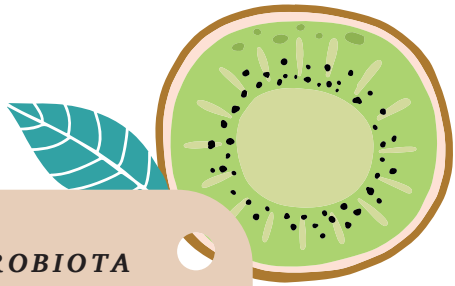
SERVES 4

- 2 DAMSONS (140G)
- 2 PEACHES (280G)
- 2 KIWIS (140G)
- 1 BANANA WITH GREEN PEEL (120G)
- BLUEBERRIES (120G)
- ELDERBERRIES (30G)
- 1 ORGANIC LEMON

1. Wash the peaches and damsons, remove the stones and cut everything into pieces leaving the peel on. **2.** Peel and cut the kiwis and banana into pieces, add the blueberries and elderberries after having carefully washed them. Remove only the yellow outer peel of the lemon, squeeze it, then finely dice the white part of the peel. **3.** Add all the cut fruit to a bowl and sprinkle with lemon juice.







FRUIT SALAD AND THE MICROBIOTA

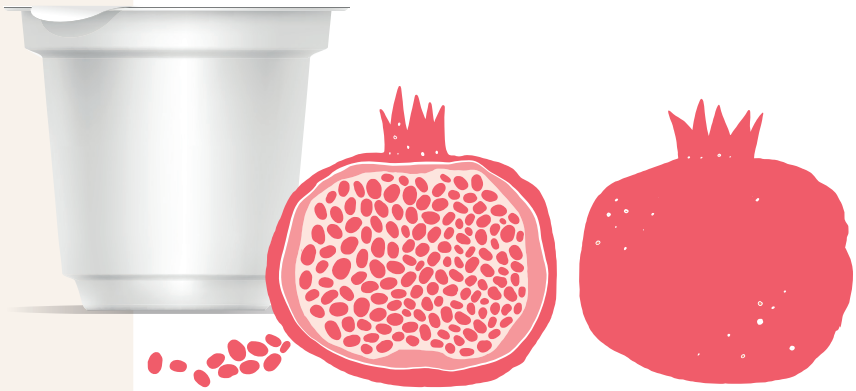
The regular consumption of fruit is of fundamental importance for the microbiota; the greater the variety of fruit eaten, the greater the variety of fibre and polyphenols which can help stimulate the microbial diversity of the microbiota. Fibre is found above all in the flesh, but keeping the peel on is a good idea, since many nutrients, including prebiotic polyphenols, are particularly concentrated in this part of the fruit. Fruits that are particularly beneficial for the microbiota have been chosen for this fruit salad: kiwis, as they contain various types of pectins with a prebiotic action and increase levels of Lactobacillus rhamnosus; and peaches are one of the fruits richest in fruit-oligosaccharides. Out of season they can be replaced with pears or apples. Plums, meanwhile (including prunes), have been shown to boost Bacteroides and reduce Firmicutes, which are considered to be beneficial changes at the metabolic level. Bananas contain resistant starch type 3, but during ripening this prebiotic starch is converted into sugars, which is why it is preferable to use bananas which are not too ripe and have a green skin. Thanks also to the particular polyphenols they contain, blueberries are good for the microbiota, and in particular for lactobacilli and biofibacteria. The particular pectins contained in lemon (and other citrus fruits) are good for the microbiota, especially if you add the peel, rich in pectic oligosaccharides, while in elderberries we find about twenty different types of anthocyanins, nutrients with documented prebiotic properties.

YOGURT
WITH RHUBARB, FOX GRAPES, POMEGRANATE AND REDCURRANTS

INGREDIENTS
SERVES 4

- 1 POT SUGAR-FREE YOGURT OR FERMENTED WHOLE MILK (125G)
- 30G CRANBERRIES OR REDCURRANTS
- 40G FOX GRAPES (*VITIS LABRUSCA*)
- 30G POMEGRANATE ARILS
- 2 TEASPOONS RHUBARB JAM

1. Vigorously mix the marmalade with the yogurt. **2.** Then add the fruit, without removing the grape seeds and using the pomegranate arils whole, without squeezing them.



YOGURT WITH FRUIT AND THE MICROBIOTA

The studies on the microbiota began more than a century ago with Élie Metchnikoff, who analysed the relationship between the consumption of fermented milk and gut health; since then a lot of research has been done. The useful consequences for the health of the microbiota attributable to the consumption of yogurt seem to derive above all from its bioactive peptides, which improve the intestinal mucosa, while as regards fermented milks, they may contain bacteria with documented probiotic action, such as bifidobacteria. In addition, in this recipe, foods have been selected on the basis of their benefits for Akkermansia muciniphila, a component of the microbiota particularly important for the metabolism and body weight regulation: rhubarb, because of its anthraquinone content; pomegranate, thanks to its ellagitannins (it is better not to squeeze the arils so as not to lose any of their precious antioxidants); cranberries, thanks to proanthocyanidins; and fox grapes, for the anthocyanins and type B proanthocyanidins contained in their seeds.

A CUP OF COFFEE
AND A PIECE OF CHOCOLATE, OR A "MOROCCAN" COFFEE

At the end of the meal, sometimes you feel the need for something gratifying: a coffee and a piece of chocolate. Not only are they widely loved, they can benefit the health of the microbiota thanks to some of their nutritional characteristics. Coffee contains caffeine and chlorogenic acid, the consumption of which can increase the production of propionate and butyrate through a positive modulation of the microbiota. Chocolate and cocoa are considered prebiotics, being rich in catechins and epicatechins, types of polyphenols whose consumption has been shown to be useful for the health of the microbiota as they produce an increase in lactobacilli and bifidobacteria and a reduction in clostridia. Also worth a mention is “Moroccan” coffee, a blend of coffee and cocoa (but without the cocoa butter used in chocolate, which is rich in saturated fats).

REFERENCES

MIXED SALAD WITH RED CABBAGE, FENNEL, CARROTS, RADICCHIO AND POMEGRANATE

P. Soundararajan, J.S. Kim *et al., Anti-Carcinogenic Glucosinolates in Cruciferous Vegetables and Their Antagonistic Effects on Prevention of Cancers*, in "Molecules", vol. 23, issue 11, 15 November 2018, doi: 10.3390/molecules23112983.

K.R. Martin *et al., Development of a novel pomegranate standard and new method for the quantitative measurement of pomegranate polyphenols,* in "Journal of the Science of Food and Agriculture", vol. 89, 2009, pp. 157-162.

SALAD OF GREEN RADICCHIO, RADISHES AND SPRING ONION, WITH PUMPKIN AND FLAX SEEDS

A.J. Moshfegh, *Presence of inulin and oligofructose in the diets of American*, in "The Journal of Nutrition", vol. 129, issue 7, July 1999, doi: 10.1093/jn/129.7.1407S.

Saleem Ali Banihani *et al., Radish (Raphanus sativus) and Diabetes*, in "Nutrients", vol. 9, issue 9, September 2017, p. 1014.

TUSCAN RIBOLLITA

G.T. Macfarlane, H. Steed, S. Macfarlane, *Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics*, in "Journal of Applied Microbiology", vol. 104, issue 2, 2008, pp. 305-344.

L. Kellingray, H.S. Tapp, S. Saha, J.F. Doleman, A. Narbad, R.F. Mithen, *Consumption of a diet rich in Brassica vegetables is associated with a reduced abundance of sulphate-reducing bacteria: A randomised crossover study*, in "Molecular Nutrition & Food Research", September 2017, vol. 61, issue 9, doi: 10.1002/mnfr.201600992.

SOUP OF LEGUMES AND BARLEY (OR ROLLED OATS)

B.S. Yadav *et al., Resistant starch content of conventionally boiled and pressure-cooked cereals, legumes and tubers*, in "Journal of Food Science and Technology", vol. 47, issue 1, January 2010, pp. 84-88.

M.P. Arena *et al., Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria*, in "International Journal of Molecular Sciences", vol. 15, issue 2, 20 February 2014, pp. 3025-3039, doi: 10.3390/ijms15023025.

CREAMY SWEET POTATO SOUP WITH BITTER ROOTS

Thatyane Mariano Rodriguesde Albuquerque *et al., Sweet potato roots: Unrevealing an old food as a source of health promoting bioactive compounds – A review*, in "Trends in Food Science & Technology", vol. 85, March 2019, pp. 277-286.

Chao Tang *et al., Effects of polysaccharides f rom purple sweet potatoes on immune response and gut microbiota composition in normal and cyclophosphamide treated mice*, in "Food & Function", vol. 9, 2018, pp. 937-950.

PASTA WITH SARDINES

L. Costantini *et al., Impact of Omega-3 Fatty Acids on the Gut Microbiota*, in "International Journal of Molecular Sciences", vol. 18, issue 12, 7 December 2017.

M. José Bagur *et al., Saffron: An Old Medicinal Plant and a Potential Novel Functional Food*, in "Molecules", vol. 23, issue 1, 23 December 2017.

PASTA AND CHICKPEAS

A.R. Bird *et al., Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics*, in "Beneficial Microbes", vol. 1, issue 4, November 2010, pp. 423-431.

W.M. Fernando *et al., Diets supplemented with chickpea or its main oligosaccharide component raffinose modify faecal microbial composition in healthy adults*, in "Beneficial Microbes", vol. 1, issue 2, June 2010, pp. 197-207.

ARTICHOKES AND POTATOES

M. Renna *et al., Faba Greens, Globe Artichoke's Offshoots, Crenate Broomrape and Summer Squash Greens: Unconventional Vegetables of Puglia (Southern Italy) With Good Quality Traits*, in "Frontiers in Plant Science", vol. 27, issue 9, March 2018, p. 378.

Suman Mishra *et al., Effect of Processing on Slowly Digestible Starch and Resistant Starch in Potato*, in "Starch", vol. 60, issue 9, 9 September 2008, pp. 500-507.

SOFFRITTO

R. Llorach *et al., Lettuce and chicory byproducts as a source of antioxidant phenolic extracts*, in "Journal of Agricultural and Food Chemistry", vol. 52, issue 16, 11 August 2004, pp. 5109-5116.

J.X. Song *et al., Dietary Capsaicin Improves Glucose Homeostasis and Alters the Gut Microbiota in Obese Diabetic ob/ ob Mice*, in "Frontiers in Physiology", vol. 8, 25 August 2017, p. 602.

BAKED ONIONS WITH CAPERS AND ALMONDS

E. Roldán-Marín *et al., Effects of an onion by-product on bioactivity and safety markers in healthy rats*, in "British Journal of Nutrition", vol. 102, issue 11, December 2009, pp. 1574-1582.

L. Marín *et al., Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties*, in "BioMed Research International", doi: 10.1155/2015/905215.

SAVOY CABBAGE AND MEAT ROLLS

S. Rath *et al., Uncovering the trimethylamine-producing bacteria of the human gut microbiota*, in "Microbiome", vol. 5, issue 1, 15 May 2017, p. 54, doi: 10.1186/s40168-017-0271-9.

Francisco J. Barba *et al., Bioavailability of Glucosinolates and Their Breakdown Products: Impact of Processing*, in "Frontiers in Nutrition", vol. 3, 2016, p. 24.

"PREBIOTIC" PIZZA WITH DOUBLE TOMATO SAUCE, TROPEA ONIONS, OLIVES, GARLIC AND CAPERS

Ning Zhang *et al., Study on prebiotic effectiveness of neutral arlic fructan in vitro*, in "Food Science and Human Wellness", vol. 2, issue 3-4, September-December 2013, pp. 119-123.

Hongxia Zhang *et al., Phytochemical and Pharmacological Properties of Capparis spinosa as a Medicinal Plant*, in "Nutrients", vol. 10, issue 2, 2018, p. 116.

FRUIT SALAD OF PLUMS, PEACHES, KIWIS, ELDERBERRIES, GREEN BANANA AND BLUEBERRIES

Derek V. Seidel *et al., Shaping functional gut microbiota using dietary bioactives to reduce colon cancer risk*, in "Seminars in Cancer Biology", vol. 46, October 2017, pp. 191-204.

Shanthi G. Parkar, *Gut health benefits of kiwifruit pectins: Comparison with commercial functional polysaccharides*, in "Journal of Functional Foods", vol. 2, issue 3, July 2010, pp. 210-218.

YOGURT WITH RHUBARB, FOX GRAPES, POMEGRANATE AND REDCURRANTS

Clara Depommier *et al., Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study*, in "Nature Medicine", vol. 25, 2019, pp. 1096-1103.

Kequan Zhou, *Strategies to promote abundance of Akkermansia muciniphila, an emerging probiotics in the gut, evidence from dietary intervention studies*, in "Journal of Functional Foods", vol. 33, June 2017, pp. 194-201.

A CUP OF COFFEE AND A SQUARE OF CHOCOLATE, OR A "MOROCCAN" COFFEE

Kazuchika Nishitsuji *et al., Effect of coffee or coffee components on gut microbiome and short-chain fatty acids in a mouse model of metabolic syndrome*, in "Scientific Reports", vol. 8, 2018.

Nabil Hayek, *Chocolate, gut microbiota, and human health*, in "Frontiers in Pharmacology", vol. 4, 2013, p. 11.

GLOSSARY

ANGIOGENESIS: *generation of new blood vessels starting from existing ones. Necessary for a wide range of physiological processes, such as normal tissue growth. Blood carries nutrients and oxygen to cells and is therefore crucial for their survival.*

ANTIBIOTIC: *substance capable of acting on microorganisms, both by blocking their growth and causing their death, depending on the operational mechanism of the antibiotic itself.*

BIODIVERSITY: *cohabitation and coexistence of different species that maintain a balance thanks to the interactions established with each other.*

BMI (BODY MASS INDEX): *Numerical index calculated on the basis of an individual's weight and height to determine whether they are underweight, normal weight, overweight or obese.*

COLOSTRUM: *liquid produced by the mammary gland in the first days of breastfeeding.*

COMMENSALISM: *relationship between two or more organisms in which one benefits from interaction with the other, which is not damaged in any way.*

DYSBIOSIS: *distortion of the normal balance between species, with qualitative and/or quantitative modifications and variations in the functional mechanisms associated with it.*

DNA: *container of all the instructions necessary for the construction and functioning of every cell and organism in our body.*

ENTEROTYPE: *type of microorganism making up the gut flora of an individual; they can be grouped into three main clusters according to the composition of their microbiota.*

ENZYME: *molecule capable of intervening in a chemical reaction to facilitate its completion. Although the enzyme intervenes in the reaction and is sometimes transformed during it, at the end of the reaction it has not undergone any change.*

EUBIOSIS: *situation of qualitative and quantitative equilibrium of the species present in our microbiota.*

EVOLUTION: *product of the interactions between external environmental factors and our genes.*

GENE: *hereditary unit containing the information necessary to control the expression of a character.*

IMMUNOSENESCENCE: *reduction in the functionality of the immune response often associated with ageing.*

INFLAMMATION: *one of the first forms of defence of the innate immune system in response to pathogens or damage, such as injuries. Inflammation is a localized reaction characterized by four aspects: swelling, redness, heat and pain.*

INFLUENCERS: *external factors capable of altering the quantitative and/or qualitative composition of the microbiota (leading to possible dysbiosis).*

INTESTINAL LUMEN: *the actual intestine canal (hollow organ).*

METABOLISM: *set of reactions that occur within an organism that allow, among other things, breathing and the production of energy.*

MICROBIOTA: *set of microorganisms situated in the various body districts that interact with the outside world.*

PATHOGENIC MICRO-ORGANISM: *microorganism capable of causing human diseases.*

MONOSACCHARIDE: *also called "simple sugar", a monosaccharide is a carbohydrate composed of a single simple unit.*

PRETERM BIRTH: *a newborn who comes into the world before 37 weeks of gestation have elapsed.*

OLIGOSACCHARIDE: *complex carbohydrate formed by two, or a few more, repeated units of simple sugars.*

HOMEOSTASIS: *tendency to obtain a situation of equilibrium.*

POLYSACCHARIDE: *complex carbohydrate formed by numerous units of simple sugars.*

PROTEIN: *an organic compound (as are carbohydrates); its "building bricks" are called amino acids.*

RESILIENCE: *cability of the microbiota to restore its composition following a disturbance. In other words, resilience is understood as the ability to face a challenge and get back "into shape", that is, in the same condition as before dealing with the challenge.*

RESISTANCE: *the gut microbiota's ability to remain stable in the face of disturbances from the outside world.*

RIBOSOME: *cellular organelle that reads the code carried by RNA and, following its instructions, forms proteins.*

VISCERAL SENSITIVITY: *sensitivity that deals with receiving and transmitting stimuli from the internal organs to the body.*

SYMBIOSIS: *relationship between two or more living beings in which all participants benefit from mutual interaction.*

STRESSOR EVENT: *external factor capable of causing stress within our body.*

VITAMIN: *term that refers to numerous organic substances essential for our life, sometimes very different from each other, which are normally assumed in the form of food.*

INDEX

SCIENTIFIC PANEL

Antonio Gasbarrini
Full Professor of Internal Medicine and Gastroenterology, Catholic University of the Sacred Heart. Director of the Digestive Disease Center at Policlinico Gemelli IRCCS, Rome

Mario Amore
Full Professor of Psychiatry, University of Genoa. Head of the Psychiatry Unit, San Martino University Hospital of Genoa

Giorgio Walter Canonica
Full Professor of Respiratory Diseases and Head of the Personalized Medicine Center: Asthma and Allergies, at the Humanitas Research Hospital

Michelangelo Giampietro
Specialist in Sports Medicine and Food Science, Sports Medicine Service, ASL Viterbo

Claudio Maffeis
Full Professor of Pediatrics, University of Verona. Director of the Pediatric Diabetes and Metabolic Disorders Unit, Regional Center for Pediatric Diabetes, University City Hospital of Verona

Lorenzo Morelli
Full Professor Department of Agricultural, Food and Environmental Sciences. Head of the Institute of Microbiology and Centre for Research into Biotechnologies, Catholic University of the Sacred Heart, Piacenza and Cremona

Michele Sculati
“M.D., Dietitian, Specialist in Food Science and Clinical Nutrition, PhD in Public Health, Professor at the University of Pavia and Milan Bicocca-School of Medicine and Surgery