Microbiota and bone health

Microbiota: a fundamental issue for research

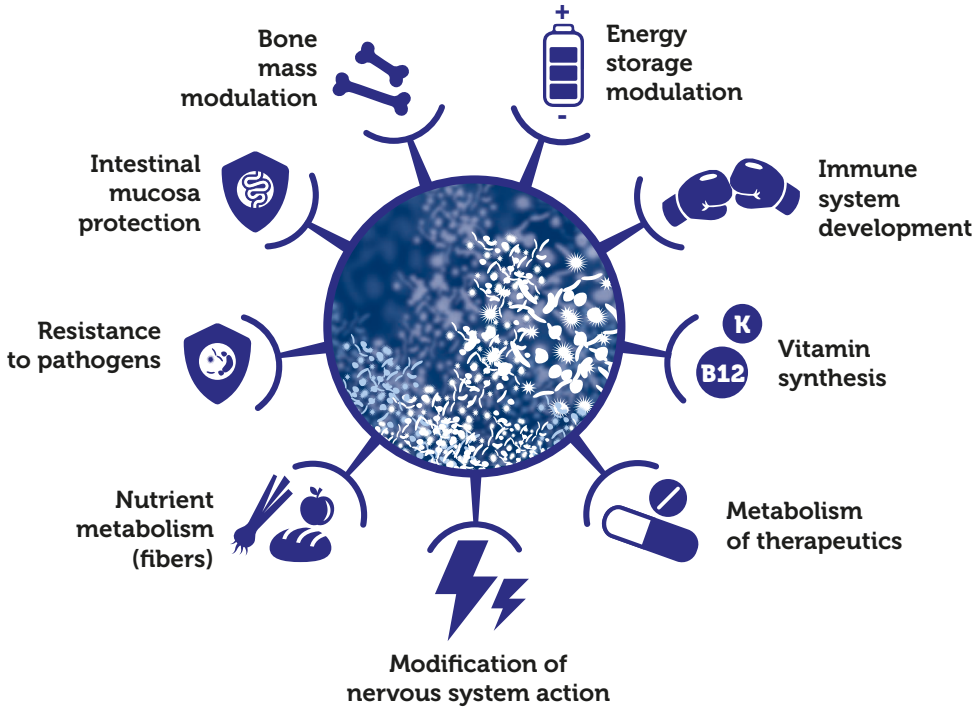
The existence of micro-organisms in the intestine has been known for over a century and it was quickly assumed that there was a genuine symbiosis between our body and this flora. But until recently available technical methods restricted investigation: only a minority of bacterial species of the microbiota could be cultivated in vitro. The development of high-speed sequencing techniques for genetic bacteria material gave new momentum to this issue.
For this reason, more and more is becoming known about the role of intestinal microbiota and that its involvement in numerous physiological functions. The functioning (and malfunctioning) of the microbiota is now better understood, as is the connection between the microbiota and its host, how micro-organisms interact with each other, and their effect on health.
All this research provides new prevention and/or treatment perspectives: how to change or re-equilibrate the microbiota in order to improve health and well-being?

The intestinal microbiota has become a real issue for health: the number of scientific publications devoted to it has increased ten-fold in 10 years.

Microbiota: how does it work?

Our intestinal microbiota (or intestinal flora) hosts approximately 100,000 billion bacteria, fungi, viruses, i.e.10 times more than the number of cells in our body, and weighs 2 kilos. It is a stable eco-system living in symbiosis with the host. Every adult hosts, in their digestive tract, 500 to 1000 different bacterial species but 4 are predominant in humans: *Firmicutes, Bacteroidetes, Proteobacteria, Actinibacteria*. The microbiota refers to all genetic material in these organisms.
Intestinal microbiota plays a fundamental role in numerous processes, either locally or remotely, thanks to molecules and metabolites produced by bacteria, which means it is worthy of being considered as a whole separate organ in the human body. It is involved in metabolism, by increasing the absorption of nutrients or by synthesizing vitamins B or K; it limits the development of pathogenic micro-organisms in intestinal mucosa; it produces bioactive substances such as short-chain fatty acids from dietary fibres or substances with oestrogenic activity; it contributes to the development and maintenance of the immune system...

Primary functions of microbiota (1)



Intestinal flora is acquired in principle from birth and remains relatively stable over time, but its equilibrium can be disturbed by age, disease, medicines, stress, dietary changes: this is referred to as dysbiosis. This increases intestinal permeability, activates the immune system and causes inflammation.
This dysbiosis may be involved in a wide variety of so-called "modern" diseases: irritable bowel syndrome, inflammatory intestinal diseases, colorectal cancer, diabetes, obesity, auto-immune diseases, atherosclerosis, auto-immune diseases and even autism or depression.

(1) Laukens D et col. FEMS Microbiol Rev. 2016 ;40(1):117-32.

Microbiota and bone

The relationship between bone and intestinal health has been restricted to the issue of intestinal absorption of calcium and protein, key nutrients for bone.
The arguments in favour of a link between microbiota and bone health are recent and are based on animal models: mice treated with antibiotics, which change the bacterial flora and sterile mice in the hygienic sense of the term, "germ free", which are free of microbiota.
In the 2 models, differences are seen in bone mass and in bone re-modelling activity compared with normal mice. This phenomenon is reversible when the intestine is re-colonized with bacteria. Even if the results obtained seem to vary according to sex, age, species, they suggest that microbiota is involved in the regulation of bone status and have generated research in how to change this microbiota.

In humans, microbiota changes with osteoporosis

This was shown in a pilot study in China which compared microbiota in normal subjects, osteopenic subjects and osteoporotic subjects by DNA analysis of bacteria[2]. For osteoporosis, and to a lesser extent for osteopenia, microbiota has 2 primary characteristics:

- If the 4 primary strains of bacteria are found in all subjects, their proportion is modified: osteoporotic subjects have more *Firmicutes* and fewer *Bacteroidetes* with the result that the *Firmicutes/ Bacteroidetes* ratio is significantly different (1.3 in normal subjects, 1.75 in osteopenia and 3.3 for osteoporosis);
- The existence, in small quantities, of bacteria that is normally absent in normal subjects, which could constitute specific markers for low bone mass.

If this association is shown to be causal, some bacterial profiles of intestinal microbiota, probably involved in the weakening of bone, could therefore be used as a diagnostic and therapeutic tool in osteoporosis.

(2) Wang J et al. PeerJ. 2017 ;5:e3450. doi: 10.7717/peerj.3450. eCollection 2017.

Can microbiota be acted on?

More and more is known about the numerous aspects of our health which may be influenced by our intestinal microbiota, i.e. all the micro-organisms which colonise our intestine. But can we effectively change it?
There are several ways of changing microbiota:

- Transplanting microbiota. In humans this radical method has only (successfully) been used in the treatment of an intestinal infection related to a specific pathogenic bacteria, Clostridium difficile
- Antibiotic treatment, which clearly has specific indications
- Prebiotics (see box)
- Probiotics: these are living micro-organisms which, when they are ingested in sufficient quantities, can interact with our microbiota and have a beneficial effect on health.

Prebiotics ([3])

These are non-digestible dietary constituents such as fibres or oligo-saccharides in breast milk which can stimulate the growth and/or activity of bacterial communities of microbiota and have a beneficial effect.

- Therefore, the absorption of inulin, a non-absorbed sugar, increases bone density; one of the mechanisms suggested is the increase in the absorption of calcium by short-chain fatty acids produced during the fermentation of inulin by microbiota.
- Another example: galacto-oligosaccharides in breast milk contribute to the growth of a child and to the development of the immune system. By acting on microbiota, they result in better use of nutrients by the body, including at a bone level.
- Finally, a study conducted over 12 weeks in Chinese subjects showed that enriching inulin and oligo-saccharides in dairy products potentiates the beneficial effect of milk on bone resorption.

(3) Whisner CM et col. Calcif Tissue Int. 2018;102(4):443-479

Probiotics

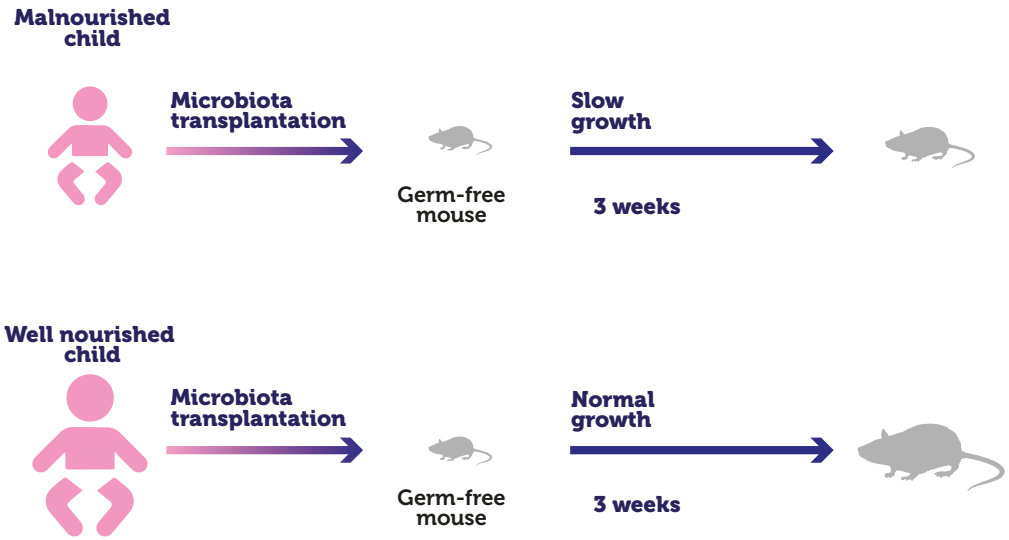
For growth...

- **In mice** [4]

- In young animals of the Balb/C germ-free line fed adequately, the absence of microbiota causes slow growth and final weight and height lower than normal animals. Mice are smaller and the length and thickness of their femur is reduced. If the quantity of proteins proposed is reduced, growth stops whereas it continues, even if in a more limited way, in others.
- If probiotics from the Lactobacillus family are given (*Lactobacillus plantarum*) to these animals, their growth becomes comparable to that of normal animals.

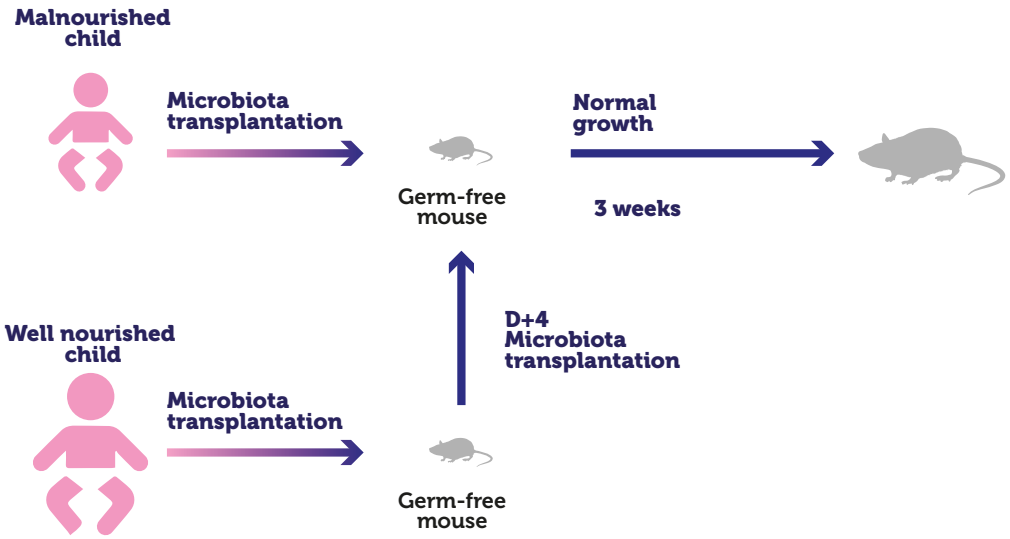
- **In children** [5]

This hypothesis that was tested by another team of researchers who compared microbiota in Malawian children aged between 6 and 18 months in good health or malnourished. Microbiota in malnourished children is very different from those in normal children; transplantation of their altered microbiota to germ-free young mice causes growth disorders and morphology anomalies in their bone.



(4) Schwarzer M et col. Calc Tissue Int 2018; 102(4):387-405)
(5)Blanton LV et al Science. 2016; 19;351(6275

But these anomalies are partly corrected if microbiota from mice who received microbiota from well-nourished children is administered to these “malnourished” mice (or some probiotic bacteria from microbiota in these mice).



2 conclusions can be drawn from these studies :

- Microbiota are necessary for optimum bone growth in mammals; this effect is related to an increase in IGF1, an essential growth factor.
- Probiotics could be part of a strategy to fight against malnutrition and its bone effects in children.

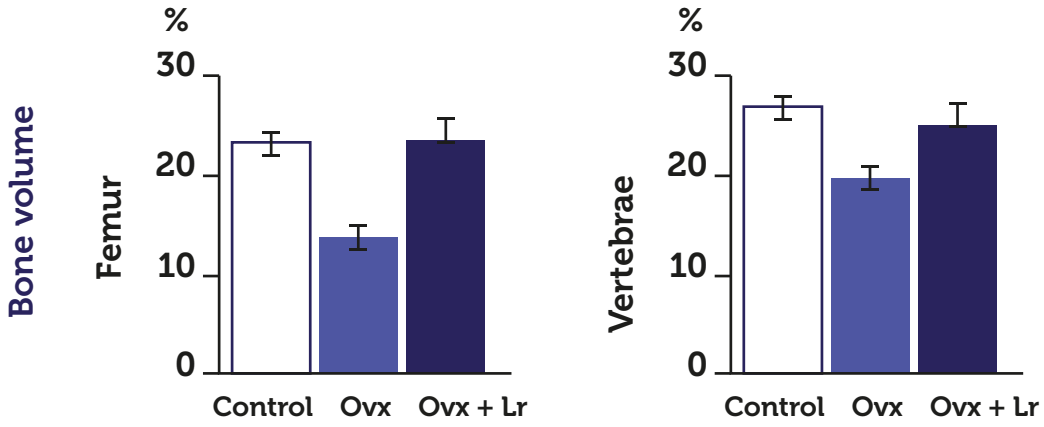
...For bone loss

Recent studies have shown the interest in probiotics on models of bone loss and in particular related to oestrogenic deficiency in the menopause.

- **In women**, it has been shown that the diversity of microbiota was associated with oestrogen levels in urine and that hormonal deficiency translates as depletion and imbalance of microbiota [(*in* 6)].

- **In ovariectomized mice** the administration of various probiotics (including *Lactobacillus reuterii*) modifies intestinal flora and prevents bone loss related to oestrogen deficiency at both a femur and vertebrae level [(7)]. This beneficial effect is related to reduced bone resorption and anti-inflammatory action.

- Control group
- Without oestrogens (Ovx)
- Without oestrogens + probiotic (Ovx + Lr)



Muscles [(8)]

A certain number of findings suggest that microbiota may also influence muscle mass and function:

- Microbiota in athletes - in this case male rugby players - is more diversified than that of sedentary people in good health, which in itself is more diversified;

- In the elderly, the diversity of microbiota is inversely associated with the level of fragility and of dependency. Fragility is accompanied by sarcopenia which is often associated or a reduction in muscle mass related to advanced age;

(6) Xu X et al. Bone Research 2017; 5,17046.
(7) Britton RA et al. J Cell Physiol 2014 ; 229 (11) : 1822-30.
(8) Grosicki GJ et al. Calcif Tissue Int 2018;102(4):433-442

- In animals, manipulating microbiota by pre- or probiotics optimizes lean body mass.

Probiotics, dairy products and bone health

In humans, the primary dietary sources of probiotics are fermented dairy products: yoghurt, fermented milk, cheese. A yoghurt contains at least 10 million live bacteria.
From a regulatory perspective, the yoghurt designation is reserved exclusively to milk fermented by 2 lactic bacteria, *Lactobacillus bulgaricus and Streptococcus thermophilus*. Fermented milk contains different bacteria; in France, the main strains are *Lactobacillus acidophilus, Lactobacillus casei and Bifidobacteria*. There is a wide variety of fermented milk globally, with the most well known being kefir typical of Caucasian region, koumis typical of Mongolia and of Kazakhstan and the leben from the Maghreb. Cheese can also act on microbiota as it contains complex eco-systems with bacteria, yeast and mould. The types of micro-organisms, their quantity and their respective proportions depend on the type of cheese. It is a new area of investigation for research and health as was seen in recent research by Inra on the effect of probiotics in cheese on animal models of inflammatory diseases of the digestive tract.

Fermented dairy products and bone mass [9]

During childhood and adolescence, interventional trials suggest a favourable effect of fermented products on bone mass and density. A study involving girls aged between 10 and 12 years concluded that calcium in the form or cheese is more effective than an equivalent quantity in the form of tablets.
In adults, the consumption of fermented dairy products may reduce age-related bone loss. A very recent study conducted in the over '60s living at home showed that consuming yoghurt is beneficial to bone health and physical abilities. For each yoghurt consumed, the risk of osteoporosis is reduced by 40% in women and by 50% in men [10].

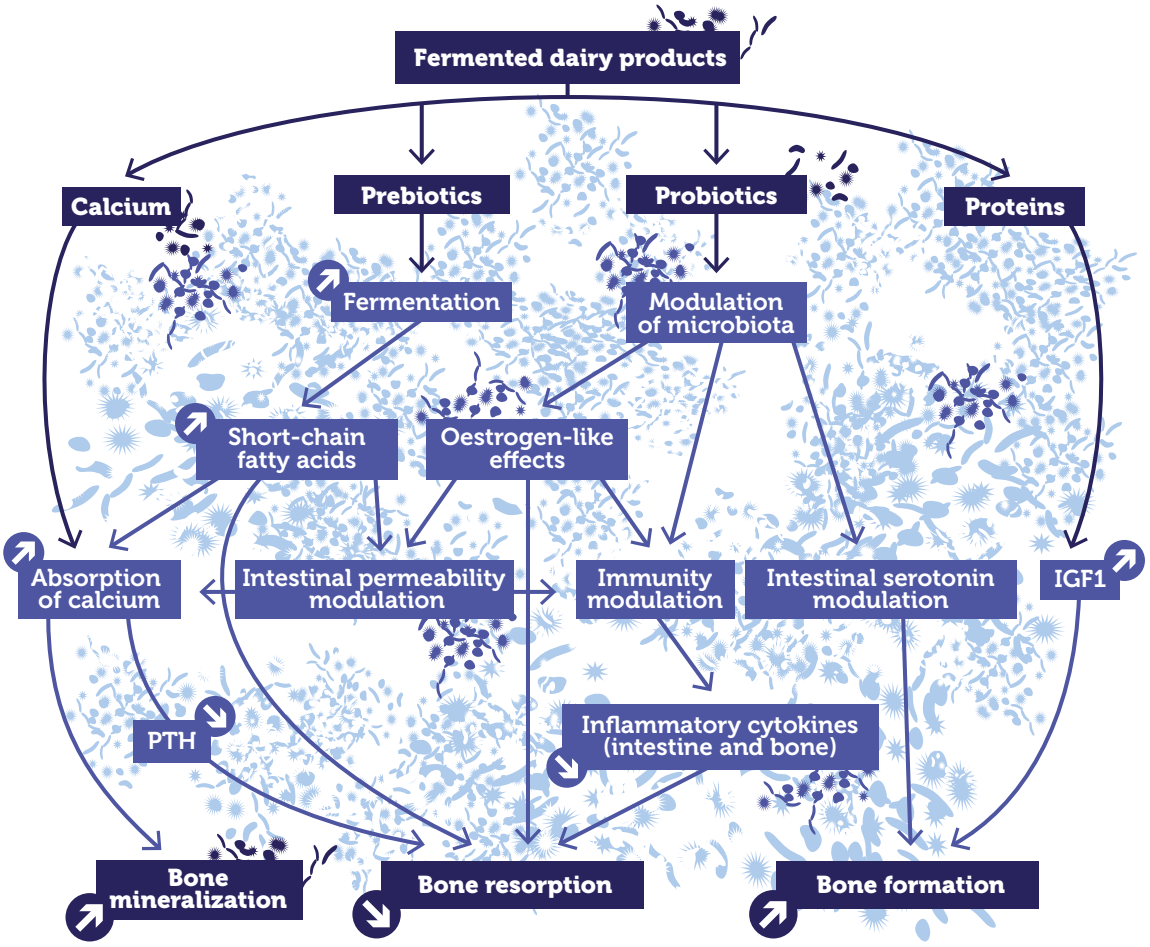
Fermented dairy products and risk of fracture

According to a Swedish study involving almost 40,000 females monitored for 22 years, the consumption of fermented dairy products and cheese is associated with a lower risk of fracture [11] : for each portion, the risk of hip fracture reduces from 10 to 15%. This reduction reaches 20% when combined with fermented dairy products (>=2/day) and fruit and vegetables >=5/d) [12], an effect which is found in another cohort in Rotterdam [13].

(9) Rizzoli R and Biver E. Calcif Tissue Int. 2018; 102(4):489-500
(10) Laird E et col. Osteoporos Int. 2017;28(8):2409-2419
(11) Michaëlsson K et col. BMJ. 2014 28;349:g6015
(12) Michaëlsson K et col J Bone Miner Res. 2018; 33(3):449-457
(13) De Jonge EA et col. Am J Clin Nutr. 2017;105(1):203-211

Mechanisms of action of fermented dairy products [9]

The consumption of fermented dairy products may positively influence growth and bone homoeostasis by different mechanisms involving essential nutrients for bone (calcium, phosphorus and proteins) in the dairy matrix but also probiotics and potentially prebiotics. Modulation of microbiota is a key vector for these beneficial effects.



(9) Rizzoli R and Biver E. Calcif Tissue Int 2018;102(4):489-500

And tomorrow?

Finally, it is not long since scientists considered microbiota to be worthy of interest. Popular wisdom, has for a long time been conscious of the close links between our head and our belly. “To have the heart in the belly, to have a knot in one’s stomach, poorly digested information or an emotion, to have fear in one’s belly, is not good...” The list is long.
Microbiota - bacteria with which the body lives in symbiosis - and their microbiome - their genome - is an emergent but fertile ground of research. Relationships between microbiota, digestive and metabolic diseases have opened the way. But microbiota do not only act “locally”. Their potential influence on bone health is a new chapter in the story.
Future research will enhance the link between diet and health, will allow better understanding of the sensitivity of an individual to a dietary model or a medical treatment, will mean that customized treatments may be offered one day, or even that foods can be used as a prevention or treatment vector. Probiotics in fermented dairy products are a perfect example of this.

Let us not forget that probiotic comes from the Greek “biotikos” and means “in favour of life”.