THEMATIC REVIEW

40 YEARS OF IGF1

The emerging connections between IGF1, the intestinal microbiome, Lactobacillus strains and bone growth

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Abstract

In most animal species, postnatal growth is controlled by conserved insulin/insulin-like growth factor (IGF) signaling. In mammals, juvenile growth is characterized by a longitudinal bone growth resulting from the ossification of the growth plate. This ossification is under IGF1 influence through endocrine and paracrine mechanisms. Moreover, the nutritional status has been largely described as an important factor influencing the insulin/insulin-like growth factor signaling. It is now well established that the gut microbiota modulates the nutrient availability of its host. Hence, studies of the interaction between nutritional status, gut microbiota and bone growth have recently emerged. Here, we review recent findings using experimental models about the impact of gut bacteria on the somatotropic axis and its consequence on the bone growth. We also discuss the perspectives of these studies in opening an entire field for clinical interventions.

Introduction

Bone growth is one of the most important features of the juvenile growth in mammals. During embryogenesis and the post-natal period, longitudinal bone growth involves three major mechanisms: multiplication of chondrocytes in the growth plate, chondrocyte hypertrophy and endochondral ossification, in which the cartilage is replaced by mineralized bone (Berendsen & Olsen 2015). Chondrocyte multiplication and hypertrophy are highly regulated by several circulating hormones and growth factors including growth hormone (GH) and the insulin-like growth factor 1 (IGF1) (Mackie et al. 2011).

Circulating IGF1 levels are highly dependent on the nutritional status. Indeed, protein calorie deficiency or total energy deficiency leads to a state of GH resistance resulting in low IGF1 circulating levels. Low circulating IGF1 levels are well described in the stunting process, which is defined by a reduced systemic growth rate and is a primary manifestation of undernutrition (Fazeli & Klibanski 2014).

For several years, the functional links between the nutritional status and the gut microbiota have been established. In humans, undernutrition has been associated
with major gut microbiota alterations, which correlate with poor growth patterns (Subramanian et al. 2014). Moreover, recently specific human microbiota patterns shaped by undernutrition have been shown to induce some of the wasting syndrome phenotypes in gnotobiotic rodents (Smith et al. 2013, Blanton et al. 2016). Even if the precise mechanisms remain unclear, there is a growing body of evidence emerging in scientific literature linking the undernutrition, gut microbiota and the regulation of the GH/IGF1 axis during juvenile growth. Here, we review recent reports, which by using gnotobiotic animal models (drosophila and mouse) established connections between the intestinal microbiome, Lactobacillus strains, IGF1 and bone growth. Finally, we provide a clinical perspective to these pre-clinical results.

**Drosophila larval growth, Lactobacillus strains and dILIns-like peptides**

The life cycle of *Drosophila melanogaster* involves three larval stages corresponding to crucial growth phases allowing the animal to enter metamorphosis and reach adulthood. This juvenile growth period is critical in determining the adult final size (Mirth et al. 2005). During these larval stages, the body increases about 200 times in mass through cell proliferation and mostly by cell growth (i.e. by increasing cell size) (Smith & Orr-Weaver 1991, Strigini & Leulier 2016). The duration of the larval stages and the larval growth rate are controlled by two major endocrine signals: the steroid hormone ecdysone and a family of eight *Drosophila* insulin-like peptides (dILPs), the analogues of mammalian insulin, IGFs and relaxins (Boulan et al. 2015). Most of the dILPs (dILPs1-7) activate a unique insulin-like receptor encoded in the fly genome (dInr), which signals through an intracellular cascade similar to the ones of the mammalian insulin-receptor (IR) and the IGF1-R (Hietakangas & Cohen 2009). dInr signaling regulates the normal growth and development of the larva and its activation leads to the activation of a cascade of intracellular phosphorylation events, leading to the phosphorylation of the dFOXO protein (Lee & Brey 2013). As shown by Brogiolo et al. *Drosophila* larvae homozygous for a partial loss-of-function mutation in dInR show an extended larval period of 10–20 days and a decrease of cell size and number by 23% and 17% respectively (Brogiolo et al. 2001). The *Drosophila* insulin-like peptides 2, 3 and 5 are mostly secreted by the neurosecretory insulin-producing cells (IPC) of the brain, the functional equivalent of beta cells in the mammalian pancreas (Nässel et al. 2013). Other dILPs are produced in several organs by muscle and epithelial cells (Brogiolo et al. 2001, Nässel et al. 2013, Strigini & Leulier 2016). dilps 1–7 encode for putative precursors that are structurally similar to preproinsulin, while dilp8 encodes a relaxin-like molecule (Colombani et al. 2012). Historically, dILPs have been identified by their sequence homology and their B-C-A domain structure, which is similar to the mammalian insulin. After cleaving on consensus cleavage sites all seven dILPs release a single peptide whose amino-acid sequence share a high degree of similarity with IGF1 and IGF2 (Grönke et al. 2010).

Similar to the insulin and IGFs in mammals, dILPs are involved in several metabolic and endocrine functions: growth and development, carbohydrate tolerance, reproduction, stress response and longevity. In 2002, Rulifson et al. highlighted the role of dILPs in the control of growth by the ablation of IPCs on *Drosophila* larvae (Rulifson et al. 2002). After IPCs ablation, larval length was decreased by 42% and entry to metamorphosis (the end of the larval period) was delayed by 7 days. Moreover, the *dilp2* over-expression in IPCs-ablated larvae rescued their length to 88% of normal length and shortened the larval period by 6 days, closely resembling the normal phenotype (Ikeya et al. 2002).

In the IPCs, the dILPs expression and secretion are regulated by complex signaling cross-talks between multiple organs informing the IPCs about the nutrient status of the animal (Boulan et al. 2015). Production and secretion of dILPs by IPCs are modulated by nutrients via signals relayed by a nutrient sensing organ, the fat-body, which is the equivalent of the mammalian liver and white adipose tissue. In this organ, dietary amino acids uptake stimulates the target of rapamycin complex (TOR), which triggers the release of systemic signals instructing the brain IPCs to express and release dILPs (Colombani et al. 2003, Géminard et al. 2009). As a consequence, upon chronic undernutrition, *Drosophila* larval growth is restricted through reduced TOR activation in the fat-body and decreased expression and release of dILPs by the IPCs (Géminard et al. 2009).

It is now well established that the gut microbial environment modulates nutrient availability (Drissi et al. 2016). The nutritional influence of the intestinal microbes has been highlighted in different studies: degradation of non-digestible starch by microbial saccharolytic activity, production of short-chain fatty acid (propionate, butyrate and acetate) and bacterial synthesis of vitamin and essential amino-acids (Shanahan et al. 2017). As such, the gut microbiota has even been qualified as a host factor that influence energy uptake (Bäckhed et al. 2004).
Hence, the gut microbiota through its nutritional influence may have a direct impact on systemic growth by modulating the nutrients availability and therefore modulating dILPs functions.

The *Drosophila*’s gut microbiota is composed of simple bacterial communities represented by the phyla *Firmicutes* and *Proteobacteria* with usually 2–3 dominating species including: *Acetobacter pomorum, Acetobacter tropicalis, Lactobacillus brevis, Lactobacillus plantarum* or *Lactobacillus fructivorans* (*Erkosar & Leulier 2014*). Storelli *et al.* reported the impact of the gut microbiota on *Drosophila*’s larval growth (*Storelli et al. 2011*). They showed that *Drosophila* symbiotic microbiota is indispensable for larval growth upon chronic undernutrition (Fig. 1). Reduction of the nutritional input results in about 2.5 day delay of adult emergence for conventional flies (i.e. animals with a microbiota); however, this delay was more than doubled for axenic (germ-free) flies as they only entered metamorphosis 2.9 days after their conventional siblings. These results established the crucial role of a resident gut microbiota on the *Drosophila* developmental timing, an observation that was also substantiated by other authors (*Shin et al. 2011, Ridley et al. 2012*). A second important finding by Storelli *et al.* was that the effect of a conventional microbiota on larval growth could be recapitulated by a mono-association of germ-free animals with a single strain

![Figure 1](http://jme.endocrinology-journals.org)  
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Impact of conventional microbiota or selected *Lactobacillus* strain on juvenile growth promotion upon undernutrition.
of Lactobacillus plantarum, one of the dominant species in the Drosophila microbiota. Lactobacillus plantarum association in poor nutritional condition was sufficient to accelerate larval growth by reducing the length of the growth phase and resulted in earlier emergence of fit and fertile adults (Fig. 1) (Storelli et al. 2011, Téfit & Leulier 2017). To probe the dILPs activity in this context, Storelli et al. used the InR gene expression as a marker since low InR expression correlates with high dILPs activity (Puig & Tjian 2005). They found that InR expression was lower in L. plantarum-associated larvae as compared to germ-free (GF) larvae. Therefore, during larval growth phase, L. plantarum association was able to increase InR signaling upon nutrient scarcity. Furthermore, Storelli et al. ectopically expressed two negative regulators of the TOR kinase, TSC1 and TSC2, in the fat body. In this context, the entry in metamorphosis (a proxy of larval growth) of mono-associated animals was no longer accelerated and was similar to the one of GF flies. Storelli et al. thus showed that optimal TOR kinase activity in the fat body is required for L. plantarum promotion of InR signaling and enhanced systemic growth upon chronic undernutrition (Storelli et al. 2011).

Further, in 2015, Erkosar et al. brought new mechanistical insights into this phenomenon and showed the impact of L. plantarum on the circulating amino acid availability upon chronic undernutrition (Erkosar et al. 2015). They showed that expression of a set of intestinal peptidases was significantly elevated in L. plantarum-associated Drosophila larvae as compared to germ-free animals. This increase in peptidase expression was associated with higher intestinal proteolytic activity, and L. plantarum's growth-promoting effect was attenuated upon treatment with peptidase inhibitors. Finally, Erkosar et al. found that the level of free amino acids and dipeptides in L. plantarum larvae was increased as compared to GF animals indicating that upon L. plantarum association, animals optimize dietary protein digestion and amino-acids uptake, a possible mechanism explaining the increased TOR activity identified by Storelli et al. upon L. plantarum association. Lastly, by searching how intestinal peptidase expression are induced upon L. plantarum association, Matos et al. recently identified that molecular motifs in L. plantarum cell walls, D-alanylated teichoic acids and peptidoglycans, are directly sensed by Drosophila enterocytes and trigger intracellular signaling cascades leading to increased intestinal peptidase expression and activity. It is clear that the PGRP-Imd-Relish signaling cascade, which is a major innate immune signaling cascade in Drosophila, contributes partly to these signaling events. Yet the exact nature of all the signaling pathways engaged in enterocytes upon cell wall motifs recognition remains elusive and requires further experiments (Erkosar et al. 2015, Matos et al. 2017).

Taken together these results establish Drosophila as a powerful model to explore the molecular underpinnings of the beneficial interactions between microbiota and its host growth and paved the way to test the role of the intestinal microbiota in juvenile growth control in mammals.

**From flies to mice**

**Involvement of the GH/IGF1 axis in juvenile growth**

The insulin/insulin like growth factor signaling (IIS) is a highly conserved pathway across the animal kingdom involved in control of growth, development and metabolic homeostasis. Despite similarities with Drosophila, the mammalian growth-axis signaling is more complex. Indeed, in mammals besides IGF1, the somatotropic axis consists of diverse family of ligands, receptors, and soluble binding's protein. The axis is centrally regulated by a 191-amino acid protein secreted by the anterior pituitary gland: the growth hormone (GH) (Butler & Le Roith 2001). The actions of GH depend on the nutritional status of the organism which dictates the induction of growth-promoting anabolic actions or lipolysis (Bergan-Roller & Sheridan 2017). Anabolic actions of GH in juvenile growth act by well-known mechanism associated with positive nitrogen balance, protein synthesis in muscle and stimulation of the longitudinal bone growth (Chikani & Ho 2014). Despite growing evidence of a direct action of GH on diverse tissues, the most significant effects of GH on bone growth plate and lean mass are mediated by an intermediate peripheral hormone: the insulin growth factor 1 (IGF1). GH mediated action starts by binding to the GH receptor (GHR), a member of the cytokine super-family associated to the tyrosine kinase JAK2. The targets of JAK phosphorylation are members of the STAT family of transcription factors. The major mediator of the GH action is identified as STAT5b (Hennighausen & Robinson 2008). The phosphorylation of STAT5b by JAK2, secondary to the binding of GH to its receptor, leads to the transcription of several target genes including Igf1. The product of Igf1 transcription is a 70-amino acid protein sharing structural similarity to insulin. The biological effects of IGF1 are mediated by specific high affinity receptor belonging to the tyrosine kinase, TSC1 and TSC2, in the fat body. In this context, the entry in metamorphosis (a proxy of larval growth) of mono-associated animals was no longer accelerated and was similar to the one of GF flies. Storelli et al. 2011, Téfit & Leulier 2017). To probe the dILPs activity in this context, Storelli et al. used the InR gene expression as a marker since low InR expression correlates with high dILPs activity (Puig & Tjian 2005). They found that InR expression was lower in L. plantarum-associated larvae as compared to germ-free (GF) larvae. Therefore, during larval growth phase, L. plantarum association was able to increase InR signaling upon nutrient scarcity. Furthermore, Storelli et al. ectopically expressed two negative regulators of the TOR kinase, TSC1 and TSC2, in the fat body. In this context, the entry in metamorphosis (a proxy of larval growth) of mono-associated animals was no longer accelerated and was similar to the one of GF flies. Storelli et al. thus showed that optimal TOR kinase activity in the fat body is required for L. plantarum promotion of InR signaling and enhanced systemic growth upon chronic undernutrition (Storelli et al. 2011).

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kinase receptor that is present on most cell type except the hepatocytes (Bartke et al. 2013). Upon activation downstream signaling pathway involves phosphorylation of Akt leading to maintenance of FOXO transcription factor in the cytoplasm thus inhibiting the transcription of FOXO regulated genes (Papaconstantinou 2009).

GH stimulated production of IGF1 induces systemic growth in a twofold manner: first, by endocrine signaling, where most of the IGF1 in the circulation originates from the liver and second, by a peripheral production through an auto/paracrine signaling (Kaplan & Cohen 2007, Mohan & Kesavan 2012). During the post-natal period, IGF1 and IGF1R play major roles in stimulating systemic growth and, more specifically, in longitudinal bone growth with evidence supporting IGF1 actions via endocrine, paracrine and autocrine systems (Baker et al. 1993, Heilig et al. 2016). Liver GH-induced IGF1 production is the main source of circulating IGF1, but IGF1 is also produced by peripheral tissues such as muscle and bone (Yakar et al. 2010). The importance of circulating IGF1 in bone development seems not to be predominant since tissue-specific deletion of IGF1 in the liver decrease circulating IGF1 levels by 70% without impairing ponderal growth in first 6 weeks of life (Yakar et al. 1999). However, a threshold of circulating IGF1 appears to be required for normal linear growth and bone turn over (Yakar et al. 2002). These data suggest circulating IGF1 plays a role in controlling bone growth. Finally, IGF1 is crucial for the skeletal muscle mass development and maintenance throughout life (Sharples et al. 2015).

**Gut microbiota is necessary for an optimal somatic and bone growth upon standard diet**

Since the beginning of the long-term gnotobiotic mouse and rats colonies in 1950s, the growth rate kinetics of juvenile germ-free and conventional (CV) animals has been a controversy, with reports stating similar or lower growth for GF animals compared to CV counterparts (Gordon 1959, Wostmann 1959). To explore this issue we recently used gnotobiotic juvenile mouse model and studied the role of the intestinal microbiota on growth kinetics of conventional (CV) mice compared to germ-free (GF) animals on standard breeding diet (Fig. 1) (Schwarzer et al. 2016). At weaning, CV and GF mice had the same weight and body length, indicating the same nutritional status. However, two months after birth the GF mice were 14.5% lighter and 4% shorter compared to CV mice. Bone growth parameters, including femur length, cortical thickness, cortical bone fraction, and the trabecular fraction of the femur were all reduced in GF animals. Importantly, the weight gain of CV mice was not due to increased adiposity or changes in the food intake. On the molecular level, CV mice showed higher level of circulating IGF1 and IGFBP-3, its major binding protein, despite similar GH levels. Interestingly, the peak of circulating IGF1 levels at day 28 corresponded to a spurt growth in CV mice, which was not observed in GF mice. Moreover, IGF1 and Igf1bp3 expression levels were higher in liver and muscle of CV mice. These data suggested higher sensitivity of conventional animals to GH actions and highlighted the major role of IGF1 in postnatal growth. To prove that IGF1 is sufficient for mediating postnatal growth, CV and GF animals were first treated with a recombinant IGF1 (rIGF1) for 10 days after weaning. Interestingly, there was no effect in CV mice, but GF mice increased their weight, length and femur length reaching the CV mice parameters. The necessity of IGF1 for post-weaning growth was confirmed by treating CV animals with a specific non-competitive inhibitor of IGF1 receptor signaling, which significantly impaired the growth gain of CV animals.

The link between the microbiota, IGF1 and bone formation in mice was recently confirmed by Yan et al. (2016). The authors studied the bone turn-over and the IGF1 secretion in young mice after 1 month and 8 months of colonization with a conventional gut microbiota compared to GF mice. Indeed, they confirmed the crucial role of microbiota in increasing the circulating IGF1 levels, which were significantly increased both 1 and 8 months after colonization despite no change in the levels of circulating GH. Interestingly, Igf1 mRNA in muscle was less expressed in mice colonized for 1 month, suggesting that muscle-derived IGF1 may not contribute to the increased circulating IGF1 pool. Discrepant results were obtained concerning the bone turn-over. At 1 month after colonization they observed a decrease of trabecular bone mass associated with a decreased body weight and unchanged body length compared to GF controls. Accordingly, the C-terminal telopeptides of type 1 collagen (CTX-I), a serum marker of bone resorption, was increased. However, markers of bone formation were concomitantly increased. Finally, the growth plate, measured by micro-tomography, was thicker in colonized mice. Hence, these results suggested a more active endochondral ossification modulated by gut microbiota, which might alter longitudinal bone growth. This was indeed true because at 8 months after colonization, bone parameters were largely improved. Compared to germ-free mice, femur length was longer and L5 vertebra was larger.
Periosteal and endosteal area were increased without changing cortical thickness. These data suggest that long-term microbiota colonization promotes radial and longitudinal bone growth and confirmed the functional link between intestinal microbiota, IGF1 production and bone growth (Yan et al. 2016).

The exact mechanisms whereby the gut microbiota sustains the GH/IGF1 axis remain unclear. Yan et al. suggested that SCFA production may be one mechanism by which microbiota increase serum IGF1. We cannot exclude that the beneficial effect of microbiota and specific bacterial strains go through an optimization of the enterocytes nutrient uptake improving the nutritional status of the animal and thus indirectly improving the somatotropic axis performance.

**Preservation of the growth promoting effect of the gut microbiota upon nutrient scarcity**

Among other factors, nutritional status is a crucial factor dictating the growth rate of juvenile mammals by modulating the somatotropic axis activity (Thissen et al. 1994). Starvation, which is defined by a global energy deficiency, result in a GH resistance state associated with elevated GH levels and a decrease in circulating IGF1 (Grinspoon et al. 1995). The mechanism of GH resistance has been studied since many years. Studies suggest that the decrease in liver GH receptor levels or an inhibition of intracellular signaling events downstream to the GHR result in the inability of GH to stimulate IGF1 production (Hintz et al. 1978). More recently, two proteins have been involved in the GH signaling dampening during starvation: Fibroblast Growth Factor 21 (FGF-21) and Sirtuin 1 (SIRT1). FGF-21 is found at high levels in the sera during starvation and it has been suggested that FGF21 signaling antagonizes STAT5 phosphorylation (Holmes 2016). Similarly, SIRT1 class III histone deacetylase is regulated by nutrient availability and its activity is increased upon nutrient scarcity. Studies have indicated that SIRT1 activity may contribute to the repression of STAT5 phosphorylation upon nutrient shortage (Yamamoto et al. 2013). Remarkably, an isolated protein deficiency is sufficient to mimic the effect of full starvation (Fazeli & Klibanski 2014). In rodent models of protein deficiency, the decrease of circulating IGF1 level is reversible by the increase in dietary proteins but not by GH injections (Thissen et al. 1990).

In the first part of the review we have seen that in the invertebrate Drosophila model, microbiota and selected bacterial strains play a decisive role in juvenile growth kinetics upon nutrient scarcity. Hence, the question arises: Is this important capacity a microbiota and specific bacterial strain contribution to the mammalian host juvenile growth upon chronic undernutrition? To this end, we challenged GF and CV mice with a low protein and low fat diet, isocaloric to the normal breeding diet. During the first week after weaning on this experimental diet, both GF and CV mice lost weight, but the weight loss was less pronounced in CV mice. Subsequently, CV mice resumed growth and significantly increase their weight albeit to a lesser extent when then bred on the normal diet. This was in stark contrast to the GF mice for which the growth stopped completely. On the molecular level, Igf1 expression was increased in liver and muscle tissues and also circulating IGF1 levels were higher in CV compared to GF animals (Fig. 1). Concomitantly, the treatment of CV mice with IGF1 receptor-specific inhibitor reduced the body and femur length gains. Furthermore, germ-free mice mono-associated with L. plantarum WJL strain previously validated for its growth-promoting capacities in Drosophila monoxenic models (Storelli et al. 2011) showed an increase of 14% size gain and 52% weight gain compared to the GF animals. These macroscopic parameters were similar to those obtained in CV mice. Femur cortical thickness and cortical bone fraction were also improved similarly to CV animals. Moreover, L. plantarumWJL-associated animals had the circulating IGF1 levels restored to the same level as CV mice. Finally, to test the degree of a GH resistance state induced by undernutrition GF, CV and L. plantarumWJL-mono-associated mice were injected with recombinant GH (rGH). CV and L. plantarumWJL-mono-associated mice showed higher phosphorylation levels of STAT5b, a direct signature of GHR activity, confirming the improved sensitivity to the GH. L. plantarumWJL effects were strictly strain dependent since mono-association with a different Lactobacillus strain L. plantarumNIZO2877 resulted in significantly lower improvement of the macroscopic growth rates and minor increase in the sensitivity to the GH.

Similar to the situation on standard diet, the exact mechanisms underlying the specific effect of gut bacteria on modulating IGF1 levels and GH sensitivity is not yet understood and is probably complex. Further experiments are warranted to determine if the microbiota interacts with the somatotropic axis directly or indirectly by modulating the nutrient uptake in the intestine or through production of metabolites such as short-chain fatty acid (Yan et al. 2016), which may result in improved nutritional status and improved GH/IGF1 axis sensitivity.
In summary, the gut microbiota in mammals is an important player in the host somatic growth-improving bone growth and modulating the GH/IGF1 axis. Moreover, in gnotobiotic settings, some specific strains are able to restore juvenile growth and somatotropic axis activity to the same extent as complete microbiota. Moving away from the gnotobiotic model toward the more relevant real-life conditions, our recent data indicate that intrinsic properties of *L. plantarum* [Witt2011] to improve juvenile growth and somatotropic axis sensitivity are also conserved in conventional animals (Schwarzer *et al.* unpublished data). These encouraging findings pave the way to intervention clinical trials involving, besides re-nutrition strategies, supplementation with specific validated bacterial strains to improve the growth in juvenile population at risk of stunting.

**Human juvenile growth: prospects for bacterial interventions in clinical nutrition**

In both the previous parts, we have brought solid proof of evidence that gut microbiota plays an important role in the stunting process in animal experimental models. Moreover, external intervention on this same microbiota could have a beneficial impact on the stunting. But actually, there is no evidence in the literature that these concepts could be extrapolated to humans. Therefore, we propose here to develop evidence-based concept about interaction between human microbiota and growth parameters.

As we saw previously, juvenile growth in mammals is highly regulated by the somatotropic axis and humans are no exception. GH is secreted by anterior pituitary gland and act mostly by its liver receptor: the GHR. GHR activation induces the expression of *Igf1* in the liver and the secretion of circulating IGF1 whose peripheral action leads to increase the longitudinal bone growth (Mohan & Kesavan 2012). IGF1 is critical to human growth in two periods: during the intrauterine period in a non-GH-dependent manner and during the post-natal period in a GH-dependent manner (Netchine *et al.* 2011). Regardless of the period of its impact on growth, all the actors of the somatotropic axis (GH/GHR; IGF1/IGF1R, IGF2) are essential to ensure on optimal growth for children (Wit & Camacho-Hübner 2011, Mohan & Kesavan 2012). This statement is consistent with the rare IGF1 molecular defects in humans described in literature and characterized by severe intrauterine growth retardation, small-for-age new borns and low height Z-score during childhood (Netchine *et al.* 2011).

The nutrient environment is an important factor regulating the GH/IGF1 axis during both periods. Nutrient deficiency (isolated protein deficiency, energy deficiency or both) leads to intrauterine growth retardation during pregnancy (Gluckman & Pinal 2003). During childhood, after an early loss of weight, chronic nutrient deficiency leads to a decrease of the systemic growth rate called ‘stunting’, which is defined by a low height for age Z-score (Hizli *et al.* 2007). At a molecular level, the stunting state is characterized by low IGF1 plasmatic level with high GH level representing a GH resistance profile (as discussed in previous section). Children undernutrition is actually a leading subject of world public health and contributes to more than 40% of death worldwide among children under 5 years old, which represent 3.1 millions of children (Horton & Lo 2013). Stunting affects 159 million children in the world (Hizli *et al.* 2007). Following the first 1000 days, stunting is commonly irreversible and leads to shorter status in adulthood (Shrimpton *et al.* 2001). Malnutrition is not an exclusive problem of developing countries. In developed countries, it is common among hospitalized children, reaching rates of up to 20% in Europe and North America (Pawellek *et al.* 2008, Joosten *et al.* 2010). Many chronic diseases may rapidly affect growth during childhood, including food allergy, inflammatory bowel disease, cardiac or respiratory insufficiency and cancer, increasing the length and cost of hospital stays while decreasing quality of life (Allison 2000).

Due to the capacity of the human microbiota to modulate the nutrient availability in the gut, the relationship between gut bacteria, nutritional status and growth in children is a promising field for research. Several studies have highlighted the capacity of the nutrient environment to shape the human microbiota. The analysis of gut microbiota profiles in undernourished children demonstrated several specific modifications. Subramanian *et al.* have compared the microbiota diversity of severely stunted children against healthy children of the same age and from the same region. They revealed that the microbiota related to stunting had a significant immaturity defined by fewer bacterial taxa and lower relative abundance (Subramanian *et al.* 2014). In another manner, the concept of environmental enteropathy (EE) is a good example of the interaction between gut microbiota, environment and stunting process. EE is characterized by decreased height of small intestinal villosity, a slower intestinal transit and increase of intestinal permeability with chronic intestinal inflammation (Keusch *et al.* 2014). This process leads to a severe malabsorption resulting in stunting. Interestingly,
EE is mainly described in parts of the world with poor hygiene, which suggests that microbial exposure or the microbiota plays a key role in its development (Ahmed et al. 2014). Finally, the microbiota modifications are not only seen in an undernutrition environment but also with an overnutrition diet, which underlines the close relationship between nutritional environment and gut microbiota composition (Turnbaugh et al. 2008).

Conversely, several other studies have shown that specific human microbiota patterns can aggravate undernutrition phenotype, thus linking gut bacteria with the juvenile growth process. However, we need to stay cautious regarding these results because according to our knowledge, findings have been only observed in a situation of human microbiota transplantation into germ-free animals. Specifically, Smith et al. transplanted gut microbiota of twins discordant for kwashiorkor into germ-free mice. Fecal transplant from stunted Malawian children to germ-free mice induced higher loss of weight when the rodents were fed a Malawian ‘wasting-triggering’ diet as compared to the co-twin’s healthy microbiota transplant (Smith et al. 2013). They were supported by Blanton et al. in 2016 where C57BL6 male germ-free mice were transplanted with fecal samples from healthy children or moderate-to-severe stunted children from another Malawian cohort (Blanton et al. 2016b). Malawian ‘wasting-triggering’ diet-fed mice colonized with microbiota form healthy donors gained significantly more weight and lean body mass than mice colonized with microbiota from stunted subjects. There was no significant difference in fat mass. Surprisingly, the trabecular bone micro CT results were discordant with the nutritional status of the donor.

In these studies, diverse bacterial interventions on the deleterious microbiota pattern improved the phenotype initially induced. First, in the Blanton et al. study, mice transplanted with stunted microbiota and dually housed with mice transplanted with healthy microbiota gained more lean mass than stunted control. The analysis of the rodent’s microbiota indicated an invasion of healthy microbiota numbers into the co-housed stunted mice showing a beneficial impact of the microbiota colonization on stunting (Blanton et al. 2016b). Furthermore, the addition of isolated strains from two specific bacterial phyla that were found to be enriched in the microbiota of a healthy twin (Ruminococcus gnavus and Clostridium symbiosum) to the immature microbiota collected from stunted twin improved the early recovery of weight gain in wasted juvenile mice and more specifically lean mass (Blanton et al. 2016b).

Next, Charbonneau et al. highlighted the properties of human milk oligosaccharides (HMOs) in the context of microbiota’s influence on juvenile growth. HMOs in mother’s breast milk act as prebiotics that promote colonization of the infant gut with bifidobacterial taxa associated with immunomodulatory beneficial functions (Charbonneau et al. 2016). In breast milk of Malawian mothers of severely stunted infants, some specific HMOs were significantly reduced. C57BL/6j germ-free mice were colonized by a defined 25 strains community from 6-month-old stunted Malawian infant and were fed with prototypic Malawian diet supplemented or not with HMOs. The juvenile mouse weight gain expressed as lean mass was significantly increased by HMOs. The femur showed significant increases in cortical thickness, cortical volumetric bone mineral density and cortical bone volume normalized to tissue volume. All the beneficial effects were microbiota dependent since they were not observed in germ-free animals. These results support the fact that bone impairment induced by malnutrition could be improved by modulation of the gut microbiota through prebiotic and/or specific bacterial strains treatment. No data about the IGF1 levels were provided in these studies, thus we can only speculate whether the improved growth was accompanied by improved somatotropic axis activity.

Few interventional trials have studied the impact of probiotic supplementation on growth in children. A systematic review of the use of probiotics and/or prebiotics in infant formula published by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition concluded the lack of proof of efficacy on childhood growth parameters. However, most of studies included small groups of healthy children who had an appropriate diet (Braegger et al. 2011). Only one study using a Lactobacillus rhamnosus strain found greater changes in length and weight in the supplemented group after the first 6 months of life (Vendt et al. 2006). To date, there is no evidence that dietary intake of probiotics is beneficial for growth in well-nourished children in developed countries. More recently, a large randomized, double-blind, placebo control trial of an oral symbiotic preparation (Lactobacillus plantarum with fructo-oligosaccharide) was conducted in 4556 newborns from rural India. The aim of this study was to evaluate the impact of the symbiotic supplementation on neonatal sepsis prevention. In addition to a large benefit in the reduction of neonatal sepsis, the authors found those in the treatment arm had a greater increase in weight at day 60 after birth compared to those given placebo (Panigrahi et al. 2008). Again, no data on linear growth or IGF1 titers were reported, but due to the early
period of life we could reasonably extrapolate these data as a hopeful sign for growth.

Despite robust and exciting data generated using animal models, until now, the clinical literature is completely lacking on the potential interactions between gut microbiota, IGF1 and bone growth in humans. Developing robust and ambitious clinical studies to evaluate the potential benefits of selected bacterial strains in the nutritional management of human undernutrition and stunting is therefore a high priority.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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