Gut microbiota: puppeteer of the host juvenile growth

Martin Schwarzer

Purpose of review
This review focuses on the recent discoveries about the impact of intestinal microbiota on mammalian host juvenile growth.

Recent findings
Intestinal microbiota is a powerful modulator of many facets of multicellular host's physiology. Recent results from human field studies and animal research have clearly shown that not only the nutrition, but also the intestinal microbiota impacts host postnatal growth kinetics. Absence of microbiome leads to stunted growth in mammalian gnotobiotic models and changes in the composition of the intestinal microbiota can impact the postnatal growth kinetics both positively and negatively under normal nutritional conditions as well as in undernutrition. Strikingly, specific bacterial strains are able to interact with GH/IGF-1 somatotropic axis activity, thus directly impacting host juvenile development.

Summary
Intestinal microbiota dictates the pace of host postnatal growth. This newly described role envisages that therapy with specific bacterial strains, together with re-nutritional strategies, might successfully alleviate the long-term sequelae of undernutrition during childhood in humans.

Keywords
IGF-1, juvenile growth, microbiota, somatotropic axis, stunting

INTRODUCTION

Growth is the fundamental physiologic process that is an intrinsic part of each individual's developmental program. In multicellular organisms the postnatal growth is achieved mostly by cellular proliferation, which ultimately leads to increase in size, both ponderal and longitudinal [1]. It is a well established common knowledge that the developmental rate and the final body size of each organism are determined by the interaction between the individual's genes and the environmental factors. Growth is by definition an anabolic process requiring sufficient supply of nutrients and energy. The undernutrition and malnutrition affect metabolism and hormonal signaling leading to altered development and altered final adult body size [2,3].

First eukaryotic organisms evolved in the world that has been dominated by bacteria and archea [4▪]. Thus, through their evolution, they were forced to interact and form diverse symbiotic relationships with bacteria that surrounded them. To satisfy their nutritional needs, multicellular organisms evolved a digestive system – a specialized organ for extracting nutrients and energy from food. Though located within the body, digestive system is *per se* a tube connected with the external environment. Accumulation of food in different stages of processing makes the digestive tube a nutrient-rich environment that is swarming with micro-organismal life. It is a home for bacteria, but also for viruses, fungi and archea that are collectively called the individual's microbiota. There is a general assumption, that the fetus is sterile and that the microbiota is acquired during and shortly after birth when bacterial colonization takes place [5▪]. Although all mucosal surfaces are colonized by distinct bacterial communities [6], the gastrointestinal microbiome is by far the most abundant. In humans the bacterial numbers in colon can reach up to the 4×10^{13}, which makes it one of the

Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Novy Hradek, Czech Republic

Correspondence to Martin Schwarzer, PhD, Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Doly 183, 549 22 Novy Hradek, Czech Republic. Tel: +420 491418535; e-mail: schwarzer@biomed.cas.cz

Curr Opin Clin Nutr Metab Care 2018, 21:000–000
DOI:10.1097/MCO.0000000000000463

KEY POINTS

- Gut microbiota influences the postnatal growth in mammals, both positively and negatively.
- Selected bacterial strains and specific prebiotic modulation of the gut microbiota ameliorate stunting induced by poor nutrition.
- Future studies in the emerging link between specific bacterial strains and host somatotropic axis activity are warranted.

most densely populated bacterial ecosystems on earth [7▪]. Thus, contrary to the first eukaryotic cells, which biological processes were modified by the interactions with bacteria that surrounded them, the physiology of recent multicellular organisms is heavily under the influence of the microorganisms that they contain within themselves [8]. It seems that the need to manage this vast microbiota community was the reason that necessitated the evolution of a complex immune system as we see it today [9].

Intestinal microbiota can be regarded as an additional organ system in the multicellular eukaryotic organism [4▪]. It is made up of a collection of cells that communicate with each other and with the host, it contains heritable components and it consumes and stores energy [10]. Due to its location, intestinal microbiota is an inseparable part of host nutritional environment. Nutrition has a direct impact on its composition and metabolism [11] and, on the other hand, microbiota takes a part in the digestion of nutrients and the energy extraction and provides the host with vitamins and various metabolic products. Viewed from this angle, multicellular organisms are no longer autonomous entities but rather a complex of the host with its associated microbes, which can be coined with the term holobiont [12]. Integrative approaches should, therefore take the intestinal microbiota and its genetic and metabolic potential as an intrinsic factor affecting all aspects of organismal biology.

The juvenile growth is one of the recently described biological traits affected by the microbiota. Evidence from human studies and experimental animal models have shown that the intestinal microbiota and its composition can impact the postnatal host growth kinetics both positively and negatively under normal nutritional conditions and in undernutrition [13▪,14,15,16▪▪,17▪▪]. Moreover, specific bacterial strains are able to interact with the growth hormone/insulin like growth factor-1 (GH/IGF-1) somatotropic axis activity, thus directly impacting the host juvenile development [13▪]. This review will concentrate on the role of the microbiota during juvenile growth in mammals and humans (Fig. 1). The link between microbiota and growth in other invertebrate and vertebrate organisms has been recently reviewed in [18].

INTESTINAL MICROBIOTA AND INCREASED HOST JUVENILE GROWTH

Investigation of the impact of gut microbiota on juvenile growth is enabled by the advances in rearing gnotobionts, that is animals devoid of their intestinal microbiota (germ-free) or intentionally colonized by selected bacterial strain or their consortium [19]. The growth rate kinetics of juvenile germ-free and conventional animals has been a contentious issue since the beginning of the long-term germ-free colonies in 1950s, with reports stating similar or lower growth for germ-free animals compared with conventional counterparts [20]. To

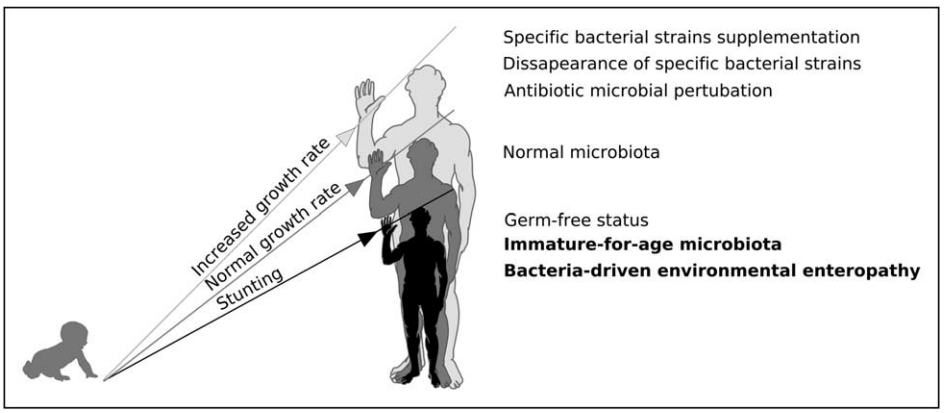

FIGURE 1. Gut microbiota and mammalian host juvenile growth. Schematic representation of juvenile growth trajectories linked with specific bacterial configurations. Configurations leading to stunting that are linked with malnutrition are depicted in bold.

investigate the contribution of the gut microbiota to the normal postnatal growth in mammals, we compared the growth kinetics of conventional and germ-free animals on nutritionally adequate breeding diet [13▪]. We showed that under normal nutritional conditions, microbiota is necessary to maximize the systemic weight gain and linear growth of weaned male BALB/c mice. Concomitantly, in comparison to the germ-free mice, microbiota improved bone growth parameters, including femur length, cortical thickness, cortical and trabecular bone fraction. It is well documented that in mammals, postnatal systemic growth is driven to a large extent by the activity of the somatotropic axis, where the pituitary gland produces growth hormone, which induces the production of IGF-1 leading to promotion of organ and systemic growth [21]. In this regard, we found that optimal growth kinetics of conventional mice was accompanied by higher level of circulating IGF-1 and IGFBP-3, its major binding protein, despite no changes in the growth hormone levels between conventional and germ-free animals. The after-weaning spurt growth period in conventional mice was accompanied by a peak of circulating IGF-1 levels at day 28 after birth, which was not observed in germ-free animals. These data suggested that intestinal microbiota is implicated in higher sensitivity of conventional animals to growth hormone actions and confirmed the importance of IGF-1 in postnatal growth. The improved growth rates of conventional animals were even more striking under undernutrition. Whenever weaned on diet low in proteins and fat, but isocaloric to the breeding diet, male germ-free mice were completely stunted with no observed gain in weight or length. On the contrary, mice harboring gut microbiota resumed to grow, although to a lesser extent compared with conventional animals on normal breeding diet [13▪].

The connection between microbiota, IGF-1 levels and skeletal growth in normal nutritional conditions was further confirmed by Yan *et al.* [22▪▪]. In their study, authors used 2 months old germ-free mice and mice conventionalized by cecal content of specific-pathogen free donor. Male mice conventionalized for 8 months were heavier and longer and their bone parameters such as femur length and periosteal/endosteal area were significantly bigger compared with the germ-free controls. These findings were accompanied by increased levels of circulating IGF-1, which could be detected as soon as 1 week after the conventionalization. Further, they showed that gut microbiota-produced short-chain fatty acid (SCFA) may be the possible mechanism behind the observed increase in serum IGF-1.

To increase the growth of mammals under normal nutritional conditions, mainly in the agricultural industry, several ways to modulate the gut microbiota have been employed: low-dose antibiotic (ATB) treatment [23], probiotics [24] or specific bacteria removal by re-derivation into sanitary conditions [25]. Recently, the most widespread method to promote the growth of farm animals is the use of antimicrobial agents administered in low doses [26]. Though widely used for more than 60 years now, the precise mechanism behind ATB-linked growth promotion is still enigmatic. In their seminal article, Cho *et al.* showed, that chronic low-dose ATB therapy to young mice induced substantial taxonomic changes in the microbiome with increased levels of colonic SCFA. Whenever initiated at weaning, the low-dose ATB treatment led to increased growth rate but also to alterations in the regulation of hepatic metabolism of lipids and cholesterol and increased adiposity. Surprisingly, no changes in the IGF-1 levels were reported [27]. More recently, the same group investigated the impact of the repeated short-term ATB treatment in a mouse model closely mimicking the pediatric antibiotic use in humans [15]. They found that early-life ATB treatment significantly accelerated juvenile growth and bone development together with long-term developmental metabolic effects. These host-related changes were associated with progressive decline in the gut microbiome diversity and changes in the population structure. In humans, ATB treatment have a documented growth promoting effect in prepubertal children in low-income and middle-income countries with the effect more pronounced for ponderal than for linear growth [28]. However, given the accumulating evidence on the long-term metabolic consequences of ATB courses during early-life, further studies to assess the potential risks are warranted.

As an alternative to antibiotics, probiotics have been described as positive modulators of juvenile growth in mammals [24]. In this regard, we have shown that *Lactobacillus plantarum* (Lp) bacterial strain has an evolutionarily conserved ability to promote juvenile growth in *Drosophila melanogaster* and in mouse [13▪]. Selected for its growth promoting properties in the gnotobiotic *Drosophila* undernutrition model, *L. plantarum* isolate WJL (Lp^{WJL}) improved the juvenile growth in monocolonized mouse both under normal nutritional conditions and in undernutrition in comparison with germ-free mice to the same extent as observed in conventional animals. This growth improvement was linked to the increased growth hormone-sensitivity and higher levels of IGF-1. The ability to improve growth was strictly strain-specific as other Lp isolates were less beneficial.

The underlying strain-specific growth-promoting mechanism remains to be elucidated. Specifically, Matos *et al.* [29] showed recently that *L. plantarum* cell walls bearing d-alanylated teichoic acids are directly sensed by *Drosophila* enterocytes and this sensing is indispensable for increased juvenile growth and maturation during chronic undernutrition. Interestingly, a meta-analysis of studies of probiotic administration to children on growth concluded that there is limited evidence suggesting the beneficial effect in developing countries and in under-nourished children. No effect of probiotics on growth was found in developed countries [30].

The link between modified gut microbiota and increased juvenile growth is compelling in connection to the secular trend in adult height of human population [31]. The trend towards increasing height began in the middle of the last century and it coincides with the onset of antibiotic era, increased sanitation standards and improved child care. As pointed out by Blaser [32], this increased hygiene might lead to the losses of particular bacterial species of our ancestral microbiota, leading to altered context in which immunological and metabolic development occur in early life. It has been suggested that the adult trend in height is already in place during the very earliest phase of childhood [31]. Thus, the disappearance of ancestral microbial strains might lead to lower stimulation of immune system during this critical period and the energy and nutrients, which would be otherwise be consumed by immune system, can be used for growth. As a consequence, the disappearance of certain bacterial strains will result in the alleviation of growth depression, but concomitantly also in the increased prevalence of chronic diseases later in life because of altered immune development.

INTESTINAL MICROBIOTA AND STUNTED HOST JUVENILE GROWTH

The germ-free status by itself is a preconditioning for decreased juvenile growth. However, also certain composition of intestinal microbiota can predispose to host growth depression [3]. Childhood undernutrition is a global health challenge and current treatment approaches, including ATB treatment and re-nutritional strategies, have only modest effect in correcting its life-long sequelae. Smith *et al.* [33] established that besides food with low-nutrient density, the gut microbiota is a causal factor in severe form of malnutrition (kwashiorkor). The authors transferred fecal microbial communities from homozygotic twins with and without kwashiorkor to germ-free mice. Whenever challenged with poor diet, microbiome derived from kwashiorkor patient induced severe weight loss and metabolic perturbations in recipient mice, which were only transiently ameliorated by re-nutrition therapy. Further, by utilizing similar approach, the same group found that the susceptibility to severe acute malnutrition is linked with the gut microbiota immaturity, that is, microbial community not containing age discriminatory taxa from normally developing gut microbiota [16▪▪]. By co-housing mice that received microbiota from healthy or severely stunted infants, they identified bacterial taxa that were able to invade the stunted microbiota-associated mice and improve body mass gain, metabolism and bone morphology. As a result, they were able to identify two of these beneficial invasive species, *Ruminococcus gnavus* and *Clostridium symbiosum*. Similarly, in a recent article, Tidjani Alou *et al.* [34▪] identified a complex of different 12 bacterial species that were present in the gut of healthy children but missing in severely malnourished children living in the same area. These findings point the way to microbiotherapy with selected bacterial strains, which will enable the improvement of the current treatment of severe acute malnutrition.

We have seen that the juvenile growth can be improved by adding certain bacterial strains to the gut consortium. But even a bacterial consortium linked to severe malnutrition can improve host juvenile growth, when its composition and metabolic output is changed by specific prebiotics. Charboneau *et al.* showed that prebiotic syalylated breast milk oligosaccharides are more abundant in milk of mothers from healthy children as compared with mothers of malnourished children. In a subsequent experiment, germ-free mice colonized with a bacterial consortium cultured from severely malnourished donor and fed poor diet supplemented with syalylated oligosaccharide showed microbiota-dependent improvement of lean body mass gain, bone morphology and a shift in metabolism, suggesting the host's greater ability to utilize nutrients for anabolism [17▪▪].

Environmental enteropathy is a major contributor to childhood malnutrition. It is a subclinical chronic inflammatory disease of the small intestine mainly observed in regions with poor sanitation and its aetiology is still obscure [35]. Brown *et al.* [14] showed recently that early life exposure to poor diet in combination with iterative oral exposure to commensal Bacteroidales species and *Escherichia coli,* remodels the small intestine to resemble all the main features of the enteropathy in humans and leads to diminished grow rates. Thus, exposure to certain bacterial strains plays a decisive role in juvenile-stunting induction. Concomitantly, this model opens up a way to test early life microbiotheraphy

and identify bacterial strains to prevent the environmental enteropathy induction and improve growth.

CONCLUSION

In recent years, unequivocal evidence both from human field studies and animal research show that the intestine and its microbial inhabitants play a decisive role in mammalian host juvenile growth. Presence or absence of specific bacterial strains leads to growth improvement or growth depression, especially in combination with food with low-nutrient density. Clinical studies incorporating prenatal and postnatal period with selected probiotics or prebiotics are required to validate the accumulating findings of experimental studies. Furthermore, defining the precise mechanisms by which the microbiota and specific bacterial strains impact the juvenile growth will likely reveal new targets to alleviate the long-term sequelae of undernutrition in early life in humans in developing countries.

Acknowledgements

I thank Associate Professor Irma Schabussova (Medical University of Vienna) for critical reading of the manuscript.

Financial support and sponsorship

Support from The Neuron Fund for the Support of Science (Neuron Fund) and grant 18–07015Y of the Czech Science Foundation is gratefully acknowledged.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Efstratiadis A. Genetics of mouse growth. Int J Dev Biol 1998; 42:955–976.
2. Cooke L, Llewellyn C. Nature and nurture in early feeding behavior. Nestle Nutr Inst Workshop Ser 2016; 85:155–165.
3. Blanton LV, Barratt MJ, Charbonneau MR. Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics. Science 2016; 352:1533.
4. ■ Rook G, Bäckhed F, Levin BR, *et al.* Evolution, human-microbe interactions, and life history plasticity. Lancet 2017; 390:521–530.

An interesting insight into the co-evolution of humans and their microbiome.

5. ■ Perez-Munoz ME, Arrieta MC, Ramer-Tait AE, Walter J. A critical assessment of the 'sterile womb' and 'in utero colonization' hypotheses: implications for research on the pioneer infant microbiome. Microbiome 2017; 5:48.

Review on the recent literature challenging the 'in utero colonization' findings.

6. Ferretti P, Farina S, Cristofolini M, *et al.* Experimental metagenomics and ribosomal profiling of the human skin microbiome. Exp Dermatol 2017; 26:211–219.
7. ■ Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell 2016; 164:337–340.

The ratio of bacteria to human cells has been revisited from 10 : 1 down to 1 : 1.

8. Leulier F, MacNeil LT, Lee WJ, *et al.* Integrative physiology: at the crossroads of nutrition, microbiota, animal physiology, and human health. Cell Metab 2017; 25:522–534.
9. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. Nature 2017; 548:43–51.
10. Marchesi JR, Adams DH, Fava F, *et al.* The gut microbiota and host health: a new clinical frontier. Gut 2016; 65:330–339.
11. Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. Curr Opin Microbiol 2017; 38:59–65.
12. Bordenstein SR, Theis KR. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol 2015; 13:e1002226.
13. ■ Schwarzer M, Makki K, Storelli G, *et al.* Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition. Science 2016; 351:854–857.

This study shows that neonatal growth and IGF-1 levels are greater in SPF mice compared with germ-free mice. Further, monocolonization of mice with *L. plantarum* impacts positively on somatotropic axis and improves growth to the same extent as complex SPF microbiota.

14. Brown EM, Wlodarska M, Willing BP, *et al.* Diet and specific microbial exposure trigger features of environmental enteropathy in a novel murine model. Nat Commun 2015; 6:7806.
15. Nobel YR, Cox LM, Kirigin FF, *et al.* Metabolic and metagenomic outcomes from early-life pulsed antibiotic treatment. Nat Commun 2015; 6:7486.
16. ■■ Blanton LV, Charbonneau MR, Salih T, *et al.* Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Science 2016; 351:; pii: aad3311.

This study shows that the undernourished infant's impaired growth phenotype can be transmitted by gut bacteria on gnotobiotic mice. Two bacterial species, *R. gnavus* and *C. symbiosum*, can invade the undernourished donor microbiota and ameliorate growth.

17. ■■ Charbonneau MR, O'Donnell D, Blanton LV, *et al.* Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. Cell 2016; 164:859–871.

This article establishes a link between consumption of prebiotic sialylated milk oligosaccharides, the gut microbiota and the host juvenile growth rate.

18. Schwarzer M, Strigini M, Leulier F. Gut microbiota and host juvenile growth. Calcif Tissue Int 2017; doi: https://doi.org/10.1007/s00223-017-0368-y. [Epub ahead of print]
19. Schoeb TR, Eaton KA. Gnotobiotics. 1st ed Elsevier, London: Academic Press; 2017; 428.
20. Gordon HA. Morphological and physiological characterization of germfree life. Ann N Y Acad Sci 1959; 78:208–220.
21. Reynolds CM, Perry JK, Vickers MH. Manipulation of the growth hormone-insulin-like growth factor (GH-IGF) axis: a treatment strategy to reverse the effects of early life developmental programming. Int J Mol Sci 2017; 18:pii: E1729.
22. ■■ Yan J, Herzog JW, Tsang K, *et al.* Gut microbiota induce IGF-1 and promote bone formation and growth. Proc Natl Acad Sci U S A 2016; 113:E7554–E7563.

This study demonstrates link between gut microbiota and increased serum levels of IGF-1, which is connected with the increased skeletal growth. Short-chain fatty acid production may be one of the mechanisms by which microbiota increase IGF-1.

23. Brussow H. Growth promotion and gut microbiota: insights from antibiotic use. Environ Microbiol 2015; 17:2216–2227.
24. Dowarah R, Verma AK, Agarwal N. The use of Lactobacillus as an alternative of antibiotic growth promoters in pigs: a review. Anim Nutr 2017; 3:1–6.
25. Dubos RJ, Schaedler RW. The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. J Exp Med 1960; 111:407–417.
26. Gonzalez Ronquillo M, Angeles Hernandez JC. Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control 2017; 72(Part B):255–267.
27. Cho I, Yamanishi S, Cox L, *et al.* Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012; 488:621–626.
28. Gough EK, Moodie EE, Prendergast AJ, *et al.* The impact of antibiotics on growth in children in low and middle income countries: systematic review and meta-analysis of randomised controlled trials. BMJ 2014; 348:g2267.
29. Matos RC, Schwarzer M, Gervais H, *et al.* D-Alanylation of teichoic acids contributes to Lactobacillus plantarum-mediated Drosophila growth during chronic undernutrition. Nat Microbiol 2017; 2:1635–1647.
30. Onubi OJ, Poobalan AS, Dineen B, *et al.* Effects of probiotics on child growth: a systematic review. J Health Popul Nutr 2015; 34:8.
31. Stulp G, Barrett L. Evolutionary perspectives on human height variation. Biol Rev Camb Philos Soc 2016; 91:206–234.
32. Blaser MJ. The theory of disappearing microbiota and the epidemics of chronic diseases. Nat Rev Immunol 2017; 17:461–463.
33. Smith MI, Yatsunenko T, Manary MJ, *et al.* Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 2013; 339:548–554.
34. ■ Tidjani Alou M, Million M, Traore SI, *et al.* Gut bacteria missing in severe acute malnutrition, can we identify potential probiotics by culturomics? Front Microbiol 2017; 8:899.

Identification of bacterial strains missing in undernourished-children microbiota and selection of potential probiotics for interventions.

35. Watanabe K, Petri WA. Environmental enteropathy: elusive but significant subclinical abnormalities in developing countries. EBioMedicine 2016; 10:25–32.