

## An Update on Gut Microbiota and Infant's Health

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Childhood malnutrition is a global problem and one of the leading cause of stunted growth, and responsible for the death of millions of children every year. Although extensive efforts have been made to promote healthy growth but results are not satisfactory and infant's health remains a challenge. Previously, it was demonstrated that undernourished children have disrupted normal pattern of intestinal microbiota and led to a proposal that it might be involved in impaired postnatal growth. Recently, various research groups focused on Malawian population and proved the role of intestinal microbiota in the stunted growth of children. In addition, one group showed the role of sialylated bovine milk oligosaccharides in promoting microbiota-dependent growth in malnourished children. Moreover, it was also revealed that *Clostridium symbiosum* and *Ruminococcus gnavus* might be used as therapeutic agent for ameliorating growth abnormalities in malnourished children. The current article summarizes the recent advancement in identifying interventions regarding health promotion of malnourished children.

**Key Words:** Microbiota, Stunted growth, Malnutrition, Infant's health

### INTRODUCTION

In developing or low income countries nutritional stunting is a serious and common health issue of the pediatric population (1). Different factors are responsible for stunting, including psychosocial, hormonal, genetic, hormonal and nutritional (1). Besides reduced height, stunted growth has also been associated with impaired intellectual development (2). Recently, it was demonstrated that undernourished child has disrupted normal pattern of intestinal microbiota and might be involved in impaired postnatal growth (3, 4). Human milk has variety of oligosaccharides known as human milk oligosaccharides (HMOs) which pass through the pro-

ximal gut without being changed and act as prebiotics for various bacterial strains (3). These bacterial strains have different beneficial effects including protection from enteropathogen, improved vaccine response and improved gut barrier function (5~7). A main feature of this review is to focus on the gut microbiota which plays a positive role in malnourished infants in developing or low income countries.

#### Role of sialylated milk oligosaccharides in promoting microbiota-dependent growth of undernourished infants

The link between growth phenotypes of infants and HMOs has not been well studied. Recently, this issue was addressed by focusing on Malawian mothers having 6-months-old infants either showing healthy growth or severely stunted (3).

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Researchers showed that mothers having stunted growth infants carry significantly less sialylated HMOs in their milk as compared to mothers of healthy infants. Breast milk were collected and analyzed for HMOs from Malawian mothers whose children showed either healthy or stunted growth. Concentrations of sialylated and fucosylated HMOs were significantly higher in mothers of healthy infants as compared to severely stunted infants (3). Consortium of various cultured strains of fecal microbiota isolated from stunted Malawian infants was transferred to germ free (GF) new born piglets and mice. Piglets mice and were fed with typical Malawian diet along sialylated bovine milk oligosaccharides (S-BMO) added or not. S-BMOs are structurally similar to sialylated HMOs. Results show that this S-BMOs preparation results in microbiota dependent encouragement of growth and metabolic changes which indicates nutrient utilization. The fecal microbiota of stunted 6-month-old Malawian infant was used to collect various bacterial strains. It is difficult to purify HMOs from milk on large scale, so monosaccharide- and lactose-free mixture of S-BMO was purified from cheese whey stream (3). A representative Malawian diet was made which contains eight principal components (M8) and this diet was not up to the recommended daily nutritional needs of mice or humans. Different groups of 5-weeks-old male GF C57BL/6J mice were transplanted with oral gavage of the defined 25 bacterial strain community. One mice group was also given M8 supplemented with inulin, a heterogeneous mixture of fructose polymers, a common ingredient of infant's formula (3). The colonization efficiency at strain level was determined by shotgun sequencing of fecal DNA. A strain achieving more than 0.1% mean relative abundance 44 days following gavage was considered successfully colonized. Among 25 bacterial strains 19 strains (76%) successfully colonized in recipient gnotobiotic mice. *Klebsiella varicola*, *Peptoniphilus harei*, *Bifidobacterium longum subsp. infantis*, and one strain each of *Olsenella uli* and *Enterococcus faecalis* failed to colonize successfully. Body composition and weight was observed for 5 weeks and there was significant weight gain by S-BMO but not in the case of inulin supplementation. This difference in weight gain was not due to differences in food consumption (3). It has been

shown that microbiota affects bone mass in mice. Using microcomputed tomography of femurs it was revealed that mice given S-BMO supplementation showed significant increase in cortical bone mineral density and thickness. Histological examination of femurs also revealed remarkably increased cortical bone volume normalized to tissue volume. *B. fragilis* act as a primary consumer of S-BMO in a food web while *E. coli* benefit as a secondary consumer. Using ultra high performance liquid chromatography-mass spectrometry, it was confirmed that *B. fragilis* degraded sialyllactos resulting in increase of free sialic acid but not *E. coli*. *B. fragilis* degrades the S-BMO and help in growth of *E. coli* (3). These studies show that microbiota dependent growth observed *in vivo* due to S-BMO supplementation might be ascribed to either primary consumers of S-BMO (*B. fragilis*) or secondary consumers (*E. coli*). Mice colonized with *E. coli* and *B. fragilis* alone supplemented with S-BMO did not show improvement in growth indicating that other members are also important for growth promotion (3). After 5-weeks gavage of 25-member bacterial culture collection, total 176 metabolites were measured in serum, liver, brain and muscle obtained from S-BMO-supplemented versus control mice. Remarkably lower level of medium- and long-chain acylcarnitines were observed in the serums of non-fasted and S-BMO supplemented animals as compared to control mice. Similarly, these animals showed increased level of serum leptin, insulin, triglycerides and non-esterified fatty acids (3). In malnourished Ugandan children, lower serum leptin levels were associated with childhood mortality (8). In women leptin levels are associated with bone mineral density (9). Collectively, these results suggest that S-BMO-supplemented mice utilize dietary components more effectively for anabolism (3). To test the effect of S-BMO supplementation in other mammalian species, piglets were selected because of their similar digestive physiology to that of humans. Similar to non-fasted gnotobiotic mice, liver profile of non-fasted, S-BMO supplemented piglets showed remarkably reduced levels of acylcarnitine and fatty acyl CoA metabolites as compared to control piglets. Collectively, these observations suggest that S-BMO supplementation has similar effect on weight gain and metabolic phenotypes in gnotobiotic piglets

and mice (3).

### **Role of microbiota in maintaining growth of under-nourished infant mice**

Malnourishment is one of the leading causes of infant and children mortality all over the world (2, 10~12). Malnutrition of children is not only due to insufficient food but other factors like gut mucosal barrier dysfunction, nutrient bio-availability and pathogen burden are also involved (13~15). When microbiota was transferred from 6- and -18 month-old healthy or malnourished Malawian children to GF mice, and mice were fed same Malawian diet, impaired growth phenotype was transmitted in the recipient animals (16). Microbiota sample from healthy or malnourished child was transferred to GF mice. Similar diet was given to mice as that of Malawian child receiving at that age. Recipient mice were observed for 4 to 5 weeks and fecal samples were obtained for bacterial 16S rRNA analysis (17). The mice transplanted with healthy donor's microbiota obtained more weight compared to colonize with malnourished donor's microbiota. Cohousing of both group mice after receiving microbiota either from healthy or severely stunted 6-month-old infants resulted in transfer of species from healthy to stunted growth showed prevention of growth impairments (16, 17). Addition of two species, *Clostridium symbiosum*, and *Ruminococcus gnavus* to the microbiota from malnourished donors also improved growth abnormalities in recipient animals (16). It was also revealed that *Clostridium symbiosum* and *Ruminococcus gnavus* might be used as therapeutic agent for ameliorating growth abnormalities in malnourished children. These results show that gut microbiota immaturity is not only related with undernutrition. Some other factors might play role in disruption of normal gut microbiota development in infants and children (16, 17).

### **Role of microbiota in maintaining infantile growth via hormonal axis**

Most of the animal species show rapid gain in body size and weight during infantile growth period (18). Recently, it has been shown that microbiota of young mice play role in maintaining longitudinal growth and weight gain irrespective

of well-balanced diet or nutritionally depleted diet. During infantile growth period, the gain in body size depends on nutrition and the animal's hormonal cues (18). Postnatal growth of mammals is under control of somatotrophic axis where growth hormone (GH) regulates peripheral tissues and liver to produce insulin-like growth factor-1 (IGF-1). IGF-1 is a critical player in controlling organ and systemic growth. Chronic malnutrition leads to a state of GH resistance which results in stunting. In contrast, acute undernutrition causes wasting which is characterized by severe weight loss (18). However, the role of gut microbiota to normal postnatal growth and its impact on activity of somatotrophic axis during chronic malnutrition has not been well studied. The growth parameters of wild-type (WT) and GF infant mice were compared until 8 weeks old. These mice were fed with standard diet. Both group consumed similar quantity of food relative to body weight. However, the weight of GF mice was 14.5% less and they were 4% shorter as compared to WT mice. The microbiota of mice using standard breeding diet ensures best weight gain as well as longitudinal growth (18). WT mice showed more weight gain in organ as compared to GF mice, establishing the fact that WT microbiota is linked with optimal systemic somatic growth. Microbiota also affects the skeletal growth as confirmed by 4% longer WT animals as compared to GF mice.

Host's microbiota supports infantile growth as confirmed by the selected strain of *L. plantarum*, which summarizes the effects of microbiota on somatotrophic axis and mouse infantile growth (18). In addition, *Lactobacilli* strains moderate the negative effects of chronic malnutrition on postnatal growth of GF mice. The authors imagine that along with nutritional therapy, use of selected bacterial strain may have a novel and beneficial effects in chronic undernutrition postnatal growth problems in children of low- and middle-income countries (18).

## **CLOSING REMARKS**

Nutrition is critical for best child development throughout the first 1000 days of life and beyond. Here, in this review we summarize that some microorganisms in the gut can pre-

vent the negative effect of undernourishment. These studies show that such microorganisms might be beneficial in the therapeutic intervention to restore growth. In addition these recent studies show that host diet, gut microbiota and health are highly interrelated. We should be careful regarding dietary intervention as it affects the whole microbiota in the gut. Moreover, as nutritional deficiencies are different among communities so in further studies this point should be considered.

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