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REVIEW ARTICLE

Establishment of an ideal gut microbiota to boost healthy growth of neonates

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ABSTRACT

For decades, supporting the optimal growth of low birth weight (LBW) infants has been considered one of the most important paediatric challenges, despite advances in neonatal intensive care technology and nutrition interventions. Since gut microbiota affects such diverse phenotypes in adults, the difference in gut microbiota composition between normal infants and LBW infants raises the possibility of gut microbiota playing an important role in different growth rates of neonates. Based on the concept that probiotics are generally beneficial to the health, numerous studies have been made on probiotics as a supplement to the diet of the LBW infants. However, clinical results on the effects of probiotics on LBW infant growth are either inconsistent or contradictory with each other, and thus the contribution of gut microbiota in neonatal growth has remained inconclusive. In this review, recent researches on neonatal gut microbiota are discussed to develop a new strategy for targeting gut microbiota as a solution to growth retardation in LBW infants. We also discuss how to establish the ideal gut microbiota to support optimal growth of LBW infants.

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Introduction

Over the recent decades, the survival rate of LBW infants, especially among preterm infants, has been increasing due to the advances in intensive neonatal care technology and nutrition interventions (Stoll et al. 2015). However, survivors have not been entirely free from complications and developmental disabilities throughout their life (Franz et al. 2009; Kumar et al. 2013). The growth restriction problem of LBW infants, compared to that of the normal foetus with the same gestation age, has not been solved even in the modern era (Bocca-Tjeertes et al. 2013; Abdeyazdan et al. 2014). Although the World Health Organization determined that the reduction of LBW is an important indicator of the Millennium Development Goal, incidents of LBW are constantly increasing in most countries according to the reliable trend data (Blencowe et al. 2012). The global prevalence of LBW is 15.5%, representing more than 20 million births each year, and thus, not only developing countries but also developed countries are facing a serious burden in the management of LBW infants (Unicef 2013). Therefore, promoting an optimal growth of LBW infants has been regarded as the most important paediatric challenge.

The gut microbiota, an organization of trillions of microbes, plays a crucial role in health and well-being of its host starting from birth (Clemente et al. 2012). Associations of gut microbiota to antimicrobial protection (Deriu et al. 2013; Kelly et al. 2016), immune modulation (Blander et al. 2017), nutrient metabolism, and energy regulation (Rosenbaum et al. 2015; Goffredo et al. 2016) in adult humans have been proven repeatedly. Recent studies on neonatal intestinal ecosystem showed that the establishment of gut microbiota in LBW infants is perturbed and differs from that of normal infants (Itani et al. 2017; Wandro et al. 2018). Indeed, the composition of gut microbiota in LBW infants lacked in diversity (Wandro et al. 2018). Furthermore, gut microbial colonization by beneficial microorganisms is delayed in LBW infants in a neonatal intensive care unit (NICU) because the LBW infants are isolated from their environment (Arboleya et al. 2012). In fact, establishment of the gut microbiota in the LBW infants is challenged due to the limited exposure to bacteria from the surrounding environment because of

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the isolation method accompanied with NICUs. Based on the concept that probiotics are beneficial to health, studies have been made on probiotics as a supplement to the diet of the LBW infants. However, according to these applications, clinical studies evaluating effects of probiotics on LBW infant growth are either inconsistent or contradictory with each other (Mohan et al. 2008; Yamasaki et al. 2012; Totsu et al. 2014; Hays et al. 2016). Obviously, the efficacy of gut microbiota on the growth of neonates still remains ambiguous. In this review, a better understanding of possible impacts of gut microbiota in neonatal growth based on immature physical characteristics in LBW will be discussed in order to evaluate gut microbiota as a potential solution to growth retardation in LBW infants by boosting healthy neonatal growth. In addition, we will discuss how to establish the ideal gut microbiota to support optimal growth of LBW infants.

Effects of gut microbiota to LBW infants

Company of gut microbiota and the host begin initially *in utero* (Moles et al. 2013). Current opinion suggests that gut microbial diversity increases rapidly after birth and fluctuates until it reaches maturity around 2 years of life (Palmer et al. 2007; Koenig et al. 2011). A basic process of gut microbial colonization that starts from facultative anaerobic microorganisms to the adult-like strict anaerobes is widely accepted because this is the adaptation to the altering oxygen environment in the gastrointestinal tract after birth (Jost et al. 2012). In healthy full-term infants, with full exposure to the mother's vaginal skin as well as the surrounding environment, this successional process occurs rapidly and thus anaerobes such as *Bifidobacterium* and *Bacteroides* may reach a high level in within the first week (Karlsson et al. 2011; Jost et al. 2012). In LBW infants, however, this switching process from facultative to strict anaerobes is significantly delayed and perturbed, mainly because current management methods under NICUs limit contact the LBW infants with their environments (Figure 1). As a result, LBW infants show higher levels of facultative anaerobic microorganisms such as *Enterobacteriaceae, Enterococcaceae, Escherichia coli, Enterococcus* sp, *Klebsiella pneumoniae, Staphylococcus* sp., etc. and reduced levels of strict anaerobes such as *Bifidobacterium, Bacteroides,* etc. (Korpela et al. 2018; Wandro et al. 2018). The bacterial species such as *Escherichia coli, Enterococcus spp,* and *Klebsiella pneumoniae* are a kind of pathogenic bacteria. These bacteria are not typically pathogenic to an individual with a normal gut microbiota. However, if these bacteria are present as a dominant species, these bacteria become pathogenic and cause enterocolitis (Packey and Sartor 2009). Considering the fact that LBW infants in NICUs are isolated from their environments, it would be

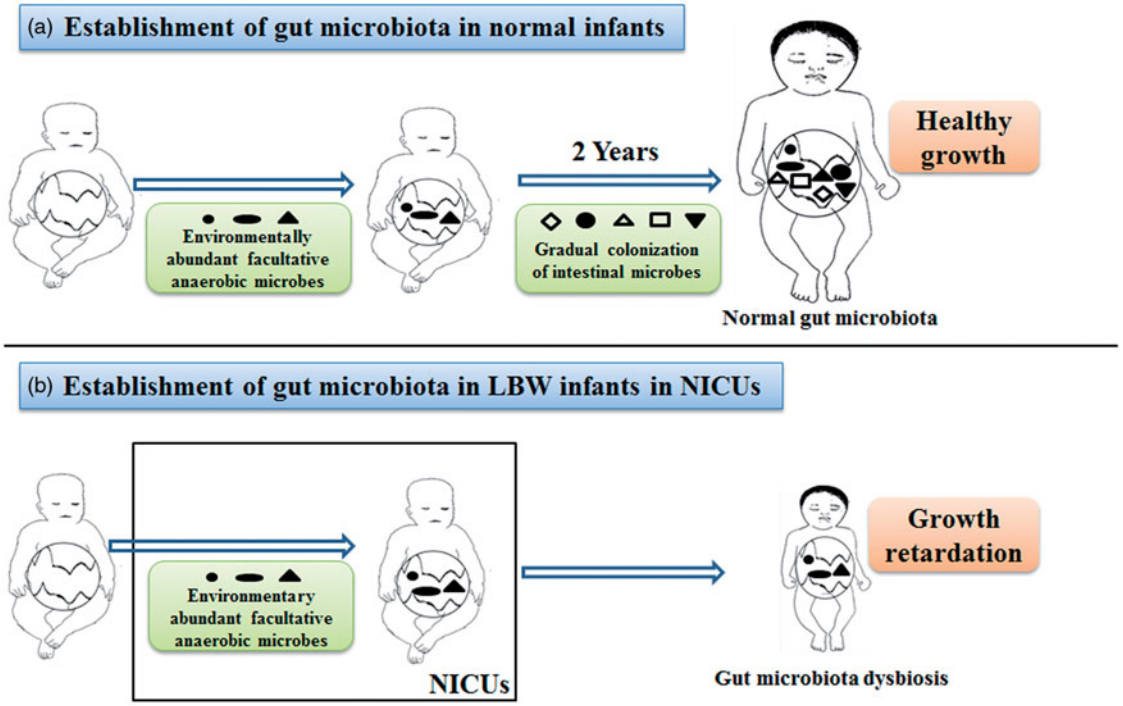

Figure 1. Schematic diagram of a gut microbiota establishment in LBW infants in NICUs in comparison to normal infants. LBW infants are typically admitted in NICUs in a modern medicine, and this strict isolation in NICUs would affect the LBW infants detrimentally.

natural that an accidental contact of LBW infants with environmentally abundant microbes is a major route of gut colonization. Because facultative anaerobes are main microbes in our environment, it is not surprising to find that pathogenic and environmentally abundant microbes such as *Escherichia coli, Enterococcus feacalis,* and *Klebsiella pneumoniae* are found as dominant microbes in LBW infants (Magne et al. 2005; Korpela et al. 2018), which affects negatively the growth of LBW infants in NICUs. In fact, recent evidence showed that infants feeding with mother's own milk rapidly developed gut microbiotas consisting of diverse intestinal microbes to stimulate the growth of LBW infants (Butcher et al. 2018). Kurath-Koller reported a similar work that environmental differences of LBW infants in NICUs such as contacts to care takers and parents influenced intestinal microbiota composition (Kurath-Koller et al. 2017; Ravi et al. 2017). These results suggest that frequent contacts of LBW infants in NICUs is helpful to colonize more diverse intestinal microbes in the guts and there must be an ideal gut microbiota for boosting optimal growth of LBW infants.

Considered as a companion of human life from birth, the gut microbiota plays a critical role in health and disease of humans. Despite evidences from numerous studies and growing appreciation for the integral role of the gut microbiota in lifelong health, relatively little is known about the efficiency of this complex microbial community during infancy. In general, the healthy development of neonates will be presented via healthy metabolism, immunity and physical including the nervous system, digestive system and others. Below we will discuss some of the main possible impacts of the gut microbiota to various associations in the growth process.

Metabolism

While LBW infants have relatively high energy requirement for development, the digestive and absorptive capabilities of their gastrointestinal system are relative low (Commare and Tappenden 2007; Hay Jr et al. 2014). Immature metabolic pathways and the deficiencies of enzymes in LBW infants predispose them into a new vicious circle of metabolic disorders that obviously contribute a significant factor to the delayed growth of LBW infants (Commare and Tappenden 2007; Clark et al. 2014).

The establishment of microbial community would shape the nutrient environment of the host by contributing to enzymatic activities. In human, the gut microbiota plays a crucial role in the metabolism of all components of food including lipid, protein, especially carbohydrates (Tremaroli and Backhed 2012). It should be noted that most indigestible carbohydrates, an important source of energy of human, would generally be lost via the stool due to lack of fermenting activities by the gut microbiota (Morrison and Preston 2016). Many studies also demonstrated that the diverse community dominant of *Bifidobacterium* and *Bacteroides* in term infants results in a higher concentration of faecal SCFAs, which is the end products of fermentation of dietary fibres, than that in LBW infants (Koenig et al. 2011; Wandro et al. 2018). Interestingly, the presence of gut microbiota mainly contributes to the digestion of more than 200 different oligosaccharide structures in breast milk since the infants lack the enzymes needed for milk glycan digestion (Ninonuevo et al. 2006; Marcobal et al. 2010; Marcobal and Sonnenburg 2012). Indeed, human milk oligosaccharide-utilizing bacteria, belonging to *Bacteroides* such as *Bacteroides fragilis* and *Bacteroides vulgatus* or *Bifidobacterium* such as *Bifidobacterium bifidum* and *Bifidobacterium infantis*, were found in high concentration in breastfed, term infants within the first day of life (Jost et al. 2012). Furthermore, some studies revealed that the genome of these bacteria encodes large number of the carbohydrate-related enzymes involved in human milk oligosaccharides consumption (Marcobal et al. 2010; Marcobal and Sonnenburg 2012).

The effects of gut microbiota on lipid metabolism have received relatively little attention. Studies on germ-free (GF) animals showed that the presence of gut microbiota is related to triglyceride level in the serum and increased the body fat content (Bäckhed et al. 2004; Velagapudi et al. 2010). In clinical studies, the gut microbiota might affect total lipid content through the production of SCFAs and some long chain fatty acids depending on gut microbial composition (Arboleya et al. 2012). In addition, through its effects on bile-acid metabolism and choline, gut microbiota is also associated with lipid-related diseases such as obesity and type 2 diabetes (Joyce et al. 2014).

In LBW infants, early amino acids intake may minimize the initial growth deficit, maintain the infantile growth rate, and affect brain growth and later life cognitive function (Tan et al. 2008; Hay and Thureen 2010). The protein is generally metabolized by both gut microbiota and the host, but without alimentary products, only gut microbiota can biosynthesize essential amino acids such as lysine, threonine, histidine, valine, etc. (Atasoglu et al. 1998; Metges 2000). Both small and large intestine harbour various bacteria involved in amino acid fermentation, belonging to the *Clostridium*

clusters, *Bacillus–Lactobacillus–Streptococcus* groups, *Proteobacteria*, and *Peptostreptococci* (Dai et al. 2011). Furthermore, gut microbiota also has the ability to produce essential vitamins. For example, the production of vitamin K in large intestine, which is a prerequisite for blood coagulation, becomes necessary in LBW infants because they are under high risk of intraventricular haemorrhage (LeBlanc et al. 2013; Kuperman et al. 2015). Or, the synthesis of vitamin B12 by small intestinal bacteria may contribute to maintenance of healthy nerve cells as well as the formation of red blood cells (Martens et al. 2002). The previous clinical study revealed that high *Bacteroides fragilis* and low *Staphylococcus* concentration of gut microbiota in the first year of life to a higher body mass index in later life (Vael et al. 2011).

On the other hand, the presence of gut microbiota may stimulate energy uptake by boosting healthy development of the gastrointestinal tract. Not only the microbiota but also its metabolites in intestine would affect substantial alterations in gut morphology, including villus width, crypt depth, rich, complex vascular network, and proliferating stem cells (Yu et al. 2016). A more recent study showed that the weight gain of the host was correlated with small intestinal growth (Yu et al. 2016). Furthermore, the different development of gut microbes between normal and LBW infants could lead to the difference in digestive tolerance (Jacquot et al. 2011). LBW infants usually have poorer digestive tolerance as compared to term infants (Jacquot et al. 2011). Overall, the gut microbiota is a host factor influencing energy regulation in neonates.

Immunity

The development of immune system begins early in the foetal period. In the first months of life, babies can be protected against opportunistic pathogens based on transferred maternal immunoglobulins including IgA and IgG through maternal–foetal circulation during last third trimester of pregnancy and breastfeed after birth. Meanwhile, neonates may develop specific protective immune responses by themselves after the exposure from the new environment (Hooper et al. 2012). However, LBW infants have not received as many antibodies passed to them from their mother due to less amount of time in the uterus and lack of chance to breastfeeding, and thus they are in a high risk of developing infection (Melville and Moss 2013).

The gastrointestinal tract harbours over 100 trillion microbial cells, which is ten times greater than the number of human cells (Qin et al. 2010). Therefore, current understanding suggests that the postnatal development of the innate and adaptive immune system depends on this highly dense microbial community (Tlaskalová-Hogenová et al. 2011). Gut microbiota makes the intestinal epithelial barrier stronger by inducing an increase of gut secretory IgA and the tightly connected intestinal epithelial cells (Sjögren YM et al. 2009). In addition, colonization with gut microbiota also increases epithelial cell proliferation, enhances intestinal epithelial integrity, and stimulates the development of gut-associated lymphoid tissues, Peyer's patches, and mesenteric lymph nodes (Bauer et al. 2006). By possessing immune-stimulating components in cell wall such as lipopolysaccharide and peptidoglycan, gut microbiota educates and stimulates the neonatal immune system. Since the intestinal mucosa is constantly exposed to commensal bacteria and pathogenic bacteria, it elicits different responses to different bacteria. While commensal or symbiotic bacteria suppress the inflammatory response and promote immunological tolerance, pathogenic bacteria trigger exaggerated immune activation that might lead to detrimental consequences such as inflammation or infectious diseases (Kamada et al. 2013). Perhaps even more importantly, gut microbiota determines the immune balance via modulating the differentiation of both anti-inflammatory T cell population such as $CD4+CD25+FOXP3+$ regulatory T(TReg) cell or pro-inflammatory T helper (Th) cells such as Th1, Th2, TH17 cells, depending on its population of cytokines and chemokines (Lee et al. 2011; Corrêa-Oliveira et al. 2016). Therefore, the gut microbiota stimulates and ensures appropriate responses of the neonatal immature immune system in its optimal composition.

Brain

The first few years of life are a critical period of time for the dramatic development of brain volume and function (Tau and Peterson 2010). There are impressive increases in the rate of myelination, synapse density, differentiation, and maturation of nerve cells to create and strengthen networks that support learning, memory and other cognitive abilities (Tau and Peterson 2010). The modulation of proteins involving synaptogenesis, myelination, and the maturation of excitatory synapses including synaptophysin and postsynaptic density protein 95 (PSD-95) by gut microbiota suggested that gut microbiota affected brain development in early life (Heijtz et al. 2011; Hoban et al. 2016). By age 3, the brain volume will double and reach about 80% of adult volume (Knickmeyer et al. 2008).

Interestingly, current microbiological knowledge suggests that the formation of gut microbiota begins rapidly after birth and reaches its maturity around 2 years of life (Koenig et al. 2011). This similarity that these early years are a critical window of opportunity for completing both neurology and digestive systems raises the intriguing possibility that there is a special connection between gut microbiota and brain development. Studies provided evidences that the premature children's brain has smaller cerebral volume, cortical grey matter, cortical white matter, and widespread microstructural abnormalities as compared with children born at term (Nosarti et al. 2014; Smyser et al. 2016). These changes in structure affect functional connectivity of neural networks, which is correlated with the increased incidence of neurodevelopmental disability including reduced social and cognitive skills later in life (Larroque et al. 2008; Nosarti et al. 2014; Bauml et al. 2015; Pierrat et al. 2017). Additionally, repetitive painful, stressful procedures in a vulnerable period during the NICU stay are associated with decreased early head growth in LBW infants (Vinall et al. 2012). Accumulating data demonstrated that the gut microbiota is associated with the development of the central nervous system via the gut microbiota-brain axis (Chen et al. 2013). Activities of gut microbiota possibly influence brain structure, function, and even behaviour through neural, endocrine, metabolism, and immune pathway (Sudo et al. 2004; Heijtz et al. 2011; Douglas-Escobar et al. 2013). Studies on GF animal have shown that the gut microbiota affects motor control, anxiety-like behaviour, and regulation of hypothalamic pituitary adrenal (HPA) axis (Dinan and Cryan 2012). Compared to specific pathogen-free mice, GF showed an increased motor activity and decreased anxiety by the combination of altered expression profiles of genes involved canonical signalling pathways, neurotransmitter turnover, and synaptic-related proteins (Heijtz et al. 2011). Postnatal microbial colonization in GF affects the postnatal development of the major neuroendocrine system and HPA system, in which a reduced stress response with the augmented levels of adrenocorticotropic hormone (ACTH) and cortisol was showed (Sudo et al. 2004). Importantly, this modulation can occur only when the gut microbiota was introduced early during postnatal development (Sudo et al. 2004). Many microbial-derived metabolites such as tyrosine, proline, arginine, tryptophan, and phenylalanine play a role in gut–brain signalling, which is suspected to contribute to diverse psychiatric and behavioural disorders including autism, schizophrenia, and depression (O'Mahony et al. 2015; Zheng et al. 2016). These studies strongly suggest that gut microbiota has significant impacts on the development of the infantile brain.

Taken together, the gut microbiota plays crucial roles in many parts of the host's physiology by expanding the neonatal metabolic capacity, improving the absorption of nutrients, contributing to the development of the brain, and greatly stimulating responses of the immune system. Currently, the associations between gut microbiota and the host such as bone formation and liver function have been elucidated (Sjögren et al. 2012; Yan et al. 2016). Therefore, the postnatal development of gut microbiota is important for achieving healthy growth.

Probiotic approach to support LBW growth: an inconclusive application

Considering the natural colonization of gut microbiota in healthy breastfed term infants, many studies showed that the initial gut microbiota community is dominated by beneficial bacteria (Jost et al. 2012). Based on these results, numerous clinical trials have been made in the hope of improving the growth of LBW infants with single or few numbers of probiotics such as *L. rhamnosus GG (LGG), L. reuteri*, *B. bifidum, B. lactis, B.longum, B. breve, LGG,* and *B. infantis, or L. acidophilus* and *B. infantis.* Although these probiotics have showed general positive effects on human health, their impacts on weight gaining or growth of neonates have been variable (Table 1). Since *LGG* has perhaps been the most studied probiotics in infants, a number of studies have analyzed the effect of administrating this probiotic on neonatal growth (Vendt et al. 2006; Scalabrin et al. 2009; Underwood et al. 2009; Chrzanowska-Liszewska et al. 2012). In 2006, Vent et al. reported the positive impact of *LGG* on neonatal growth in an intervention randomized controlled prospective trial (IRCT) (Vendt et al. 2006). In this study, infants who received *LGG* until 6 months of age displayed significantly increased weight gain and body length compared with the placebo (Vendt et al. 2006). In contrast, another IRCT with different results reported equivalent growth rate, body weight, length, and head circumference in the *LGG* and placebo group (Scalabrin et al. 2009). The outcomes of other intervention studies conducted in LBW infants have also shown that *LGG* had no effect on body weight gain (Underwood et al. 2009; Chrzanowska-Liszewska et al. 2012). ***L. reuteri* is another example of inconsistent effects of using probiotics on growth of LBW infants. While administration of *L. reuteri* compared with placebo reduced the amount of time to regain birth weight and had significantly higher body weight

Table 1. Effects of probiotics on neonatal growth in clinical studies.

Study design	Study participants	Probiotics used, dose	Duration of treatment	Host	Reported outcomes	Reference
Double-blinded placebo-controlled randomized prospective clinical trial	Probiotic group: $n = 51$ Placebo group: $n = 54$	*L. rhamnosus GG*, 10^7 cfu, once daily	Until 6 months of age	Term infants	Increased weight, body length No alteration in count of total *Lactobacilli, Enterococcus, Bifidobacterium, Clostridia*	Vendt et al. (2006)
Double-blinded, randomized, controlled, parallel, prospective study	Probiotic group : $n = 194$ Placebo: $n = 95$	*L. rhamnosus GG*, 10^8 cfu, once daily	Until 150 days of age	Term infants	No significant differences in growth rates, body weight, length and head circumference	Scalabrin et al. (2009)
Double-blinded randomized control trial	Probiotic group: $n = 21$ Placebo: $n = 26$	*L. rhamnosus*, 6×10^9 cells, once daily	42 days	LBW infants: <32 weeks	No effect on weight gain Not decrease the amount of pathogenic organisms	Chrzanowska-Liszewska et al. (2012)
Randomized, blinded, placebo-controlled trial	Probiotic group 1: $n = 30$ Probiotic group 2: $n = 31$ Placebo: $n = 29$	Group 1: *L. rhamnosus* GG, 5×10^8 Group 2: *L. acidophilus, B. longum, B. bifidum,* and *B. infantis* , 5×10^8 of each organism, twice daily	28 days	LBW infants: <35 weeks	No effect on weight gain	Underwood et al. (2009)
Randomized triple-blinded clinical trial	Probiotic group: $n = 30$ Placebo: $n = 30$	*L. reuteri DSM 17938*, minimum 2×10^7/kg, twice daily	Until reach full enteral feeding	LBW infants: 28–34 weeks	Reduce the time to reach full enteral feeding No effect on weight gain	Shadkam et al. (2015)
Randomized, double-blinded, clinical, and placebo-controlled trial	Probiotic group: $n = 30$ Placebo: $n = 30$	*L. reuteri DSM 17938,* 1×10^8 cfu, once daily	Until 30 days of life	LBW infants <37 weeks	Reduced time to regain birth weight Increased body weight at the end of the study	Indrio et al. (2017)
Cluster-randomized, double-blinded, placebo-controlled trial	Probiotic group: $n = 153$ Placebo group: $n = 130$	*B. bifidum*, 2.5×10^9 cells, twice daily	Until body weight reached 2 kg	VLBW infants (birthweight <1500 g)	No effect on body weight gain, head circumference	Totsu et al. (2014)
Randomized control trial	Probiotic group: $n = 18$ Placebo group: $n = 18$	*B. bifidum*, 2.5×10^9 viable cells, twice daily	Until body weight reached 2 kg	VLBW infants: had birthweights <1500 g	Increased body weight No differences in the population of *Bifidobacterium, Staphylococcus, Bacteroides* or total bacteria.	Yamasaki et al. (2012)
Double-blinded, randomized, placebo-controlled trial	Probiotic group: $n = 147$ Placebo group: $n = 52$	*B. lactis*, 10^9 cfu, or *B. longum* , 10^9 cfu, or *B. lactis* and *B. longum*, 10^9 cfu of each organism, once daily	4–6 weeks	LBW infants: (25–31 weeks Birth weight: 700 -1600 gram	No effect on body weight, length, or head circumference	Hays et al. (2016)
Double-blinded, placebo controlled randomized clinical study	Probiotic group: $n = 37$ Placebo: $n = 32$	*B. lactis Bb12*, 1.6×10^9 cells on day 1–3 and 4.8×10^9, once daily	21 days	LBW infants <37 weeks	Increased body weight gain	Mohan et al. (2008)
Randomised double-blinded placebo controlled trial	Probiotic group: $n = 77$ Placebo group: $n = 76$	*B. breve*, $1.5–3 \times 10^9$ cfu, once daily	Until the corrected age 37 weeks	LBW VLBW neonates	No effect on body weight	Patole et al. (2014)
Randomized control study	Probiotic group: $n = 108$ Placebo group: $n = 100$	*B. breve*, 1×10^9 cfu, twice daily	Until body weight reached 2300 g or conception age of 37 weeks	LBW infants (VLBW infants)	Body weight on the original expected date was significantly higher	Hikaru et al. (2010)
Prospective randomized clinical study	Probiotic group: $n = 45$ Placebo group: $n = 46$	*B. breve*, 10^9 cfu, once daily	28 days	VLBW infants	Increased body weight between 4 and 8 weeks of life	Kitajima et al. (1997)
Parallel, partially randomized, controlled trial	Probiotic group: n=41 Placebo group: n=39	*B. longum* sp. *Infantis*, minimum 1.8×10^{10}cfu, once daily	21 days	Term infants	No effect on body weight	Smilowitz et al. (2017)

(*continued*)

Table 1. Continued.

Study design	Study participants	Probiotics used, dose	Duration of treatment	Host	Reported outcomes	Reference
Randomized controlled double-blinded clinical study	Probiotic group: $n = 50$ Placebo group: $n = 51$	*L. rhamnosus* GG and *B. infantis*, 5×10^8 cfu of each organism, once daily	Until 34 weeks	LBW infants: birth weight $\leq$ 1000 g	No effect on improving growth (including daily weight gain and growth velocity)	Al-Hosni et al. (2012)
Observational, population-based study	Probiotic group: $n = 6229$ Placebo group: $n = 2305$	*L. acidophilus and B. infantis*, 10^9 cells of each organism, once daily	During primary stay in hospital	LBW infants: birth weight $<$1500 g and gestational $\leq$32 6/7 weeks	Increased growth: weight gain, body length, head circumference	Härtel et al. (2017)
Double-blinded, randomized controlled clinical trial	Probiotic group: $n = 45$ Placebo group: $n = 49$	*L. rhamnosus* GG and *B. longum* , 10^8 lyophilized cells of each probiotics, daily	Until discharge (probiotic group 60.7±28.8 d) Placebo group : 65.6 ± 30.0 d	LBW infants: $<$32 weeks, a birth weight $<$1500 g, a postnatal age $\leq$2 week	No effect on daily weight gain No alteration in the composition of *Staphylococcus, Enterococcus, Enterobacteria, Clostridium*	Rougé et al. (2009)

B: Bifidobacterium; L: Lactobacillus; VLBW: very low birth weight; cfu: colony forming unit.
The placebo group received no probiotic during duration of intervention.

in LBW population at the end of intervention in Indrio's study (Indrio et al. 2017), Shadkam et al. reported in recent IRCT that supplementation with *L. reuteri* has no effect on weight gain even though it reduced the time to reach full enteral feeding in LBW infants (Shadkam et al. 2015). For *Bifidobacteria*, the contrasting results among clinical studies were also reported in impact of *B. bifidum* (Yamasaki et al. 2012; Totsu et al. 2014), *B. lactis* (Mohan et al. 2008; Hays et al. 2016), or *B. breve* (Kitajima et al. 1997; Hikaru et al. 2010; Patole et al. 2014) on administration on weight and growth of LBW infants.

Obviously, the inconsistency and contradiction in clinical results of probiotic applications have made determining the role of gut microbiota on neonatal growth more complicated than ever. It had been clinically proven that there was a positive relationship between weight gain and the diversity of gut microbiota (Jacquot et al. 2011). However, in LBW infants, many clinical trials showed that probiotic supplementation by itself did not significantly alter the composition and number of total bacterial population of gut microbiota (Rougé et al. 2009; Yamasaki et al. 2012; Hays et al. 2016) even if the duration of supplementation lasted 6 months (Vendt et al. 2006). Especially, previous studies also reported that single or mixture of probiotic strains failed in inhibiting the growth of potential pathogens such as Enterobacteriaceae, *Clostridium*, *Enterococcus sp*. in the intestine (Rougé et al. 2009; Chrzanowska-Liszewska et al. 2012). Indeed, probiotic administration did not present an anti-inflammatory effect in clinical studies (Rougé et al. 2010; Hays et al. 2016). Perhaps, since LBW infants are predisposed to early gut dysbiosis, the interactive metabolic activities between probiotics with pre-existing intestinal bacteria might determine the influence of community on body weight gain in LBW infants. In this point, the concentration of SCFAs, which are known to express the efficiency of metabolic activity of the intestinal microbiota, is higher in term infants (Arboleya et al. 2012) whose gut microbiota composition is more diverse than in LBW infants (Koenig et al. 2011). Once again, the SCFAs content was not improved during the duration of intervention in the group receiving probiotic supplementation (Underwood et al. 2009).

The recent study of the Human Microbiome Project Consortium suggested that the microbial communities of individuals most certainly exhibit notable differences at the species levels (Vendt et al. 2006; Chrzanowska-Liszewska et al. 2012), but there is a core microbiota, which may cover major metabolic activities and are evenly distributed and prevalent across all healthy

individuals (Huttenhower et al. 2012). Thus, the possibility that only probiotics could not support to format the core microbiota in neonates might explain the inconsistent or contradictory results of probiotic interventions for neonatal growth. Therefore, the application of probiotics in an attempt to promote neonatal growth should be reconsidered.

Conclusions and future perspectives to establish an ideal gut microbiota for LBW infants

Optimal postnatal growth is obviously essential for LBW infants (Leppanen et al. 2014; Ong et al. 2015). Current studies are highlighting the importance of the postnatal development of the gut microbiota for achieving healthy growth of neonates (Subramanian et al. 2015; Yang et al. 2016; Tanaka and Nakayama 2017). Interpreting the efficacy of probiotic supplementation on neonatal growth has provided us a new direction to establish an ideal gut microbiota for LBW infants. Although gut microbiota is the complex community consisting of trillion microbes, the entire bacterial community only harbours between 1000 and 1150 prevalent bacterial species (Qin et al. 2010; Methé et al. 2012). Furthermore, a common set of microbial species composing of 40–60 bacteria exists in the gut microbiota of most individuals that accounts for more than 99% of gut microbiota and thus may cover most interactions and functions between the gut microbiota and the host (Qin et al. 2010). It will, therefore, be important to characterize the main representatives of the gut microbial community. The selection should also consider the immune-tolerance that determines the colonization ability of bacteria. Since these common bacteria, including commensal or symbiotic bacteria, have been proved of their roles in suppressing the inflammatory response and promoting immunological tolerance in the early colonization, the selected common bacteria will have a higher opportunity to colonize in gut (Hansen 2012). On the other hand, diversity is the key requirement for the influence of gut microbiota in a host (Lozupone et al. 2012). Indeed, Jacquot et al. (2011) demonstrated that an increased diversity of gut microbiota was associated with weight gain and digestive tolerance in LBW neonates. Therefore, an early establishment with many common bacteria instead of one or some probiotics may be more effective in LBW infants whose gut microbiota could not reach to necessary diversity after birth. In addition, these commensal pioneers may facilitate the establishment of metabolic networking and shape intestinal physiology and environment to protect the host against pathogens in neonatal gut (Houghteling and Walker 2015). Thus, early establishment of gut microbiota is the basis to the development of bacterial community and rapid growth of host. In fact, different bacteria show varying effect on weight because their genomes encode different proteins during metabolism (Drissi et al. 2014). Since body weight gain during early life is the most important indicator of healthy growth and development in LBW infants, we propose that the selected gut microbiota should be composed of common intestinal bacteria with weight gain effect.

Since the foetal gut is considered sterile, the GF animal is the ideal model for assisting the selection of representatives with a high potential contribution to the neonatal growth. To prevent the interaction with pre-existing intestinal bacteria that may perturb the actual effects of a bacterium on the host, the newborn GF mice should be colonized immediately right after birth with single bacterial species. A GF model of a premature neonate will also be required to determine whether selected bacteria can grow in a more aerobic neonate gut.

Since no comprehensive solution can manage all problems of LBW infants, considering every aspect and chance in all methods is a prerequisite to design an effective strategy. The combination of the advances in technology supports in respiratory, cardiovascular functions, appropriate nutritional interventions, professional caring, and, finally, healthy gut microbiota in early life is necessary to achieve better prognosis for LBW infants. Defining an ideal gut microbiota to boost LBW infant growth should be the prime objective in future pharmabiotics.

Disclosure statement

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