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Review

Development of the gut microbiota and dysbiosis in children

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The gut microbiota resides in the human gastrointestinal tract, where it plays an important role in maintaining host health. Recent advancements in next-generation sequencing methods have revealed the link between dysbiosis (imbalance of the normal gut microbiota) and several diseases, as this imbalance can disrupt the symbiotic relationship between the host and associated microbes. Establishment of the gut microbiota starts *in utero* or just after birth, and its composition dramatically changes to an adult-like composition by 3 years of age. Because dysbiosis during childhood may persist through adulthood, it is crucial to acquire a balanced gut microbiota in childhood. Therefore, current studies have focused on the factors affecting the infant gut microbiota. This review discusses recent findings, including those from our studies, on how various factors, including the delivery mode, feeding type, and administration of drugs, including antibiotics, can influence the infant gut microbiota. Here, we also address future approaches for the prevention and restoration of dysbiosis in children.

Key words: gut microbiota, dysbiosis, 16S rRNA gene sequencing, mode of delivery, feeding type, antibiotics

INTRODUCTION

The adult human harbors 100 trillion gut bacteria, comprising more than 1,000 different species, and approximately 160 species per fecal sample, outnumbering the somatic cells by a ratio of 1.3:1 [1]. While commensal bacteria reside on the human skin and in the colon, oral cavity, male and female genital tracts, and respiratory system, most of them inhabit the colon [2]. Advancements in genome sequencing have enabled us to understand the microbial composition and functions of the gut microbiota. As the understanding of the relationship between the gut microbiota and several human health problems has deepened, it has been revealed that the balance of the gut microbiota in early life plays an important role in human health, and its imbalance, called dysbiosis, is associated with the development of diverse diseases.

In this review, we describe the basic concepts of gut microbiota development during the infantile period, the factors affecting the gut microbiota composition, and some interventions for maintaining a balanced gut microbiota or restoring dysbiosis, based on the latest studies, including our own data.

DEVELOPMENT OF THE GUT MICROBIOTA AND DISEASES

In 2016, Odamaki *et al.* reported that the gut microbiota composition changes with age [3]. By analyzing stool samples from 367 healthy Japanese, aged between 0–104 years, and using 16S rRNA sequencing, they found that the microbial composition was stable during adulthood. Firmicutes, including Lactobacillales and Clostridiales, was the most predominant phylum in the adult gut microbiota, whereas Actinobacteria, including Bifidobacteriales, was more abundant in samples from one-year-old individuals. The relative abundance of Actinobacteria in children decreased after weaning, and the gut microbiota developed closer to an adult-like gut microbiota by the age of three.

Recent studies have found microbial DNA in the placenta [4], amniotic fluid [5], and meconium of neonates born through cesarean section [6], thus altering the widely accepted notion that the intrauterine environment is sterile. Furthermore, the maternal gut microbiota may determine the transcriptional profile of the fetal intestinal microbiota [7]. However, the latest evidence suggests that the detected bacteria are not viable for establishing the fetal gut microbiota [8–10].

Immediately after birth, establishment of the infant gut microbiota starts through exposure to microbes from the maternal birth canal, maternal skin biota, and the environment and it subsequently develops into an adult-like gut microbiota.

Dysbiosis, or imbalance of the gut microbiota, is associated with a wide range of health problems. Dysbiosis is associated with increased risk of gastrointestinal diseases, such as inflammatory

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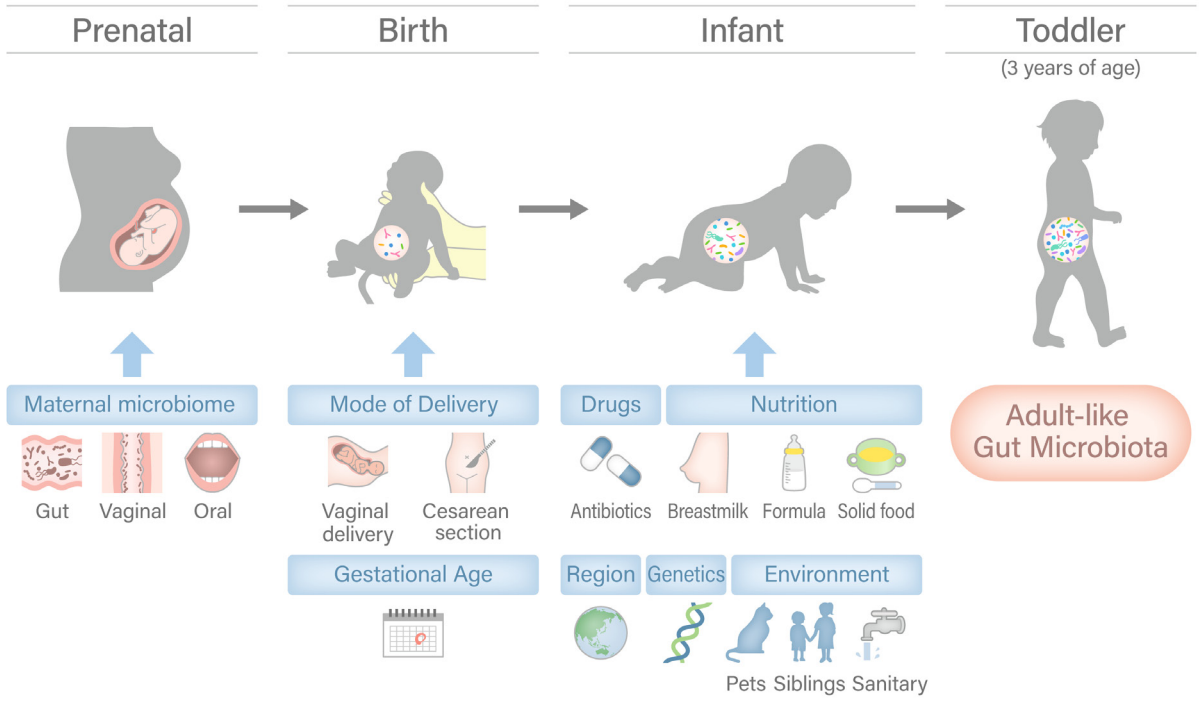

Fig. 1. Factors affecting the gut microbiota in children.

Various factors affect the development of the gut microbiota, leading to the establishment of an adult-like microbiota at approximately 3 years of age. Maternal microbiota potentially influence the fetal gut microbiota and immunity. The mode of delivery influences the initial acquisition of the gut microbiota. Breast milk has a high abundance of Bifidobacteriales and human milk oligosaccharides.

intestinal disorder [11, 12], irritable bowel syndrome [13], and necrotizing enterocolitis [14]; allergic diseases [15, 16]; diabetes [17]; obesity [18, 19]; cardiovascular disease [20]; autism spectrum disorder [21]; and sudden infant death syndrome [22]. We propose that dysbiosis may also be present in children with idiopathic nephrotic syndrome [23–25] and Kawasaki disease [26].

FACTORS AFFECTING THE GUT MICROBIOTA IN CHILDREN

As mentioned above, because gut microbiota changes drastically toward an adult-like composition during the first three years of life [27] and dysbiosis that develops during the early stages of life may remain until adulthood [3, 28], it is important to establish a favorable gut microbiota during infancy [29]. There are several factors that affect the gut microbiota of newborns and infants, such as maternal microbiota from the vagina and intestine [30, 31], the mode of delivery [32–35], feeding type [32–34], use of antibiotics [34, 36] and other drugs [37, 38], gestational age [39, 40], siblings and pets [41], and regional differences, including diet and sanitary conditions [42] (Fig. 1). Here, we discuss current evidence regarding the effects of these factors on the gut microbiota among children, primarily focusing on the mode of delivery, feeding type, and antibiotics.

Mode of delivery and feeding type

Vaginally delivered newborns acquire Bifidobacteriales and Bacteroidales from the maternal birth canal or intestinal tract, whereas neonates born through cesarean section delivery (CD) acquire bacteria from the maternal skin, mouth, and the hospital environment, causing dysbiosis compared with newborns born by vaginal delivery (VD) [43]. Bifidobacteriales are also present in breast milk. Additionally, human milk contains oligosaccharides (human milk oligosaccharides; HMOs) that reach the colon without being digested and are therefore known as prebiotics, which are defined as non-digestible dietary substances benefiting the host by promoting the growth of beneficial intestinal microbes. More than 200 HMOs have been reported to date, and they account for one-third of the solid components in breast milk, following lactose and lipids in terms of abundance of solid components in breast milk. Colostrum contains as much as 20 g/L of HMOs [44]. Although formulas include galacto-oligosaccharides and fructo-oligosaccharides, their HMO compositions differ from that of breast milk.

We conducted a study to determine whether Japanese neonates born by CD show dysbiosis and whether dysbiosis can be corrected by breast milk [35]. The subjects were 36 healthy full-term neonates with normal birth weights who were born to Japanese parents in the same hospital in Osaka, Japan, between September 2015 and August 2016. Stool samples were collected at day 4 after birth and at a 1-month checkup. DNA hypervariable regions were amplified, and 16S rRNA gene sequencing was performed. To assess the effect of delivery mode (VD or CD) on the gut microbiota, stool samples collected at 4 days of age were compared between neonates born by VD and CD. To compare the effect of feeding type (breastfed [BF] or formula-fed [FF]) on the gut microbiota, stool samples collected at the 1-month checkup were also analyzed by dividing the subjects into four groups as follows: 10 infants vaginally delivered and breastfed for 1 month

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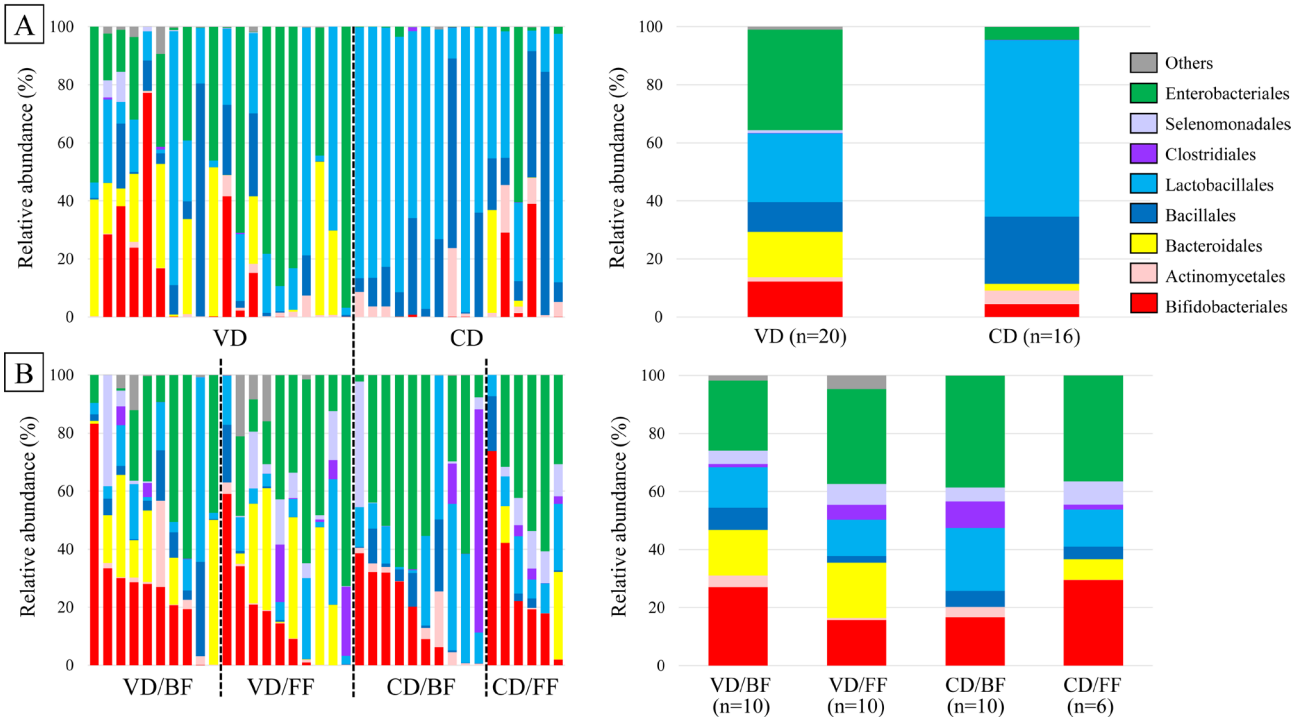

Fig. 2. Composition at the order level of the gut microbiota in 36 healthy infants aged 4 days (A) and 1 month (B).

A: The relative abundances of Bacteroidales and Enterobacteriales were significantly higher in the VD group compared with the CD group, whereas those of Bacillales and Lactobacillales were significantly higher in the CD group in 4-day-old infants compared with the VD group. B: Only Bacteroidales showed a significant difference among the four groups in 1-month-old infants. VD: vaginal delivery; CD: cesarean section delivery; BF: breastfeeding; FF: formula feeding. Adapted from "Effect of Delivery Mode and Nutrition on Gut Microbiota in Neonates", by Akagawa *et al.*, 2019. Annals of Nutrition and Metabolism, 74: 132−149. Modified with permission from S. Karger AG, Basel. Original figure Copyright© 2020 by S. Karger AG, Basel.

(Group VD/BF), 10 infants born by CD and breastfed for 1 month (Group CD/BF), 10 infants vaginally delivered and formula fed for 1 month (Group VD/FF), and 6 infants born by cesarean section and formula fed for 1 month (Group CD/FF). There were no significant differences among the four groups in terms of sex or gestational age. Feeding types were classified in conformity with the Japan Pediatric Society's recommendations, with BF classified as more than 80% of total feeding being breastfeeding until the 1-month checkup and FF classified as more than 80% of total feeding being formula feeding. According to these recommendations, all of the formula-fed neonates belonging to Group VD/FF and Group CD/FF in our study were defined as "partially" breastfed.

At 4 days of age, the relative abundances of the Lactobacillales and Bacillales orders were lower, whereas those of Enterobacteriales and Bacteroidales were higher in the gut microbiota of VD infants (n=20) compared with CD infants (n=16; Fig. 2A). The median Shannon index was significantly higher for VD compared with CD (2.20 vs 1.79, p=0.04). However, at 1 month of age, there was no significant difference in the median Shannon index among the groups (2.0 in group VD/BF, 2.4 in group VD/FF, 2.3 in group CD/BF, and 2.1 in group CD/FF). Regarding the microbial composition, vaginally delivered infants showed a higher abundance of Bacteroidales, regardless of feeding type (p=0.0033, Fig. 2B).

Our results clearly demonstrated that at 4 days of age, differences associated with the mode of delivery included a reduced abundances of Lactobacillales and Bacillales and reduced diversity in CD neonates compared with VD neonates, resulting in dysbiosis (Fig. 2A). However, at 1 month of age, there was little difference associated with the mode of delivery or feeding type (Fig. 2B). The similarity of gut microbiota among the four groups could be explained by the fact that all subjects were at least partially breastfed, including infants grouped as formula fed (Group VD/FF and Group CD/FF), which suggests that even a small consumption of breast milk might restore a balanced gut microbiota in cases of dysbiosis caused by CD [35].

In 2019, a larger study by Shao *et al.* presented results similar to our study [45]. They performed a whole-genome shotgun metagenomic analysis on 596 stool samples from full-term babies born in UK hospitals. At 4 days of age, the mode of delivery affected the gut microbiota significantly; VD neonates showed higher abundances of Bifidobacteriales and Bacteroidales, whereas CD neonates showed higher abundances of Lactobacillales and Bacillales. However, this difference between VD and CD neonates diminished over time towards 7 and 21 days of age, showing a microbial composition similar to that in infancy (8.75 ± 1.98 months) [45]. Their findings suggest that the mode of delivery and/or feeding type during the neonatal period might have a greater effect on the gut microbiota than ethnic differences.

Antibiotics

It is well known that antibiotics affect both pathogenic and

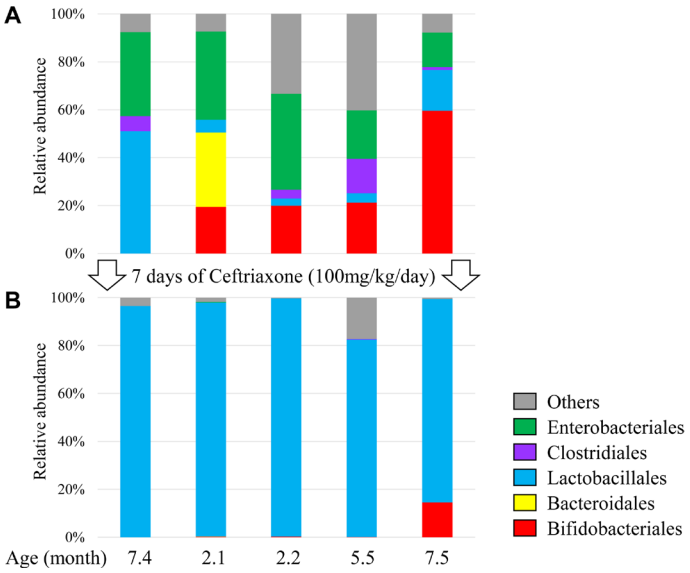

Fig. 3. Changes in gut microbiota profiles before (A) and after (B) antibiotic treatment.

Seven days of intravenous ceftriaxone treatment to treat upper urinary tract infections in five infants altered the gut microbiota: the most abundant commensal bacteria order was changed to Lactobacillales, and microbial diversity as assessed by the Shannon index decreased significantly.

commensal gut bacteria, resulting in dysbiosis. However, little is known about how antibiotics affect the infant gut microbiota. We analyzed the gut microbiota of five infants (2 boys, median months of age of 5.5 [2.1–7.4]) who were diagnosed and treated for upper urinary tract infection. Stool samples were collected before and after treatment with intravenous ceftriaxone and subjected to perform 16S rRNA gene sequencing. After 7 days of antibiotic treatment, we found a significant predominance of the Lactobacillales order and decreased overall microbial diversity (Fig. 3), as shown by a Shannon index of 2.53–3.25 (mean, 3.06) before treatment compared with a Shannon index of 0.12–1.96 (mean, 1.12) after treatment (p=0.009).

Even though ceftriaxone has broad-spectrum activity, it does not affect *Enterococcus faecium* and *Enterococcus avium* [46], the most abundant enterococcal species belonging to the Lactobacillales order, thus potentially accounting for the significant dominance of Lactobacillales in the gut microbiota after antibiotic treatment. We found that significant dysbiosis may be induced even through short-term administration of an antibiotic.

Dethlefsen *et al.* studied the dysbiosis caused by antibiotics in three adults after ciprofloxacin treatment and reported that gut microbial diversity began to be restored 1 week after the end of treatment and subsequently resembled the pretreatment microbial profile by the fourth week [47, 48]. However, studies in children (108 newborns) conducted by Martin *et al.* using qPCR revealed that the use of antibiotics after 3 months of age correlated with a significant decrease in *Staphylococcus* and *Bifidobacterium* at 6 months of age [49]. Furthermore, by following 39 children from birth to 3 years of age, Yassour *et al.* found that 20 children treated with antibiotics during the study period presented significant dysbiosis, with decreased microbial diversity, compared with 19 controls [50]. These studies suggest that dysbiosis caused by antibiotics may continue for a longer period in children compared with adults. Further investigation is required on this subject.

Non-antibiotic drugs

Recent studies reported that non-antibiotic drugs targeting human cells, but not microbes, are associated with changes in gut microbial composition. Hakim *et al.* analyzed fecal samples collected from children undergoing chemotherapy for acute lymphoblastic leukemia. Following chemotherapy, microbial diversity decreased significantly, and the relative abundances of certain bacterial taxa were altered [37]. Furthermore, the effect of proton-pump inhibitors (PPIs) on the pediatric gut microbiota was assessed among 12 infants with gastroesophageal reflux disease. Treatment with PPIs did not affect microbial diversity; however, it decreased the relative abundance of *Lactobacillus* and *Stenotrophomonas* and increased the relative abundance of *Haemophilus* [38]. Although the effects of other non-antibiotic drugs on the gut microbiota are still unknown, *in vitro* data indicate that up to 24% of 1,000 marketed drugs inhibit the growth of at least one strain [51].

Gestational age

Gestational age at birth is another important factor affecting the gut microbiota. Preterm infants delivered between 22 weeks and 36 weeks of gestation have immature gut barrier function and immunity, thus increasing the risk of sepsis and necrotizing enterocolitis. Korpela *et al.* recently analyzed 262 fecal samples from 45 preterm infants to visualize the properties of the gut microbiota and its development in preterm infants. The preterm infant gut microbiota displayed decreased microbial diversity with certain predominant genera (*Bifidobacterium, Enterobacter, Staphylococcus*, or *Enterococcus*), with the predominant genera changing within a few days. The microbial composition was altered from *Staphylococcus-Enterococcus*-dominated gut microbiota towards *Bifidobacterium*-dominated microbiota, and this was associated with postnatal age [40].

Environment

Several environmental factors also affect the infant gut microbiota. On analyzing stool samples of 24 four-month-old healthy infants, it was found that the microbial diversity increased in infants living with pets and decreased in infants with siblings. Furthermore, the bacterial composition was altered, with an increase in the relative abundance of Peptostreptococcaceae and a reduction in that of Bifidobacteriaceae, among infants living

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with pets, while it was demonstrated that there was a reduction in the relative abundance of Peptostreptococcaceae among infants living with siblings [41]. Interactions with animals have protective effects on preclinical type I diabetes [52], allergies, and asthma [53]. Rural differences may also influence the gut microbiota. De Fillippo *et al.* compared the gut microbiota of 29 children from Europe and rural Africa and reported a significant enrichment in Bacteroidetes and depletion in Firmicutes among African children. In addition, fecal short-chain fatty acid levels were found to be higher in African children, probably owing to differences in diet and sanitation [42].

INTERVENTIONS FOR DYSBIOSIS IN CHILDREN

As the knowledge concerning the relationship between the gut microbiota and health problems has deepened, research has also focused on the prevention and restoration of dysbiosis, by improving the gut microbiota.

Probiotics, prebiotics, synbiotics, and biogenics

Probiotics are defined as "microorganisms that promote a balanced gut microbiota", and they can be found in yogurt, cheese, fermented foods, and dietary supplements. Prebiotics are defined as non-digestible dietary substances that benefit the host by promoting the growth of beneficial intestinal microbes. Prebiotics include oligosaccharides, dietary fiber, and other non-digestible carbohydrates [54]. The concept of synbiotics was introduced by Gibson and Roberfroid, who described them as combinations of prebiotics and probiotics synergistically promoting gastrointestinal health by improving the survival and adherence of live microbial dietary supplements in the gastrointestinal tract [55]. On the other hand, biogenics are defined as substances that benefit the host by directly or indirectly modulating the gut microbiota, resulting in the improvement of some biological functions and biophylaxis, the prevention of diseases, the promotion of recovery, or anti-aging effects. Among the biogenics are vitamins, eicosapentaenoic acid, docosahexaenoic acid, flavonoids, and bacteriocins [56]. Although probiotics, prebiotics, synbiotics, and biogenics are used extensively, there is no conclusive evidence to confirm their suggested benefits on human health [57].

Fecal microbiota transplantation (FMT)

FMT is intended to restore a patient's gut microbiota to a healthy state through a transfer of feces from a healthy donor. The effectiveness of FMT has become largely evident among adults with recurrent *Clostridioides* (formerly *Clostridium*) *difficile* infections (CDI) [58], ulcerative colitis [59], and treatment-resistant functional dyspepsia [60]. Although the data regarding FMT among children are still limited and preliminary, FMT could be helpful in establishing a treatment alternative for recurrent CDI. A recent multi-center retrospective cohort study in the United States reported that FMT was successful in 272 of 336 (81%) children with CDI [61].

Vaginal microbial transfer to infants born by cesarean section

CD-born infants acquire microbiota from maternal skin because they are not exposed to beneficial maternal vaginal microbiota [43]. A novel method of exposing CD-born infants to maternal vaginal microbiota consists of wiping the infants with gauze previously incubated in the maternal vagina before birth. The gut microbiota of CD-born infants treated with this procedure showed microbiota similar to those of VD infants at 1 month of age [62].

CONCLUDING REMARKS

Due to recent advances in the detection of intestinal commensal bacteria using culture-independent techniques, the relationships between the gut microbiota and many diseases are being elucidated. Furthermore, studies on how to prevent dysbiosis and restore a balanced gut microbiota are being conducted, even in children [63–65].

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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