Breastfeeding, Breast Milk Composition, and Growth Outcomes

Mads Vendelbo Lind · Anni Larnkjær · Christian Mølgaard · Kim F. Michaelsen

Department of Nutrition, Exercise, and Sports, University of Copenhagen, Frederiksberg, Denmark

Abstract
Breastfed infants have a growth pattern that is different from formula-fed infants, which is regarded as the optimal growth pattern. Breastfed infants increase more in weight, length, and BMI during the first 2–3 months of life and then have a slower growth velocity up to 12 months. They also have a higher accumulation of fat during early infancy. Breastfed infants have lower levels of circulating IGF-I and insulin, which could be part of the explanation of their growth pattern. Many studies and meta-analyses have examined the association between breastfeeding and later obesity. Most find a moderate reduction in the risk of later obesity, but it has been argued that this could be biased due to residual confounding and reverse causation. From studies in low- and middle-income countries randomizing women to breastfeeding promotion, there was only little effect on early growth. Recent studies have found associations between breast milk composition (total fat, protein, human milk oligosaccharides, adiponectin, leptin, and insulin) and growth. However, the studies are few, and the results are inconsistent. More studies, including studies of maternal factors influencing breast milk composition, are needed to better understand how breastfeeding influences current and later growth and thereby short- and long-term health.

Introduction

During infancy, breastfed infants have a growth pattern which is different from formula-fed infants. This is regarded as the optimal growth pattern. Emerging evidence also indicates that the pattern of lean and fat mass (FM) accretion differs between breastfed and formula-fed infants. Many studies have examined the
effect of breastfeeding on growth patterns later in childhood and up to adulthood, including linear growth and risk of later obesity. Although some studies have shown beneficial effects of breastfeeding, other studies could not show any effects. Uncontrolled confounding and reverse causation could cause some of the associations found as the majority of studies are not randomized. Although there seems to be a special growth pattern in breastfed infants, there are also large differences in growth within those being breastfed. The composition of breast milk, including nutrients, appetite, and growth-related hormones, as well as other bioactive components, have also been associated with short- and long-term growth patterns, suggesting causative effects.

The aim of this short narrative review is to discuss the issues mentioned above and highlight some of the recent studies and reviews covering these topics.

**Methodological Issues: Randomization, Reverse Causality, and Residual Confounding**

It is difficult to explore how breastfeeding is influencing growth as both observational and randomized controlled trials (RCTs) within the field have methodological challenges. It is unethical to randomize infants to breastfeeding or no breastfeeding. Thus, RCTs, randomizing mothers to a breastfeeding promotion intervention, is the preferable study design when trying to explore how breastfeeding is influencing growth. A systematic review/meta-analysis including such studies has been published [1], but several considerations regarding this type of RCT need to be taken into account. Noncompliance with the allocated intervention may become a problem, and large overlaps in the duration and the degree of breastfeeding are highly likely. This might limit the ability to find potential differences between groups as the effective difference between groups becomes small. Further, if the analysis is based on the intention to treat, the research question changes to the effect of the breastfeeding promotion intervention and not about the effect of breastfeeding per se [2]. Furthermore, infant feeding and growth are dynamic processes, each affecting each other [2].

Some researchers have demonstrated reverse causality by investigating the influence of prior growth on subsequent feeding choices [2–4]. Rapid weight gain in UK infants from birth to 3 months of age predicted earlier age of weaning [4]. In PROBIT (Promotion of Breastfeeding Intervention Trial), a study from Belarus, different results were found [5]. A high weight-for-age z-score at 1 month of age was associated with lower numbers of weaning and stopping exclusive breastfeeding by 2 months of age. In line with this, Eriksen et al. [3] re-
ported that a higher mean weight-for-length z-score at 3 months of age predicted longer duration of exclusive breastfeeding in a rural Gambian setting. This could potentially mean that infant size impacts breastfeeding practices in these settings and not the other way around.

Another problem in studies of the effect of breastfeeding on growth is residual confounding. Factors associated with breastfeeding like maternal obesity, education, socioeconomic position, and smoking are also likely to be associated with growth, and it is not always that all such factors are controlled for in large observational studies.

Thus, exploring the associations between breastfeeding and growth and the potential mechanisms behind such an association is challenging.

**Breastfeeding and Growth during Infancy**

In order to study the link between breastfeeding and growth, growth reference data are important. The old WHO growth reference was based on data from Ohio collected from 1929 to 1975. In that study, few children were breastfed, which was the reason why Dewey et al. [6] analyzed the growth pattern of infants from 7 cohorts who were exclusively breastfed for at least 4 months and partially breastfed up to at least 12 months, and compared them to the old WHO reference. The breastfed infants grew more rapidly during the first 2 months and then slower up to 12 months. At 12 months, they had lower values of weight, length, and weight for length compared to the old reference. Because of these marked differences, the WHO decided to develop the WHO growth standards based on data from 6 centers including high-, middle-, and low-income countries. Only mothers with high socioeconomic status and only infants following the WHO recommendation on breastfeeding practices were included [7]. Between the 6 centers, length was almost identical during the first 5 years, supporting that children have the same growth potential if they are given optimal conditions [8]. Despite some potential limitations, the WHO standards have been promoted widely [9–11]. In 2011, the WHO growth standards were adopted in 125 countries and were considered to be used in 25 countries [12].

Several cohort studies have confirmed that breastfed infants have higher growth velocity during the first few months followed by a slower growth period compared to formula-fed infants. In PROBIT, the largest difference in growth was between 3 and 6 months [13], and in the Dutch GECKO (Groningen Expert Center for Kids with Obesity) cohort, the difference persisted up to 6 months [14]. In the ALSPAC (Avon Longitudinal Study of Parents and Children) cohort
from the UK, the slower growth in breastfed infants was significant up to the age of 31 months [15].

Fewer high-quality studies investigating breastfeeding and infant growth have been conducted in resource-poor areas, where poor growth is the nutritional problem rather than overweight and obesity. A recent meta-analysis of studies on breastfeeding counseling included 11 studies from middle-income countries [1]. The effects were modest but positive for weight (+0.11 z-score, borderline significant) and for length (+0.07 z-score). However, in the majority of these studies, growth was measured only up to 6 months. A few studies have also investigated the effect of exclusive breastfeeding until 6 months on infant growth in resource-poor settings where no infant formula was given. Kramer and Kakuma [16] reanalyzed growth data from 3 studies (2 randomized trials from Honduras and 1 observational study from Senegal) and found higher mean z-scores (weight-for-age, weight-for-length, and length-for-age z-scores) in infants at 6 months of age who had been exclusively breastfed to 6 months compared with infants exclusively breastfed to 4 months (with continued breastfeeding and complementary foods); however, these differences were not significant. The same conclusion was reached in a recent observational study in rural Gambia, where exclusive breastfeeding to 6 months had limited influence on infant growth [3].

The evidence that breastfeeding reduces the incidence and severity of acute infections, especially diarrhea and lower respiratory tract infections, is strong and convincing [17]. Such infections are likely to have a negative effect on growth, especially in resource-poor settings. It is therefore surprising that there were only modest effects of breastfeeding promotion interventions on growth in low- and middle-income countries in a meta-analysis of breastfeeding intervention studies [1]. However, it should be kept in mind that the meta-analysis assessed the impact of the interventions and not the effects of breastfeeding, as also pointed out by the authors [1]. Promotion of breastfeeding should remain a high priority because of the many benefits to both the infant and the mother [17], and because it is likely to have also a positive effect on growth in resource-poor settings.

**Breastfeeding and Growth-Related Hormones in the Infant**

Hormones involved in growth during infancy are influenced by the infant feeding mode and may provide a mechanistic link behind the different growth patterns of breastfed and formula-fed infants. According to the “early protein hypothesis,” the higher protein content in infant formula compared to breast milk stimulates the production of IGF-I and insulin which promotes growth [18]. In accordance with this hypothesis, several studies have reported higher
levels of insulin and IGF-I in formula-fed infants in early infancy compared to breastfed infants [19–21]. Also, in later infancy (around 9 months of age), which is well into the complementary feeding period, infants who were still breastfed showed lower levels of IGF-I, IGFBP-3 [19, 22], and insulin [23] than infants no longer breastfed. In the Danish SKOT I cohort, both IGF-I and insulin concentrations showed a reverse dose-response relationship with the numbers of daily breastfeedings indicating that partial breastfeeding may also have a modulating effect on growth-related hormones and hence growth [22, 23].

Appetite-related hormones, which play a central role in the regulation of food intake and body composition, may also have an effect on growth. These hormones are likely to be affected by breastfeeding, and an example is the hormone leptin, which has been suggested to play a role in the regulation of pre- and postnatal growth [24]. However, studies have reported conflicting results regarding leptin levels in breastfed infants compared to formula-fed infants. In newborns up to 5 days of age and at 3–4 months of age, leptin levels were lower in breastfed than formula-fed infants [20, 25]. Contrary to this, Savino et al. [21] found lower levels of leptin in formula-fed infants at 3–4 months of age, and a study by Gruszfeld et al. [26] detected no difference in leptin levels between breastfed and formula-fed infants at 6 months of age. Breastfeeding also seems to reduce the levels of ghrelin compared to formula feeding [20, 21].

Breastfeeding, Body Composition, and Early BMI Peak

Compared to formula feeding, the effects of breastfeeding on body composition in infancy have been evaluated in a systematic review and meta-analysis by Gale et al. [27], identifying 15 studies for the systematic review and 11 studies for the meta-analysis. One of the challenges was the use of different methods for assessing body composition, but subgroup analyses, including only studies using the same techniques, showed comparable results. The overall conclusion was that formula-fed infants have a higher fat-free mass during the 1st year of life. The comparison of FM and FM percentage was, however, more complicated. FM and FM percentage were higher in breastfed infants aged 3–6 months, while the opposite was seen at 12 months of age with a higher FM in formula-fed infants. There were no gender differences in the effects. These results support the suggestion that breastfed infants accumulate fat during the first months of life. In a study from the Danish SKOT cohort, Jensen et al. [28] analyzed the relationship between infant peak BMI and duration of breastfeeding. They found that longer duration of exclusive breastfeeding was associated with an earlier peak in infant BMI which also indicates a higher fat accumulation early in infancy in breastfed compared to formula-fed infants.
Overweight and Extreme Weight Gain during Breastfeeding

Despite the fact that breastfed infants, on average, have a slower gain in weight and length, and weight-for-length z-scores after the first 2–3 months than formula-fed infants, as described above, some infants experience a very high weight gain during exclusive breastfeeding. There is a tendency among health personnel not to worry about such a large weight gain during the exclusive breastfeeding period as it is believed that these infants will normalize their weight once complementary feeding starts along with more active movements. However, this is not evidence based. Two cases with very high weight gain in infancy have been published [29, 30]. Both infants reached a weight-for-age SD score above +4 during exclusive breastfeeding and decreased thereafter. However, they were only followed up until 12 and 19 months of age. In both cases, there was no clear explanation for the high weight gain, but it was suggested that a high protein content in the breast milk could be part of the explanation. In one of the cases, breast milk adiponectin was higher than previously reported, which could also play a role [30]. In a paper based on a Dutch cohort, it was concluded that exclusively breastfed overweight infants are at the same risk of overweight at age 5–6 years as formula-fed overweight infants [31]. However, in this study, infant overweight was defined as a BMI only above +1 SD (using the WHO growth standards) at the age of 6 months. The authors concluded that prevention of overweight should also include exclusively breastfed infants, without specifying how it should be done. As there is increasing evidence that a high weight gain during infancy is associated with an increased risk of later obesity [32], it is important to investigate the mechanisms behind and the consequences of an extreme weight gain during exclusive breastfeeding.

Effects of Breastfeeding on Growth Later in Childhood

Breastfeeding and Stature Later in Life

Some studies have suggested that breastfeeding has a programming effect on the IGF axis and thereby could have a positive effect on later linear growth. In the ALSPAC cohort, IGF-I was measured at the age of 7–8 years, and it was found that those who had been breastfed had higher levels than those who had never been breastfed [33]. This is interesting, as breastfed infants have generally been found to have lower IGF-I levels in infancy, as described above. This programming pattern has been supported by data from a Danish cohort. IGF-I values at 9 months were negatively associated with IGF-I values at 17 years, suggesting a programming of the IGF-I axis [34]. In line with this, the Boyd-Orr cohort reported that boys who had been breastfed were 2.5 cm taller as
adults than those not breastfed [35]. However, the cohort was born around 1920–1930, when alternatives to breast milk were far from optimal. The hypothesis that breastfeeding is associated with increased height later in life, despite slower linear growth during breastfeeding, was not supported by data from PROBIT, the RCT from Belarus [36]. At the age of 11.5 years, there were no effects of the breastfeeding intervention on height and IGF-I values [37].

Breastfeeding and Later Overweight and Obesity

In the *Lancet* series on breastfeeding from 2016 [17], it was concluded, based on the review by Horta et al. [38], that there was suggestive evidence for a protective effect of breastfeeding against later overweight and obesity. Of the 113 studies included, the effect in the 23 high-quality studies was a risk reduction of 13%. The authors also concluded that it was difficult to exclude residual confounding by socioeconomic group, but as the effect size was the same in studies from low- and middle-income countries, where the association between obesity and socioeconomic group is often opposite to what is seen in high-income countries, a true effect was supported. In studies from high-income countries, the risk reduction was 17%, and it was 14% in middle-/low-income countries. Another meta-analysis including 25 studies from 12 countries, not including any studies from low-income countries, found an obesity risk reduction of 22% [39]. Based on 17 of the studies, the authors showed a dose-response effect of breastfeeding with a risk reduction of 10% for those breastfed less than 3 months and of 21% if breastfed for 7 months or longer.

In the PROBIT intervention, which had a cluster randomized design, there was no effect of the breastfeeding intervention on BMI, waist circumference, waist:hip ratio, or skinfold thickness when the children were followed up at 6.5 years of age [36]. At the 11.5-year follow-up, the authors further concluded that the intervention had no effect on the prevention of overweight or obesity, despite a slight increase in overweight in the intervention group, which the authors found likely to be a chance finding [37]. Kramer et al. [40] argued that although many of the published studies on breastfeeding and obesity have controlled for relevant confounding, there is still residual confounding. Furthermore, another explanation for the dose-response effect observed in some studies can be that slower-growing infants are satisfied by exclusive breastfeeding while those growing rapidly may crave additional formula or food earlier. An analysis of data from the ALSPAC cohort in the UK and the Pelotas cohort from Brazil also concluded that the effects of breastfeeding on the risk of obesity most likely reflect residual confounding [41].

Considering that studies from low- and middle-income countries, where breastfeeding is typically more frequent among the lower social classes, also
show a protective effect of breastfeeding, it seems plausible that breastfeeding has an overall modest protective effect against obesity, especially in obesogenic societies with longer duration of breastfeeding [17].

Breast Milk Composition and Growth

Many bioactive components in breast milk, such as hormones and some nutrients, are considered to play multiple roles in infant growth. They support digestive functions, gut development, immune responses, and the development of the immune system, and influence the regulation of appetite and energy homeostasis. To have an impact on the infant’s physiology, these components have to be bioavailable, and some need to enter the infant’s circulation. Thus, these compounds need to bypass the infant’s gastrointestinal system intact, and several factors, such as protease inhibitors in breast milk, high gut pH, and immature pancreatic enzymatic activity, have been suggested to make this plausible [42, 43]. In addition to nutrients, cytokines, and hormones, other components of breast milk, such as microRNAs and microbes, are increasingly receiving attention as potential links between breastfeeding and growth [44, 45]. Even though it is a new research area, there are multiple studies indicating that breast milk composition might play an important role for growth during infancy and also later, and might influence the risk of developing overweight and obesity.

Breast Milk Macronutrient Composition and Infant Growth

Breast milk contains multiple nutritional compounds such as fat, carbohydrates, proteins, and human milk oligosaccharides (HMOs), all of which are likely to play a central role in determining infant growth (Table 1). The distribution of macronutrients in complementary foods has been studied widely with regard to the risk of later obesity. High-protein intake early in life seems to increase the risk of obesity in later life [46], and it has been suggested that a high fat intake might reduce the risk of later obesity [56]. Recently, Prentice et al. [54] examined breast milk macronutrient composition in a large cohort of children and found that higher total energy content of hindmilk samples was associated with lower BMI at 12 months and weight and BMI gains from 3 to 12 month of age. Furthermore, the breast milk fat percentage was inversely associated with gains in weight, BMI, and skinfold thickness at 3 to 12 months, while the carbohydrate percentage showed opposite associations. The breast milk protein content (percentage) was positively associated with BMI at 12 months of age but not with
Breastfeeding and Growth

Table 1. Overview of particularly interesting compounds in breast milk with regard to growth

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Suggested role in growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macronutrients</strong></td>
<td>Overall higher protein content might be related to rapid growth while early fat intake might be related to slower growth [46]</td>
</tr>
<tr>
<td>Overall protein and fat content</td>
<td>Intact proteins such as haptocorrin are important for binding vitamin B₁₂ in breast milk, which might be important for growth [43]</td>
</tr>
<tr>
<td>Intact proteins, such as haptocorrin and α-lactalbumin</td>
<td>HMOs are part of the carbohydrate components in breast milk, and HMO composition has been linked to growth patterns, both positively and negatively [47]</td>
</tr>
<tr>
<td>Human milk oligosaccharides (HMOs) such as lacto-N-fucopentaose</td>
<td></td>
</tr>
</tbody>
</table>
| **Fatty acid composition** | EPA and DHA have effects on growth in some studies, but no firm conclusions have been established yet [48]  
Other fatty acids might also play a role in infant growth [49] |
| Long-chain polyunsaturated fatty acids such as EPA and DHA |  |
| **Amino acid composition** | Leucine might stimulate the release of growth hormones such as insulin and IGF-1  
Glutamic acid and glutamine might be involved in the satiety regulation [50] |
| Branched-chain amino acids, e.g., leucine or others such as glutamic acid and glutamine |  |
| **Hormones** | Leptin might induce satiety in the infant, and adiponectin might have insulin-sensitizing effects  
Growth hormones as well as leptin and adiponectin are involved in the regulation of energy homeostasis [42, 51, 52]  
A few studies have found associations between human milk IGF-1 and insulin and growth, suggesting a stimulating effect [53] |
| Adipokines such as leptin and adiponectin |  |
| Growth hormones such as IGF-1 and insulin |  |
| **Bioactive factors** | TNF-α and IL-6 are both proinflammatory cytokines that might be involved in immune defense of the breastfed infant and thereby could have an effect on growth [42, 52]  
Breast milk microbes might play a role in infant gut function and in the establishment of the microbiome, which is hypothesized to affect growth  
MicroRNAs might be involved in programming of the epigenome and thus in long-term programming of growth [44] |
| Cytokines such as TNF-α and IL-6 |  |
| Breast milk microRNAs and microbes |  |

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IGF-1, insulin-like growth factor 1; TNF-α, tumor necrosis factor-α. References used were Alsaweed et al. [44], 2015; Alderete et al. [47], 2015; Delgado-Noguera et al. [48], 2015; Fields and Demerath [52], 2012; Fields et al. [42], 2016; Fields et al. [51], 2017; Gomez-Gallego et al. [45], 2016; Haschke et al. [43], 2016; Kon et al. [53], 2014; Larnkjær et al. [50], 2016; Lind et al. [46], 2017; Much et al. [49], 2013; Prentice et al. [54], 2016; and Savino et al. [55], 2009.

any of the other indicators measured. Even though the study lacks information on total milk intake, and thereby total energy and macronutrient intake, and the analyses were based on hindmilk samples, the study still provides interesting insights into breast milk macronutrient composition and growth.

It is not only the total protein, fat, and carbohydrate intake in breast milk that might be related to infant growth. In regard to the association between high-
protein intake and later risk of obesity, it is interesting that a higher concentration of branched-chain amino acids has been found in the breast milk of obese mothers than lean mothers [57]. Potentially, this could predispose these infants to rapid growth through an increased stimulation of IGF-I and insulin by leucine, for example [46], which in turn could increase the later risk of obesity. Free amino acids in breast milk, especially glutamic acid and glutamine, could have an effect on growth [50]. In a small observational study, free glutamine in breast milk was associated with length at the age of 4 months [50]. Besides lactose as a carbohydrate source, breast milk also contains HMOs, which have also been proposed to play a role in infant growth and body composition. Alderete et al. [47] showed that higher HMO diversity at 1 month of age was associated with lower total and percent FM at 1 month while individual HMOs, e.g., lacto-N-fucopentaose, were associated with fat and fat-free mass at 6 months of age. Furthermore, differences in the fatty acid composition of breast milk and the use of maternal supplements to alter this, especially ω-3 long-chain fatty acids, have been proposed to have protective effects on early obesity development, too [48]. One study found that breast milk DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), and total ω-3 long-chain polyunsaturated fatty acids at 6 weeks but not 4 months were positively related to the sum of 4 skinfold measurements at age 1 year [49]. However, a 2015 Cochrane systematic review concluded that there was inconclusive evidence to support or refute the practice of supplementation with long-chain fatty acids to breastfeeding mothers in order to improve growth [48]. They reported a 0.75 cm lower length in the intervention group compared to controls at the follow-up of >24 months, but head circumference was 0.69 cm higher. They found no differences in weight. However, very few studies were included and hence the conclusion on the general lack of evidence.

**Breast Milk Hormones and Infant Growth**

Hormones in breast milk might also play a role in determining infant growth [42, 55]. The hormones most studied in breast milk in regard to growth are leptin and adiponectin. There is still some controversy regarding the link between breast milk leptin and adiponectin concentrations and growth mainly because of few and small studies [42]. A recent study by Brunner et al. [58] showed positive associations between breast milk adiponectin at 6 weeks and 4 months and infant growth and adiposity up to 2 years of age. Breast milk leptin was at these time points unrelated to growth and body composition except for an inverse correlation between milk leptin and infant weight at 4 months of age [58].
Some less-studied hormones in breast milk, such as cortisol and insulin, might also play a role in infant growth. It has been shown that breast milk cortisol at 3 months of age was inversely associated with BMI gains up to 2 years, and that breast milk cortisol might be a stronger BMI predictor in girls than boys [59]. Higher breast milk insulin at 1 month of age has been associated with lower infant total lean mass and weight-for-length and BMI z-scores [52], while insulin was not associated with body composition in the first 6 months of life [51]. Furthermore, a study found that infants with higher weight gain in the first 3 months of life were fed breast milk with a higher content of IGF-I [53]. Additionally, a positive correlation was observed between the breast milk IGF-I level and infant weight gain, suggesting that IGF-I in breast milk might also influence early growth in infants [53].

**Breast Milk Cytokines and Infant Growth**

Cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α are also present in breast milk and might play a role in infant growth [42]. Both IL-6 and TNF-α have been reported to be negatively associated with total lean mass at 1 month of age, while IL-6 was also associated with a lower relative weight, percentage of fat, and FM at 1 month as well as lower weight gain at 0–1 months of age [52]. This can possibly be attributed to the involvement of cytokines in infant immune maturation, but the exact mechanisms linking these to growth are not known. However, in a recently published study, IL-6 and TNF-α did not show any association with body composition or growth at 1 and 6 months of lactation [51].

**Methodological Considerations and Determinants of Breast Milk Composition**

Several methodological considerations must be taken into account when exploring the impact of breast milk composition on growth. Getting comparable determinations of breast milk composition across studies is difficult as there are differences in sampling methods. The fat content differs considerably between fore- and hindmilk, and there can be differences in the content of many substances in a small sample taken by hand expression and a sample from a full emptying of a breast with a milk pump. Content might also change with the time of day. The method of analysis is also important. For example, leptin levels in breast milk are considerably higher in whole- versus skimmed milked samples,
possibly because a subset of the leptin is trapped in the milk fat droplets or in proteins associated with milk fat [55]. Thus, standardized study protocols for measuring breast milk composition are needed.

In addition to the methodological issues, some substances in breast milk seem also to be influenced by maternal factors. These include genes, maternal BMI, parity, mode of delivery, and lifestyle factors such as nutrition, including the time of the last meal before sampling, and smoking [42, 60, 61]. For example, mothers with high BMI have also higher levels of breast milk leptin as well as insulin, while maternal fish intake is associated with breast milk DHA content [42, 60, 61]. In some studies, some of these maternal factors are also related to infant growth, which can render it difficult to conclude about causality.

**Conclusion**

Breastfeeding has a marked effect on early growth, and the growth pattern of breastfed infants is regarded as an optimal growth pattern. Many factors are influencing growth of breastfed infants. The levels of growth factors and growth-related hormones differ between breastfed and formula-fed infants. Appetite regulation is also different. Breastfed infants also experience many different flavors in breast milk, and fat content changes considerably during emptying of the breast, which is likely to influence satiety and thereby milk intake [62].

It is difficult to evaluate the exact effect of breastfeeding on growth, because the vast majority of studies are observational and influenced by potential residual confounding and reverse causality, as described above. In addition, RCTs of breastfeeding promotion have methodological challenges as well, which makes the study of a causal effect of breastfeeding on growth challenging. However, there is increasing interest in the relation between the composition of breast milk, including macronutrients, HMOs, appetite-regulating hormones, and other bioactive substances, and growth. This is likely to improve our understanding of how breastfeeding is influencing and regulating both short- and possibly also long-term growth. Future studies should also include the assessment of body composition, as breastfeeding is also influencing the pattern of lean mass and FM accretion, and because it is likely that breast milk composition has an effect on body composition.

**Disclosure Statement**

None of the authors have anything to disclose.
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