Increasing evidence suggests that nutrition and growth velocity during infancy is related to later obesity and thereby the risk of chronic diseases, such as type 2 diabetes and cardiovascular diseases. However, it is still not clear what mechanisms are behind these associations. Furthermore, there is limited evidence that interventions directed towards nutrition and growth during infancy can reduce the risk of later obesity and its complications. During the last few years there have been a large number of publications focusing on specific aspects of the associations between nutrition and growth during infancy and obesity later in life, which have increased our understanding of the area. Recent studies have also shown that it is a complex area where the state of the art continues to be challenged by new findings. The first thousand days, from conception to age 2 years, seems to be a sensitive window, but there is still uncertainty about the most sensitive periods for the early-life factors of nutrition and growth.

In our selection of papers, we have included studies and reviews which have a focus on early nutrition (mainly breastfeeding and complementary feeding) and early growth. There are a number of new and promising areas, such as microbiota, epigenetics and metabolomics, which are likely to play a role in the interaction between early nutrition, early growth and later obesity. However, papers on these potentially important areas have not been included as we could only cover a limited area. We have included 11 papers published during the 2-year period from mid-2013 to mid-2015. We found these to be of special interest in shedding light on the association between early nutrition, growth and later obesity.

Breastfeeding has a marked effect on early growth, which could be an important mediator of the protective effect of breastfeeding against later obesity seen in many studies. It is therefore of interest to better understand how breast milk intake is regulated. The first two papers address appetite hormones in breast milk, exploring maternal...
factors related to milk hormone levels and how these hormones in breast milk are related to weight gain and body composition in infants. The next three papers describe different aspects of the association between breastfeeding and later body composition, overweight and obesity. After these, four papers are included which deal with the effects of complementary feeding, with a focus on the effects of protein and fat intake, on later obesity. Most studies on early growth and later obesity focus on weight gain. Therefore, we included a large cohort study which compared both how early weight gain and how early linear growth is associated with later body composition.

The strong evidence that high early weight gain, especially during the first 6 months of life, is positively associated with later overweight and obesity is difficult to translate into public health interventions. As far as we know, there is no evidence that interventions aiming at reducing high weight gain during the first half of infancy can modify the risk of later overweight and obesity. We therefore found the last paper of interest as it focuses on modifiable early-life risk factors for later obesity, although it addresses mainly maternal risk factors, and only 1 of the 5 modifiable factors are on early nutrition.

It is difficult to present an overall conclusion from the 11 papers we have included in this chapter, as they cover many areas. Although it is still being discussed, we find that there is convincing evidence that breastfeeding protects against later overweight and obesity, although the protective effect is not strong. We also find it convincing that high protein intake during the first years increases the risk of later obesity, and that a high fat intake does not seem to be a problem. Several of the papers mention promising areas which should be explored further in future studies. These include how appetite regulation during infancy and body composition can influence long-term health. Although it may be difficult, studies testing the effects of early interventions on the risk of overweight and obesity during childhood should be given high priority.

Breast milk leptin and adiponectin in relation to infant body composition up to 2 years

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**Background:** Human milk contains bioactive components not present in formula milk, which may play a role in the suggested protective effect of breastfeeding against later obesity. This includes the
appetite-related hormones leptin and adiponectin. The level of these adipocyte-secreted hormones in human milk seems to be related to early weight gain and BMI, but the relation with fat mass and fat free mass is less clear. The objective of the study was to investigate the associations of leptin and adiponectin in breast milk with weight gain and body composition up to the age of 2 years.

Methods: Fasting milk samples from a complete breast expression were collected at 6 weeks (n = 152) and 4 months (n = 120) postpartum. Leptin and adiponectin content was analyzed in skimmed milk samples. Infants were examined at birth, 6 weeks, 4 months, and 1 and 2 years (n = 118), including weight, length and skinfold thicknesses at four sites. Body fat was calculated from an algorithm using the skinfold measurements. Information on breastfeeding was also collected. The relationship between adipokines and growth and body composition up to the age of 2 years was explored.

Results: Leptin, but not adiponectin, was strongly positively correlated with maternal BMI, skin folds and fat mass. The concentrations of the adipokines decreased slightly from 6 weeks to 4 months postpartum, but only significantly for adiponectin. The majority of the infants were exclusively breastfed (85% at 6 weeks, 83% at 4 months). Leptin was not associated with any anthropometric measures in the infants up to 2 years, except for an inverse association with weight and lean body mass at 4 months in the adjusted model. Milk adiponectin at 6 weeks was or tended to be inversely related to lean body mass (p = 0.015), weight and BMI (p ≥ 0.054) up to 4 months, but was significantly positively associated with skin folds and fat mass at 1 year, and weight gain from 6 weeks to 1 year. Adiponectin at 4 months was also positively associated with weight gain and fat mass measures up to 2 years.

Conclusions: The results suggest that high concentrations of breast milk adiponectin up to 4 months might be associated with greater weight gain and higher fat mass in the offspring up to the age of 2 years. Interestingly, breast milk leptin did not seem to be associated with infant anthropometry.

Comments
It has been speculated that breast milk hormones are related to infant appetite and energy homeostasis, and thereby may be related to later weight gain and gain in fat mass. This could be one of the mechanisms through which breastfeeding seems to protect against obesity later in life. However, the impact of breast milk hormones depend on the bioavailability, and it is not known to what extent hormones from breast milk reach the infant circulation or receptors in the gastrointestinal (GI) tract of the infant. Furthermore, bioavailability may also change over time as the GI tract matures. Therefore, longitudinal studies like this one are important for exploring associations between these hormones and later infant growth. Even if associations can be shown, the causality and the mechanisms behind them need to be explored further.

In the Discussion, the authors summarized studies which have examined the association between breast milk leptin and adiponectin and later growth. A few have found positive associations between leptin and growth, which could not be supported by this study. The finding that milk adiponectin is positively associated with weight gain is supported by a number of other studies. This study expands this knowledge by showing that there was a positive effect of adiponectin on not only BMI, but also on fat mass accretion.

An additional finding of the study was that breast milk leptin levels were positively associated with maternal BMI and fat mass. This is also the main finding in the systematic review by Andreas et al., also included in this chapter, of the effect of maternal BMI on hormones in breast milk.

A recent study from Russia examined ghrelin and IGF-I in milk, in addition to milk leptin and adiponectin, in relation to infant weight gain [1]. Leptin, and to some extent ghrelin, was higher among those with the highest weight gain, but the most signifi-
A significant effect was that milk IGF-I was higher in the group with the highest weight gain. Also, it is not known if breast milk IGF-I content has a direct effect on growth or if it is only an association. Understanding appetite regulation in infants is important. This study provides some insight, but more studies are needed.

Effect of maternal body mass index on hormones in breast milk: a systematic review

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Background: Nutrition during infancy may have long-term effects on the risk of adult obesity. Breast milk contains many appetite regulatory hormones that may have an effect on infant metabolism during the neonatal period, thereby influencing the risk of obesity in later life. Maternal BMI is also positively associated with infant obesity risk. However, factors influencing the levels of hormones in breast milk are unclear, and maternal BMI has been suggested to play a role. The objective of this systematic review was to explore the relationship between maternal BMI and the concentration of appetite-regulating hormones in breast milk.

Method: Studies including breast milk concentration of at least one of the following hormones, leptin, adiponectin, insulin, ghrelin, resistin, obestatin, peptide YY and glucagon-like peptide 1, and the associations with maternal BMI before, during or after pregnancy were searched in PubMed. The quality of the studies was scored in terms of design, sample size and sample preparation, which were considered necessary to achieve reliable hormone measurements.

Results: Twenty-six studies were identified and included in the systematic review. There was a high degree of variability between studies with regard to time of collection, preparation and analysis of breast milk samples, and regarding measurement of maternal BMI. Eleven of 15 studies reporting breast milk leptin found a positive association between maternal BMI and milk leptin concentration, but they differed regarding stage of lactation. Only two of nine studies investigating adiponectin found a positive association between maternal BMI and breast milk adiponectin concentration; however, after controlling for time postpartum in one of the studies, the correlation was no longer significant. No association was seen between maternal BMI and milk adiponectin in the other seven studies identified. The studies differed regarding measurement of maternal BMI and stage of lactation when samples were taken; six included analysis of colostrum. Two of four studies found a positive correlation between insulin and maternal BMI. For ghrelin, resistin, peptide YY and glucagon-like peptide 1, there were no associations with maternal BMI. No relevant studies for obestatin were found.

Conclusions: Of all the hormones investigated in this systematic review, the only consistent association found was a positive relation between leptin in breast milk and current maternal BMI, despite variable methodology used in the studies. For the other hormones, additional studies with larger sample sizes and improved study designs regarding standardization on sample collection, hormone analyses and measurement of current maternal adiposity are warranted.
The levels of hormones in breast milk may depend on maternal factors such as maternal BMI. Maternal BMI is associated with infant weight gain and later risk of obesity. As genetics may also play a role in this association, it is difficult to determine whether hormones in breast milk have an effect on infant weight gain. The association between maternal BMI and hormones in breast milk were explored in this comprehensive review of many published studies, and the difficulties in comparing and achieving consistency in the results were underlined. The literature search included many hormones: leptin, adiponectin, insulin ghrelin, resistin, obestatin, peptide YY and GLP1. Except for leptin and adiponectin, there were few studies or only one study measuring the other hormones. Thus, it is difficult to draw conclusions on those hormones. The quality of the included studies was adequately estimated, and the relevant limitations were pointed out. In particular, the heterogeneity in study design and the small sample size in many studies were highlighted. The influence of possible confounders, such as a shared obesogenic environment, especially as infants born to obese mothers have increased risk of becoming obese themselves, is underlined, but genetic aspects should be considered as well.

Exclusively breastfed overweight infants are at the same risk of childhood overweight as formula fed overweight infants

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Background: Rapid weight gain and overweight in infancy increases the risk of childhood overweight, while breastfeeding seems to protect against childhood overweight. There is a tradition not to worry about exclusively overweight breastfed infants, as it is considered that they are not at high risk of later overweight and obesity. However, there is not much evidence behind this. So, should we worry about exclusively breastfed overweight infants? The aim of this study was to examine the association of feeding type [exclusive breast feeding (EBF), formula feeding or mixed feeding] and overweight at the age of 6 months with the risk of overweight at the age of 5–6 years.

Methods: The Amsterdam Born Children and Their Development study is a large prospective population-based birth cohort study that was conducted in the Netherlands. Children with complete information pertaining to feeding type and weight status at the age of 6 months and 5–6 years were included (n = 3,367). EBF was defined as receiving only breastfeeding for at least 3 months. This group was divided into those with EBF for 3–6 months and those with EBF ≥6 months. Overweight at the age of 6 months was defined as a BMI ≥1 SD according to the WHO growth standards and overweight at age 5–6 years was defined according to the International Obesity Task Force as an iso-BMI of 25. The association of feeding type and overweight at 6 months with overweight at 5–6 years was assessed using logistic regression analyses.

Results: Overweight infants had 4.10-fold (95% CI: 2.91–5.78) higher odds of childhood overweight compared with those who were not overweight, independent of feeding type. EBF did not affect the association between infant overweight and childhood overweight.
Conclusions: Overweight in infancy increases the odds of childhood overweight equally for exclusively breastfed and formula-fed infants. Overweight prevention should start before or at birth and applies to formula-fed children as well as exclusively breastfed children.

Comments: This study showed that even in infants exclusively breastfed for 3–6 months, the association between early high weight gain and later overweight was equal to formula-fed infants. Thus, the common belief that there is no reason to worry if an overweight infant is exclusively breastfed for a considerable period could not be confirmed. In the analysis, EBF ≥6 months reduced the risk of later overweight with borderline significance by 35% in the crude model. In the full model the reduction is 15%, but this is far from being significant. The authors concluded that there is a need to implement prevention of overweight also in overweight breastfed infants, and that overweight prevention should start before birth. There is no evidence that breastfeeding modifications, such as reducing the number or duration of breastfeeding sessions, have any effect on later overweight, and this is not suggested by the authors. However, in formula-fed infants, the usual advice is to try to reduce the intake even if there is no evidence that this will have an effect on later obesity. In the population examined in this study, it is remarkable that among those exclusively breastfed for ≥6 months there were significantly more infants that became overweight (14.4%) compared to those who were formula fed (11%). The opposite would be expected. There is a need for more studies to explore if exclusively breastfed infants with a high weight gain during the first months of life have an increased risk of later overweight and obesity.

General and abdominal fat outcomes in school-age children associated with infant breastfeeding patterns

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Background: Breastfeeding may have a protective effect on the development of obesity in later life, but not much is known about the effects of infant feeding on more specific fat measures. The aim was to examine associations of breastfeeding duration and exclusiveness and age at the introduction of solid foods with general and abdominal fat outcomes in children.

Methods: This was a population-based, prospective cohort study of 5,063 children. Information about infant feeding was obtained by using questionnaires at 2, 6 and 12 months of age. Information on breastfeeding duration, introduction of complementary foods, and types of milk and solids were collected. Fully adjusted models were additionally adjusted for family-based sociodemographic factors (maternal age, ethnicity and education), maternal lifestyle-related factors (prepregnancy BMI, parity and smoking during pregnancy) and childhood factors (gestational age at birth, birth weight and television watching). At a median age of 6.0 years (95%
range: 5.7–6.8), childhood anthropometric measures, total fat mass and the android:gynoid fat ratio using dual-energy X-ray absorptiometry and preperitoneal abdominal fat using ultrasound were measured.

**Results:** In the models adjusted for child age, sex and height only, shorter breastfeeding duration, nonexclusive breastfeeding and younger age at the introduction of solid foods were associated with higher childhood general and abdominal fat measures (p trend < 0.05), but not with higher childhood BMI. The introduction of solid foods at a younger age, but not breastfeeding duration or exclusivity, was associated with a higher risk of overweight or obesity (OR = 2.05, 95% CI: 1.41, 2.90). After adjustment for family-based sociodemographic, maternal lifestyle and childhood factors, the introduction of solid food between 4 and 4.9 months of age was associated with higher risks of overweight or obesity, but the overall trend was not significant.

**Conclusions:** Associations of infant breastfeeding and age at the introduction of solid foods with general and abdominal fat outcomes are explained by sociodemographic and lifestyle-related factors. Whether infant dietary composition affects specific fat outcomes at older ages should be further studied.

**Comments** By using dual-energy X-ray absorptiometry scans and ultrasound to measure preperitoneal abdominal fat in a large cohort of children, this study was able to test if early diet influenced body fat at school age. In studies only using BMI as the outcome and not showing the effects of early diet, it has been speculated whether BMI is a poor indicator of adiposity and if there might have been effects on body fat. Duration of breastfeeding and age at introduction of solid foods were the main characteristics of early feeding examined in this study, and the findings in the crude analysis were as expected, with beneficial effects of longer breastfeeding and later introduction of complementary feeding. However, as these significant associations disappeared after adjustment for the factors mentioned in the Methods section above, the question arises as to whether apparent associations between early feeding and later overweight and adiposity reported in other studies would have disappeared if controlled for some of the factors controlled for in this study. Thus, it cannot be excluded that some of the findings of a preventive effect of early feeding on later obesity may be caused by confounding. The discussion of which potential confounders to control for and whether there is a risk of missing relevant associations if controlling for a wide range of factors is important. The problems related to confounding in studies of infant feeding and later adiposity are discussed in detail in an editorial in the same issue [2].

The authors finish by speculating that breast milk composition could have an effect on later obesity, and that differences in the composition between populations could cause some of the differences in effects of breastfeeding between populations. It is mentioned that n-3 fatty acids and especially DHA content may reduce the risk of later obesity. The potential effects of early DHA intake on later obesity are discussed later in this chapter when the study by Zheng et al. is reviewed.
The association between breastfeeding and childhood obesity: a meta-analysis
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Background: The increase in childhood obesity is a serious public health concern. Several studies have indicated that breastfed children have a lower risk of childhood obesity than those who were not breastfed, while other studies have provided conflicting evidence. This article refers to three large reviews published in 2004–2005, which all showed a protective effect of breastfeeding. The objective of this meta-analysis was to make an updated analysis of the association between breastfeeding and the risk of childhood obesity.

Methods: The PubMed, EMBASE and CINAHL Plus with Full Text databases were systematically searched from the start date to August 1, 2014. Based on the meta-analysis, the pooled adjusted odds ratio (AOR) and 95% CI were calculated. The I² statistic was used to evaluate between-study heterogeneity. Funnel plots and fail-safe N were used to assess publication bias and reliability of results, and results from both Egger’s and Begg’s tests were reported.

Results: Twenty-five studies with a total of 226,508 participants were included in this meta-analysis. The studies’ publication dates ranged from 1997 to 2014, and they examined the populations of 12 countries. Results showed that breastfeeding was associated with a significantly reduced risk of obesity in children (AOR = 0.78, 95% CI: 0.74, 0.81). Categorical analysis of 17 studies revealed a dose-response effect between breastfeeding duration and reduced risk of childhood obesity. The AOR (95% CI) was 0.90 (0.84, 0.95) for <3 months, 0.88 (0.79, 0.97) for 3–4.9 months, 0.83 (0.76, 0.90) for 5–6.9 months and 0.79 (0.70, 0.88) for ≥7 months.

Conclusions: Results of the meta-analysis suggest that breastfeeding is a significant protective factor against obesity in children. The analysis showed a strong dose-response effect with increasing protection up to 7 months.

Comments: This is an important and comprehensive update of the previous meta-analysis on the topic published about 10 years ago. With a quarter of a million individuals included from 25 studies in 12 countries and advanced statistical analysis, this study provides strong evidence that breastfeeding protects against later obesity. However, the results are in contrast with the only large randomized study of effects of breastfeeding from Belarus, the PROBIT study, which found no effect of breastfeeding on obesity at 6.5 years [3]. A recent article in the New England Journal of Medicine discussing myths associated with obesity also concluded that it was a myth that breastfeeding protects against later obesity [4]. What could the reasons be that quite a few studies have shown no effects of breastfeeding when there is an effect in this large meta-analysis? Part of the explanation could be that some studies have been conducted in populations with short durations of breastfeeding. Other reasons could be that some of the studies are from societies that are not obesogenic. There are several reasons why breastfeeding can protect against obesity, e.g. protein content is lower in breast milk compared to infant formula, and the content of appetite-regulating hormones in breast milk could also play a role. Both aspects are discussed in other parts of this chapter. Furthermore, breast milk composition and taste changes during a feeding,
which could provide satiety signals for the infant. Several studies show that some aspects of appetite regulation is different in breastfed compared to formula-fed infants.

**Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial**

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**Note:** This article is discussed also in the chapter by Shalitin et al. [this vol., pp. 21–49].

**Background:** Early nutrition is recognized as a target for the effective prevention of childhood obesity. In this intervention study, previous publications based on a 2-year follow-up have shown that those in a higher-protein formula group had a more rapid weight gain during the first year of life and were heavier at 2 years compared to those receiving a lower-protein formula. In this study with follow-up at 6 years of age, it was tested whether the reduction of protein in infant formula reduces BMI and the prevalence of obesity at 6 years of age.

**Methods:** The Childhood Obesity Project was conducted as a European multicenter, double-blind, randomized clinical trial that enrolled healthy infants born between October 2002 and July 2004. Formula-fed infants (n = 1,090) were randomly assigned to receive higher-protein (HP)- or lower-protein (LP)-content formula in the first year of life; breastfed infants (n = 588) were enrolled as an observational reference group. The protein content in the formula used from 0 to 4 months was 1.25 g/100 ml in the LP formula group and 2.05 g/100 ml in the HP group. The protein content in the formula used from 4 to 12 months was 1.6 g/100 ml in the LP formula group and 3.2 g/100 ml in the HP formula group. At the 6-year follow-up, the weight and height of 448 (41%) formula-fed children were measured and compared with the breastfed reference group. BMI was the primary outcome.

**Results:** HP formula children had a significantly higher BMI (by 0.51, 95% CI: 0.13, 0.90; p = 0.009) at 6 years of age. The risk of becoming obese in the HP formula group was 2.43 times (95% CI: 1.12, 5.27; p = 0.024) that of the LP formula group. There was a tendency for a higher weight in HP formula children (0.67 kg, 95% CI: −0.04, 1.39; p = 0.064), but no difference in height between the intervention groups. Anthropometric measurements were similar in the LP formula and breastfed groups.

**Conclusions:** Infant formula with a lower protein content reduces BMI and obesity risk at school age. Avoidance of infant foods that provide excessive protein intake could contribute to a reduction in childhood obesity.

**Comments** This study suggests that a high protein intake during the first year of life has a programming effect on later weight gain, as it increases both the median BMI and the risk of obesity at the age of 6 years. As the study is randomized, it provides strong evidence for the early-protein hypothesis. It would have been interesting to study to
which degree the higher weight and BMI in the HP group was caused by increases in fat mass or fat-free mass. In relation to the question if breastfeeding protects against later obesity, it was of interest that there were no differences in BMI and prevalence of obesity between the LP formula and the breastfed reference group.

The protein content in the LP and HP formulas were within the recommended content at the time the intervention study was performed. However, European regulations changed in 2006 and the upper limit of infant formula was reduced from 2.25 to 1.8 g/100 kcal, and the upper limit for follow-up formulas was reduced from 4.5 g/100 kcal to 3.5 g/100 kcal. Calculated as g/100 kcal, the protein content in the HP formula was 4.4 g/100 kcal. The protein content in cow’s milk is about 5.4 g/100 kcal. Thus, the protein content of the HP infant formula, which the infants received from the age of 4 months, was quite high – about 80% of the content in cow’s milk. Furthermore, it is of interest that the content of casein in the formulas was high, as in cow’s milk (casein:whey 80%:20%). It is especially casein which stimulates IGF-1 [5]. Most infant formulas contain 40% casein and 60% whey.

As the LP and HP formulas were isocaloric, the fat content in the HP formulas was lower to compensate for the higher protein content. In the HP formula used from 4 to 12 months, the fat content was approximately 15% lower than in the LP formula. As pointed out by the authors, it is not likely that such a difference could have an influence on growth. However, it is difficult to exclude that there could be a minor effect on growth, as some data suggest that a low fat intake could result in an increased risk of obesity, as discussed below in this chapter.

As the largest difference in protein intake was during the age from 4 to 12 months, these data are also relevant for the complementary feeding period, suggesting that throughout this period it is important to avoid a high protein intake. During this period, the average protein intake increases gradually to three times the physiological needs, typically to a median intake of 15% energy from protein (PE%). However some children will then have a very high intake, e.g. above 20 or 25 PE% [6]. The Nordic Nutrition Recommendations suggest a protein content in the diet from 12 to 24 months of 10–15 PE% and thus to avoid a diet with a protein PE% above 15% [7].

Association of nutrition in early life with body fat and serum leptin at adult age

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Int J Obes (Lond) 2013; 37:1116–1122

Background: There is considerable evidence that nutrition during early life has long-term health consequences. However, the role of diet composition during the complementary feeding period in programming obesity and leptin resistance is not well described. This study aimed at determining whether nutritional intake at 10 months and 2 years was associated with body composition and leptin resistance at 20 years.
Methods: Healthy infants participating in the prospective ELANCE (Etude Longitudinale Alimentation Nutrition Croissance des Enfants) cohort study were included. The data used in this analysis were dietary intake at 10 months and 2 years, and at the 20-year follow-up the following data were included: weight, height, subscapular and triceps skinfold thicknesses, fat mass and fat-free mass assessed via bioelectrical impedance analysis, and serum leptin concentration.

Results: Data from 73 subjects were included. In adjusted linear regression models, an increase by 100 kcal in energy intake at 2 years was associated with higher subscapular skinfold thickness (β = 6.4%, 95% CI: 2.53–10.30, p = 0.002) and higher fat-free mass (0.50 kg, 0.06–0.95, p = 0.03) at 20 years. An increase of 1% energy from fat at 2 years was associated with lower subscapular skinfold thickness (~2.3%, ~4.41 to ~0.18, p = 0.03), lower fat mass (~0.31 kg, ~0.60 to ~0.01, p = 0.04) and lower serum leptin concentration (~0.21 μg l−1, ~0.39 to ~0.03, p = 0.02) at 20 years. At 10 months, fat intake was also negatively associated with BMI and fat mass. Protein intake either at 10 months or at 2 years was not associated with any of the anthropometric measures at 20 years.

Conclusions: Fat intake in early life was negatively associated with body fat (particularly at the trunk site) and serum leptin concentration at 20 years, suggesting that early low-fat intake could increase the susceptibility to develop overweight and leptin resistance at later ages. The authors suggest a ‘low fat programming’ hypothesis that restriction of dietary fat could decrease serum leptin levels in early life, resulting in adaptive mechanisms later in life. These findings substantiate current recommendations against restricting fat intake in early life and open new directions for investigating the origin of obesity.

Comments: The comment on this paper is combined with the comment on the following paper.

Breastfeeding, early nutrition, and adult body fat

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Note: This article is discussed also in the chapter by Shalitin et al. [this vol., pp. 21–49].

Background: This article presents further analysis of data from the cohort described above which showed that those who had low fat intake early in life had more body fat and insulin resistance at 20 years. The aim of the present paper was to explore the effect of breastfeeding on body fat at 20 years, adjusting for nutritional intake in early childhood.

Methods: Nutritional intake of the infants born in 1984 who participated in the ELANCE cohort (Etude Longitudinale Alimentation Nutrition Croissance des Enfants) was estimated at 10 months of age and again at 2 years of age. Breastfeeding was defined as any breastfeeding, including partial breastfeeding, regardless of duration. At 20 years of age, weight, height, age, subcapular skinfold thickness (SF) and fat mass (assessed via bioelectrical impedance analysis) were measured.
**Results:** In the 73 individuals included in the analysis, 64% of the children had been breastfed. In linear regression models adjusted for the mother’s BMI and the father’s profession, breastfeeding was not associated with any of the body fat measurements at 20 years (all \( p > .05 \)). After adding nutritional intake variables (total energy and % energy from macronutrients at 10 months and 2 years) to the models, breastfeeding became significantly associated with lower SF at 20 years. In particular, breastfed subjects had a significantly lower percent of SF at 20 years after adjustment for energy and percentage of fat intake at 2 years of age (\( \beta = -28.25\% \) SF, 95% CI: -50.28 to -6.21, \( p = 0.013 \)) or when adjusting for energy and percentage of carbohydrates at 2 years of age (\( \beta = -28.27\% \) SF, 95% CI: -50.64 to -5.90, \( p = 0.014 \)). There was also a tendency that those who were breastfed had lower fat mass when controlling for lipids and carbohydrates (\( p = 0.066 \) and 0.079, respectively).

**Conclusions:** Breastfeeding was not associated with adult body fatness when the usual confounding factors were taken into account. However, after also adjusting for nutritional intake covariates, a protective effect of breastfeeding emerged. Early nutrition needs to be taken into account when examining the long-term health effects of breastfeeding. It is suggested that the reason that some studies cannot show an effect of breastfeeding on later obesity could be because early fat intake was not considered.

**Comments**

It is interesting that these two papers, which are based on the same small cohort with only 73 individuals with a full data set, have been able to show that body fat at 20 years was higher if fat intake at 2 years was low and if the infant was not breastfed. Concerning breastfeeding, an effect could be shown even when considering the short duration of breastfeeding among the 64% of the cohort who were breastfed (median 2.5 months). Several meta-analyses have shown a protective effect on breastfeeding on later obesity, and one of the latest and most convincing of these is the meta-analysis by Yan et al. (discussed earlier in this chapter). The effect of breastfeeding was only shown when controlling for intake of energy and macronutrients at 10 months and 2 years. Thus, it could be that some of the studies showing no effect of breastfeeding on later obesity could have found an effect if they had controlled for dietary intake during the complementary feeding period.

As far as we know this is the only study that has indicated low fat intake during the first years of life is associated with increased BMI and body fat later in life. However, several other studies have indicated that there is no association between early fat intake and later adiposity [8]. The fat content of the diet (28 PE% at 10 months and 32% at 2 years) was low compared to recommendations. With a mean content at 10 months of 28 PE% and a standard deviation of 4.5, there must have been some infants with very low intake (e.g. ≤20 PE%), which has been shown to result in a relative energy deficit that can result in slower growth [9]. As pointed out by the authors, very low fat intake early in life may promote adaptive metabolism which could increase the risk of obesity later in life. Such a pattern is seen in undernourished populations in low-income countries, where the combination of early undernutrition and later overnutrition increases the risk of lifestyle diseases, such as obesity and type-2 diabetes. The findings that early low fat intake also results in relative leptin-resistance at 20 years further underlines that the low fat intake appears to have long-term metabolic consequences.

Combining the finding of this study that low fat intake is associated with later obesity with the convincing data that high protein intake early in life is associated with an increased risk of later obesity suggests that the macronutrient composition found in breast milk with low protein and high fat should be kept during the complementary feeding period for up to about 2 years. However, the time window when this pattern is beneficial is not known and should be addressed in future studies.
Complementary feeding and childhood adiposity in preschool-aged children in a large Chinese cohort

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Background: To examine the association between the timing and type of complementary feeding and childhood adiposity in mainland China.

Methods: During 1999–2009, 97,424 singletons were enrolled in the Jiaxing Birth Cohort, a population-based prospective cohort study in Southeast China. Of these children, 43,848 children provided complementary diet information and anthropometric measurements at visits at 1, 3 and 6 months of age, and were followed up until 4–5 years of age. Dietary data was collected by asking parents at the 1-, 3- and 6-month visits if their child had been introduced to the following 10 foods or supplements: fish liver oil, rice cereal/porridge, egg yolk, fish paste, liver paste, tofu, animal blood, bread/steamed buns/fine dried noodles, ground meat/soy products and pureed noodles/cookies. Obesity and overweight were identified as a BMI-for-age z-score (SD) ≥2 and between 1 and 2, respectively, based on the WHO growth standards.

Results: Among the 40,510 children in the statistical analysis, 3.18% were overweight and 64.8% were fed complementary food before 3 months of age. Early introduction of complementary foods was associated with a greater BMI z-score (p trend < 0.001) and higher risk of overweight (p trend = 0.033). Compared with introduction of complementary foods between 4 and 6 months of age, introduction before 3 months of age was associated with 11% greater risk of overweight (OR = 1.11, 95% CI: 1.03–1.19). No significant association between timing of introduction of complementary foods and obesity was observed. Fish liver oil was the major type of complementary food associated with adiposity. Early introduction of fish liver oil was associated with a greater BMI z-score (p < 0.001) and greater risk of overweight (p trend = 0.004). Introduction of animal blood after 6 months of age showed lower BMI z-scores compared with introduction between 4 and 6 months of age (p = 0.044). Furthermore, early introduction (<3 months of age) of egg yolk was marginally associated with a lower BMI z-score (p = 0.046) compared with introduction of egg yolk between 4 and 6 months of age.

Conclusions: Early introduction of fish liver oil is associated with greater childhood BMI and risk of overweight in Chinese children at 4–5 years of age. Introduction of complementary foods before 3 months was associated with later overweight but not obesity.

Comments: In the discussion of the association of early diet and later obesity, it is interesting to have a large study from China where timing of introduction and the types of complementary foods used are different from what has been examined in many studies from populations in Western industrialized populations. Such studies open the possibility to generate new hypotheses about the mechanisms linking early nutrition to later overweight and obesity. The most consistent finding was that early introduction of fish liver oil, which is given as a supplement to provide vitamin A and D, was associated with an increase in BMI and risk of overweight at 4–5 years. As suggested by the authors, the reason could be that the fish oil supplement increases energy intake in early life. However, it has also been suggested that a high n-3 long-chain polyunsaturated fatty acid intake (e.g. from fish oil) may reduce weight gain and thereby prevent adiposity, compared to other oils [10, 11].
The finding that early introduction of egg yolk was associated with a lower BMI is difficult to explain. It does not fit with the early protein hypothesis, but the amounts given might have been small. Late introduction of animal blood which is used as an iron supplement was related to lower BMI. A negative effect of iron supplements on linear growth has been shown in iron-replete children [12], but it is difficult to know if an effect of iron on growth can explain the association in this population.

**Associations of infant feeding and timing of linear growth and relative weight gain during early life with childhood body composition**

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**Background:** Growth and feeding during infancy have been associated with later life BMI, but BMI is a combination of fat-free mass (FFM) and fat mass (FM), which may have different influences on later health. The associations of infant feeding, linear growth and weight gain relative to linear growth with these separate components of body composition, however, remain unclear.

**Methods:** Of 5,551 children with collected growth and infant-feeding data in a prospective cohort study (Amsterdam Born Children and Their Development), body composition measured using bioelectrical impedance analysis at the age of 5–6 years was available for 2,227 children. Feeding (duration of full breastfeeding and timing of introduction of complementary feeding) was assessed and conditional variables representing linear growth and relative weight gain were associated with childhood FFM and FM.

**Results:** Birth weight was positively associated with both FFM and FM in childhood, and more strongly with FFM than FM. Faster linear growth and faster relative weight gain at all ages in infancy were positively associated with childhood FFM and FM. The associations with FM were stronger for relative weight gain than for linear growth (FM z-score: β coefficient 0.23, 95% CI: 0.19–0.26, p < 0.001, and 0.14, 0.11–0.17, p < 0.001 per SD change in relative weight gain and linear growth between 1 and 3 months, respectively). Compared with full breastfeeding <1 month, full breastfeeding >6 months was associated with lower FM (FM z-score: –0.17, –0.28 to –0.05, p = 0.005) and lower FFM (FFM z-score: –0.13, –0.23 to –0.03, p = 0.015), as was the introduction of complementary feeding >6 months (FM z-score: –0.22, –0.38 to –0.07, p = 0.004) compared with <4 months.

**Conclusions:** Early growth in weight and length seems to have different effects on later body composition as faster infant weight gain is associated with a healthier childhood body composition when it is caused by faster linear growth. Full breastfeeding >6 months and introduction of complementary feeding >6 months are associated with lower childhood FM.

**Comments**

Several studies have focused mainly on weight gain when evaluating the influence of early growth on later weight and body composition, often expressed as BMI. It is interesting that this study also measured early linear growth, a parameter that may be
influenced by other nutrients (e.g., minerals and vitamins), than just weight gain, which depends more on energy-supplying macronutrients. Furthermore, it is a clear advance that the end point measure is not only weight and BMI, but also the body composition split up in FFM and FM. It was shown that both higher birth weight and faster early linear growth was associated with higher FFM and to a lesser extent higher FM, a body composition believed to be healthier in relation to later diseases. With the focus on linear growth, it is a pity that the authors do not know birth length and thereby do not have the possibility to analyze whether the positive effect of birth weight is mainly through an effect of birth length. It will be interesting to see if the association between higher birth weight, higher early linear growth and body composition also exist later in childhood and adulthood. Thus, a follow-up later in childhood of this cohort would be interesting.

The authors suggest that linear growth velocity should be taken into consideration when evaluating the risk of later obesity in infants with high weight gain. This can be done by plotting and evaluating the BMI growth pattern of the infant.

In relation to the other papers on effects of breastfeeding discussed in this chapter, breastfeeding >6 months was associated with lower FM as well as lower FFM at the 5- to 6-year follow-up. Thus, breastfeeding does not seem to have a strong influence on the relation between the two.

A recent study also examined the association of breastfeeding duration and early weight gain with body composition at 3 years measured by bioimpedance. There was a significant positive association between high weight gain from birth to 5 months, but not later, on fat mass index at 3 years. Longer duration of breastfeeding attenuated this effect, and the effect was eliminated in infants fully breastfed for 6 months.

These studies further underline that body composition should be included in studies exploring the effect of early nutrition and early growth on later obesity. Such data provide both a better understanding of the mechanisms involved and of the long-term health aspects of overweight and obesity.

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**Modifiable early-life risk factors for childhood adiposity and overweight: an analysis of their combined impact and potential for prevention**

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**Note:** This article is discussed also in the chapter by Shalitin et al. [this vol., pp. 21–49].

**Background:** Factors in early life are likely to be important for the risk of later diseases including obesity. As prevention is a priority, insight into the potential impact of modifying early-life risk factors on later obesity is interesting and can be gained by evaluating their combined effects. The objective was to examine the relation between early-life risk factors and obesity outcomes among children in a prospective birth cohort (Southampton Women’s Survey).
Methods: Five potentially modifiable risk factors, which have been shown to be related to obesity in this study or in other studies, i.e. maternal obesity (prepregnant BMI >30), excess gestational weight gain (Institute of Medicine, 2009), smoking during pregnancy, low maternal vitamin D status (<64 nmol/l) and short duration of breastfeeding (none or <1 month), were defined. Obesity outcomes at age 4 and 6 years were BMI, dual-energy X-ray absorptiometry-assessed fat mass, overweight or obesity (International Obesity Task Force cutoffs). Data were available for 991 mother-child pairs, with children born between 1998 and 2003.

Results: At 6 years, 12.9% were overweight and 3.3% obese. Of the children, 148 (15%) had no early-life risk factors, 330 (33%) had 1 risk factor, 296 (30%) had 2 risk factors, 160 (16%) had 3 risk factors and 57 (6%) had 4 or 5 risk factors. At both 4 and 6 years, there were positive graded associations between the number of early-life risk factors and each of the five obesity outcomes (all p < 0.001). After taking confounders into consideration, the relative risk of being overweight or obese for children who had 4 or 5 risk factors was 3.99 (95% CI: 1.83, 8.67) at 4 years and 4.65 (95% CI: 2.29, 9.43) at 6 years, compared with children who had none (both p < 0.001). Those with 4 or 5 risk factors had 19% more body fat mass at 4 years and 47% more at 6 years. Adjustment for physical activity in childhood and a prudent diet score attenuated the associations somewhat, but there was still a relative risk of about 3.5.

Conclusions: A greater number of early-life risk factors were associated with large differences in adiposity and risk of overweight and obesity in later childhood. These findings suggest that early intervention to change these modifiable risk factors may make a significant contribution to the prevention of childhood obesity.

Comments: This study followed a relatively large group of mother-child pairs from before pregnancy until the children were 6 years of age. Focus was on so-called potential modifiable early risk factors for later obesity and body composition. Several systematic reviews have identified early-life risk factors for overweight and obesity [14, 15]. The authors selected 5 risk factors based on earlier analyses from the same cohort and other studies, showing that they were independently associated with a higher risk of childhood obesity. Four are well-known risk factors, maternal obesity, excess gestational weight gain, smoking during pregnancy and short duration of breastfeeding (none or <1 month), while the fifth, low maternal vitamin D status (<64 nmol/l), seems less well established. The strengths of the study are that the authors use dual-energy X-ray absorptiometry state-of-the-art measurements for fat mass assessment and analyzed the combined potential impact of the 5 risk factors, and thereby estimated the potential for prevention of obesity and fatness by intervening on these 5 factors. With a 4-fold increase in risk in children having 4–5 risk factors, there is certainly potential for prevention. The authors claim that the risk factors are modifiable; however, some of them may be easier to change, like low maternal vitamin D status by vitamin D supplements, while it might be difficult to change prepregnancy BMI. A recent study has shown that in obese mothers, who often have difficulties breastfeeding, a low-cost intervention with increased telephone support could increase the duration of breastfeeding considerably [16]. The study is observational and the results show associations and thereby not necessarily causality, as pointed out by the authors. We are still waiting for intervention studies showing that a reduction of so-called early risk factors result in less obesity. If society is to use resources on prevention of childhood obesity, it is important to establish evidence for an effect.
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References