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ORIGINAL RESEARCH

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Different protein intake in the first year and its effects on adiposity rebound and obesity throughout childhood: 11 years follow-up of a randomized controlled trial

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Summary

Background and Objectives: Infant feeding affects child growth and later obesity risk. We examined whether protein supply in infancy affects the adiposity rebound, body mass index (BMI) and overweight and obesity up to 11 years of age.

Methods: We enrolled healthy term infants from five European countries in a double blind randomized trial, with anticipated 16 examinations within 11 years follow-up. Formula-fed infants ($n = 1090$) were randomized to isoenergetic formula with higher or lower protein content within the range stipulated by EU legislation in 2001. A breastfed reference group ($n = 588$) was included. Adiposity rebound and BMI trajectories were estimated by generalized additive mixed models in 917 children, with 712 participating in the 11 year follow-up.

Results: BMI trajectories were elevated in the higher compared to the lower protein group, with significantly different BMI at adiposity rebound (0.24 kg/m^2, 0.01–0.47, $p = 0.040$), and an increased risk for overweight at 11 years (adjusted Odds Ratio 1.70; 1.06–2.73; $p = 0.027$) but no significant difference for obesity (adjusted Odds Ratio 1.47; 0.66–3.27). The two formula groups did not differ in the timing of

Clinical Trial Registration
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adiposity rebound, but all children with obesity at 11 years had an early adiposity rebound before four years.

Conclusions: Compared to conventional high protein formula, feeding lower protein formula in infancy lowers BMI trajectories up to 11 years and achieves similar BMI values at adiposity rebound as observed in breastfed infants.

KEYWORDS
adiposity rebound, BMI trajectory, breastfeeding, obesity, protein

1 | INTRODUCTION

Childhood obesity is a major public health concern: Children with obesity are five times more likely to become adults with obesity,[1] and even if they do not become adults with obesity, they have an increased risk for later type 2 diabetes and cardiovascular disease.[2] Although the combined prevalence of overweight and obesity in 2- to 13-year-old children stabilized in most European countries (1999–2016), the prevalence of about 19 to 25% across European regions is still alarmingly high.[3] Therefore, effective early prevention of overweight and obesity is urgently needed.[4] Critical growth periods in childhood have been linked to later obesity risk.[5] Early weight gain results in a first adiposity peak around the age of 1 year,[6,7] followed by the adiposity rebound between 2 and 9 years, a second rise in body mass index (BMI) after its nadir in the second year of life.[8,9,10] Both of these changing points have been proposed as critical risk predictors for later overweight and obesity depending on their timing and their intensity (BMI at adiposity peak and at adiposity rebound).Of interest, these characteristic changing points in BMI trajectories have also been linked to early nutrition.[11,12] Breastfeeding has been associated with a significantly lower later obesity risk compared to formula feeding in numerous observational studies, with one meta-analysis of 25 studies reporting a pooled adjusted odds ratio of 0.78 with 95% confidence interval of 0.74–0.81.[13] This protective effect of breastfeeding might in part be attributable to lower protein supply, as compared to conventional infant formula feeding. The "early protein hypothesis" suggests, that higher protein intake in the first year(s) of life enhance adipogenic activity as a result of increased levels of insulin-like-growth-factor-1 and insulin.[14,15] The effect of early protein supply on early weight gain was confirmed in controlled intervention trials comparing formulas with different protein contents, including previous results from our Childhood Obesity Project[16,17,18]: lower protein supply by infant formula results in significantly lower BMI z-score at 2 years compared to higher protein supply (estimated difference 0.23, 95% CI 0.089, 0.36, $p = 0.001$). Previously, we reported a marked impact of infant protein supply on obesity prevalence[19] and body fatness[20] at the age of 6 years. However, evidence from controlled intervention trials on possible longer term effects of early nutrition on the timing of and the BMI at adiposity rebound and on the BMI in later childhood is lacking, and results of observational cohort studies are inconsistent.[21,22,23] We followed participants of the double-masked randomized CHOP trial with a dietary intervention in the first year of life (higher vs. lower protein content formula, compared to a reference group of breastfed children) up to 11 years of age and analysed BMI trajectories, timing of and BMI at adiposity rebound, and the risk for overweight and obesity at 11 years. We hypothesized that the early intervention with lower protein content formula in the first year lowers BMI trajectories to a level comparable to those of breastfed infants, leads to differences in timing of and BMI at adiposity rebound, and lowers the risk for overweight and obesity at 8 and 11 years.

2 | SUBJECTS AND METHODS

2.1 | Study design

The CHOP study is a multicentre double blind randomized controlled intervention trial that enrolled 1678 healthy term infants born with an appropriate for gestational age birthweight in Belgium (Brussels, Liège), Germany (Munich, Nuremberg), Italy (Milano), Poland (Warsaw), and Spain (Reus, Tarragona). Details of the study design and conduct were previously published.[17] Within the first two months of life (median age 14 days), formula fed infants were randomized to infant and follow-on formula with higher protein (HP) content (1.6 g/dl in infant and 3.2 g/dl in follow-on formula) or lower protein (LP) content (1.25 g/dl and 2.05 g/dl) provided for the first year of life. Compliant infants received study formula for at least 90% of the feedings. The respective protein levels were chosen to be similar to the range of protein contents stipulated by European Union legislation at the time. Adjustment of fat contents achieved isoenergetic formulas in the two randomized arms.[17] Exclusively breastfed (BF) infants (for at least 3 months) were included as a reference.[17]

2.2 | Outcome measurements

Repeatedly trained study personnel performed anthropometric measurements following carefully designed standard operating procedures[24] during study visits at the ages of 3 and 6 months, 1 and 2 years, biannually until 6 years, at 7, 8 and 11 years. These anthropometric measurements included body weight, body height and skinfold thicknesses. BMI and fat mass index (FMI, based on the Slaughter equation for skinfolds) were calculated.[20,25] Overweight and obesity were defined based on International Obesity Task Force BMI cut offs.[26]

2.3 | Statistical methods

BMI trajectories from the age of 1 year (end of the intervention) to 11 years were compared between higher and lower formula fed and breastfed infants using a generalized additive mixed model (GAMM) including random intercept and slope for age and a feeding group (HP, LP, BF) specific thin plate regression spline for age with 7 knots and penalty 3. Wald confidence regions were plotted based on the bayesian posterior covariance.

Timing of and BMI at adiposity rebound were assessed as follows: We estimated BMI trajectories from age 1 to 11 years for all children with more than 3 observations between 1 and 7 years (GAMM with thin plate regression spline for age, basis dimension 5, grade of penalty 2, individual random intercept and slope for age). Model-based predicted BMI at and timing of adiposity rebound were defined at the first increase of 0.2 BMI-units after the minimum estimated BMI (see online supporting information for more details, graphical examples and R-code). We categorized the adiposity rebound as very early (≤3.5 years), early (>3.5 and ≤5 years) or normal and late (>5 years) as previously proposed.[10,23]

Infant feeding effects (HP, LP and BF) on and associations with continuous outcomes (age at adiposity rebound and BMI at adiposity rebound) were estimated by linear regression models; on and with dichotomous outcomes (overweight, obesity) by generalized linear regression models (with logit link).

Generally, we adjusted for BMI z-score at inclusion (within the first 8 weeks of life) and sex ("crude model"), further for smoking in pregnancy (yes/no), maternal prepregnancy BMI, parental highest education (low, middle, high), mode of delivery (Caesarean section or vaginal birth) and study country ("adjusted", only exceptions are generalized linear models for the risk of overweight and obesity at 8 and 11 years, where study country was eliminated from the adjustment due to low prevalences). Interactions with sex were included but discarded when they were not significant. Confounding effects of adjustment factors were reported in case of relevant effect changes.

Stratified sample mean and quantiles of BMI and FMI were plotted for visualization.

Significance was assumed at an error probability of 0.05. Analyses were carried out with R 3.5.3 (The R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org).

2.4 | Ethics statement

All study procedures were in line with the Declaration of Helsinki and were reviewed by the competent local ethical committees of all participating study centres. Parents gave written informed consent. Breastfeeding was actively encouraged and supported during recruitment. Intervention formulas complied in all aspects to the 1991 EU Directive on Infant and Follow-on Formulae.[27]

3 | RESULTS

3.1 | Study population

Of the originally enrolled 1678 infants, 1066 participated in measurements at 1 year and 917 were included in analyses of the adiposity rebound. Some 712 children (42%) participated in the 11 year follow-up (Figure 1).

Cohort characteristics at study start and dropout-reasons until 6 years of age were previously reported.[19] We summarized characteristics and changes to 11 years of age in Tables S1 and S2. While the breastfed group differs in several aspects from the formula fed group (higher parental education, lower smoking rate in pregnancy, lower rate of Caesarean delivery), formula groups showed no differences at

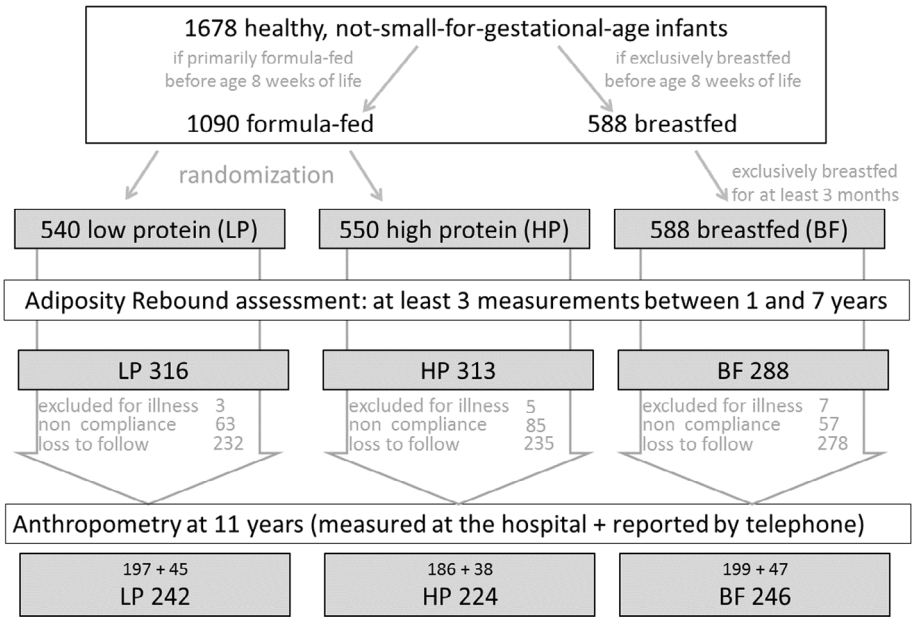

FIGURE 1 Flowchart

randomization. At 11 years, there is a higher rate of Caesarean delivery and maternal smoking in pregnancy in LP. Dropout is highest in families with lower education level.

After the intervention period, dietary intake assessed by 3-day weighed food protocols showed no group differences in macronutrient intakes (protein, carbohydrates and fat in grams per day) at all ages up to 8 years (data not shown).

3.2 | BMI trajectories

BMI trajectories vary between the feeding groups (Figure 2). At the end of the intervention period (age 1 year), HP starts with significant higher BMI levels than LP and BF as a result of increased early weight gain in HP compared to LP and BF during the intervention.[17] At 2 years, BMI in HP is also significantly higher than in LP. From 2 to 8 years, the differences steadily increase: HP shows highest and BF lowest predicted BMI trajectories. The 95%-confidence region indicates a pointwise significant lower BMI trajectory of LP compared to HP (Figure 2, HP black line outside of the confidence region for LP) across ages and significant lower BMI trajectory of BF compared to HP (Figure S1B, BF grey dotted line outside of the confidence region for HP). The results are hardly changed after adjusting for maternal and baseline characteristics (Figure S1A and B). Stratification by sex yield a stronger long-term effect of the protein intervention in boys but main interaction effects were not significant (Figure S1C).

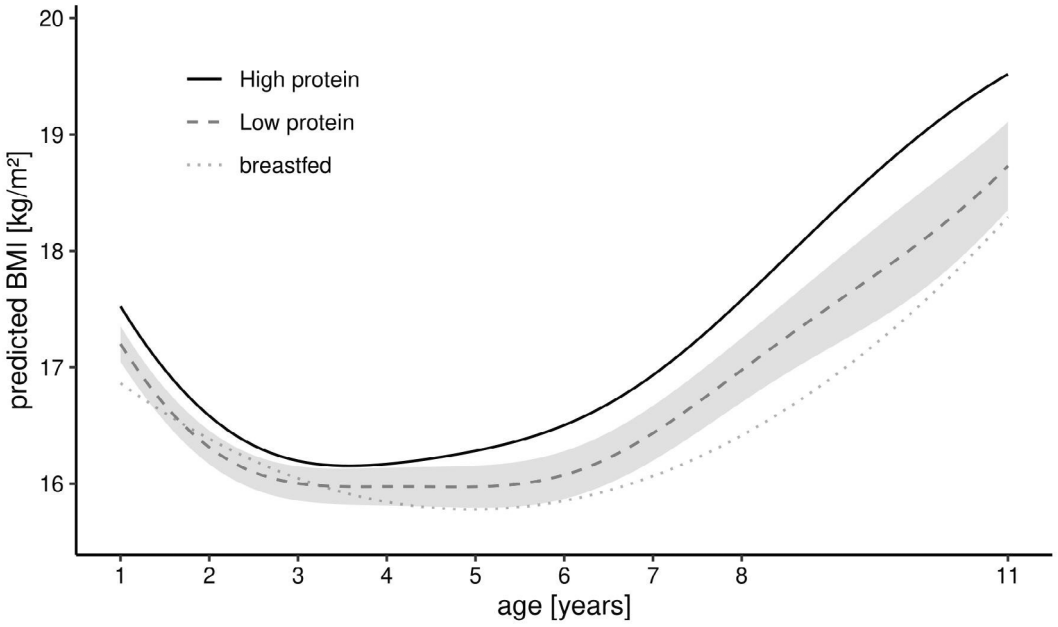

FIGURE 2 Predicted BMI trajectories from 1 to 11 years by feeding group. Crude predicted mean BMI trajectories by generalized additive mixed model with 95% confidence region (grey) for the lower protein group. The model includes an individual random intercept and slope for age, feeding group specific thin plate regression spline of age (with 7 knots and penalty of 3) adjusted for BMI z-score at inclusion, sex and feeding group as fixed effects.

TABLE 1 Number of children, timing of and BMI at adiposity rebound stratified by feeding group and gender

	Higher protein		Lower protein		Breastfed	
Adiposity rebound	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Number of children	158	155	146	170	124	164
Mean (SD) of						
Timing of adiposity rebound (years)	5.6 (1.9)	5.6 (1.9)	5.7 (1.7)	5.6 (1.7)	6.0 (1.6)	5.8 (1.7)
BMI at adiposity rebound (kg/m^2)	15.86 (1.36)	15.88 (1.35)	15.70 (1.30)	15.61 (1.13)	15.54 (1.28)	15.57 (1.13)
Number of children with adiposity rebound						
Very early (<=3.5 years)	22	24	12	22	10	17
Early (>3.5 and < =5 years)	34	32	45	41	23	35
Normal/late (>5 years)	102	99	89	107	91	112

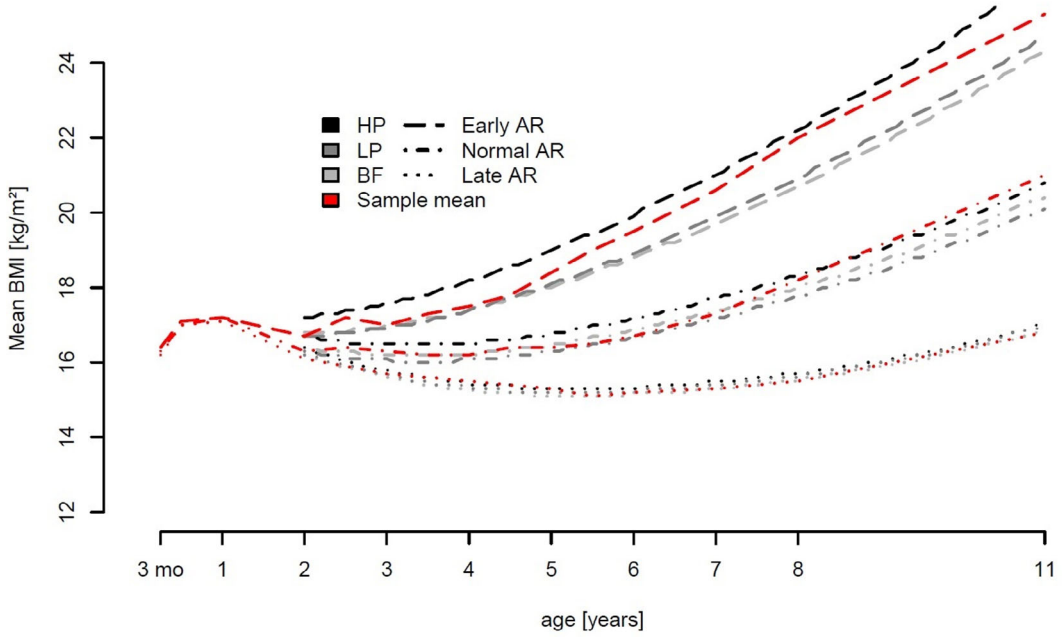

FIGURE 3 BMI trajectories by the categorized timing of adiposity rebound (very early, early, normal or late) stratified by feeding group. Predicted mean trajectories from crude assessment models (GAMM with thin plate regression spline for age, basis dimension 5, grade of penalty 2, individual random intercept and slope for age, as used for the adiposity rebound assessment) are depicted in black for the higher protein group, dark grey for the lower protein group and light grey for the breastfed group. Dotted lines represent the group with normal and late adiposity rebound (>5 years), dash - dotted those with early adiposity rebound (<=5 years and > =3.5 years) and long dashed lines those with very early adiposity rebound (<3.5 years). For comparisons we added the stratified sample means in red, respectively.

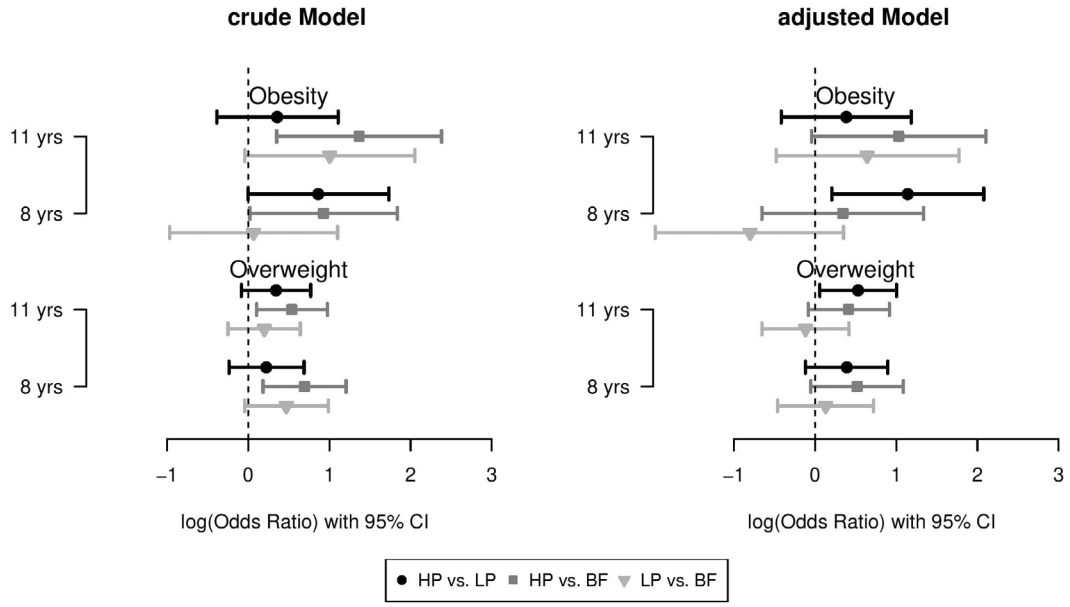

FIGURE 4 Odds ratios for the risk of overweight (including obesity) and obesity at age 8 and 11 years. Crude and adjusted odds ratios were estimated by generalized linear models. The crude model includes BMI z-score at inclusion and sex, adjusted model includes additionally smoking in pregnancy (yes/no), maternal prepregnancy BMI, parental highest education (low, middle, high), and mode of delivery (Caesarean section/vaginal birth), due to low prevalences study country was eliminated from the adjustment factors.

The upper distribution quantiles are particularly affected by the intervention. Group specific trajectories of the mean, the 80th and the 95th BMI percentile are plotted in Figure S2.

3.3 | Adiposity rebound

Feeding group specific means of BMI at and timing of adiposity rebound are listed in Table 1 stratified by sex.

We did not observe differences in the timing of adiposity rebound between HP and LP. Both formula groups together show an about 9 months earlier adiposity rebound than breastfed infants (−0.28 years, −0.52 to −0.03, $p = 0.03$, unadjusted). In adjusted analyses, maternal BMI (−0.10 years, −0.13 to −0.08, $p < 0.001$) and Caesarean section (−0.43 years, −0.71 to −0.15, $p = 0.003$) have the highest impact on timing of adiposity rebound, and the feeding effect of formula versus breastfeeding vanished. BMI at adiposity rebound is significantly higher in HP compared to LP (0.24 kg/m^2, 0.01–0.47; $p = 0.04$) and BF (0.34 kg/m^2, 0.10–0.57; $p = 0.002$). There were no differences between BF and LP. Adjustment with maternal and baseline characteristics lowered the effect size between HP and BF (−0.28 kg/m^2, 0.01–0.47, $p = 0.07$) but the effect between the formula groups was unchanged.

Predicted BMI trajectories for very early, early, normal or late rebounder stratified by feeding group are depicted in Figure 3.

3.4 | Overweight and obesity

Numbers of children with overweight (including obesity) and obesity at 8 and 11 years are listed in Table S2. Odds ratios between the feeding groups on log-scale are depicted in Figure 4.

The risk for overweight and obesity at 8 and 11 years tends to be higher in HP compared to LP and BF (all Odds ratios >1) with significant differences in the adjusted model for obesity at 8 years (adjusted OR 3.13; 1.23–7.99; $p = 0.017$) and for overweight at 11 years (adjusted OR 1.90; 1.12–3.21; $p = 0.017$) (Figure 4). At 8 and 11 years, no child with late adiposity rebound (after 5 years) is considered as child with obesity but 94% of all children with obesity at 8 years (96% at 11 years) were very early rebounders (before 3.5 years). All children with obesity showed an adiposity rebound younger than 4 years.

4 | DISCUSSION

This study shows infant feeding to have a significant and long-lasting impact on BMI trajectories. Higher protein intake with a cow's milk-based formula in infancy induced higher early weight gain to a higher adiposity peak and higher BMI at adiposity rebound, compared to lower protein intakes. Timing of and BMI at adiposity rebound are strongly associated with BMI, overweight and obesity at 11 years. Up to early adolescence, the effects of the early protein intervention were most pronounced in the upper quantiles of the BMI distribution. Consequently, higher protein intake in infancy induces an increased risk for overweight at 11 years of age.

Considering the BMI trajectory from birth to 11 years, the evolution of obesity seems to start with high early weight gain, resulting in higher BMI at adiposity peak, followed by a less emphasized nadir to an earlier adiposity rebound with again higher BMI. Although we did not observe an association between protein intake in the first year and timing of adiposity rebound, both characteristic periods (adiposity peak and adiposity rebound) showed increased BMI levels in the HP group resulting in higher rates of overweight and obesity at 11 years of age.

4.1 | Impact of infant feeding

Nutrition in the first year of life, as evaluated in our randomized trial, modifies early weight gain and has long-lasting effects on growth and BMI until 11 years of age. Evidence has accumulated demonstrating that limiting protein intake in early life is a promising strategy to normalize early weight gain and to attenuate later obesity risk.[16,18,19,28,29,30]

Effects of a nutritional intervention in infancy on the later adiposity rebound have not been studied previously in a randomized controlled intervention trial. Observational cohort studies have explored the hypothesis that protein intake can have a programming effect on early adiposity rebound and subsequent obesity, as first proposed by Rolland-Chachera in 1995,[22] but results were inconclusive: In the ALSPAC study,[23] protein intake at 18 months was not associated to the timing of adiposity rebound but the association to BMI at adiposity rebound was not examined.[23] However, the ALSPAC study in the UK shows the predictive value of adiposity rebound for later obesity risk.[10] The DONALD study in Germany[21] found an association of protein intakes at the ages of 12 to 24 months with BMI at adiposity rebound in girls but not in boys.[21] No association to the timing of adiposity rebound was reported. Both these observational studies focused on protein intake in the second year of life. In the GenerationR study in the Netherlands, total protein intake and particularly animal protein at 12 months were associated with higher BMI and FMI trajectories up to the age of 10 years.[31,32,33] Other studies also reported an association of protein intake in early childhood (between 1.5 and 4 years) with BMI or obesity around or after the age of adiposity rebound (between 4 and 8 years).[34,35,36]

Less evidence is available on effects of protein supply during the first year of life. Scaglioni et al. reported that protein intake at 12 months was the only macronutrient associated to the risk of overweight at 5.5 years.[37] A small study in Iceland showed associations of animal protein intake at 12 months with higher BMI at 6 years.[38] Further studies on protein intake in the first year lack long-term follow-up.[16,18,29] An association of longer breastfeeding duration with later adiposity rebound was reported in an Australian[39] and in a Swedish study[40] (for Swedish mothers, not for immigrants).

Overall, the available data suggest that protein intake in early life may impact on BMI during childhood and the onset of obesity, but

conclusive evidence from controlled intervention trials with long-term follow-up has been lacking.

4.2 | Adiposity rebound predicts later obesity

Timing of and BMI at the adiposity rebound are strongly associated with later obesity. In our study, each year earlier adiposity rebound accounts for an increase of 1.7 BMI-units (−1.72 kg/m^2/year; −1.80 to −1.66; $p < 0.001$, −1.68 adjusted) at 11 years of age; an increase in BMI at adiposity rebound of 1 BMI-unit doubles at 11 years (1.99; 1.85 to 2.14; $p < 0.001$, adjusted 1.91). To which extent modifiable factors such as early nutrition matter has been controversial. Cole et al.[41] suggested that there is no critical predictive period, but rather that early rebounders would represent children whose BMI centiles are following an upward crossing pattern. In a commentary,[8] Rolland-Cachera and Cole proposed that only those children whose BMI increase predominantly after adiposity rebound have an increased risk for later metabolic disorders, whereas high early weight gain would play a subordinated role.[8] In contrast, high early weight gain is one of the best predictors for later obesity.[5,6,7]

Of interest, early rebounders show a disproportionally high acquisition of fat mass, which occurs primarily about 1–2 years after BMI rebound.[42,43] In our study, the difference in fat mass gain from 5.5 years to 11 years in children with early adiposity rebound, compared to children with a normal/late adiposity rebound, is 12 kg representing about 70% of total weight gain during this time (Figures S3 and S4). Avoiding excessive fat mass acquisition related to early adiposity rebound may be an important opportunity for targeted effective strategies for promotion of later health and the prevention of insulin resistance related to increased body fat deposition, and of related cardio-metabolic risks.

4.3 | Public health impact

In view of the high burden of obesity and its long-term consequences for children in their later life, the preventive potential of early interventions has received considerable attention and has been highlighted by a respective report of the World Health Organization.[44] Considering accumulating indications of possible adverse effects of high protein intakes in infancy, the protein contents of infant formula have been lowered in many products used around the world over the last two decades. In the European Union, the maximum permitted protein content in regular cow's milk protein infant formula has been reduced from previously 3 g/100 kcal[27] to now 2.5 g/100 kcal[45] in infant formulas and from previously 4.5 g /100 kcal[27]to 2.5 g/100 kcal[45] in follow-on formulas, while the required minimum protein content in follow-on formulas has recently been reduced from 1.8 g/100 kcal[45] to now 1.6 g/100 kcal.[46] Therefore, today, the protein supply of formula fed infants following current European standards or similar concepts in other parts of the world will not reach the intake of infants receiving higher protein formula in the CHOP trial. However, very high protein intakes can still occur in infancy by feeding unmodified cow's milk and complementary foods with high content of (dairy) protein. The possible adverse effect of a high infant protein intake is underlined by the following fact in the CHOP trial: 55% of early rebounders in the lower protein group and 52% in the breastfed group became children with overweight or obesity at 6 years of age, in the higher protein group a much higher proportion of 70% of those with very early and early adiposity rebound suffer from overweight and obesity from age 6 years onwards. It appears that early detection of growth trajectories with an upward crossing pattern might offer added opportunities for early targeted interventions to reduce later obesity risk.

4.4 | Strength and limitations

Our study has some major strengths compared to other studies. Due to the longitudinal design with sequential follow-up examinations, we could analyse the BMI development during the course of pre-pubertal childhood, including early weight gain, adiposity rebound and BMI at the onset of puberty, in one model without the necessity of cross-sectional analysis. Further, the double-masked randomization to higher and lower protein formula feeding in the first year ensured consistent differences in protein intake over the whole intervention period,[17] and not only at selected cross-sectional time points with dietary assessments that can be deducted from observational studies. The multi-centre design is both a strength and a limitation. Data on children from five European countries are more generalizable, but at the same time, confounding dietary and lifestyle effects may partly impede analysis and interpretation. Timing of adiposity rebound has been reported to be quite different across studies.[21,23,39,42,47] Due to the lack of a commonly used definition of adiposity rebound, we referred to previous published categorization but used continuous data in most analyses. The loss-to follow-up (follow-up rate of 42.4%) is a limitation, but is not unusual for trials offering no major benefits for the individual participants and with such a long follow-up time.[48] Due to the large initial sample size ($N = 1678$), the final results are still based on a rather large sample of 712 children at 11 years of age. The age of 11 years may be less than ideal for evaluating BMI and obesity risk since it is prior to or during the early stages of puberty, and hence added variability of related growth data may be expected. Therefore, a further follow-up of these children at a time point after puberty is highly desirable.

5 | CONCLUSION

Providing infant formula with reduced protein contents during the first year of life induced a lower trajectory of BMI evolution from 3 months to 11 years, compared to a conventional infant formula with a high protein content. Both breastfeeding and formula with lower protein content lead to significantly lower BMI at adiposity rebound and to lower BMI trajectories thereafter compared to feeding high

protein formula. In this study, all children with obesity at 11 years had an adiposity rebound before 4 years, with very high fat mass acquisition thereafter. We conclude that avoidance of excessive protein intakes in infancy can contribute to reducing the burden of childhood obesity.

AUTHOR CONTRIBUTIONS

Martina Totzauer was responsible for data management, analysis, literature search, drafted the manuscript and generated the figures. Prof Koletzko conceptualized and initiated the CHOP trial and acts as Principal Investigator and Guarantor. Prof Escribano, Prof Closa-Monasterolo, Prof Verduci, Prof Jean-Paul Langhendries, Prof Gruszfeld, Prof Socha, Prof Koletzko jointly designed the CHOP trial, acted as Principal Site Investigators. Dr Luque, Mrs ReDionigi, Mrs Xhonneux, Mrs Martin designed the data collection instruments, and collected data. Dr Grote coordinated and supervised data collection, management, and the data analysis reported here. All authors reviewed and contributed to the revision of the manuscript, and all approved the final manuscript as submitted.

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CONFLICT OF INTEREST

All authors declare: no support from any organization other than the Commission of the European Community for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years The participating company had no decisive role in the conduct and analysis of the study.

DATA AVAILABILITY STATEMENT

Deidentified individual participant data will not be directly made available due to the details of consent and personal data protection, but study data can be accessed through the EU Child Cohort Network as part of the EU lifecycle project (https://lifecycle-project.eu).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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