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Original Research Article

Infant Milk Formula with Large, Milk Phospholipid-coated Lipid Droplets Enriched in Dairy Lipids Affects Body Mass Index Trajectories and Blood Pressure at School Age: Follow-up of a Randomized Controlled Trial

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A B S T R A C T

Background: Human milk comprises large fat globules enveloped by a native phospholipid membrane, whereas infant formulas contain small, protein-coated lipid droplets. Previous experimental studies indicated that mimicking the architecture of human milk lipid droplets in infant milk formula (IMF) alters lipid metabolism with lasting beneficial impact on later metabolic health.

Objectives: To evaluate in a follow-up (FU) study of a randomized, controlled trial whether a Concept IMF with large, milk phospholipid-coated lipid droplets enriched with dairy lipids beneficially impacts long-term body mass index (BMI in kg/m^2) trajectories and blood pressure at school age.

Methods: Fully formula-fed infants were randomly assigned to Concept IMF ($n = 115$) or Control IMF with conventional, small lipid droplets containing vegetable oils ($n = 108$) for the first 4 mo of age. A group of 88 breastfed infants served as a reference. During FU, anthropometrics were collected at 1, 3, 4, and 5 y of age, and blood pressure only at the last visit.

Results: Compared to Control, Concept group children had consistently lower mean BMI values during FU, with the most marked difference at 1 y of age (difference in means –0.71 kg/m^2, 95% confidence interval (CI): –1.13, –0.29; $P = 0.001$); mean values were close to the breastfed group ($P > 0.05$). Contrary, the mean BMI values of the Control group were higher compared with the breastfed group during FU from 1 to 5 y of age (differences in means from 0.59 to 0.96 kg/m^2, respectively; $P < 0.02$). At 5 y of age, the Concept group had a lower mean diastolic and arterial blood pressure compared with the Control group; –4.3mm Hg (95% CI: –7.3, –1.3; $P = 0.005$) and –3.7 mm Hg (95% CI: –6.5, –0.9; $P = 0.01$), respectively.

Conclusions: Early life feeding of an innovative IMF with large, milk phospholipid-coated lipid droplets enriched with dairy lipids results in a BMI trajectory closer to breastfed infants and a lower blood pressure at school age.

This trial was registered at the Dutch Trial Register as NTR3683 and NTR5538.

Keywords: infant nutrition, dietary lipid quality, growth trajectories, nutritional programming, milk fat globule membrane (MFGM), metabolic health, childhood

Abbreviations: ABP, arterial blood pressure; ANCOVA, analysis of covariance; CHOP, European childhood obesity project; DBP, diastolic blood pressure; FFM, fat-free mass; FM, fat mass; FMI, fat mass index; FU, follow-up; IMF, infant milk formula; ITT, intention-to-treat; MMRM, a linear mixed effects model for repeated measurements; PP, per protocol; SBP, systolic blood pressure.

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Introduction

The global prevalence of overweight and obesity has rapidly increased over the past decades, reaching epidemic proportions, and is therefore considered a major public health threat [1]. An early onset of overweight and obesity is paralleled by early signs of metabolic syndrome [2,3] and is associated with increased metabolic and cardiovascular disease risks throughout childhood and adulthood [4,5]. Accelerated weight gain during early infancy is associated with increased childhood overweight risk [6,7] and cardiovascular disease risk in early adulthood [8]. This underlines the relevance of early excess weight gain prevention as a strategy to counteract this emerging public health threat.

Exclusive human milk is the preferred feeding for infants and provides a complete supply of nutrients to support infant development. Compared with formula feeding, human milk feeding is associated with dissimilar growth and adiposity patterns during infancy [9–11] and may have a protective effect on childhood overweight and adverse metabolic health outcomes [12–14]. Given the complexity of human milk, the mechanism is most likely multifactorial [15–17], and the causal factors and biological mechanisms for this long-term health impact are still to be unraveled [18].

One specific contributing factor could be the distinct differences in the supramolecular structure of the lipid droplets between human milk, consisting of large fat globules enveloped by a tri-layered, native phospholipid membrane, and infant milk formula (IMF), containing small lipid droplets primarily coated by proteins [19,20]. A concept IMF was developed with large, milk phospholipid-coated lipid droplets, more closely mimicking the characteristics of human milk fat globules [21]. In our previous experimental studies, we confirmed that introducing large, milk phospholipid-coated lipid droplets alters the physiologic response to IMF, i.e., altered digestion kinetics, lipid metabolism, and nutritional programming effects on metabolic and cognitive outcomes [22–25]. Consequently, we postulated that this concept of IMF could also exert a beneficial programming impact on infant growth and adiposity outcomes, further narrowing the gap in (long-term) metabolic health outcomes between formula-fed and breastfed infants.

Originally, the Mercurius study, a multicenter, randomized controlled equivalence trial designed to confirm the nutritional adequacy and safety of the Concept IMF, demonstrated that the concept IMF with large, milk phospholipid-coated lipid droplets enriched with dairy lipids provided during the first months of age, was safe and well-tolerated with an equivalent daily weight gain (primary outcome), daily length gain and daily head circumference gain from baseline to 4 mo of age compared to a Control IMF [26]. The present research reports in the follow-up (FU) of the Mercurius study with a particular interest on later (infancy) weight gain and BMI outcomes until 5 y of age and blood pressure at school age as potential biomarkers for a metabolically healthier trajectory. Since standard infant formulas are typically associated with greater infant weight gains, growth outcomes of the included breastfed reference group and the WHO growth standards were considered indicators of optimal growth patterns.

Study design

From October 2012 to January 2014, a total of 17 study centers from 4 countries (Netherlands, Belgium, France, and Singapore) participated in the Mercurius study: a multicenter, double-blind, randomized controlled clinical equivalence trial to evaluate the safety and tolerance of a concept IMF. The details of the design, as well as primary, safety, and tolerance study outcomes were reported previously [26]. In short, formula-fed infants were enrolled ≤5 wk of age and randomly assigned to receive either Concept ($n = 115$) or Control IMF ($n = 108$) until 4 mo of age. A group of fully breastfed infants ($n = 88$) was included as a reference. After initiation and before completion of subject enrolment of the Mercurius study, it was decided to amend the protocol and to include an additional (optional) visit at 12 mo of age in the study design, requiring additional informed consent from the parents. Subsequently, the present Mercurius (FU) study was initiated, inviting the participants who at least completed the Mercurius study until the end of the intervention period at 4 mo of age to attend study visits at 3, 4, and 5 y of age. A total of 10 study centers from 3 countries agreed to participate in this FU study, in which subjects were enrolled between January 2016 and August 2018: the Netherlands (Erasmus University Medical Centre/Sophia Children's Hospital, Rotterdam; Albert Schweitzer Ziekenhuis, Dordrecht; Amphia Ziekenhuis, Breda; Isala Klinieken Zwolle, Zwolle; Medisch Spectrum Twente, Enschede), Belgium (Algemeen Stedelijk Ziekenhuis, Aalst; Universitair Ziekenhuis, Brussel; Clinique et Maternité Sainte-Elisabeth, Namur; Centre Hospitalier Régional de la Citadelle, Liège) and Singapore (KK Women's and Children's Hospital, Singapore). All participating centers obtained approval from the relevant ethical review board. The Mercurius study and its FU were conducted according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use-Good Clinical Practice principles, in compliance with the principles of the Declaration of Helsinki and with the local laws and regulations of the countries where the study was performed. The original 4-mo intervention study, including the optional visit at 12 mo, was registered in the Dutch Trial Register (Mercurius study; https://trialresearch.who.int) as NTR3683. The Mercurius FU study from 3 to 5 y of age was registered in the Dutch Trial Register as NTR5538. Written informed consent was obtained from all parents/guardians before enrolment into both studies.

Subjects

Healthy, term-born infants, either fully formula-fed or fully breastfed, were eligible for participation in the intervention study if they had a gestational age between 37 and 42 wk, postnatal age ≤35 d, a birth weight between the 10th and 90th percentiles according to the Dutch Growth Charts [27] and a head circumference within the normal range for age and sex (within 2 SDs according to WHO Growth Standard [28]). Infants with illnesses that could interfere with the study, special dietary needs, a mother diagnosed with hepatitis B or HIV, participation in any other study, or having parents who might not be able to comply with the protocol requirements were excluded from participation in the original study. Enrolled formula-fed infants were randomly assigned to 1 of the 2 intervention formulas with sex, continent (Europe or Asia), and age at randomization (≤14 d or >14 d) as strata. The intervention formulas were provided from baseline to 4 mo of age. IMFs were coded by the sponsor as letter codes (A, B, C, or D) and provided in otherwise identical tins; investigators and parents were blinded to the formulas. The database was unblinded after the database lock of the Mercurius study (the last subject completed the last visit at 12 mo of age). However, to secure (single) blinding during the Mercurius FU study, only information on the group level was shared with the investigators and parents. All subjects of the participating study centers that took part in the Mercurius study until 4 mo of age were eligible for participation in the FU study until 5 y of age. No further in- or exclusion criteria were defined for participation in the FU study. To facilitate enrolment, parents who were not reachable when

their child turned 3 (or 4) y of age could start participating when the child turned 4 (or 5) y of age.

Intervention

The randomly assigned formula-fed infants received 1 of the 2 study formulas, manufactured by Danone Nutricia Research per good manufacturing practices (ISO 22000) and compliant with Directive 2006/141/EC, during the intervention period from randomization until 4 mo of age. The intervention formulas differed in the size and coating of their lipid droplets and the origin of their lipid sources but were isocaloric, i.e., containing a similar amount of protein, lipids, and fatty acid profile, carbohydrates as well as the specific prebiotic mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (ratio of 9:1) (Supplemental Table 1). Like most conventional IMFs, the lipid droplets in the Control IMF comprised vegetable oil and had a volume-based mode diameter of 0.5 μm and proteins as main emulsifiers. In contrast, the lipid droplets in the Concept IMF contained a mixture of vegetable (52%) and milk fat (48%) and had a volume-based mode diameter of 3–5 μm with an interface predominantly composed of milk phospholipids that are generated within an innovative production process [21] (Nuturis, patent EP2825062A1).

Parents were instructed to provide the study formulas ad libitum and as the sole source of nutrition until the end of the intervention period at 4 mo. Lactating mothers were asked to fully breastfeed (or provide expressed milk) until ≥3 mo of age. Indeed, in the original Mercurius study, ~75% of the lactating mothers continued exclusive human milk feeding until 3 mo of age. The median duration of exclusive human milk feeding was 127 d (interquartile range 92.5–135 d of age) for the breastfed reference group.

Methods

Demographic data and infant characteristics were collected via interview using a questionnaire at the enrolment visit of the original Mercurius study. Weight, length (height as from 3 y of age), and head circumference were measured at randomization (baseline), monthly ≤4 mo of age, and thereafter at 12 mo, and at 3, 4, and 5 y of age. Early infancy rapid weight gain, a risk factor for childhood overweight and adverse metabolic outcomes, was defined as an increase in weight SD score of ≥0.67 SD from baseline to 4 mo of age. Anthropometric data were converted to (normalized) *z*-scores using the macro provided by the WHO growth standards [28]. Overweight and obese were defined as having a BMI-for-age *z*-score ≥85th percentile and ≥97th percentile of the WHO Growth standards, respectively.

Starting at 3 mo of age, supra-iliac, subscapular, biceps, and triceps skin folds were measured at each visit. The percentage of fat mass (% FM) was derived from the skinfold thicknesses using the Slaughter equation formulated for fatness predictions in children [29]. This %FM was used to derive the total fat mass (FM in kg; weight (kg) multiplied by %FM), which was used to define FM Index (FMI, kg/m^2, FM (kg) divided by height (m) squared). Similarly, the fat-free mass (FFM, kg) was calculated based on weight minus FM, and subsequently, the % FFM and FFM index were calculated. Waist circumference was measured at 3, 4, and 5 y of age. Each planned study visit had to take place within a ±5 d window for the monthly visits, within ±14 d window at the 12 mo visit, and within ±60 d window for all later time points. All measurements were taken twice, and in case of substantial differences between the measurements (as specified in the protocol), a third measurement was obtained; the mean of the 2 closest measurements was used as the outcome value for the anthropometrical parameter at a time point.

Systolic (SBP) and diastolic (DBP) blood pressure outcomes were measured at the right brachial artery, 4 times at 1-min intervals, using a sphygmomanometer while the subject was in a sitting position after 5 min of rest. The mean of the 4 measurements was used as the outcome value. Arterial blood pressure (ABP) was defined based on SBP and DBP as follows: ABP = (1/3 * SBP) + (2/3 * DBP). According to the AAP Clinical Practice Guidelines, blood pressure outcomes were defined according to age-, sex-, and height-standardized SBP and DBP values as normal (SBP and DBP <90th percentile), elevated (SBP or DBP values ≥ 90th and ≤ 95th percentile) or hypertensive (SBP or DBP ≥95th percentile) [30].

All measurements were performed by trained study personnel using calibrated equipment and according to standard protocols.

Statistical analysis

We hypothesized that children in the Concept group would have growth patterns persistently closer to the breastfed group, resulting in a lower weight gain during infancy (0–12 mo) and lower BMI values up to 5 y of age than the Control group. As such, we defined the weight gain during infancy (0–12 mo) as a key secondary outcome parameter of the Mercurius study. Given the nature of the Mercurius FU study, no primary or secondary outcomes were defined for this FU study. However, growth outcomes were considered as key parameters, with BMI as 1 of the main key outcome parameters of interest, whereas SBP and DBP outcomes were considered as exploratory outcome parameters.

In the Mercurius FU study (until 5 y), for the evaluation of differences between Concept and Control groups in BMI and the anthropometric *z*-scores at time points as well as between time points, a linear mixed effects model for repeated measurements (MMRM) was applied with *1*) fixed effects for visit, sex, age at study entry (≤14 d, >14 d), continent (Europe, Asia), birthweight and treatment; *2*) fixed effects interaction terms of: sex by visit, treatment by visit and birthweight by visit; and *3*) unstructured covariance matrix for the repeated measurements within subjects. For the evaluation of differences in growth outcomes between either formula groups with the breastfed group, the model was extended with additional terms: *1*) fixed effects for smoking during pregnancy (yes/no), education of the mother (none/primary, high/trade school, at least university) and maternal prepregnancy BMI; and *2*) fixed effects interaction terms of these additional terms by visit.

Given the strong impact of maternal BMI on offspring growth outcomes, a stratified analysis of BMI-for-age *z*-score was done for infants born from mothers with a normal weight or underweight before pregnancy (BMI ≤25 kg/m^2) compared with mothers with overweight or obesity (BMI >25 kg/m^2; with 23 kg/m^2 as cut-off used for the population from the Asian continent). For the evaluation of between-group differences, the MMRM model was extended with additional terms of maternal weight category (normal weight or underweight, overweight or obese), its interaction with treatment, with visit, and with treatment by visit (stratified MMRM).

Potential differences in incidences of overweight or obesity in infants between intervention formula groups were analyzed by Firth's penalized likelihood logistic regression method, with group, sex, continent, age at study entry, and weight at birth as covariates. For comparing the formula groups to the breastfed reference group, the same model was used with the addition of maternal education, maternal BMI, and maternal smoking as covariates.

For the evaluation of differences between Concept and Control groups in weight, length, and head circumference, a linear mixed

effects model was applied with *1*) fixed effects for sex, age at study entry (≤14 d, >14 d), continent (Europe and Asia), birthweight, treatment, age, and age squared until 4 mo of age and thereafter piecewise linear (from 4 mo to 12 mo, from 12 mo to 3 y, from 3 to 4 y and from 4 to 5 y of age); *2*) fixed effects interaction terms of sex by (each of the) age terms, treatment by age terms, and birthweight by age terms; and *3*) random effects for (each of the) age terms with the unstructured covariance matrix. For the evaluation of differences in growth outcomes between either formula groups with the breastfed group, the model was extended with additional terms: *1*) fixed effects for smoking during pregnancy (yes/no), education of the mother (none/primary, high/trade school, at least university) and maternal prepregnancy BMI; and *2*) fixed effects interaction terms of these additional terms by age-related fixed effects. Differences in the incidence of early infancy rapid weight gain from baseline to 4 mo of age or incidence of overweight and obesity were evaluated using a logistic regression model with terms for sex, age at study entry, continent, treatment, and birthweight for all comparisons, and for the comparison with the breastfed group, additionally education of the mother (none/primary, high/trade school, at least university) and maternal prepregnancy BMI.

For the evaluation of differences between the groups in blood pressure outcomes at 5 y (SBP, DBP, and ABP), an analysis of covariance (ANCOVA) was used with terms for the treatment group, maternal education, continent, maternal prepregnancy BMI, sex, child BMI at 5 y; to evaluate group differences for boys and girls, this model was additionally extended with the group by sex interaction. For the evaluation of differences between the groups in prevalence of elevated blood pressure (including hypertensive, i.e., SBP or DBP ≥90th percentile), a Firth's penalized likelihood logistic regression model was used, with the same factors as used for the ANCOVA analyses except for sex by group interaction.

For the evaluation differences between the formula groups in FMI and %FM, an ANCOVA was used per year, with terms for sex, age at study entry (≤14 d, >14 d), continent (Europe and Asia), birth weight, and treatment. For the evaluation of differences between the formula groups and the breastfeeding reference, the ANCOVA model additionally adjusted for smoking during pregnancy (yes/no), education of the mother (none/primary, high/trade school, at least university), and maternal prepregnancy BMI. These analyses were performed on a log-transformed FMI and %FM.

Although the intention-to-treat (ITT) population was defined as the leading population, we also evaluated the outcomes for the per-protocol (PP) population as a supportive analysis. The PP population was primarily defined for the equivalence analyses of the primary parameter in the Mercurius study (daily weight gain from randomization to 4 mo of age); thus, subjects with major protocol violation before the first postbaseline visit were excluded; for others, only data collected during visits after a major violation (e.g., other IMFs or solid food consumption) were excluded. Subjects included in the PP population until 4 mo of age were included in the PP dataset for all attended visits thereafter (12 mo until 5 y).

After fitting each model, residual diagnostics were reviewed. A sensitivity analysis was performed, excluding subjects who were identified as outliers or influential during statistical evaluation. For the longitudinal linear mixed effects model, subjects with scaled residuals outside [–4, +4] were identified as outliers, and the ones with large Cook's D and Covariance Ratio statistics as influential.

The sample size of the original 4-mo intervention study was based on the primary parameter defined on (growth) data ≤17 wk of age. The FU study was not powered to detect any particular differences in the explored parameters (at various time points and time intervals). The analyses were considered to be a hypothesis-generating nature, with the main focus on estimating the between-group differences rather than on statistical significance testing. However, for completeness, unadjusted *P* values were provided without multiplicity adjustments performed. All statistical analyses were performed using SAS (SAS version 9.4_TS1M3 or higher in SAS Life Science Analytics Framework version 4.7.3 or higher) for LIN X64 (SAS Institute Inc).

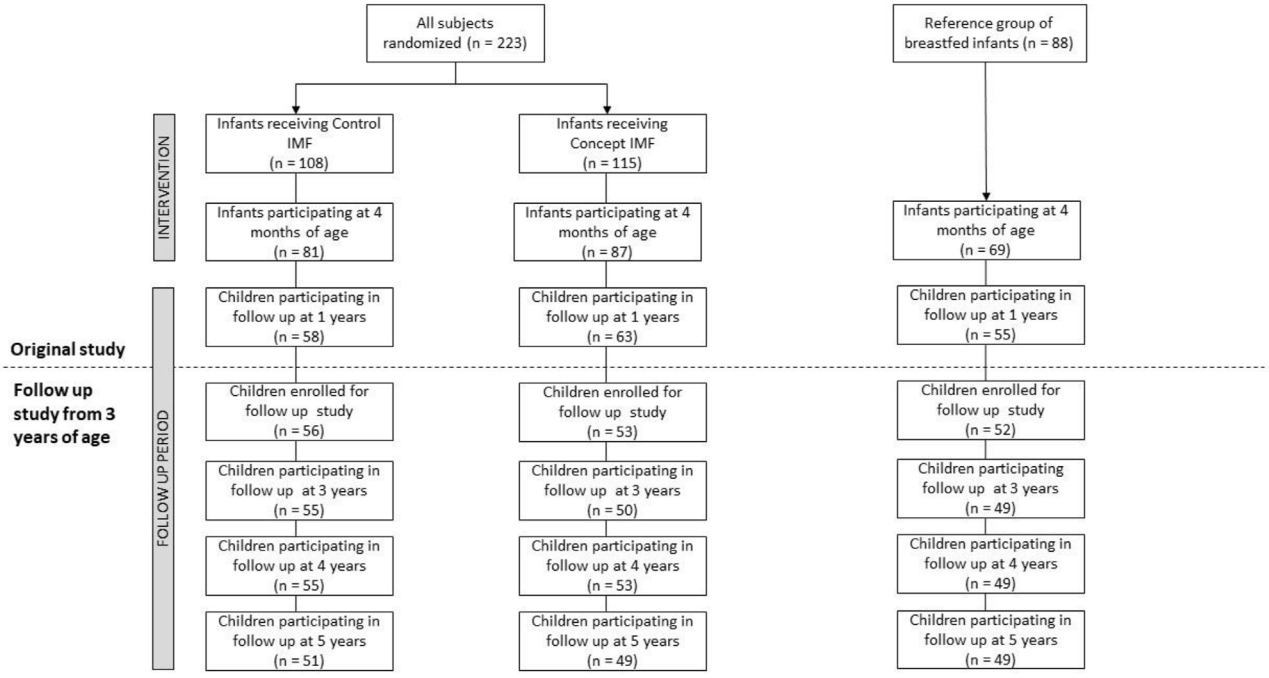


FIGURE 1. Flow chart of subjects enrolled in Mercurius and Mercurius FU study. IMF, infant milk formula; FU, follow-up.

Results

Study population

A total of 237 (76%) of the 311 infants enrolled in the Mercurius study completed the last visit of the intervention period at 4 mo of age, and 176 infants attended the optional FU visit at 12 mo of age (Figure 1). A total of 97 originally enrolled subjects were not eligible for participation in the FU study, of which 74 subjects did not complete the Mercurius study ≤4 mo of age, and for 23 subjects, their respective study site did not participate in the FU study. Hence, a total of 214 children were eligible for participation in the Mercurius FU study, of which 3 quarters ($n = 161$) consented to be enrolled. Upon enrolment in the FU study, a remarkably high retention rate was accomplished until 5 y of age (149 subjects attended the 5 y visit; 7.45% drop out; Figure 1). Twelve subjects terminated the FU study early (4 subjects in the Concept group, 5 in the Control group, 3 in the breastfed group), all because of parental lack of time or interest. The participation rate in the FU study across countries was as follows: in the Netherlands, 74 subjects were recruited by 5 sites (1 site from the original study did not participate in the FU); in Belgium, 68 subjects were recruited in 4 sites (3 sites did not participate), in Singapore 19 subjects were recruited by 1 site, in France none of the 3 sites participated in the FU study.

Demographic data recorded at enrolment into the original Mercurius study for the subjects participating in the FU study were not apparently different between both formula groups (Table 1). Compared to both formula groups, infants in the breastfed group were older at enrolment into the original study [mean (SD) age of 10.0 (9.4) and 12.1 (10.1) d of age compared with 19.8 (10.9) d of age], had a higher percentage of mothers with a university degree and the group included a higher percentage of subjects from the Asian continent (Table 1). The infant population of the FU study had more infants older than 14 d of age at enrolment compared with the full infant population that participated in the original study in the formula groups (32% in FU compared with 24% in the original study) as well as in the breastfed group (71% in FU compared with 61% in the original study) (Supplemental Table 2). The circumstance that 7 European study sites did not participate in the FU study and the very successful retention of subjects enrolled in Singapore (from 21 subjects, only 2 subjects from the Concept group did not participate in the FU) resulted in a relatively increased contribution of infants from the Asian continent in the FU study, particularly in the breastfed group (from 4.1% to 6.4% for the randomly assigned groups and from 13.6% to 23.1% in the breastfed group). In the breastfed group, the percentage of mothers with a normal prepregnancy BMI increased from 50% to 62% for the FU compared to the original study. None of the other demographic characteristics of the participants in the FU study were apparently different from the original equivalence study population (Table 1 and Supplemental Table 2). Demographics of the PP population of the FU study participants were highly similar to those of the ITT population (Supplemental Table 3).

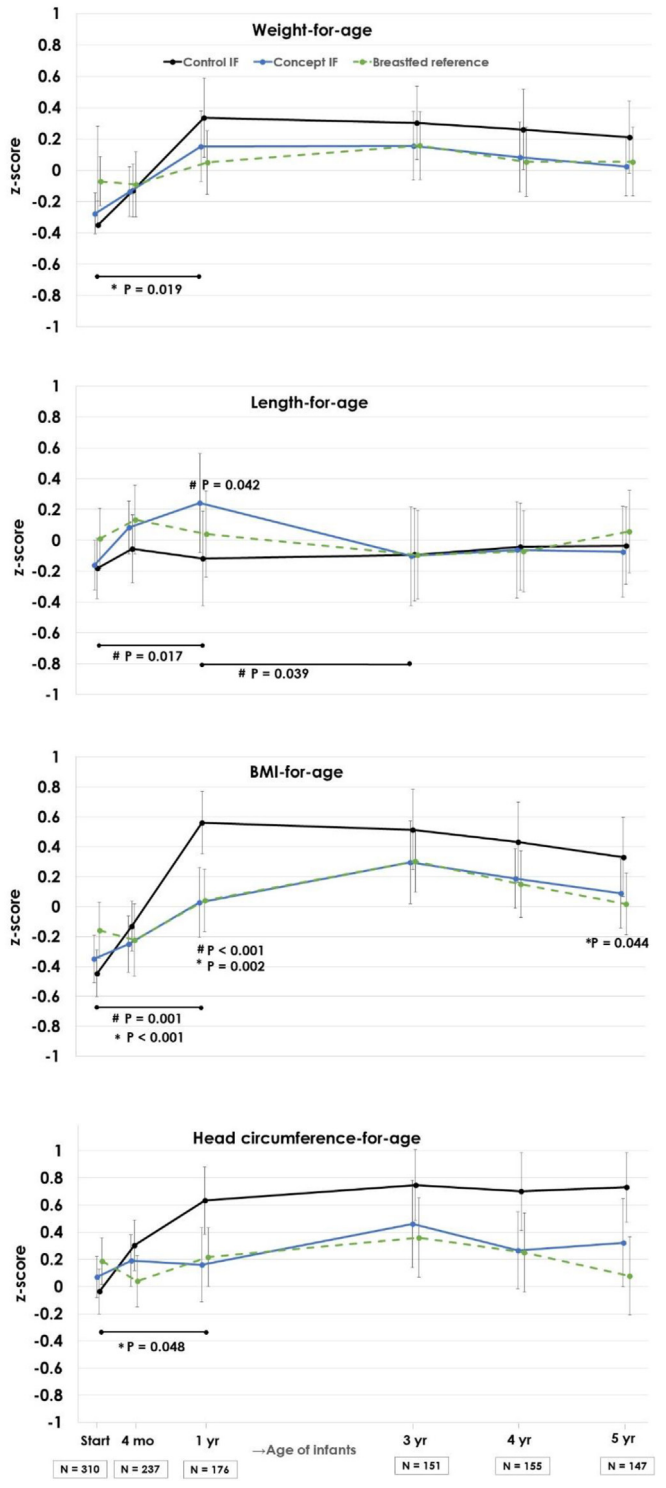
Weight and BMI gain during infancy

The estimated mean weight-for-age z-score change during the full first year of life, from baseline to 12 mo of age, was comparable for Concept compared with the Control group but higher in both formula groups compared to the breastfed group ($P = 0.073$ for Concept and $P = 0.019$ for Control group; Supplemental Table 4 and Figure 2). Moreover, the current research shows that the incidence of early infancy rapid weight gain (≥0.67 SD) between baseline and 4 mo of age, a risk factor for childhood overweight and adverse metabolic outcomes, was 23.3% ($n = 20$) in the Concept and 28.8% ($n = 23$) in the Control group and 20.3% ($n = 14$) for the breastfed group. The estimated odds ratios (ORs) for all pairwise comparisons lay between 0.92 and 0.97 (P

TABLE 1
Demographic characteristics collected at enrolment in the original Mercurius study of infants participating in the follow-up study (intention-to-treat population)[1]

	Statistic	Concept group ($N = 53$)	Control group ($N = 56$)	Breastfed group ($N = 52$)
Sex				
Male	n (%)	27 (51)	28 (50)	24 (46)
Female	n (%)	26 (49)	28 (50)	28 (54)
Continent				
Asia	n (%)	3 (6)	4 (7)	12 (23)
Europe	n (%)	50 (94)	52 (93)	40 (77)
Age at baseline (d)				
Age ≤14	n (%)	34 (64)	40 (71)	15 (29)
Age ˃14	n (%)	19 (36)	16 (29)	37 (71)
Birth characteristics				
Weight (g)	Median (Q1,Q3)	3350 (3100, 3562)	3380 (3020, 3545)	3370 (3135, 3654)
Length (cm)	Median (Q1,Q3)	50.0 (49.0, 50.0)	49.0 (48.0, 51.0)	50.0 (49.0, 51.0)
Head circumference (cm)	Median (Q1,Q3)	34.5 (33.8, 35.0)	35.0 (34.0, 35.5)	34.0 (33.0, 35.0)
Vaginal delivery	n (%)	33 (62)	40 (71)	36 (69)
Cesarean section	n (%)	20 (38)	16 (29)	16 (31)
Gestational age (wk)	Median (Q1,Q3)	39.4 (38.4, 40.4)	39.5 (38.4, 39.9)	39.6 (38.7, 40.9)
Parental characteristics				
Maternal age (y)	Median (Q1,Q3)	31.0 (29.0, 35.0)	30.5 (27.0, 34.5)	31.5 (29.0, 35.0)
Maternal university education	n (%)	22 (42)	23 (41)	31 (60)
Maternal prepregnancy BMI (kg/m^2)	Median (Q1,Q3)	23.4 (21.2, 28.7)	23.8 (21.4, 27.2)	22.4 (21.0, 25.6)
Paternal BMI (kg/m^2)	Median (Q1,Q3)	24.3 (22.6, 27.7)	25.9 (23.1, 28.5)	24.8 (22.9, 26.3)
Maternal weight status				
Underweight	n (%)	4 (7.5)	1 (1.8)	2 (3.8)
Normal	n (%)	26 (49.1)	38 (67.9)	32 (61.5)
Overweight	n (%)	12 (22.6)	12 (21.4)	13 (25.0)
Obese	n (%)	11 (20.8)	5 (8.9)	5 (9.6)

[1] Demographic data was collected at the enrolment visit from the original Mercurius study (0–35 d of age).



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> 0.05) after adjustment for sex, age at study entry, continent, and birthweight for the comparison of Concept compared with Control and additional adjustment for maternal education and maternal prepregnancy BMI for the comparison of each intervention group compared with the breastfed group.

In addition to weight gain, measures of proportionate growth such as weight-for-length or BMI-for-age *z*-score and their gains are considered (more) appropriate outcomes to assess adiposity during infancy [31]. Interestingly, although the BMI-for-age *z*-score increased from baseline to 12 mo of age in both formula groups, the estimated mean increase for the Concept was markedly smaller than the Control group ($P = 0.001$; Supplemental Table 4 and Figure 2). Moreover, compared to the breastfed group, the mean BMI-for-age *z*-score change from baseline to 12 mo of age was higher in the Control group ($P < 0.001$; Supplemental Table 4 and Figure 2). Contrary, the mean BMI-for-age *z*-score change from baseline to 12 mo of age in the Concept group was close to that in the breastfed group (Supplemental Table 4 and Figure 2).

The described findings for BMI-for-age *z*-score and weight-for-age *z*-score changes were confirmed for the PP population and after the exclusion of influential subjects (data not shown).

Longitudinal anthropometric measurements ≤5 y of age

Throughout the study and its FU, a consistently lower BMI and BMI-for-age *z*-score was observed in the Concept compared to the Control group (Figure 2 and Table 2), reaching statistical significance with a mean estimated difference [95% confidence interval (CI)] of –0.71 kg/m^2 (–1.13, –0.29; $P = 0.001$), and –0.49 SD score (–0.77, –0.21; $P < 0.001$) at 12 mo of age. Notably, in the PP population, the difference between the Concept and Control groups in BMI and BMI-for-age *z*-score development was even more apparent and statistically significant at 4 mo, 1, 4, and 5 y of age (Supplemental Table 5 and Supplemental Figure 1). During sensitivity analysis, the exclusion of 3–4 influential subjects from the Control group in this PP population resulted in smaller estimated mean differences in BMI and BMI-for-age

FIGURE 2. Mean *z*-score (with 95% CIs) for weight-for-age, length-for-age, BMI-for-age, and head circumference-for-age per intervention group and in the breastfed reference group of the ITT population from baseline to 5 y of age. Control IF (black line): intervention with standard infant formula from start to 4 mo of age. Concept IF (blue line): intervention with infant formula containing large, milk phospholipid-coated lipid droplets and dairy lipids from start to 4 mo of age. Breastfed reference (green dotted line): infants that were exclusively breastfed at enrolment from mothers with the intention to continue full human milk feeding for at least up to 3 mo of age. A linear mixed effects model for repeated measurements was applied with *1*) fixed effects for a visit, sex, age at study entry (≤14 d, >14 d), continent (Europe and Asia), birth weight, and treatment; *2*) fixed effects interaction terms of: sex by visit, treatment by visit and birthweight by visit; and *3*) unstructured covariance matrix for the repeated measurements within subjects. For the evaluation of differences in growth outcomes between either formula groups with the breastfed group, the model was extended with additional terms: *1*) fixed effects for smoking during pregnancy (yes/no), education of the mother (none/primary, high/trade school, at least university) and maternal prepregnancy BMI; and *2*) fixed effects interaction terms of these additional terms by visit. Interaction effect for treatment by visit for weight-for-age $P < 0.0001$; length-for-age $P = 0.0727$; BMI-for-age $P = 0.0002$; head circumference-for-age $P = 0.0987$. In case P values <0.05, the specific P value is provided for group comparisons at specific time points and for the time intervals baseline-12 mo, 1–3 y, and 3–5 y. $^{\#}P < 0.05$ between Concept and Control group. $^{*}P < 0.05$ between the Control and breastfed reference group.
Abbreviations: CI, confidence interval; ITT, intention-to-treat; IF, infant formula.

TABLE 2
Mean anthropometric measures (SD) and estimated LS mean differences (95% confidence interval) between the groups of the intention-to-treat population

Measure	Timepoint	Concept group ($N = 115$) Mean (SD)	Control group ($N = 107$) Mean (SD)	Breastfed group ($N = 77$) Mean (SD)	Estimated difference for concept vs. control[1] LS mean (95% CI), P value		Estimated difference for concept vs. breastfed [2] LS Mean (95% CI) P value		Estimated difference for control vs. breastfed [2] LS Mean (95% CI), P value	
Weight (kg)	Baseline	3.35 (0.36)	3.30 (0.37)	3.38 (0.36)	–0.00 (–0.04, 0.03)	$P = 0.788$	–0.08 (–0.14, –0.02)	$P = 0.013$	–0.07 (–0.13, –0.01)	$P = 0.026$
	4 mo	6.58 (0.67)	6.60 (0.67)	6.64 (0.77)	–0.06 (–0.22, 0.10)	$P = 0.446$	–0.06 (–0.25, 0.13)	$P = 0.533$	0.01 (–0.18, 0.20)	$P = 0.938$
	12 mo	9.52 (1.10)	9.74 (1.19)	9.46 (0.87)	–0.17 (–0.47, 0.14)	$P = 0.285$	0.07 (–0.25, 0.39)	$P = 0.683$	0.24 (–0.09, 0.56)	$P = 0.152$
	3 y	14.59 (1.45)	14.89 (1.57)	14.67 (1.44)	–0.18 (–0.69, 0.32)	$P = 0.477$	0.04 (–0.51, 0.58)	$P = 0.895$	0.23 (–0.31, 0.78)	$P = 0.402$
	4 y	16.52 (1.80)	16.99 (2.18)	16.47 (1.82)	–0.29 (–0.96, 0.38)	$P = 0.402$	0.01 (–0.68, 0.69)	$P = 0.988$	0.35 (–0.34, 1.03)	$P = 0.323$
	5 y	18.44 (1.71)	18.94 (2.25)	18.60 (2.04)	–0.40 (–1.18, 0.38)	$P = 0.315$	–0.21 (–0.99, 0.58)	$P = 0.604$	0.28 (–0.51, 1.07)	$P = 0.486$
Length (cm)	Baseline	50.82 (2.31)	50.73 (2.22)	52.35 (2.70)	–0.08 (–0.41, 0.26)	$P = 0.656$	–0.31 (–0.71, 0.08)	$P = 0.122$	–0.21 (–0.61, 0.19)	$P = 0.304$
	4 mo	62.97 (2.06)	62.73 (2.33)	63.14 (2.05)	0.08 (–0.43, 0.59)	$P = 0.754$	0.05 (–0.50, 0.61)	$P = 0.851$	–0.07 (–0.63, 0.49)	$P = 0.804$
	1 y	75.50 (3.42)	74.68 (3.16)	75.28 (2.66)	0.93 (0.04, 1.81)	$P = 0.041$	0.54 (–0.38, 1.47)	$P = 0.245$	–0.41 (–1.34, 0.52)	$P = 0.387$
	3 y	95.67 (4.38)	95.76 (4.30)	96.02 (3.91)	0.23 (–1.17, 1.63)	$P = 0.751$	0.53 (–0.91, 1.98)	$P = 0.467$	0.15 (–1.30, 1.60)	$P = 0.843$
	4 y	102.85 (4.84)	103.01 (4.56)	102.82 (3.89)	0.30 (–1.21, 1.81)	$P = 0.699$	0.36 (–1.17, 1.89)	$P = 0.647$	–0.04 (–1.58, 1.50)	$P = 0.960$
	5 y	109.34 (4.96)	109.39 (4.22)	110.09 (4.38)	0.42 (–1.18, 2.03)	$P = 0.605$	0.06 (–1.59, 1.71)	$P = 0.940$	–0.47 (–2.13, 1.18)	$P = 0.574$
BMI (kg/m^2)	Baseline	13.16 (1.14)	13.03 (1.11)	13.86 (1.45)	0.02 (–0.20, 0.24)	$P = 0.869$	–0.42 (–0.70, –0.13)	$P = 0.005$	–0.42 (–0.72, –0.13)	$P = 0.005$
	4 mo	16.57 (1.29)	16.74 (1.12)	16.63 (1.56)	–0.24 (–0.60, 0.11)	$P = 0.179$	0.13 (–0.29, 0.55)	$P = 0.542$	0.34 (–0.09, 0.77)	$P = 0.119$
	1 y	16.68 (1.31)	17.41 (1.24)	16.67 (1.09)	–0.71 (–1.13, –0.29)	$P = 0.001$	0.24 (–0.21, 0.70)	$P = 0.296$	0.96 (0.49, 1.42)	$P < 0.001$
	3 y	15.94 (1.29)	16.24 (1.36)	15.90 (0.97)	–0.28 (–0.75, 0.19)	$P = 0.238$	0.26 (–0.23, 0.75)	$P = 0.293$	0.59 (0.10, 1.08)	$P = 0.018$
	4 y	15.60 (0.99)	15.98 (1.46)	15.55 (1.10)	–0.36 (–0.81, 0.09)	$P = 0.114$	0.26 (–0.21, 0.74)	$P = 0.271$	0.68 (0.20, 1.15)	$P = 0.005$
	5 y	15.44 (1.17)	15.81 (1.50)	15.31 (1.00)	–0.41 (–0.93, 0.10)	$P = 0.114$	0.23 (–0.28, 0.74)	$P = 0.369$	0.73 (0.22, 1.24)	$P = 0.005$

Data are presented as means (SD).

Abbreviations: CI, confidence interval; SD, standard deviation; LS, least squares.

[1] Derived for weight and length from a linear mixed effects model with *1*) fixed effects for sex, age at study entry (≤14 d, >14 d), continent (Europe and Asia), birthweight, treatment, age, and age squared until 4 mo of age and thereafter piece-wise linear (from 4 mo to 12 mo, from 12 mo to 3 y, from 3 to 4 y and from 4 to 5 y of age); *2*) fixed effects interaction terms of: sex by (each of the) age terms, treatment by age terms and birthweight by age terms; and *3*) random effects for (each of the) age terms with unstructured covariance matrix and for BMI – from a linear mixed effects model for repeated measurements with *1*) fixed effects for a visit, sex, age at study entry (≤14 d, >14 d), continent (Europe and Asia), birthweight and treatment; *2*) fixed effects interaction terms of: sex by visit, treatment by visit and birthweight by visit; and *3*) unstructured covariance matrix for the repeated measurements within subjects.

[2] For the comparison to the breastfed reference group, the models as listed[1] were extended with additional terms, fixed effects for maternal smoking during pregnancy, prepregnancy BMI, and education level, as well as their interaction with age-related fixed effect terms (for weight and length) and with visit (for BMI); The P value for the treatment by visit interaction for the BMI outcomes was $P = 0.0003$. The 95% CIs in parentheses, followed by the P value.

z-scores for Concept compared to the Control group; $P < 0.05$ at 4 mo and 1 y of age only (Supplemental Table 5).

Strikingly, throughout the study, mean BMI values and BMI-for-age *z*-scores observed in the Concept group were much closer to the breastfed group. In contrast, from 12 mo of age onwards, the Control group had consistently higher mean absolute BMI and BMI-for-age *z*-scores compared to the breastfed group in the ITT (Figure 2 and Table 2) as well as in the PP population (Supplemental Figure 1 and Supplemental Table 5). During sensitivity analysis, the exclusion of 3–4 influential subjects from the Control group in the PP population did not affect the findings on absolute BMI outcomes, but for the BMI-for-age *z*-scores, only the differences in means at 1 y remained significant (Supplemental Table 5).

Throughout the study period until 5 y of age, the weight-for-age and head circumference-for-age *z*-scores were not statistically significantly different at any time point between any of the study groups (in pairwise comparisons; Figure 2). In parallel to the change in weight-for-age *z*-scores, a notable change in head circumference-for-age *z*-score was observed in the first year of life for the Control compared to the breastfed reference group ($P = 0.048$; Figure 2). Interestingly, a transient higher mean length-for-age *z*-score was observed in the Concept compared to the Control group, with the most marked difference at 12 mo of age (estimated difference in mean length-for-age *z*-score of 0.37 SD, $P = 0.042$; Figure 2). The breastfed group had intermediate length outcomes. No apparent differences in waist circumference at 3, 4, and 5 y of age or in skinfolds from 3 mo to 5 y of age or their derived parameters, i.e., sum of skinfolds, calculated %FM or FMI, were observed between any of the study groups (Supplemental Table 6). Moreover, no remarkable differences in child overweight or obesity incidences were observed at the time points from 1 to 5 y of age between any of the study groups (Supplemental Table 7).

BMI development in infants at risk for childhood overweight

Given the known substantial impact of maternal BMI on offspring growth trajectories, a stratified analysis was performed on BMI-for-

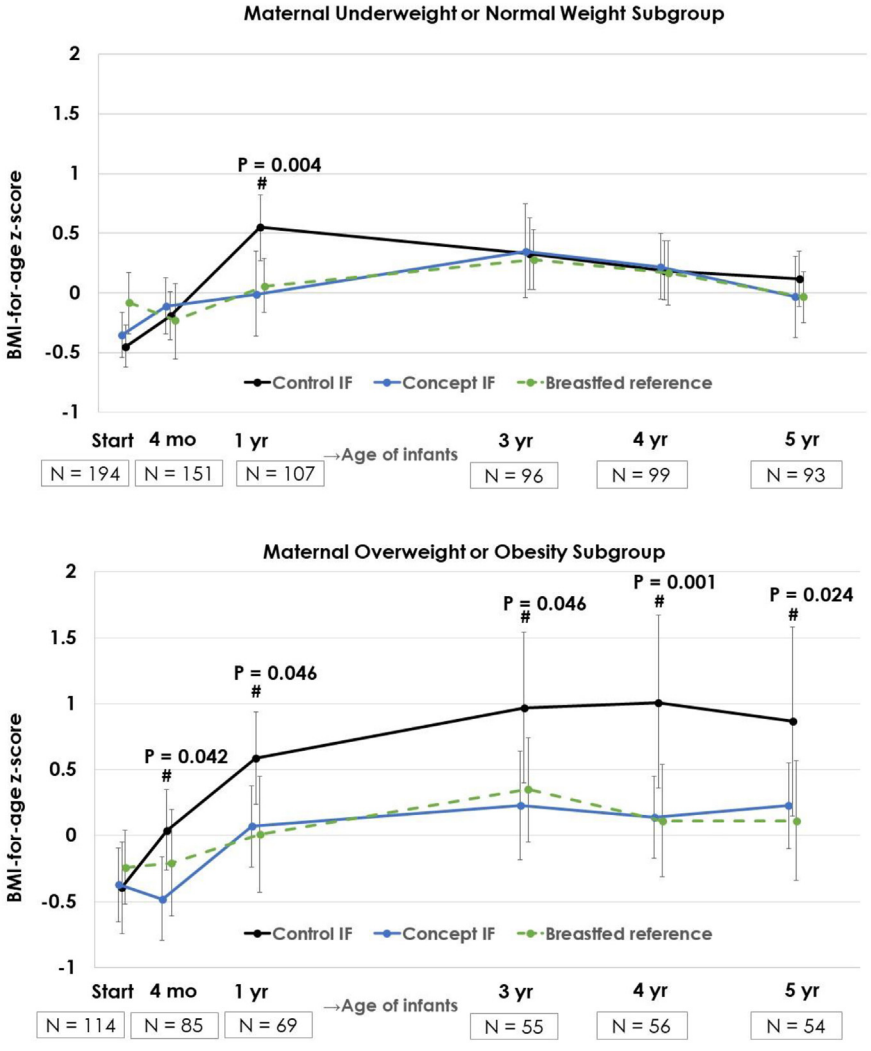

FIGURE 3. Mean (95% CI) BMI *z*-score per intervention group stratified according to maternal BMI status (ITT population). #$P < 0.05$ between Concept and Control group. No statistical comparisons with the breastfed reference group are provided. Maternal weight categories for mothers from the European continent: normal/underweight (BMI <25) and overweight/obese (BMI ≥25). Maternal weight categories for mothers from the Asian continent: normal/underweight (BMI <23) and overweight/obese (BMI ≥23). At baseline, the number of infants from mothers with normal weight compared with overweight/obesity was 70 and 44 for the Concept, 77 and 29 for the Control, and 47 and 41 for the breastfed reference group. At 5 y of age, these numbers decreased to 26 and 22, 36 and 14, and 31 and 18 for each of these groups, respectively. Abbreviations: CI, confidence interval; ITT, intention-to-treat population.

TABLE 3
Blood pressure outcomes at 5 y of age per study group of the intention-to-treat population population[1]

Blood pressure (mm Hg)	Concept group ($N = 47$)	Control group ($N = 46$)	Breastfed group ($N = 47$)	Estimated difference for concept vs. control	Estimated difference for concept vs. breastfed	Estimated difference for control vs. breastfed
Diastolic blood pressure	57.9 (6.0)	61.7 (6.9)	59.5 (8.8)	–4.2 (–7.1, –1.3) $P = 0.006$[2] –4.3 (–7.3, –1.3) $P = 0.005$[3]	–2.6 (–5.6, 0.4) $P = 0.094$[2] –2.6 (–5.6, 0.4) $P = 0.093$[3]	1.6 (–1.4, 4.6) $P = 0.298$[2] 1.7 (–1.4, 4.8) $P = 0.276$[3]
Systolic blood pressure	95.5 (7.3)	98.0 (10.0)	97.5 (8.8)	–2.8 (–6.1, 0.5) $P = 0.098$[2] –2.4 (–5.7, 0.9) $P = 0.154$[3]	–4.8 (–8.2, –1.4) $P = 0.006$[2] –4.7 (–8.1, –1.3) $P = 0.007$[3]	–2.0 (–5.4, 1.4) $P = 0.249$[2] –2.3 (–5.7, 1.1) $P = 0.186$[3]
Arterial blood pressure	70.4 (5.7)	73.8 (7.2)	72.2 (8.0)	–3.7 (–6.5, –1.0) $P = 0.008$[2] –3.7 (–6.5, –0.9) $P = 0.010$[3]	–3.3 (–6.1, –0.5) $P = 0.022$[2] –3.3 (–6.1, –0.5) $P = 0.023$[3]	0.4 (–2.4, 3.2) $P = 0.778$[2] 0.4 (–2.5, 3.2) $P = 0.801$[3]

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; SD, standard deviation.
[1] Limited to the subjects with blood pressure measurement at 5 y of age. Data are presented as means (SD).
[2] Derived from an ANCOVA model with maternal education, continent, prepregnancy maternal BMI, and sex as covariates.
[3] Derived from an ANCOVA model with maternal education, continent, prepregnancy maternal BMI, sex, and child BMI at 5 y as covariates; 95% CIs in parentheses, followed by the *P* value.

age *z*-score patterns. Interestingly, in offspring from mothers with overweight or obesity, a substantially lower mean BMI-for-age *z*-score was observed for the Concept group compared to the Control group from 4 mo onwards (estimated difference in means ranging from –0.43 to –0.81 SD, $P < 0.05$; Figure 3). In contrast, in offspring from mothers with a normal weight or underweight, only a markedly lower mean BMI-for-age *z*-score in the Concept group was observed at 12 mo of age (estimated difference in means of –0.53 SD, $P = 0.004$; Figure 3) but not at later time points.

Blood pressure at 5 y

Blood pressure measurements were collected in the vast majority of subjects completing the final visit at 5 y of age, with only 2 subjects out of 49 in the Concept, 5 out of 51 in the Control, and 2 out of 49 in the breastfed group lacking measurements. No apparent differences were found in the demographic data of subjects with blood pressure assessments at 5 y of age compared to the overall ITT population of the Mercurius FU study (data not shown).

Mean DBP was lower in the Concept group than in the Control group (estimated mean difference of –4.3 mm Hg; 95% CI: –7.3, –1.3); $P = 0.005$, Table 3). Mean SBP was also lower, but with a smaller (absolute) estimated difference in means between Concept compared with Control group (–2.4 mm Hg; 95% CI: –5.7, 0.9); $P = 0.154$). As a result, the mean ABP was lower in the Concept group compared to the Control group (difference in means –3.7 mm Hg 95% CI: –6.5, –0.9; $P = 0.010$; Table 3). Compared to the breastfed group, the mean blood pressure parameters were lower in the Concept group ($P < 0.05$ for SBP and ABP; Table 3), whereas for the Control group, the mean SBP was lower, but the DBP was slightly higher ($P > 0.05$ for all estimated differences in blood pressure outcomes for Control compared with the breastfed group; Table 3). Although the interaction of sex by intervention group was not statistically significant, our analysis suggested that the treatment effect on DBP and ABP may have been more pronounced in girls (difference in estimated means of –5.7 mm Hg; 95% CI: –10.1, –1.4); $P = 0.010$ and –4.8 mm Hg 95% CI: –8.9, –0.8; $P = 0.020$) compared to boys (difference in estimated means of –3.0 mm Hg; 95% CI: –7.2, 1.2; $P = 0.159$ and –2.6 mm Hg 95% CI: –6.5, 1.3); $P = 0.184$), respectively.

Subjects with elevated blood pressure or hypertension were identified across all 3 study groups (9 in Concept, 17 in Control, and 14 in breastfed group). In the Concept group, the prevalence of elevated blood pressure (including hypertension) tended to be lower than in the Control group (OR: 0.38; 95% CI: 0.13, 1.07; $P = 0.066$) and in the breastfed group (OR: 0.34; 95% CI: 0.12, 0.99; $P = 0.049$). No relevant differences in the prevalence of elevated blood pressure were observed when comparing the Control and breastfed group (OR: 0.91; 95% CI: 0.35, 2.38; $P = 0.855$).

Discussion

The current research provides the first clinical investigation of the potential long-term programming impact of lipid droplet characteristics in IMF on BMI patterns and childhood blood pressure. This FU of a randomized, controlled trial suggests that infants consuming an innovative IMF with large, milk phospholipid-coated lipid droplets enriched with dairy lipids in early life have a differential BMI trajectory up to school age, particularly if their mother had overweight or obesity, with values closer to those observed in fully breastfed infants. Moreover, a lower childhood blood pressure was observed at 5 y of age.

Compared to human milk feeding, formula-fed infants typically display a higher infant weight gain and adiposity during later infancy and, as such, a higher obesity risk in later life [32–35]. Based on our previous experimental research [22–25], we hypothesized that bringing the lipid quality of IMF closer to human milk fat globules could optimize growth patterns of formula-fed infants, considering the breastfed reference group and the WHO growth standards as indicators

of optimal growth patterns. Indeed, in our current research, during the infancy period, a significantly lower mean gain in BMI-for-age *z*-score was observed in the Concept group compared to the Control group, with values close to the breastfed group. Length growth appeared to be the strongest contributor to these observed differences in BMI, with a remarkably higher gain in mean length-for-age *z*-score in the Concept compared to the Control group. Body length (gain) has been shown to be the most dominant predictor of lean body mass during infancy [36] and has been positively associated with bone mass in childhood and adolescence [37,38], postulating that the observed differences in length gain may be reflective of higher lean body growth.

FM and BMI outcomes during infancy have been shown to be closely related to their respective values in childhood [39]. Accordingly, in the current research, the differential pattern in infant BMI with a markedly lower value for the Concept group at 12 m ($P < 0.001$) remained during the FU from 3 to 5 y of age, although the effect size was smaller. These group differences were even more apparent in the PP population, suggesting that these can be (partly) attributed to early life exposure to the intervention formula. Remarkably, and in line with our hypothesis, the BMI (*z*-score) trajectory of the Concept group was much closer to the breastfed group. Previous studies evaluating milk fat globule membrane enriched IMFs have confirmed their nutritional adequacy [40,41] but did not result in differential growth outcomes in the first 12–18 mo of life [42–47]. Moreover, the presence of palm oil, palm olein, or sn-2 palmitate in formulas did not seem to influence the anthropometric measures of infants strongly [48]. Our findings are compatible with previous experimental studies, which demonstrated that early life exposure of mice to an IMF diet containing large, milk phospholipid-coated lipid droplets prevented excessive fat accumulation and adverse metabolic outcomes in later life [22,49,50], whereas the mere addition of milk fat globule membrane did not [51]. Thus, it is plausible that the supramolecular structure of the lipid droplets of the Concept formula may have had a definable impact on infant growth trajectories in the current research. Although one could speculate on potential mechanisms for the observed impact, i.e., differential digestion and postprandial lipid kinetics impacting metabolic regulation, fate of nutrients, or energetic efficiency [23,24,52], the precise mode of action remains to be elucidated.

Interestingly, the early life BMI outcomes in the current research equated to reported findings in an intervention study evaluating the impact of a high protein intake level during the first year of life on infant growth [European Childhood Obesity Project (CHOP) study], which ultimately led to a 2.43 times higher risk of obesity during school age [34,53]. Of note, both the Concept and Control formula used in the current research has a protein concentration (1.97 g/100 kcal) quite in range with the lower protein formula (1.77 g/100 kcal) rather than, the higher protein formula (2.9 g/100 kcal) of this CHOP study. Although the difference in *z*-scores for BMI at 5 y of age between the intervention groups of the current research were highly comparable to those reported at 6 y in the CHOP study (0.30; 95% CI: 0.09, 0.52), we did not confirm their associated difference in obesity risk at school age neither for the intervention groups nor for the comparison with the breastfed group. As a final consideration of the potential clinical relevance of any of the aforementioned group differences, all mean values for body weight, length, head circumference, and BMI remained within the adequate growth ranges of the WHO standards.

Interestingly, in the current research, the observed intervention effect on the BMI trajectory was much more pronounced in the offspring of mothers with overweight or obesity compared to offspring of mothers with a normal weight (with differences for mean BMI-for-age *z*-scores between 0.4 SD and 0.8 SD) and persisting from early infancy onwards. Previously, both the impact of early protein intake [53] and maternal BMI [54,55] have been reported to be more pronounced in infants with higher BMI *z*-scores. It is plausible that certain subgroups, such as children from mothers with overweight or children with a high BMI *z*-score, might be more sensitive to nutritional interventions.

Although the reported blood pressure measurements were within reference ranges for all groups, the Concept group had a substantially lower mean blood pressure at 5 y of age than the Control and breastfed reference group. One limitation in the interpretation of these outcomes is the potential challenge of obtaining appropriate and accurate blood pressure measurements in children at the age of 5 y, especially when performed during a clinic visit [56]. Although this setting may have influenced the validity of the measurements, despite the usage of calibrated equipment and standard protocols by trained study personnel in the current research, it seems highly unlikely that this has led to the observed effect sizes between both randomly assigned formula groups. The reported effect size in our research is in line with the beneficial impact reported for consuming long-chain PUFA-supplemented formula during infancy on childhood blood pressure [57]. Interestingly, in the current research, erythrocyte long-chain PUFA concentrations were higher in the Concept compared with the Control group [58] despite their similar content in the IMFs, potentially indicating differences in lipid bioavailability. Since elevated blood pressure during childhood may magnify over time, it is considered a cardiovascular disease risk factor, and as such, early intervention is key [59]. Hence, the findings in the current FU study are considered clinically relevant, although further studies and confirmation are warranted.

The key strengths of the current research were the stringent trial design, including the randomization, multi-country setting, and prospective, long-term FU. During the execution of the trial, all investigators received training and strict manuals for the assessments. Despite the exploratory nature of the FU study, key outcome parameters and their statistical analyses were predefined. Close to 3-quarter of the study population with a completed visit at the end of the intervention period at 4 mo agreed to participate in the FU study, with only 15% dropouts between 1 and 5 y of age. No marked differences in demographics were observed between the participants of the original and FU study, even though only about half of the infants enrolled in the original study participated. Moreover, potentially of even greater importance, no apparent differences in demographic characteristics were observed between the randomly assigned formula groups of the FU study. Hence, we did not find any indication of bias related to the reduced study population size over time. A group of breastfed infants was included as a reference in the current research, and despite the inclusion of influential variables such as maternal smoking, education level, and prepregnancy BMI during statistical evaluation, residual confounding may have existed in comparison with the randomly assigned formula groups. Inspired by human milk composition, the lipid moiety of the Concept formula differed in multiple aspects from the Control formula. Consequently, the findings of the current research cannot be specifically attributed to either the lipid droplet structure or lipid composition per se. Moreover, another limitation of the current study is that we did not adequately collect data on foods consumed in the postintervention period, which may have influenced the anthropometric status of the children during the FU until 5 y of age. Lastly, it is important to emphasize that the current research is a FU of a randomized, controlled trial, which was, as such, not a priori designed or

powered to evaluate the potential long-term impact of the nutritional interventions applied during the infancy period.

In conclusion, the current exploratory FU study suggests that the presence of large, milk phospholipid-coated lipid droplets enriched with dairy lipids in IMF may have a lasting beneficial impact on BMI trajectories and childhood blood pressure at 5 y of age. This new dimension of lipid quality in IMF may further narrow the gap in functional health outcomes compared to breastfed infants. Future longitudinal, larger-scaled clinical studies are required to confirm this potential programming impact on growth, body composition, and metabolic health outcomes.

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Author contributions

The authors' responsibilities were as follows– MA-B, SNJJ, PCK, VR, SMKP, RHTvB, OFN, SS, MM-P, EMvdB, GMSJS, YV, and ACSH-K: designed the trial or substantially contributed to the interpretation of data for the work; SNJJ, PCK, VR, SMKP, RHTvB, OFN, GMSJS, YV, and ACSH-K: conducted the trial; SS, MM-P, and MA-B: were responsible for the statistical analysis; MA-B: wrote all versions of the paper; MA-B and ACSH-K: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

At the time of study conduct, MA-B, SS, MM-P, and EMvdB were employees of Danone Nutricia Research. All other authors report no conflicts of interest.

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Data availability

Data described in the manuscript can be made available upon request, pending application and approval.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.10.017.

References

[1] GBD 2015 Obesity Collaborators, A. Afshin, M.H. Forouzanfar, M.B. Reitsma, P. Sur, K. Estep, et al., Health effects of overweight and obesity in 195 countries over 25 years, N. Engl. J. Med. 377 (1) (2017) 13–27, https://doi.org/10.1056/NEJMoa1614362.

[2] P. Zimmet, K.G. Alberti, F. Kaufman, N. Tajima, M. Silink, S. Arslanian, et al., The metabolic syndrome in children and adolescents - an IDF consensus report, Pediatr. Diabetes. 8 (5) (2007) 299–306, https://doi.org/10.1111/j.1399-5448.2007.00271.x, 2007.

[3] W. Ahrens, L.A. Moreno, S. Mårild, D. Molnár, A. Siani, S. De Henauw, et al., Metabolic syndrome in young children: definitions and results of the IDEFICS study, Int. J. Obes. (Lond.). 38 (Suppl 2) (2014) S4–14, https://doi.org/10.1038/ijo.2014.130.

[4] J.L. Baker, L.W. Olsen, T.I. Sørensen, Childhood body-mass index and the risk of coronary heart disease in adulthood, N. Engl. J. Med. 357 (23) (2007) 2329–2337, https://doi.org/10.1056/NEJMoa072515.

[5] N.E. Thomas, S.M. Cooper, S.P. Williams, J.S. Baker, B. Davies, Relationship of fitness, fatness, and coronary-heart-disease risk factors in 12- to 13-year-olds, Pediatr. Exerc. Sci. 19 (1) (2007) 93–101, https://doi.org/10.1123/pes.19.1.93.

[6] C. Druet, N. Stettler, S. Sharp, R.K. Simmons, C. Cooper, G.D. Smith, et al., Prediction of childhood obesity by infancy weight gain: an individual-level meta-analysis, Paediatr. Perinat. Epidemiol. 26 (1) (2012) 19–26, https://doi.org/10.1111/j.1365-3016.2011.01213.x.

[7] J.A. Woo Baidal, L.M. Locks, E.R. Cheng, T.L. Blake-Lamb, M.E. Perkins, E.M. Taveras, Risk factors for childhood obesity in the first 1,000 days: A systematic review, Am. J. Prev. Med. 50 (6) (2016) 761–779, https://doi.org/10.1016/j.amepre.2015.11.012.

[8] R.W. Leunissen, G.F. Kerkhof, T. Stijnen, A. Hokken-Koelega, Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood, JAMA 301 (21) (2009) 2234–2242, https://doi.org/10.1001/jama.2009.761.

[9] M.S. Kramer, T. Guo, R.W. Platt, I. Vanilovich, Z. Sevkovskaya, I. Dzikovich, et al., Feeding effects on growth during infancy, J. Pediatr. 145 (5) (2004) 600–605, https://doi.org/10.1016/j.jpeds.2004.06.069.

[10] C. Gale, K.M. Logan, S. Santhakumaran, J.R. Parkinson, M.J. Hyde, N. Modi, Effect of breastfeeding compared with formula feeding on infant body composition: a systematic review and meta-analysis, Am. J. Clin. Nutr. 95 (3) (2012) 656–669, https://doi.org/10.3945/ajcn.111.027284.

[11] L.M. Breij, M. Abrahamse-Berkeveld, D. Acton, E. De Lucia Rolfe, K.K. Ong, A.C.S. Hokken-Koelega, Impact of early infant growth, duration of breastfeeding and maternal factors on total body fat mass and visceral fat at 3 and 6 months of age, Ann. Nutr. Metab. 71 (3–4) (2017) 203–210, https://doi.org/10.1159/000481539.

[12] K.G. Dewey, D. Güngör, S.M. Donovan, E.M. Madan, S. Venkatramanan, T.A. Davis, et al., Breastfeeding and risk of overweight in childhood and beyond: a systematic review with emphasis on sibling-pair and intervention studies, Am. J. Clin. Nutr. 114 (5) (2021) 1774–1790, https://doi.org/10.1093/ajcn/nqab206.

[13] L. Wisnieski, J. Kerver, C. Holzman, D. Todem, C. Margerison-Zilko, Breastfeeding and risk of metabolic syndrome in children and adolescents: A systematic review, J. Hum. Lact. 34 (3) (2018) 515–525, https://doi.org/10.1177/0890334417737038.

[14] B.L. Horta, C. Loret de Mola, C.G. Victora, Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: a systematic review and meta-analysis, Acta. Paediatr. 104 (467) (2015) 30–37, https://doi.org/10.1111/apa.13133.

[15] A. Mazzocchi, M.L. Giannì, D. Morniroli, L. Leone, P. Roggero, C. Agostoni, et al., Hormones in breast milk and effect on infants' growth: A systematic review, Nutrients 11 (8) (2019), https://doi.org/10.3390/nu11081845.

[16] S.E. Maessen, J.G.B. Derraik, A. Binia, W.S. Cutfield, Perspective: human milk oligosaccharides: fuel for childhood obesity prevention? Adv. Nutr. 11 (1) (2020) 35–40, https://doi.org/10.1093/advances/nmz093.

[17] B. Koletzko, R. von Kries, R. Closa, J. Escribano, S. Scaglioni, M. Giovannini, et al., Can infant feeding choices modulate later obesity risk? Am. J. Clin. Nutr. 89 (5) (2009) 1502S–1508S, https://doi.org/10.3945/ajcn.2009.27113D.

[18] M. Lemaire, I. Le Huërou-Luron, S. Blat, Effects of infant formula composition on long-term metabolic health, J. Dev. Orig. Health Dis. 9 (6) (2018) 573–589, https://doi.org/10.1017/S2040174417000964.

[19] B. Delplanque, R. Gibson, B. Koletzko, A. Lapillonne, B. Strandvik, Lipid quality in infant nutrition: current knowledge and future opportunities, J. Pediatr. Gastroenterol. Nutr. 61 (1) (2015) 8–17, https://doi.org/10.1097/MPG.0000000000000818.

[20] M.C. Michalski, V. Briard, F. Michel, F. Tasson, P. Poulain, Size distribution of fat globules in human colostrum, breast milk, and infant formula, J. Dairy Sci. 88 (6) (2005) 1927–1940, https://doi.org/10.3168/jds.S0022-0302(05)72868-X.

[21] S. Gallier, K. Vocking, J.A. Post, B. Van De Heijning, D. Acton, E.M. Van Der Beek, et al., A novel infant milk formula concept: mimicking the human milk

fat globule structure, Colloids. Surf. B Biointerfaces. 136 (2015) 329–339, https://doi.org/10.1016/j.colsurfb.2015.09.024.
[22] A. Oosting, D. Kegler, H.J. Wopereis, I.C. Teller, B.J. van de Heijning, H.J. Verkade, et al., Size and phospholipid coating of lipid droplets in the diet of young mice modify body fat accumulation in adulthood, Pediatr. Res. 72 (4) (2012) 362–369, https://doi.org/10.1038/pr.2012.101.
[23] E. Abrahamse, G. Thomassen, B. Van De Heijning, M. Balvers, J. Knol, I.B. Renes, In vitro lipid digestion of infant formula with large milk phospholipid-coated fat droplets is slower than standard infant formula and closer to human milk, J. Pediatr. Gastroenterol. Nutr. (2021), 72(6th World Congress of PGHAN):N-ePwP-001.
[24] S. Baumgartner, B.J.M. van de Heijning, D. Acton, R.P. Mensink, Infant milk fat droplet size and coating affect postprandial responses in healthy adult men: a proof-of-concept study, Eur. J. Clin. Nutr. 71 (9) (2017) 1108–1113, https://doi.org/10.1038/ejcn.2017.50.
[25] L. Schipper, G. van Dijk, L.M. Broersen, M. Loos, N. Bartke, A.J. Scheurink, et al., A postnatal diet containing phospholipids, processed to yield large, phospholipid-coated lipid droplets, affects specific cognitive behaviors in healthy male mice, J. Nutr. 146 (6) (2016) 1155–1161, https://doi.org/10.3945/jn.115.224998.
[26] L.M. Breij, M. Abrahamse-Berkeveld, Y. Vandenplas, S.N.J. Jespers, A.C. de Mol, P.C. Khoo, et al., An infant formula with large, milk phospholipid-coated lipid droplets containing a mixture of dairy and vegetable lipids supports adequate growth and is well tolerated in healthy, term infants, Am. J. Clin. Nutr. 109 (3) (2019) 586–596, https://doi.org/10.1093/ajcn/nqy322.
[27] G.H. Visser, P.H. Eilers, P.M. Elferink-Stinkens, H.M. Merkus, J.M. Wit, New Dutch reference curves for birthweight by gestational age, Early Hum. Dev. 85 (12) (2009) 737–744, https://doi.org/10.1016/j.earlhumdev.2009.09.008.
[28] WHO Multicentre Growth Reference Study Group, WHO Child growth standards based on length/height, weight and age, Acta Paediatr Suppl 450 (2006) 76–85.
[29] M.H. Slaughter, T.G. Lohman, R.A. Boileau, C.A. Horswill, R.J. Stillman, M.D. Van Loan, et al., Skinfold equations for estimation of body fatness in children and youth, Hum. Biol. 60 (5) (1988) 709–723, 1988.
[30] J.T. Flynn, D.C. Kaelber, C.M. Baker-Smith, D. Blowey, A.E. Carroll, S.R. Daniels, et al., Clinical practice guideline for screening and management of high blood pressure in children and adolescents, Pediatrics 140 (3) (2017), https://doi.org/10.1542/peds.2017-1904.
[31] S.M. Roy, J.G. Spivack, M.S. Faith, A. Chesi, J.A. Mitchell, A. Kelly, et al., Infant BMI or weight-for-length and obesity risk in early childhood, Pediatrics 137 (5) (2016), https://doi.org/10.1542/peds.2015-3492.
[32] K.G. Dewey, M.J. Heinig, L.A. Nommsen, J.M. Peerson, B. Lönnerdal, Growth of breast-fed and formula-fed infants from 0 to 18 months: the DARLING Study, Pediatrics 89 (6 Pt 1) (1992) 1035–1041, https://doi.org/10.1542/peds.89.6.1035.
[33] A. Singhal, Does breastfeeding protect from growth acceleration and later obesity? Nestlé Nutr. Workshop Ser. Pediatr. Program. 60 (2007) 15–29 [discussion] -9.
[34] B. Koletzko, R. von Kries, R. Closa, J. Escribano, S. Scaglioni, M. Giovannini, et al., Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial, Am. J. Clin. Nutr. 89 (6) (2009) 1836–1845, https://doi.org/10.3945/ajcn.2008.27091.
[35] M. Zheng, K.J. Campbell, L. Baur, C. Rissel, L.M. Wen, Infant feeding and growth trajectories in early childhood: the application and comparison of two longitudinal modelling approaches, Int. J. Obes. (Lond.). 45 (10) (2021) 2230–2237, https://doi.org/10.1038/s41366-021-00892-5.
[36] W.W. Koo, J.C. Walters, E.M. Hockman, Body composition in human infants at birth and postnatally, J. Nutr. 130 (9) (2000) 2188–2194, https://doi.org/10.1093/jn/130.9.2188.
[37] L. Vidulich, S.A. Norris, N. Cameron, J.M. Pettifor, Infant programming of bone size and bone mass in 10-year-old black and white South African children, Paediatr. Perinat. Epidemiol. 21 (4) (2007) 354–362, https://doi.org/10.1111/j.1365-3016.2007.00806.x.
[38] C. Mølgaard, A. Larnkjær, A.B. Mark, K.F. Michaelsen, Are early growth and nutrition related to bone health in adolescence? The Copenhagen Cohort Study of infant nutrition and growth, Am. J. Clin. Nutr. 94 (6) (2011) 1865S–1869S, https://doi.org/10.3945/ajcn.110.001214. Suppl.
[39] I.A.L.P. van Beijsterveldt, K.S. de Fluiter, L.M. Breij, M. van der Steen, A.C.S. Hokken-Koelega, Fat mass and fat-free mass track from infancy to childhood: new insights in body composition programming in early life, Obesity (Silver Spring) 29 (11) (2021) 1899–1906, https://doi.org/10.1002/oby.23271. PMID 34549538.
[40] C. Billeaud, G. Puccio, E. Saliba, B. Guillois, C. Vaysse, S. Pecquet, et al., Safety and tolerance evaluation of milk fat globule membrane-enriched Infant Formulas: a randomized controlled multicenter non-inferiority trial in healthy term infants, Clin. Med. Insights Pediatr. 8 (2014) 51–60, https://doi.org/10.4137/CMPed.S16962. PMID 25452707.
[41] Y. Li, X. Peng, Z. Li, B. Christensen, A.B. Heckmann, H. Stenlund, et al., Feeding infants formula with probiotics or milk fat globule membrane: A double-blind, randomized controlled trial, Front Pediatr 7 (2019) 347, https://doi.org/10.3389/fped.2019.00347.
[42] N. Timby, E. Domellöf, O. Hernell, B. Lönnerdal, M. Domellöf, Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: a randomized controlled trial, Am. J. Clin. Nutr. 99 (4) (2014) 860–868, https://doi.org/10.3945/ajcn.113.064295.
[43] J. Hedrick, M. Yeiser, C.L. Harris, J.L. Wampler, H.E. London, A.C. Patterson, et al., Infant formula with added bovine milk fat globule membrane and modified iron supports growth and normal iron status at one year of age: A randomized controlled trial, Nutrients 13 (12) (2021), https://doi.org/10.3390/nu13124541.
[44] B. Jiang, Y. Xia, L. Zhou, X. Liang, X. Chen, M. Chen, et al., Safety and tolerance assessment of milk fat globule membrane-enriched Infant Formulas in healthy term Chinese infants: a randomised multicenter controlled trial, BMC Pediatr 22 (1) (2022) 465, https://doi.org/10.1186/s12887-022-03507-8.
[45] A. Nieto-Ruiz, J.A. García-Santos, M.G. Bermúdez, F. Herrmann, E. Diéguez, N. Sepúlveda-Valbuena, et al., Cortical visual evoked potentials and growth in infants fed with bioactive compounds-enriched infant formula: results from COGNIS randomized clinical trial, Nutrients 11 (10) (2019), https://doi.org/10.3390/nu11102456.
[46] M. Venkat, L.W. Chia, T.T. Lambers, Milk polar lipids composition and functionality: a systematic review, Crit. Rev. Food Sci. Nutr. (2022) 1–45, https://doi.org/10.1080/10408398.2022.2104211.
[47] D. Ambrożej, K. Dumycz, P. Dziechciarz, M. Ruszczyński, Milk fat globule membrane supplementation in children: systematic review with meta-analysis, Nutrients 13 (3) (2021), https://doi.org/10.3390/nu13030714.
[48] M.E. Smith, G. Cisbani, R.J.S. Lacombe, R.P. Bazinet, A scoping review of clinical studies in infants fed formulas containing palm oil or palm olein and Sn-2 palmitate, J. Nutr. 151 (10) (2021) 2997–3035, https://doi.org/10.1093/jn/nxab246.
[49] A. Oosting, N. van Vlies, D. Kegler, L. Schipper, M. Abrahamse-Berkeveld, S. Ringler, et al., Effect of dietary lipid structure in early postnatal life on mouse adipose tissue development and function in adulthood, Br. J. Nutr. 111 (2) (2014) 215–226, https://doi.org/10.1017/S0007114513002201.
[50] A. Baars, A. Oosting, E. Engels, D. Kegler, A. Kodde, L. Schipper, et al., Milk fat globule membrane coating of large lipid droplets in the diet of young mice prevents body fat accumulation in adulthood, Br. J. Nutr. 115 (11) (2016) 1930–1937, https://doi.org/10.1017/S0007114516001082.
[51] A. Oosting, L. Harvey, S. Ringler, G. van Dijk, L. Schipper, Beyond ingredients: supramolecular structure of lipid droplets in infant formula affects metabolic and brain function in mouse models, Plos One 18 (8) (2023) e0282816, https://doi.org/10.1371/journal.pone.0282816 eCollection 2023.
[52] A. Smolinska, A. Baranska, J.W. Dallinga, R.P. Mensink, S. Baumgartner, B.J.M. van de Heijning, et al., Comparing patterns of volatile organic compounds exhaled in breath after consumption of two infant formulae with a different lipid structure: a randomized trial, Sci. Rep. 9 (1) (2019) 554, https://doi.org/10.1038/s41598-018-37210-5.
[53] M. Weber, V. Grote, R. Closa-Monasterolo, J. Escribano, J.P. Langhendries, E. Dain, et al., Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial, Am. J. Clin. Nutr. 99 (5) (2014) 1041–1051, https://doi.org/10.3945/ajcn.113.064071.
[54] A. Beyerlein, A.M. Toschke, R. von Kries, Breastfeeding and childhood obesity: shift of the entire BMI distribution or only the upper parts? Obesity (Silver Spring) 16 (12) (2008) 2730–2733, https://doi.org/10.1038/oby.2008.432.
[55] A. Beyerlein, A.M. Toschke, R. von Kries, Risk factors for childhood overweight: shift of the mean body mass index and shift of the upper percentiles: results from a cross-sectional study, Int. J. Obes. (Lond). 34 (4) (2010) 642–648, https://doi.org/10.1038/ijo.2009.301.
[56] M. Hosaka, K. Asayama, T. Ohkubo, K. Nakai, Y. Imai, Children's home blood pressure and growth environment, Hypertension 61 (4) (2013) e33, https://doi.org/10.1161/HYPERTENSIONAHA.111.00843.
[57] J.S. Forsyth, P. Willatts, C. Agostoni, J. Bissenden, P. Casaer, G. Boehm, Long chain polyunsaturated fatty acid supplementation in infant formula and blood

pressure in later childhood: follow up of a randomised controlled trial, BMJ 326 (7396) (2003) 953, https://doi.org/10.1136/bmj.326.7396.953.
[58] L. Schipper, N. Bartke, M. Marintcheva-Petrova, S. Schoen, Y. Vandenplas, A. Hokken-Koelega, Infant formula containing large, milk phospholipid-coated lipid droplets and dairy lipids affects cognitive performance at school age, Front Nutr 5 (10) (2023) 1215199, https://doi.org/10.3389/fnut.2023.1215199, eCollection 2023.
[59] X. Chen, Y. Wang, Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis, Circulation 117 (25) (2008) 3171–3180, https://doi.org/10.1161/CIRCULATIONAHA.107.730366.