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The Groningen LCPUFA Study: No Effect of Short-Term Postnatal Long-Chain Polyunsaturated Fatty Acids in Healthy Term Infants on Cardiovascular and Anthropometric Development at 9 Years

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ABSTRACT: Conflicting evidence exists on the effect of long-chain polyunsaturated fatty acid (LCPUFA) formula supplementation on cardiovascular health in term infants. It is known that LCPUFA supplementation does not affect infant growth, but long term outcome data are not available. The current study investigates whether 2 mo LCPUFA formula supplementation affects cardiovascular and anthropometric development at 9 y. A prospective, double-blind, randomized trial was performed in healthy term infants: a standard formula control group (CF, $n = 169$) and a LCPUFA-supplemented group [LF, $n = 145$; 0.45% (by wt) AA and 0.30% (by wt) docosahexaenoic acid (DHA)]. A breastfed group (BF; $n = 159$) served as reference. At the age of 9 y, systolic and diastolic blood pressure, heart rate, head circumference, weight, and height were measured. Univariate and multivariate analyses were performed; 63 to 79% of children were assessed. None of the cardiovascular or anthropometric measurements differed between the formula groups. Breastfed children had a marginally lower heart rate than formula-fed children, in particular compared with children fed control formula. Blood pressure and parameters of growth including BMI of breast and formula-fed children did not differ. In conclusion, the study suggests that short-term LCPUFA supplementation does not influence cardiovascular and anthropometric development at 9 y. **(*Pediatr Res* 70: 411–416, 2011)**

The notion that breast feeding has consistently been associated with a modest beneficial effect on blood pressure in later life is relevant for public health (1,2). The underlying mechanisms of the association are not clear. Long-chain polyunsaturated fatty acids (LCPUFAs) may be candidates.

Until recently, LCPUFAs were not present in standard formula. Formula only contained the precursor essential fatty acids, alpha-linolenic acid (ALA,18:3n-3), and linoleic acid (LA, 18:2n-6). As young infants have a limited capacity to synthesize docosahexaenoic acid (DHA) and arachidonic acid (AA) from these precursors (3), LCPUFA levels in plasma or red blood cell membrane gradually decline in infants fed nonsupplemented formulae indicating a relative LCPUFA deficiency. Based on the advantageous effect of LCPUFA supplementation on neurodevelopment assessed at young age, it is advised that infant formulae should contain LCPUFA in quantities comparable with breast milk (4). Consequently, several of the formulas currently on the market contain LCPUFAs. However, there is still an ongoing debate concerning the long-term consequences of dietary LCPUFAs, including the effect on blood pressure regulation.

Studies on the effects of early LCPUFA supplementation on cardiovascular indices in humans, such as blood pressure and heart rate, are limited in number and heterogeneous in methodological quality (Table 1). They also differ in the duration of supplementation, fatty acid dosage, and lipid composition. Two studies on early postnatal supplementation with LCPUFAs (5,6) and one in late infancy (7) reported beneficial effects of LCPUFAs on blood pressure and heart rate in later life. Two other studies however found no effect of LCPUFA supplementation in early life (8,9) and one study even found a negative effect in school age boys (10).

Two recent meta-analyses indicated that early postnatal LCPUFA supplementation in term infants does not affect growth measures such as head circumference, weight, length, and the resulting BMI until the age of 18 mo (11,12). Breastfeeding, on the other hand, is associated with a small but consistent reduction in obesity risk in later childhood (13). However, it is debated whether the effect is mediated by the nutritional contents of human milk or by associated factors, such as less maternal smoking during pregnancy and a maternal BMI <25 (Ref. 14).

The current study is part of the Groningen LCPUFA study, a randomized controlled trial on the effect of supplementation of formula with LCPUFAs during the first two postnatal months in healthy term infants. The focus of the study is neurodevelopmental outcome, but also some other indicators of child health are evaluated (15). Previously, the data of our study groups were used in a meta-analysis, which indicated

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Abbreviations: AA, arachidonic acid; **BF,** breastfed; **CF,** control formula; **DHA,** docosahexaenoic acid; **LCPUFA,** long-chain polyunsaturated fatty acids; **LF,** LCPUFA supplemented formula; **OOS,** obstetric optimality score

Table 1. *Overview studies on the relationship between LCPUFA supplementation and cardiovascular parameters*

Study set-up	Study group	Supplementation with	Follow-up age	Parameters used at evaluation	Effect of LCPUFA on cardiovascular health (95% CI)	References
Follow-up of RCT	n = 147, healthy full terms	DHA 0.15–0.25 g; AA 0.3–0.4 g from egg yolk per 100 g fat via formula birth till 4 mo	6 y	Blood pressure	Lower mean blood pressure: 3 mm Hg (5.4–0.5); lower diastolic blood pressure: 3.6 mm Hg (6.5–0.6)	(5)
Observational study	n = 79, healthy full terms	Contrasting: DHA 0.15% + AA 0.40%; DHA 0.32% + AA 0.64% from unprovided source via formula; from 2 mo until 12 mo	6 mo	Resting heart rate, heart rate variability	Lower heart rate: presented in figure, numerical, values not provided; lower values for heart rate variability: presented in figure, numerical values not provided	(6)
RCT	n = 83, healthy full terms	Fish oil 5ml daily; from 9mo till 12 mo	12 mo	Blood pressure, erythrocyte fatty acid composition and plasma lipid profile	Lower systolic blood pressure: 6.3 mm Hg (0.9–11.7); increased erythrocyte (n-3) LCPUFA content; higher plasma total cholesterol: 0.51 mmol/L (0.07–0.95); higher LDL cholesterol: 0.52 mmol/L (0.02–1.01)	(7)
RCT	n = 98, healthy full terms	Maternal fish oil: 1.5g/day n-3, during the first 4 mo of lactation	7 y	Blood pressure and body composition	Higher diastolic blood pressure: 6 mm Hg (CI not provided), boys only mean arterial blood pressure: 6 mm Hg (CI not provided), boys only	(10)
RCT	n = 73, healthy full terms	Maternal fish oil: 4.5 g, during the first 4 mo of lactation	2.5 y	Blood pressure and anthropometry	No effect	(9)
RCT	n = 616	Dietary intervention, additional n-3 fatty acids and reduced n-6 fatty acids in nutrition from time of weaning until 5 y	8 y	Blood pressure, arterial structure and function	No effect	(8)

RCT, randomized controlled trial.

that LCPUFA supplementation and breastfeeding were not associated with growth advantages at 18 mo (12). Recently, the participants of the study were reassessed at 9 y. Here, we report on cardiovascular health expressed as blood pressure and heart rate and anthropometrics in terms of weight, length, BMI, and head circumference.

The primary aim of the current article is to explore whether the short-term postnatal LCPUFA intervention was associated with blood pressure, heart rate, and anthropometric development at 9 y. In addition, we explored whether breast feeding was associated with a beneficial effect on blood pressure, heart rate, and anthropometric development.

METHODS

Subjects. The study is part of a double-blind randomized controlled trial investigating the effect of LCPUFA supplementation on the development of healthy term infants (the Groningen LCPUFA study). Details on the study design, including exact diet composition, have been described previously (15). Between 1997 and 1999, expecting mothers were recruited during pregnancy checkup visits at the University and Martini Hospitals in Groningen and at midwife clinics in and near Groningen. Final enrollment occurred in the neonatal period. All infants were born at 37–42 wk of gestation. Mothers of 314 infants chose to bottle feed their child and 160 opted for breastfeeding. The infants receiving formula were randomized into a standard formula group [control formula (CF; n = 169)] and a LCPUFA-supplemented formula group (LF, n = 145). Standard formula consisted of Nutrilon Premium. For the supplemented formula, the lipid fraction of Nutrilon Premium was enriched with 0.45% (by wt) AA and 0.30% (by wt) DHA. DHA was derived from egg yolk and tuna oil that was low in eicosapentaenoic acid, and the source of AA was egg yolk and a single cell oil produced by a common soil fungus, *Mortierella alpina*. The LCPUFAs were provided as mix of phospholipids (15%) and triglycerides (85%) to mimic the composition of breast milk. All infants received their assigned diets beginning at age 1–5 d. Supplementation lasted till the end of the second postnatal months. In case breastfeeding stopped before 2 mo, the infant received LCPUFA-supplemented formula till the full age of 2 mo. All formula-fed infants received CF from two completed months until the age of 6 mo. All 436 children (92% of the original groups) assessed at 18 mo were eligible for re-examination at 9 y. At the 9 y follow-up, both parents and examiners were unaware of the type of formula feeding the infant had received. The examiners were also blind to formula *versus* breast status.

Protocol and measurements. In the current follow-up, the children and their parents were contacted, and depending on the wish of the participants, assessments were carried out either at the hospital or at home. Three hundred forty-one children agreed to participate: 91 in the LF group (63%), 123 in the CF group (73%), and 127 in the breastfed group (BF group; 79%; Fig. 1). During the study, type of feeding was assessed prospectively by daily diaries filled in by the mothers until the infant was 8 mo. Duration of breastfeeding varied from 1 to 56 wk; 50% of mothers exclusively breastfed beyond 2 mo. The assessments of cardiovascular parameters and anthropometrics were part of a more extensive evaluation focusing on neurodevelopmental outcome, see Ref. 16.

Blood pressure and heart rate were measured immediately after a 15-min rest period, measurement took place on the left arm with the child sitting on a chair and the arm resting on a table. An automated blood pressure monitor was used (Datascope Accutorr plus; Datascope corporation, Mahwah) with a small adult cuff (10.6–23.9 cm size bladder; measuring with a precision of micrometers Hg). This procedure was performed twice during the assessment

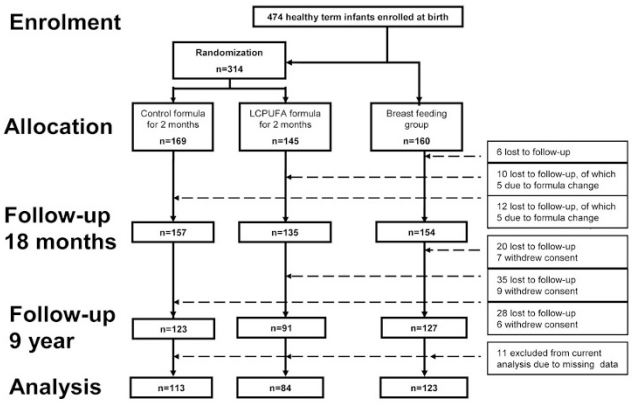

Figure 1. Flow diagram of children enrolled in the study and followed up until 9 y of age.

with an interval of an hour. In all children, heart rate and systolic and diastolic blood pressure were recorded as the mean of the two readings.

Weight was measured using a calibrated Radwag scale (Radwag; Radom, Poland; which measured with a precision of 500 mg) and body length using a Seca stabilometer (Seca Deutschland, Hamburg, Germany; which measured with a precision of millimeters). BMI was determined using international standards, taking gender and age into account (17). Occipitofrontal head circumference was assessed using a nonstretchable "lasso" tape with a precision of millimeters.

Data on pre- and perinatal conditions had been collected during enrollment with the help of the obstetric optimality score (OOS).The OOS describes the obstetric conditions ranging from the parents' socioeconomic status to the infant's condition immediately after birth (18). Anthropometrics had been recorded at birth and at 3 and 18 mo (12). At the 9-y follow-up, information was collected on parental education and profession, the child's medical history, nutritional habits, and family composition (Table 2).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Groningen University Hospital. Written informed consent was obtained from all subjects. The trial is registered under ISRCTN52788665.

Data analysis. The study was originally designed to detect a difference of 7.5 on the mental developmental index of the Bayley test of infant development at the age of 18 mo between the LF and CF groups (assuming SD = 15, power 80% at 5% significance level). Accordingly, at least 57 children had to be included in each formula group.

Statistical analysis focused on differences between the two randomized groups; in addition, differences between the formula groups and the BF group were analyzed. The anthropometric and blood pressure data, apart from the children's BMI, were normally distributed. We used independent samples *t* tests for crude comparison of the feeding groups and linear regression analysis for comparison adjusted for potential confounders. The children's BMI was dichotomized into normal ($\leq$25)/overweight ($>$25), χ^2 tests were used for crude comparisons between the feeding groups and logistic regression analyses for comparisons adjusted for potential confounders.

Factors included into the multivariate analyses were variables associated with outcome at $p < 0.20$: gender, maternal level of education (1 = low, 2 = medium, and 3 = high), smoking during pregnancy (yes or no), duration of the second stage of delivery, Apgar score at 1 and 3 min, birthweight, OOS, and prepregnancy maternal BMI. In line with other developmental studies, smoking was dichotomized into no/minimal smoking defined as $<$5 cigarettes per day and evident smoking defined as $\geq$5 cigarettes per day (19). Specific attention was paid to duration of exclusive breastfeeding as potential confounder. Anthropometric parameters at 3 and 18 mo were not included in the multivariate analyses as they were not regarded as confounders but as outcome variables.

Relative risk analyses were used to compare risk between the two formula groups and between the formula and BF groups for having a high blood pressure (diastolic or systolic blood pressure at or above the 90th percentile) and for being overweight (BMI $>$25). A *p* value of 0.05 or less was considered as statistically significant. Statistical analyses were performed using SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

In general, obstetric and social characteristics of children assessed at 9 y were comparable with those not participating in the follow-up at 9 y (data not shown). Nonetheless some selective attrition was present, the children who took part in the follow-up showed a less optimal neuromotor condition at 3 mo ($p = 0.003$). Moreover, part of the attrition was group specific, more boys in the LF group did not partake (35 boys and 17 girls) compared with both the BF group (15 boys and 17 girls) and the CF group (24 boys and 17 girls). In addition, in the LF group, more children with a lower mental development index at 18 mo were lost to attrition ($p = 0.007$; see Ref. 16). Attrition was, however, not selective for parameters of growth at birth and during infancy (data not shown).

Cardiovascular and anthropometric outcomes in the LF and CF groups were similar (Tables 3 and 4). Multivariate analyses confirmed the absence of differences in systolic and diastolic blood pressure, heart rate, weight, height, BMI, and

Table 2. *Obstetrical and social characteristics of the three groups assessed at 9 y*

	CF group (n = 123)	LF group (n = 91)	BF reference group (n = 127)
Gender, boys/girls	71/52	42/49	64/63
Maternal BMI, median (range)	24.7 (17.7–40.9)	23.7 (18.2–35.9)	22.6 (17.2–38.2)
Weight gain in kilograms during pregnancy*, median (range)	13 (0–46)	13 (0–26)	14 (0–31)
Presence of maternal smoking during pregnancy*, *n* (%)	28 (23)	17 (19)	13 (10)
Presence of maternal hypertension during pregnancy, *n* (%)	21 (17)	11 (12)	11 (9)
Birth weight (g), mean (SD)	3518 (473)	3527 (498)	3588 (436)
Apgar score after 3 min, median (range)	10 (7–10)	10 (7–10)	10 (5–10)
OOS, median (range)	59 (46–67)	59 (50–67)	60 (43–69)
Maternal education†			
High‡, *n* (%)	15 (13)	13 (15)	60 (49)
Medium§, *n* (%)	85 (72)	52 (60)	57 (46)
Low‖, *n* (%)	18 (15)	22 (25)	6 (5)

* Significant difference between BF and formula fed in children assessed at 9 y: $p < 0.05$.

† Significant difference between all nutritional groups found both in the groups lost to attrition and the groups assessed at 9 y: $p < 0.01$.

‡ University education or vocational college.

§ College graduate or junior vocational college.

‖ No education or primary education.

Table 3. *Growth, height, and head circumference in the three nutritional groups*

	CF		LF		BF reference group	
	n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
Weight (kg)						
At birth	118	3.518 (0.473)	88	3.527 (0.50)	127	3.588 (0.436)
3 mo	95	6.306 (0.744)	80	6.323 (0.687)	120	6.253 (0.764)
18 mo	71	12.040 (1.40)	66	11.910 (1.40)	80	12.020 (1.430)
9 y	116	34.0 (7.0)	85	33.70 (5.40)	124	33.10 (6.50)
Height (cm)						
At birth	62	51.0 (2.2)	40	51.0 (2.2)	58	51.0 (2.0)
3 mo	94	63.1 (2.6)	80	63.1 (2.6)	120	62.6 (2.6)
18 mo	121	85.7 (3.5)	90	85.4 (3.7)	126	85.4 (3.8)
9 y	123	139.8 (5.3)	90	139.8 (5.9)	126	139.8 (6.2)
Head circumference (cm)						
At birth	57	35.0 (1.6)	38	34.6 (1.4)	43	34.9 (1.5)
3 mo	96	41.2 (1.5)	80	40.9 (1.3)	119	40.8 (1.3)
18 mo	119	48.4 (1.6)	90	48.1 (1.4)	125	48.4 (1.5)
9 y	122	53.5 (1.7)	88	53.2 (1.6)	127	53.7 (1.6)

Table 4. *Blood pressure, heart rate, and anthropometrics at 9 y*

	CF group		LF group		BF reference group	
	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*
Systolic BP (mm Hg)	104.59 (7.9)	117	104.53 (9.0)	90	105.17 (7.8)	124
Diastolic BP (mm Hg)	63.78 (7.6)	117	62.27 (8.5)	90	63.77 (8.0)	124
Heart rate beats/minute	78.72 (10.3)	113	78.20 (9.2)	88	76.19 (9.6)	124
Head circumference (cm)	53.40 (1.6)	122	53.17 (1.6)	88	53.70 (1.6)	127
BMI (% normal)	72%	116	76%	85	79%	123

BMI was determined using international standards by Cole (17) taking age and gender into account.
BP, blood pressure.

Table 5. *Results of linear regression analysis of factors contributing to systolic blood pressure, diastolic blood pressure, and heart rate*

Contributing factors	Heartrate		
	Effect	95% CI	*p*
Type of feeding			
LF *vs* CF	−0.540	3.33 to 2.24	0.70
BF *vs* CF	−2.550	−58.09 to −0.009	0.049
BF *vs* LF	−2.0	−4.71 to 0.70	0.146
Covariates			
Neonatal jaundice	7.54	−0.40 to 15.48	0.063

Heart rate: reference CF: 78.57; $n = 325$; $R^2 = 0.24$.

head circumference between the two formula groups. Relative risk analysis demonstrated no difference between the groups for high blood pressure [diastolic: CF, 0.875 (95%CI, 0.622–1.23); LF, 1.219 (95%CI, 0.685–2.168) and systolic: CF, 1.094 (95%CI, 0.725–1.650); LF, 0.898 (95%CI, 0.568–1.420)] nor for being overweight [CF, 0.881 (95%CI, 0.677–1.146); LF, 1.209 (95%CI, 0.787–1.856)].

The observational part of the study indicated that children of the BF group had a slightly lower heart rate than the children of the CF group ($p = 0.04$; effect size 0.914). Multivariate analyses confirmed the marginal difference ($p = 0.049$, 95%CI: −58.09 to −0.009; Table 5). Systolic and diastolic blood pressure, weight, height, BMI, and head circumference of the BF group were similar to those of the two formula groups (Tables 3 and 4). The lack of difference in these outcome parameters was confirmed in the multivariate analyses. Relative risk analysis demonstrated no difference between the groups for high blood pressure [diastolic: BF, 0.915 (95%CI, 0.602–1.389); formula, 1.058 (95%CI, 0.80–1.40) and systolic: BF, 1.104 (95%CI, 0.682–1.786); formula, 0.946 (95%CI, 0.733–1.221)] nor for being overweight [BF, 1.133 (95%CI, 0.787–1.633); formula, 0.930 (95%CI, 0.763–1.135)].

DISCUSSION

Our data indicated that LCPUFA supplementation for the duration of 2 mo in healthy term infants was not associated with a change in blood pressure, heart rate, weight, height, BMI, or head circumference nor with an enhanced risk of high blood pressure or overweight at 9 y. The study also revealed that children who were breastfed had a slightly lower heart rate at 9 y than children who had received formula.

A major limitation of the study is its attrition, which was 28%. However, considering the duration of the follow-up period, 9 y, this may be regarded as relatively favorable (20). Attrition was selective with respect to gender and cognitive development at 18 mo in the LF group suggesting that LF participants were a positive selection of the original LF group. However, attrition was not selective with respect to social class and parameters of growth at birth and during infancy (Table 3). A second limitation of the study is its relatively weak power. Group sizes had been determined on the basis of neurodevelopmental outcome at 18 mo measured with the Bayley Scales of Infant Development (21). *Post hoc* power analyses indicated that with α set at 0.05 current sample sizes

allowed for a detection of small effect sizes (0.20) with a power of 0.80, *i.e.* the groups allowed for the detection of differences in blood pressure of 1.5 mm Hg, in heart rate of 2 beats per minute, in body weight of 1.5 kg, and in height of 1 cm. This means that this study was not able to detect more subtle effects on growth, blood pressure, and heart rate. It may also be regarded as a limitation that our parameters of cardiovascular health and anthropometrics were restricted to blood pressure, heart rate, bodyweight, length, BMI, and head circumference and did not encompass body composition, ECG, and blood lipid profile. A third limitation is the relatively short duration of supplementation, *i.e.* 2 mo. Some indications have been found that LCPUFA supplementation for 9 to 12 mo may affect visual and cognitive development (22,23), whereas supplementation for a few months does not affect developmental outcome (11). Further research is required to assess whether prolonged supplementation is associated with improved outcome, including cardiovascular and anthropometric outcome. The strengths of the study are its randomized design, the presence of information on a wide range of possibly confounding variables, and its assessor-blinded evaluation.

Previous studies on the relationship between LCPUFA supplementation and blood pressure reported inconsistent findings: LCPUFAs were associated with a positive effect on blood pressure, no effect, or even a negative effect. This heterogeneity presumably is the result of the large variation in methodological quality of the studies resulting from the use of high-risk populations, highly selective attrition, and cointervention with other nutrients. In addition, there is large variation between the studies in dosage and duration of the intervention and the ages during which the intervention was applied (Table 1). The current study concludes that LCPUFA supplementation of formula for 2 mo is not associated with blood pressure improvements of more than 1.5 mm Hg and a decrease in heart rate of more than 2 beats per minute. This also implies that smaller effect sizes or larger effects of longer periods of supplementation are not precluded. Originally, our study was one of a series in which the effect of longer periods of supplementation was also evaluated. However, our short-term supplementation study was the only one that was completed.

Breastfeeding, on the other hand, has shown a relatively consistent relation with blood pressure: it is associated with a modest decrease in blood pressure, *i.e.* a reduction of around 1.4 mm Hg in systolic blood pressure and 0.4 mm Hg in diastolic blood pressure (1). These breastfeeding effects are smaller than the current study was able to detect. However, we did find suggestions of a minor advantage for breastfeeding in heart rate, the other parameter of cardiovascular health. The minor reduction in heart rate may reflect a slight shift in autonomic control involving a minor decrease of sympathetic dominance. This may be associated with a minor reduction in risk for cardiovascular diseases in adulthood (24). The small size of the effect is illustrated by the fact that the advantage only reached statistical significance in the comparison with CF. Our finding of a minor beneficial effect of breastfeeding on cardiovascular indices is in line with other reports but somewhat smaller than often reported. This might be explained by the relatively short average period of breastfeeding, 53% of breastfed infants received a maximum of 8 wk exclusive breastfeeding (25), which is representative of Dutch breast feeding habits (26).

None of the anthropometric measures in this study were associated with early postnatal nutrition. The lack of effect of postnatal LCPUFA supplementation is in line with existing literature, but the lack of difference between the breastfed and the formula-fed groups is not. This might be explained by the limited ability to detect differences between the groups and to the above mentioned breastfeeding practices in the Netherlands.

To summarize, the current study suggests that LCPUFA supplementation of formula in healthy term infants for the duration of 2 mo does not influence cardiovascular and anthropometric development at the age of 9 y. Breastfeeding is found to have a marginal beneficial effect on cardiovascular development. The study underscores the need for carefully designed and well-controlled studies on the effect of LCPUFA supplementation on cardiovascular development.

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