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ORIGINAL ARTICLE

Long-chain polyunsaturated fatty acids in infant formula and cardiovascular markers in childhood

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Abstract

To investigate whether children who consumed infant formula supplemented with long-chain polyunsaturated fatty acids (LCPUFAs) had a more favourable cardiovascular profile than children who consumed formula without these fatty acids, we used the Wheezing Illnesses Study Leidsche Rijn, a birth cohort that included 2,468 newborns between 2001 and 2014. Data on infant feeding were obtained by questionnaires. At age 5, blood pressure, carotid intima-media thickness (CIMT), and carotid distension were measured. We used multivariable linear regression analysis to compare levels of cardiovascular markers in formula-fed children born before and after the LCPUFA supplementation. To account for secular trends, we compared levels of cardiovascular markers in a control group of breastfed children from the same cohort born before and after the supplementation. Formula-fed children born after the LCPUFA supplementation (n = 48) had no different systolic blood pressure (−2.58 mmHg, 95% confidence interval, CI [−5.5, 0.30]), diastolic blood pressure (−0.13 mmHg, 95% CI [−2.3, 2.1]), or carotid distension (24.8 MPa^{-1}, 95% CI [−47.1, 96.6]) and had a higher CIMT (18.6 μm, 95% CI [3.7, 33.5]) than formula-fed children born before the supplementation (n = 163). In the control group, children born after the LCPUFA supplementation (n = 98) had no different systolic- or diastolic-blood pressure, or CIMT, and a higher carotid distension than children born before the supplementation (n = 142). In conclusion, children who consumed infant formula supplemented with LCPUFAs did not have a more favourable cardiovascular profile in early childhood than children who consumed formula without LCPUFAs.

KEYWORDS

blood pressure, cardiovascular health, DHA, fatty acids, IMT, infant formula

1 | INTRODUCTION

Consumption of long-chain polyunsaturated fatty acids (LCPUFAs), especially those found in fatty fish, has a favourable effect on blood pressure and serum markers of cardiovascular disease risk in adults (Balk et al., 2004; Miller, Van, & Alexander, 2014). Until around 2006 in the Netherlands, infant formulae did not contain these LCPUFAs, and it has therefore been hypothesized that supplementing formulae with LCPUFAs will improve the cardiovascular profile in formula-fed children. The evidence for a beneficial effect on blood pressure in childhood, however, is conflicting (de Jong, Boehm, Kikkert, & Hadders-Algra, 2011; Forsyth et al., 2003).

In 2006, Dutch nutrition companies have started supplementing retail formulae with LCPUFAs based on evidence of beneficial effects on visual development in infancy (Birch et al., 2005; Hoffman et al., 2003). We used this formula supplementation as a natural experiment to investigate our hypothesis that children who consumed formula with LCPUFAs had a more favourable cardiovascular profile than children who consumed formula without LCPUFAs. We used data from the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), a prospective birth cohort study that enroled newborns between 2001 and 2014. To take account of secular trends in related exposures during this period, we compared levels of cardiovascular markers in a control group of breastfed children in the same cohort who were born before and after the supplementation.

2 | SUBJECTS AND METHODS

2.1 | Study design

The WHISTLER study is a large single-centre prospective birth cohort initiated in December 2001 in the Netherlands. Details of the study design and rationale are described elsewhere (Katier et al., 2004). Briefly, 2,468 healthy newborns whose parents lived in Leidsche Rijn (area in the city of Utrecht) were enrolled in the study between 2001 and 2014. Newborns were recruited via the municipality's civil affairs department. In the Netherlands, parents must register a new birth at the municipality within 3 days. Exclusion criteria were gestational age < 36 weeks, major congenital abnormalities, and neonatal respiratory disease. Monthly questionnaires until the child's age of 1 year were filled out by the parents. Although the WHISTLER study originally aimed to study determinants of wheezing illnesses, cardiovascular research questions have always been of interest. Non-invasive ultrasonographical measurements of the carotid artery were considered feasible when the first participating children reached the age of 5 years in 2007 (WHISTLER–Cardio). These ultrasound measurements were part of a physical examination at the local outpatient clinic for which all 5-year-old children were invited (n = 1,861 until January 2016, see Figure 1). Of those invited, 251 children (13.5%) were lost to follow-up because of incorrect telephone numbers, nonresponses despite mailings, or incorrect addresses. Of the remaining 1,610 participants, 992 were accepted. Presently, 942 children visited the outpatient clinic. The WHISTLER study was approved by the paediatric Medical Ethical Committee of the University Medical Centre Utrecht. Parental written informed consent was obtained.

The current study was a natural experiment with a before–after formula supplementation comparison of levels of cardiovascular markers in formula-fed children and in a control group of breastfed children.

2.2 | Study population

Of the 942 children who visited the outpatient clinic at age 5 years, 822 children had complete data on the type and duration of infant feeding and were eligible to participate (Figure 1). Of these 822 children, 232 children (28.2%) received exclusive formula feeding, 163 children received formula without LCPUFAs (blood pressure measurements in 143 children, carotid intima-media thickness [CIMT] in 147 children, and carotid distension in 131 children), 48 children received formula with LCPUFAs (blood pressure measurements in 47 children, CIMT in 46 children, and carotid distension in 39 children), and 21 children were excluded because of uncertainty about LCPUFA supplementation at the date of birth. Another 275 children (33.5%) received exclusive breastfeeding for at least 3 months and were defined as the control group, 142 children were born before formula feedings were supplemented with LCPUFAs (blood pressure measurements in 130 children, CIMT in 126 children, and carotid distension in 115 children), 98 children were born after the supplementation (blood pressure measurements in 96 children, CIMT in 94 children, and carotid distension in 81 children), and 35 children were excluded because of uncertainty about LCPUFA supplementation at the date of birth. The remaining 315 children (38.3%) received both breastfeeding and formula feeding simultaneously and were excluded from this study.

Key messages

- Since 2006/2007, infant formulas are supplemented with long-chain polyunsaturated fatty acids (LCPUFAs) based on evidence related to visual and cognitive development.
- LCPUFAs are associated with cardiovascular health in adults, but it is unknown whether the current supplementation of infant formulas with LCPUFAs is also beneficial for cardiovascular health in children.
- Our results show that children who received infant formula with n-3 LCPUFAs had no different blood pressure or carotid distension and had a higher intima-media thickness at age 5 than children who received infant formula without LCPUFAs. Whether this higher CIMT is a consequence of the LCPUFA supplementation, or whether unknown secular trends have confounded the association remains to be answered.

2.3 | Collection of data on infant feeding

At baseline when infants were 4 weeks old and in the 11 subsequent months, the type of infant feeding was ascertained via parental questionnaires by asking “Has your child been breastfed this month?” Answering options were “yes, exclusively breastfed,” “yes, both breastfed and formula fed,” and “no, exclusively formula fed.” In case the child received formula feeding, the brand of the infant formula was inquired. All questionnaires were returned by mail, and parents received monthly reminders to return them.

2.4 | Definition of infant feeding

Formula feeding was defined as receiving exclusive formula feeding from birth onwards (n = 162) or receiving both breastfeeding and formula feeding in the first week(s), but switching to exclusive formula feeding in the first month after birth (n = 70). In total, 232 children were classified as being formula fed. Breastfeeding was defined as receiving exclusive breastfeeding from birth until at least 3 months of age (n = 275).

2.5 | Definition of exposure: Infant formula supplemented with LCPUFAs

The type of infant formula that each child received was identified by extracting the formula brands that were reported in the baseline questionnaire. Nutritionists of the two infant feeding companies that formulas were most often used by our study population (Table 1) provided information on the year and month in which LCPUFAs were added to their formulas. For the six children who received other

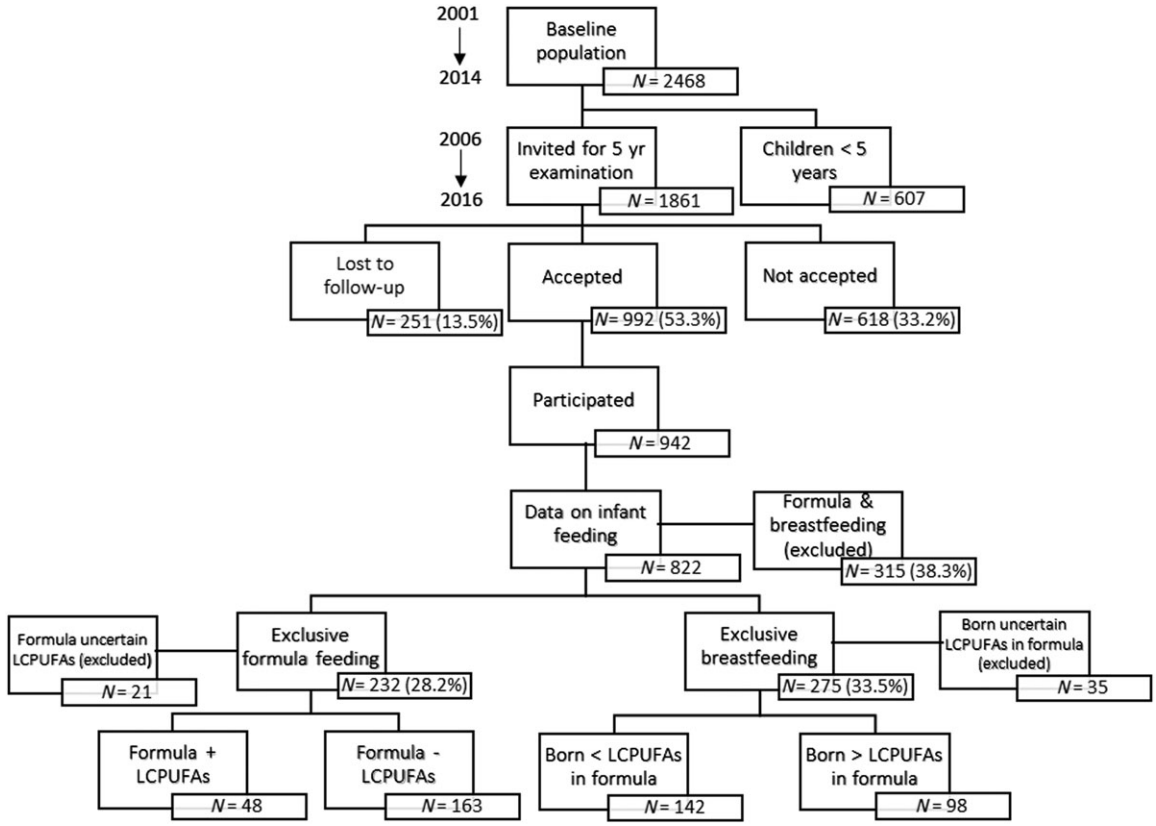

FIGURE 1 Flow chart of the study population. LCPUFA = long-chain polyunsaturated fatty acid

brands, it was determined from the "Compendium on dietary products and supplemental foods" that no LCPUFAs were present. Nutricia started distributing supplemented formula feedings in the spring of 2006. Friso supplemented their formula feedings in 2007 but could not be specific about the month or season in which their formula feedings were distributed. Therefore, 21 children who were born during these "transition" periods and who received Nutricia formula feeding (n = 7) or Friso formula feeding (n = 14) were excluded from the analysis (Figure 2). The presence of LCPUFA supplementation could thus be identified in 211 of the 232 formula-fed children; 163 children received formula without LCPUFAs and 48 children received formula with LCPUFAs. The composition of fatty acids in the infant formulae is shown in Table S1. Besides the LCPUFAs supplementation, no other nutrients were added to or removed from the formulae over the duration of the study. In Nutrilon, there were small increases in the amount of proteins (1.4–1.6 g) and carbohydrates (7.5–7.7 g). In Nutrilon and Friso formula, there were small changes in the amount of vitamins and minerals.

2.6 | Measurement of the outcome variables: Blood pressure, CIMT, and carotid distension during follow-up visit at 5 years of age

When children became 5 years old, they were reinvited to the outpatient clinic to participate in anthropometric and vascular measurements, performed by a trained paediatric research nurse. Vascular conditions of the right common carotid artery were studied ultrasonographically by using high-resolution echotracking technology (Art.lab; Esaote, Italy). This technology gives access to study CIMT and carotid distension, a measure of arterial stiffness. CIMT is the thickness of the inner wall of the carotid artery, and carotid distension is the change in systolic diameter relative to the diastolic diameter during the cardiac cycle. CIMT was measured with 2.1-μm resolution, and distension was measured with 1.7-μm resolution. Subjects were placed in supine position with their head turned to the left after at least 10 min of resting. Measurements were repeated a maximum of 4 times. During the measurements, children could watch their favourite animation movie to facilitate laying still during measurements. CIMT measurements were successful in 193/232 (83.1%) formula-fed children and in 220/275 (80%) breastfed children. Reproducibility was evaluated. Coefficients of variation based on measurements by one observer in 10 subjects at two occasions for distension and CIMT were 7.1% and 4.3%. During ultrasonography, blood pressure was recorded twice in the brachial artery using a semiautomatic oscillometric device with cuff size adjusted to the children (DINAMAP; Criticon, Tampa, FL, USA).

TABLE 1 Distribution of used infant formula brands in 232 formula-fed children

Brand	*N* children	% of total
Nutrilon	138	59.5
Friso	82	35.2
Nutrilon and Friso	6	2.6
Nestle	2	0.9
Similac	2	0.9
Kruidvat	2	0.9
Total	232	100

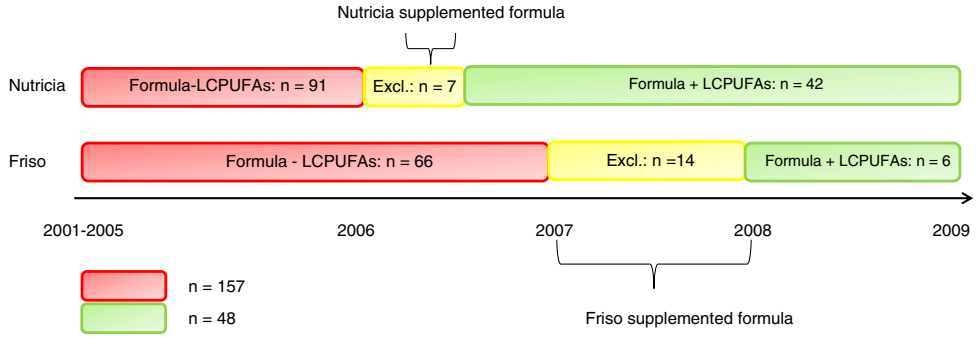

FIGURE 2 Number of children who received Nutricia or Friso formula with and without long-chain polyunsaturated fatty acids (LCPUFAs)

2.7 | Definition of the outcome variables

Outcome variables were defined as mean levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), CIMT, and carotid distension. In each individual child, SBP and DBP were calculated as the mean from the two repeated measurements. Mean CIMT and mean carotid distension were calculated from the maximum of four repeated measurements. Mean carotid distension was adjusted for mean arterial pressure (systolic blood pressure + 2 * (diastolic blood pressure)/3) and for the end-diastolic diameter of the common carotid artery.

2.8 | Covariates

Based on differences observed between children born before and after the supplementation of formula with LCPUFAs, we included maternal educational level and maternal smoking during pregnancy as potential confounders in the analyses. We also included body mass index (BMI) at 5 years of age because this factor may lie on the causal pathway in the associations between formula supplemented with LCPUFAS and SBP, DBP, CIMT, and carotid distension. BMI was calculated from measured weight and height (weight in kg/height squared in m) during the 5-year visit at the outpatient clinic. In the analysis of the control group of breastfed children, we also included maternal age at birth as a potential confounder.

2.9 | Statistical analysis

Means (*SD*) and percentages (*N*) of maternal and child characteristics were described in children born before and after the supplementation of formula with LCPUFAs. Linear regression analysis was used to investigate differences in mean levels of SBP, DBP, CIMT, and carotid distension between children who received formula with LCPUFAs and children who received formula without LCPUFAs (=reference category). In multivariable models, we adjusted for potential confounders. In separate models, we added BMI at 5 years of age. To take account of secular trends in relevant exposures over the 8-year measurement period, for example, changes in active or passive smoke exposure due to the introduction of a smoking ban in public places in 2008, we also compared cardiovascular markers in a control group of breastfed children who were born before (n = 142) and after (n = 98) supplementation of formula with LCPUFAs.

Furthermore, to investigate whether the 70 children who switched from (partial) breastfeeding to exclusive formula feeding in the first month after birth have influenced the associations, we performed a sensitivity analysis in which we excluded these children. In this restricted study population, 111 children received formula without LCPUFAs and 33 children received formula with LCPUFAs.

Results are expressed as mean differences with 95% confidence intervals. An alpha level of <0.05 was considered statistically significant. All analyses were performed with SAS version 9.2 (SAS Institute, Cary, North Carolina, USA). For simplicity, we will refer to the before–after comparison as formula-fed children or control children born before 2006 (before supplementation of formula with LCPUFAs) and after 2006 (after supplementation of formula with LCPUFAs).

As this natural experiment is part of an ongoing study with a broader research question on determinants of cardiovascular health, we performed a post hoc power calculation as the sample size was already established for this research question. We calculated that our sample size was adequate to detect a statistically significant difference of 4 mmHg between the group of children who received infant formula with and without LCPUFAs (assuming 80% power and two-tailed p value .05).

3 | RESULTS

3.1 | Baseline characteristics

Formula-fed children born after 2006 more often had highly educated mothers than formula-fed children born before 2006 (64.1% vs. 51.1%) but the difference was not statistically significant, (p = .15). No differences in other characteristics were observed between the two formulae fed groups (Table 2). In the control group, children born after 2006 had older mothers (33.6 vs. 32.5 years, p < .05) than children born before 2006.

3.2 | Differences in cardiovascular markers between children born before and after 2006

In formula-fed children born before 2006, the mean SBP was 103.1 mmHg, mean DBP was 53.7 mmHg, mean CIMT was 380.9 μm, and mean carotid distension was 788.5 MPa^{-1} (Table 3). After adjustment for confounders, formula-fed children born after 2006 had no different SBP (−2.58 mmHg, 95% CI [−5.5; 0.30], p = 0.07), DBP (−0.13 mmHg, 95% CI [−2.3; 2.1], p = .90), or carotid distension

TABLE 2 Characteristics of formula-fed and control children born before and after 2006

Variables[a]	Formula <2006[b] (*n* = 163)	Formula >2006[b] (*n* = 48)	Control <2006[c] (*n* = 142)	Control >2006[3c] (*n* = 98)
Male sex, % (*n*)	46.6 (76)	43.8 (21)	47.9 (68)	39.8 (39)
Birth weight in gram	3508 (557)	3480 (624)	3575 (444)	3574 (516)
Gestational age in week	39.3 (1.6)	39.2 (1.4)	39.7 (1.3)	39.5 (1.4)
Maternal age at birth in year	32.2 (3.5)	32.5 (3.3)	32.5 (3.3)	33.6 (3.1)[d]
Maternal smoking during pregnancy, % (*n*)	11.0 (18)	8.3 (4)	4.2 (6)	2.0 (2)
Maternal educational level, % (*n*)				
Low	7.5 (10)	5.1 (2)	1.5 (2)	2.1 (2)
Intermediate	41.4 (55)	30.8 (12)	25.2 (33)	19.0 (18)
High	51.1 (68)	64.1 (25)	73.3 (96)	79.0 (75)
Mother Dutch ethnicity, % (*n*)	94.1 (127)	88.1 (37)	90.2 (119)	93.6 (87)
Age at 5 year measurements in year	5.4 (0.3)	5.5 (0.3)	5.4 (0.2)	5.6 (0.3)
Body mass index at 5 years in kg/m^2	15.0 (1.5)	15.2 (1.6)	15.2 (1.4)	15.0 (1.3)

[a]Values are means (*SD*) unless stated otherwise.

[b]Formula-fed children born before and after formula feedings were supplemented with long-chain polyunsaturated fatty acids.

[c]Control group of breastfed children born before and after formula feedings were supplemented with long-chain polyunsaturated fatty acids.

Maternal educational level was defined as follows: Low level (until lower secondary professional education), intermediate level (higher general secondary education, pre-university education), and high level (higher professional education, university).

[d]Significantly different from control <2006 (*t* test *p* value <.05)

TABLE 3 Means of cardiovascular markers in formula-fed and control children born before and after 2006

Cardiovascular makers	Formula <2006[a]		Formula >2006[a]		Control <2006[b]		Control >2006[b]	
	N	Mean (*SD*)	*N*	Mean (*SD*)	*N*	Mean (*SD*)	*N*	Mean (*SD*)
SBP, mmHg	143	103.1 (7.8)	47	100.9 (6.7)	130	103.3 (7.5)	96	103.0 (6.1)
DBP, mmHg	143	53.7 (6.4)	47	53.5 (4.8)	130	53.7 (6.5)	96	53.6 (6.8)
CIMT, µm	147	380.9 (39.6)	46	402.6 (44.3)	126	387.8 (37.6)	94	399.2 (51.5)
Carotid distension, MPa^{-1}	131	788.5 (128.7)	39	772.7 (121.8)	115	811.1 (117.5)	81	794.4 (133.9)

[a]Formula-fed children born before and after formula feedings were supplemented with long-chain polyunsaturated fatty acids.

[b]Control group of breastfed children born before and after formula feedings were supplemented with long-chain polyunsaturated fatty acids. SBP = systolic blood pressure; DBP = diastolic blood pressure; CIMT = carotid intima-media thickness.

(24.8 MPa^{-1}, 95%CI [−47.1; 96.6], p = .49) than formula-fed children born before 2006 (Table 4). Formula-fed children born after 2006 had an 18.6 µm (95% CI [3.7; 33.5], p < .05) higher CIMT than formula-fed children born before 2006. In the control group, children born after 2006 had no different SBP (−0.40 mmHg, 95% CI [−2.3; 1.5], p = .67), DBP (−0.49 mmHg, 95% CI [-2.3; 1.3], p = .58), or CIMT (10.2 µm, 95% CI [−1.9; 22.2], p = .09), and a higher carotid distension (56.7 MPa^{-1}, 95% CI [6.7; 106.7], p < .05) than children born before

TABLE 4 Differences in cardiovascular markers between formula-fed and control children born before and after 2006

	Formula >2006 versus formula <2006[b]				Control >2006 versus control <2006			
	Crude	Adjusted[d]			Crude	Adjusted[e]		
	β	β	95% CI	*p* value	β	β	95% CI	*p* value
SBP, mmHg	−2.15	−2.58	[−5.5, 0.30]	.07	−0.36	−0.40	[−2.3, 1.5]	.67
DBP, mmHg	−0.18	−0.13	[−2.3, 2.1]	.90	−0.19	−0.49	[−2.3, 1.3]	.58
CIMT, µm	21.7	18.6[e]	[3.7, 33.5]	.01	11.3	10.2	[−1.9, 22.2]	.09
Carotid distension, MPa-1	36.9	24.8	[−47.1, 96.6]	.49	49.4[e]	56.7[e]	[6.7, 106.7]	.02

Note. SBP = systolic blood pressure; DBP = diastolic blood pressure; CIMT = carotid intima-media thickness; CI = confidence interval.

[a]Formula-fed children born after versus before formula feedings were supplemented with long chain polyunsaturated fatty acids.

[b]Control group of breastfed children born after versus before formula feedings were supplemented with long-chain polyunsaturated fatty acids.

Carotid distension was adjusted for mean arterial pressure and for the end-diastolic diameter of the common carotid artery

[c]Adjusted for maternal educational level and maternal smoking during pregnancy.

[d]Adjusted for maternal educational level, maternal smoking during pregnancy, and maternal age at birth.

[e]Significant at alpha <0.05.

2006. Additional adjustment for BMI at 5 years of age did not alter the associations (data not shown). Associations were similar when we excluded children who switched from (partial) breastfeeding to exclusive formula feeding in the first month (data not shown).

4 | DISCUSSION

Children who consumed infant formula supplemented with LCPUFAs (born after 2006) had no statistically significant different levels of SBP or DBP and had a higher level of CIMT than children who consumed formula without LCPUFAs (born before 2006). No statistically significant differences in SBP, DBP, and CIMT were observed in the control group of breastfed children born before and after the supplementation of formula with LCPUFAs, indicating that the results are less likely to be explained by time-dependent changes in confounders. For carotid distension, no statistically significant difference was observed between children who consumed formula with and without LCPUFAs. In the control group, breastfed children born after the supplementation had a higher level of carotid distension than breastfed children born before the supplementation.

A strength of our study is the reliable data on exposure because we prospectively assessed the type and duration of infant feeding, as well as the used formula brands, by using monthly questionnaires in the child's first year of life. Another strength is the long period of subject enrolment in WHISTLER, which allowed us to design a study in which time was the only factor that determined whether a child received formula with or without LCPUFAs. Our long subject enrolment also has a limitation. Secular trends may have occurred during the same period when infant formulae were supplemented with LCPUFAs, and this may be accompanied by time-dependent confounding factors that may not have been present in the collected data. Although we consider it unlikely that the cardiovascular health of children born in 2006–2008 was influenced by very different risk factors than the cardiovascular health of children born in 2001–2005, we tried to account for potential trends in unmeasured confounding factors by including a control group of breastfed children from the same cohort, as these children have been exposed to similar secular changes. Nevertheless, unmeasured confounding cannot be completely ruled out, and a causal relationship between infant formula with LCPUFAs and CIMT in childhood cannot be proven with this study. Another limitation of our study is the lack of power to perform subgroup analyses, that is, to assess whether associations differ between boys and girls.

Children who received formula supplemented with LCPUFAs tended to have a lower SBP than children who received formula without LCPUFAs, but the difference was not statistically significant. No difference in DBP was observed between those who consumed formula with and without LCPUFAs. In the control group, no differences in SBP or DBP were observed between children born before and after the supplementation. In a European multicenter trial, consumption of formula supplemented with LCPUFAs until 4 months of age was associated with a similar decrease in SBP as in our study at 6 years of age, which was accompanied by a 3.6 mmHg lower DBP (Forsyth et al., 2003). In another study, consumption of formula with LCPUFAs until 2 months of age was not associated with either SBP or DBP at the age of 9 years (de Jong et al., 2011). Because only a few studies investigated an association between formula supplemented with LCPUFAs and blood pressure in childhood, more studies are needed to reach a final conclusion of the association.

To our knowledge, no previous study has investigated an association between formula supplemented with LCPUFAs and CIMT in childhood. Our observed higher CIMT in children fed supplemented formula seems unexpected, because a higher CIMT in adults indicates atherosclerotic plaque progression (Bots & Grobbee, 2002). In the paediatric population, CIMT is also regarded as an indicator of vascular health (Urbina et al., 2009). Statistically significant increases in CIMT of 0.2 , 0.05 , and 0.03 mm were already observed in, respectively, obese (Reinehr, Kiess, de Sousa, Stoffel-Wagner, & Wunsch, 2006), diabetic (Järvisalo et al., 2002), and hypercholesterolemic children (Pauciullo et al., 1994). In a previous study conducted within WHISTLER, with follow-up before LCPUFA supplementation in formula was introduced, we showed that children who received breastfeeding had a higher CIMT at 5 years of age than children who received formula feeding (Evelein et al., 2011). Breast milk contains LCPUFAS, whereas at that time, infant formulae did not. We discussed that the higher CIMT might reflect a short-term effect of high cholesterol exposure due to a high cholesterol concentration in breast milk. In the current study, cholesterol unlikely explains the observed higher CIMT because its level in formula feeding has not changed throughout the study period. Besides the LCPUFA supplementation, small changes were made in the amount of proteins and carbohydrates (Nutrilon) and in the amount of some vitamins and minerals (Nutrilon and Friso). We assume that this has not affected our results as these small changes are unlikely to have clinical relevance, and most of these nutrients are irrelevant for the outcomes of our study.

We can only speculate about the implications of a higher CIMT in childhood for future cardiovascular risk. Thickening of the inner walls of the arteries is a slow process that tracks over time (Salonen & Salonen, 1991). Follow-up measurements in WHISTLER at 8 years of age and beyond will clarify whether children who received formula supplemented with LCPUFAs will continue to have a higher CIMT throughout childhood and will help to understand the implications of these findings for future health. Nevertheless, more studies are needed to investigate an association between formula supplemented with LCPUFAs and CIMT in childhood before firm conclusions can be made.

In accordance with the observed higher CIMT, children fed formula with LCPUFAs tended to have a higher carotid distension than children fed formula without LCPUFAs, but the difference was not statistically significant. In the control group, breastfed children born after the supplementation had a statistically significantly higher carotid distension than those born before the supplementation. If this underlies a secular trend for carotid distension, this may actually mean that carotid distension did not improve in formula-fed children born after 2006, in a similar direction as for CIMT.

5 | CONCLUSION

This natural experiment showed that children who consumed infant formula supplemented with LCPUFAs had no different levels of blood

pressure and carotid distension and had a higher CIMT than children who consumed infant formula without LCPUFAs. Randomized controlled trials need to study whether the higher CIMT is a consequence of the LCPUFA supplementation or whether changes in unknown risk factors over time have confounded the observed associations.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

LP contributed to design of the study, data analysis, and interpretation of data and drafted the article. GD contributed to design of the study, analysis and interpretation of data, and revising the article for important intellectual content. HS contributed to design of the study, interpretation of data, and revising the article for important intellectual content. KE and CU contributed to conception and design of the study, acquisition of data, and revised the article critically for important intellectual content. LR contributed to design of the study, and analysis and interpretation of data, revising the article for important intellectual content. All authors approved the current version of the manuscript to be published.

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REFERENCES

Balk, E., Chung, M., Lichtenstein, A., Chew, P., Kupelnick, B., Lawrence, A., … Lau, J. (2004). Effects of omega-3 fatty acids on cardiovascular risk factors and intermediate markers of cardiovascular disease. *Evidence Report/Technology Assessment (Summary)*, 1–6.

Birch, E. E., Castañeda, Y. S., Wheaton, D. H., Birch, D. G., Uauy, R. D., & Hoffman, D. R. (2005). Visual maturation of term infants fed long-chain polyunsaturated fatty acid–supplemented or control formula for 12 mo. *The American Journal of Clinical Nutrition*, *81*, 871–879.

Bots, M. L., & Grobbee, D. E. (2002). Intima media thickness as a surrogate marker for generalised atherosclerosis. *Cardiovascular Drugs and Therapy*, *16*, 341–351.

de Jong, J. C., Boehm, G., Kikkert, H. K., & Hadders-Algra, M. (2011). 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. *Pediatric Research*, *70*, 411–416.

Evelein, A. M., Geerts, C. C., Visseren, F. L., Bots, M. L., van der Ent, C. K., Grobbee, D. E., & Uiterwaal, C. S. (2011). The association between breastfeeding and the cardiovascular system in early childhood. *The American Journal of Clinical Nutrition*, *93*, 712–718.

Forsyth, J. S., Willatts, P., Agostoni, C., Bissenden, J., Casaer, P., & Boehm, G. (2003). Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: Follow up of a randomised controlled trial. *BMJ*, *326*, 953.

Hoffman, D. R., Birch, E. E., Castañeda, Y. S., Fawcett, S. L., Wheaton, D. H., Birch, D., & Uauy, R. (2003). Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: A randomized clinical trial. *The Journal of Pediatrics*, *142*, 669–677.

Järvisalo, M. J., Putto-Laurila, A., Jartti, L., Lehtimaki, T., Solakivi, T., Ronnemaa, T., & Raitakari, O. T. (2002). Carotid artery intima-media thickness in children with type 1 diabetes. *Diabetes*, *51*, 493–498.

Katier, N., Uiterwaal, C. S., de Jong, B. M., Kimpen, J. L., Verheij, T. J., Grobbee, D. E., … van der Ent, C. K. (2004). The Wheezing Illnesses Study Leidsche Rijn (WHISTLER): Rationale and design. *European Journal of Epidemiology*, *19*, 895–903.

Miller, P. E., Van, E. M., & Alexander, D. D. (2014). Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: A meta-analysis of randomized controlled trials. *American Journal of Hypertension*, *27*, 885–896.

Pauciullo, P., Iannuzzi, A., Sartorio, R., Irace, C., Covetti, G., Di Costanzo, A., & Rubba, P. (1994). Increased intima-media thickness of the common carotid artery in hypercholesterolemic children. *Arteriosclerosis and Thrombosis*, *14*, 1075–1079.

Reinehr, T., Kiess, W., de Sousa, G., Stoffel-Wagner, B., & Wunsch, R. (2006). Intima media thickness in childhood obesity: Relations to inflammatory marker, glucose metabolism, and blood pressure. *Metabolism*, *55*, 113–118.

Salonen, R., & Salonen, J. T. (1991). Determinants of carotid intima-media thickness: A population-based ultrasonography study in eastern Finnish men. *Journal of Internal Medicine*, *229*, 225–231.

Urbina, E. M., Williams, R. V., Alpert, B. S., Collins, R. T., Daniels, S. R., Hayman, L., … McCrindle, B. (2009). Noninvasive assessment of subclinical atherosclerosis in children and adolescents: Recommendations for standard assessment for clinical research: A scientific statement from the American Heart Association. *Hypertension*, *54*, 919–950.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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