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The 10-year follow-up of a randomised trial of long-chain polyunsaturated fatty acid supplementation in preterm infants: effects on growth and blood pressure

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ABSTRACT

Objective To test the hypothesis that consumption of infant formulas containing long-chain polyunsaturated fatty acids (LCPUFAs) by preterm infants would favourably influence growth, body composition and blood pressure (BP) at age 10 years.

Methods This was a follow-up study of a preterm cohort (<35 weeks and birth weight <2000 g) randomly assigned to unsupplemented or LCPUFA-supplemented formulas to 9 months post term. The setting was a research clinic at Yorkhill Hospital for Sick Children, Glasgow, UK. A total of 107 children aged 9–11 years who participated in the original randomised controlled trial (45% follow-up) took part. Main outcome measures were: (1) anthropometry, (2) body composition and (3) BP.

Results There were no differences in growth or BP between randomised groups for the whole cohort. However, girls who had received LCPUFA-supplemented formula were heavier (42.20 (SD 9.61) vs 36.94 (9.46) kg, p=0.05), had greater skin fold thicknesses (biceps 10.7 (3.3) vs 8.5 (3.6) mm, p=0.03; suprailiac 16.7 (8.2) vs 12.0 (7.5) mm, p=0.03) and higher BP (mean 82.2 (8.4) vs 78.1 (6.2) mm Hg, p=0.04: systolic 111.4 (10.1) vs 105.9 (9.0) mm Hg, p=0.04: diastolic 64.8 (8.4) vs 61.1 (5.4) mm Hg, p=0.05). Differences in weight SD score (0.85 (95% CI 0.13 to 1.58), p=0.02), *Ln sum of* skin fold thicknesses (0.27 (0.02 to 0.52), p=0.04) and BP (mean 4.6 mm Hg (0.43 to 8.84), p=0.03; systolic 6.1 (0.45 to 11.7), p=0.04) remained after adjustment for prerandomisation confounders. Differences in BP were not significant following adjustment for current weight.

Conclusions Girls born preterm and randomised to LCPUFA-supplemented formula showed increased weight, adiposity and BP at 9–11 years, which might have adverse consequences for later health. No effects were seen in boys. Long-term follow-up of other LCPUFA supplementation trials is required to further investigate this finding.

What is already known on this topic

- Systematic reviews of long-chain polyunsaturated fatty acid (LCPUFA) supplementation of preterm infant formula have found no effect on growth or cognition up to the age of 2 years.
- One randomised controlled trial (RCT) of LCPUFA supplementation of term infant formula has shown a reduction in blood pressure (BP) at age 6 years in children born at term.

What this study adds

- We found no long-term effect on LCPUFA supplemented formula on growth, body composition or BP in boys born preterm at age 10 years.
- Girls who were born preterm and received LCPUFA supplemented formula showed increased weight, adiposity and BP at age 10 years, with potential consequences for later health.

INTRODUCTION

Long-chain polyunsaturated fatty acids (LCPUFAs) are structural molecules found in cell membranes in neural and retinal tissues and vascular endothelium, but are also functional compounds, acting as precursors for eicosanoids, signalling molecules that are known to control inflammatory processes,[1] immunity and adipogenesis.[2] Importantly, the two predominant LCPUFA families (n-6 and n-3) often have opposing actions; thus, arachidonic acid (AA; n-6) is adipogenic and docosahexaenoic acid (DHA; n-3) antiadipogenic.[3] Breast milk contains LCPUFAs and, based largely on short-term biochemical studies, they have been added to infant formula, mainly in the expectation that this would improve cognitive outcomes.

The recently updated Cochrane systematic reviews on LCPUFA supplementation in preterm and term infants,[4 5] based on 15 and 14 studies respectively, concluded that there was no evidence that supplementation of infant formula affected the cognitive outcome or growth of infants up to 24 months of age. Very few longer-term data have been published from randomised trials of LCPUFA supplementation, and only a single 12-month follow-up study has reported specifically on body composition; Groh-Wargo *et al*[6] reported no effect of LCPUFA supplementation of preterm formula

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with DHA and AA on overall growth, but supplementation was associated with the likely beneficial effects of increased fat free mass (FFM) and reduced fat mass (FM) at 12 months.

In adults, n-3 fatty acid supplementation (usually from fish oil) has been shown to reduce blood pressure (BP)[7] and heart rate.[8] There are few available data investigating effects of LCPUFAs on cardiovascular outcome in healthy children born at term and none in those born preterm. Damsgaard *et al*[9] showed reduced systolic BP in 9-month-old infants following a 3-month fish oil intervention. However, of greater interest is whether effects of supplementation persist beyond the period of intervention, which may suggest a long-term programming effect. Forsyth *et al*[10] reported that LCPUFA supplementation during the first 4 months of life in term infants reduced mean and diastolic BP at 6 years. In contrast, a Danish randomised controlled trial (RCT) of fish oil supplementation given to lactating mothers for 4 months found that the supplemented boys (but not girls) had higher BP at age 7 years.[11]

This paper addresses the paucity of data regarding the potential long-term effects of LCPUFAs on outcomes other than cognition. We report the findings from a 10-year follow-up of a trial in preterm infants randomised to standard or LCPUFA-supplemented formulas from birth until 9 months post term. LCPUFA supplementation in this study was associated with greater weight and length gain by 9 months, with a stronger effect in boys which remained at 18 months.[12] However, there were no differences in skin fold thicknesses (as a measure of body composition; unpublished data). Here, we test the hypotheses that LCPUFA supplementation of infant formula would have favourable effects on later body composition, growth and BP.

SUBJECTS AND METHODS

Original trial

Preterm infants with birth weights <2000 g were recruited from five UK neonatal intensive care units in Glasgow between April 1995 and July 1997. All had gestational age <35 weeks and were free from congenital malformations. Infants were eligible if they received some of their feeds as formula milk while in hospital. After discharge, infants were seen at home at 6, 12 and 26 weeks and at 9 and 18 months post term. The primary outcome for the study was neurodevelopment at 18 months post term. Secondary outcome measures were (1) neurodevelopment at 9 months and (2) neurological impairment and growth at 9 and 18 months. Growth was considered a secondary outcome measure and a safety outcome.

Trial diets

Infants were randomly assigned a trial formula using random permuted block allocation with assignments kept in sealed opaque envelopes and opened at the point of randomisation, after informed consent was obtained. Subjects received either LCPUFA-supplemented or unsupplemented formulas once formula feeding commenced, until 9 months post term. Twins and triplets received the same formula. Preterm formula was used until the infant reached 2 kg or was discharged from hospital (Osterprem±LCPUFA; HJ Heinz Co Ltd, Hayes, UK), then a nutrient-enriched postdischarge formula (Farley's PremCare ± LCPUFA). LCPUFAs were sourced from borage (starflower) oil (γ-linolenic acid (GLA), C18:3 n-6 a precursor of AA) and tuna fish oil (DHA, C22:6 n-3). Trial formulas were identical in appearance and smell (table 1). Blinding was maintained until after the 18-month data analysis.

Table 1 Composition of trial formulas per 100 ml

Composition	Formula	
	Preterm infant formula*	Postdischarge formula*
Protein, g	2.0	1.85
Casein, g	0.77	0.72
Whey, g	1.23	1.13
Carbohydrate, g	7.65	7.24
Fat, g	4.6	3.96
Energy		
kJ	334	301
kcal	80	72
Minerals		
Calcium, mg	110	70
Phosphorus, mg	63	35
Sodium, mg	42	22
Potassium, mg	72	78
Iron, mg	0.04	0.65
Zinc, mg	0.88	0.6
Vitamins		
A (retinol), μg	100	100
D_3, μg	2.4	1.3
K, μg	7.0	6.0
E, mg	10	1.5
Carnitine, mg	1.0	1.1
Choline, mg	5.6	5.1
	Control formulas†	LCPUFA-supplemented formulas
Fatty acid composition, g/100g fat		
C10:0 capric	1.6	1.5
C12:0 lauric	12.9	12.0
C14:0 myristic	6.9	6.6
C16:0 palmitic	11.6	12.0
C18:0 stearic	5.6	5.6
Other saturated	3.7	3.9
Total saturated	42.3	41.6
C16:1 palmitoleic	0.7	0.8
C18:1 oleic	43.0	41.0
Other monounsaturated	0.9	1.2
Total monounsaturated	44.6	43.0
C18:2 n-6 linoleic	11.5	12.3
C18:3 n-6 γ-linolenic	Trace	0.9
C18:3 n-3 α-linolenic	1.6	1.5
C20:5 n-3 eicosapentaenoic	0.0	0.1
C22:6 n-3 docosahexaenoic	0.0	0.5
C20:4 n-6 arachidonic	0.0	0.04

*Values apply to control and LCPUFA-supplemented formulas.
†Values apply to preterm and postdischarge formula.
LCPUFA, long-chain polyunsaturated fatty acid.

Follow-up study at 10 years

All surviving children from the original study were invited to join the follow-up study which consisted of one visit to the Royal Hospital for Sick Children, Glasgow for cognitive assessment (data not shown) and measurement of anthropometry, body composition and BP. Written informed consent was obtained from the carer and assent from the child. Ethics approval was obtained from the Yorkhill Local Research Ethics Committee (reference no.: 06/S0708/05 May 06).

Weight was measured using digital scales, height using a stadiometer (Leicester height measure; Child Growth Foundation, London, UK) and head circumference (HF) and mid-upper arm circumference using a tape measure (Lasso tape; Child Growth Foundation). SD scores (SDS) for weight, height and body mass index were calculated using the 1990 British growth reference.[13] Skin fold (SF) thicknesses (four sites) were measured using Harpenden callipers (Holtain Instruments Ltd, Crymych, UK). Social and demographic factors were assessed by collecting data on mother's age, highest educational attainment and the occupation of the parent

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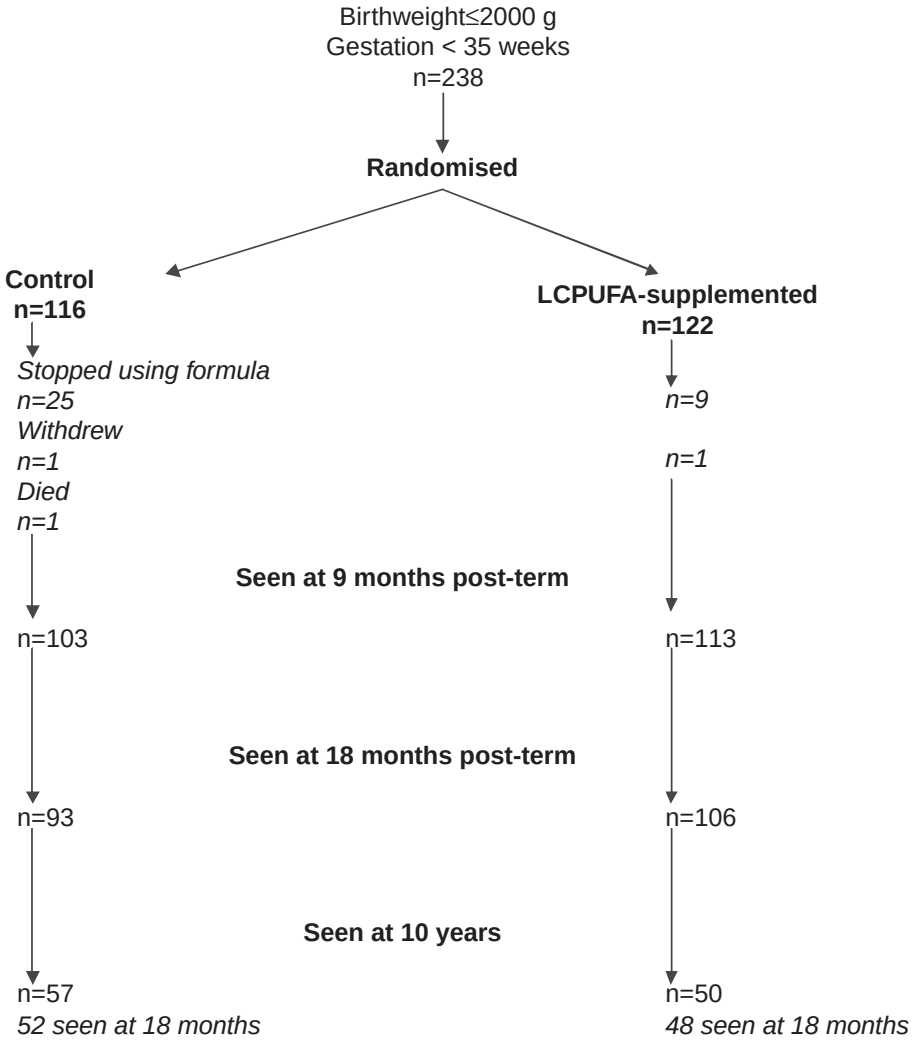
providing the main financial support; social code was derived using occupation code from the Standard Occupational Classification for the UK.[14]

Body composition was measured using two-component models providing measures of FM and FFM: (1) bioelectric impedance (BIA) with a single frequency (50 kHz) analyser (model BIM4, impedance range 10–2000 Ohms; SEAC, St Lucia, Queensland, Australia); (2) deuterium dilution and (3) skin fold equations. Body composition from BIA was calculated using the equations of Hautkooper *et al*.[15] For deuterium dilution, the subject provided a baseline saliva sample before drinking a dose of diluted deuterium. After 5 h a second saliva sample was obtained. Total body water (TBW) was estimated using an equilibration method (Thermo Finnigan Delta Plus XP; Bremen, Germany[16]), with values adjusted for fluid intake during the 5 h period; specific hydration factors (unpublished data) were used to calculate FFM in kg, as follows: FFM=TBW/hydrFFM. Body composition was also calculated from triceps and subscapular skin fold thicknesses using the gender and size specific skin fold equations of Slaughter *et al*.[17] FM and FFM values were normalised by height squared to create a fat mass index (FMI) and a fat free mass index (FFMI): FMI=FM/height^2; FFMI=FFM/height^2.[18]

BP was measured using an automated device (Accutorr; Datascope, Fairfield, New Jersey, USA), systolic, diastolic and mean arterial (MAP) BP and pulse rate were collected (MAP=(((2×diastolic)+systolic)/3). Socioeconomic data were collected by interview. Subjects assessed their pubertal status



Reason for non-participation at 10 years	Control (n = 59)	LCPUFA supplemented (n = 72)
No response to 3 letters	*36*	*43*
Did not wish to take part	*6*	*3*
Moved away / abroad	*4*	*6*
GP advised not to contact family	*2*	*8*
DNAs (at least twice)	*6*	*7*
Not registered with a GP	*1*	*2*
Not known	*4*	*3*

Figure 1 Flow chart of progress of subjects through study.

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themselves using diagrams showing the Tanner stages of development.

Sample size

We aimed to study 64 subjects per randomised group (requiring a 66% follow-up rate), which would allow us to detect a 0.5 SD difference in outcome variables with 80% power and 5% significance. This effect size was considered to be plausible and biologically relevant.

Statistics

Outcome measures were compared between randomised groups using the Student's t test (normally distributed outcomes) or non-parametric equivalent (outcomes with non-normal distribution). Categorical variables were analysed by χ^2 test.

Planned subgroup analyses by gender were conducted in view of the gender-specific effects of LCPUFA found in the original study. Multiple regression analyses were used to examine the effects of prerandomisation variables that differed between groups at follow-up, considered as potential confounders. We also examined whether the effect of LCPUFA supplementation on later BP might be mediated by current weight.

RESULTS

In all, 107 children were seen at 10-year follow-up; 57 previously randomised to control formula and 50 to LCPUFA-supplemented formula (see figure 1). Compared to subjects not seen for follow-up, those seen had significantly higher birthweight SD scores, lower gestational age, were significantly older at randomisation and were more likely to have required ventilation and to have received maternal breast milk (table 2). Of subjects seen at follow-up (table 3), the LCPUFA-supplemented group had older mothers (30.1 (3.8) years vs 27.9 (6.0), p=0.03) and higher socioeconomic status (social codes 1 and 2; 36% vs 12%), p=0.006). These factors were included as confounders in subsequent analyses.

There were no significant differences for any outcome measure between control and LCPUFA-supplemented groups for the whole cohort (boys and girls combined). However, in preplanned analyses by gender (table 4), LCPUFA-supplemented girls had significantly higher weight, weight SD score, height, head circumference (and SD score), suprailiac and biceps skin fold thicknesses and BP, with trends in the same direction for most other outcome measures. Significance values for tests of interaction between gender and intervention group were as follows: weight 0.12, weight SDS 0.21, HC 0.06, HC SDS 0.059, suprailiac SF 0.042, biceps SF 0.083, systolic BP 0.047, mean BP 0.14.

Analyses were repeated after adjusting for prerandomisation confounders (social code and mother's age), and differences in weight SD score, height, head circumference, biceps skin fold, log sum skin fold thicknesses and BP remained (table 5). We did not add current weight to our initial adjusted analysis, as this postrandomisation measurement may be a consequence of the intervention rather than a confounder. However, in view of the recognised relationship between current weight and BP, we performed an additional explanatory analysis, including current weight in the model as a potential mediator of the observed effect of LCPUFA supplementation on later BP. In this model, differences in BP between the LCPUFA-supplemented groups and control groups were reduced (table 5). Pubertal stage was not added to the model, as there were no differences between groups.

DISCUSSION

Our findings, from the longest follow-up to date of a randomised trial of LCPUFA supplementation of preterm infant formula, suggest that supplementation using DHA from fish oil and GLA from borage oil (as a precursor of AA) has no effect on growth, body composition or BP at 10 years

Table 2 Baseline characteristics for those seen and not seen at 10-year follow-up

	Seen, n=107)	Not seen, n=131	p Value
Birth weight, g	1485 (352)	1509 (318)	0.58
Birth weight SD score	−0.41 (1.07)	−0.70 (0.95)	0.03
Gestational age, weeks	30.8 (2.2)	31.3 (1.9)	0.03
Boys, n (%)	51 (48)	66 (50)	0.39
From multiple pregnancy, n (%)	38 (36)	62 (47)	0.07
Delivered by caesarean section, n (%)	70 (65)	73 (56)	0.083
Social class 1 or 2, n (%)	25 (23)	29 (22)	0.48
Mother with degree	4 (4)	7 (5)	0.40
Mother's age, years	28.9 (5.2)	28.6 (5.2)	0.59
Paternal non-smokers	62 (55)	61 (47)	0.23
Maternal non-smokers first trimester	59 (51)	64 (48)	0.40
Maternal non-smokers beyond 12 weeks	66 (57)	68 (51)	0.21
Maternal BMI,* kg/m^2	24.0 (5.1)	22.8 (4.4)	0.05
Paternal BMI,* kg/m^2	25.1 (3.6)	24.8 (4.3)	0.62
Mother of European ethnicity, n (%)	106 (99)	126 (95)	0.16
Age at randomisation, days	16 (11)	12 (8)	0.002
Days in hospital	43 (22)	39 (26)	0.23
Required ventilation, n (%)	49 (46)	41 (31)	0.03
Days of ventilation	5.1 (5.4)	6.4 (7.9)	0.39
Received MBM, n (%)	68 (64)	62 (47)	0.01
Proportion of enteral intake as MBM	28.8 (31.1)	21.9 (23.6)	0.15

* Parental anthropometry collected during first 6 months of original study.
BMI, body mass index; MBM, maternal breast milk.

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Table 3 Comparison of groups at follow-up, according to randomised diet group.

	Control, n=57	LCPUFA-supplemented, n=50	p Value
Age, years	10.8 (0.7)	10.8 (0.6)	0.73
Boys, n (%)	26 (46)	25 (50)	0.40
Pubertal status, n* (%)			
1	36 (66)	33 (67)	
2	14 (26)	13 (27)	
3	4 (7)	2 (4)	
4	1 (2)	1 (2)	0.50
Mother's age,† years	30.1 (3.8)	27.9 (6.0)	0.03
Mother's weight,‡ kg	69.67 (16.05)	69.85 (14.01)	0.88
Mother's height,‡ cm	161.0 (8.6)	161.8 (5.40)	0.56
Mother's BMI,‡ kg/m^2	26.8 (5.4)	26.6 (4.7)	0.93
Father's weight,‡ kg	86.3 (17.4)	85.7 (15.7)	0.78
Father's height,‡ cm	175.3 (6.4)	177.7 (9.3)	0.16
Father's BMI,‡ kg/m^2	28.1 (5.0)	27.0 (4.3)	0.22
Social code 1 and 2, n (%)	7 (12)	18 (36)	0.006
Mother of European ethnicity, n (%)	57 (100)	49 (98)	0.29
Received human milk, n (%)	34 (60)	34 (68)	0.24
Percentage human milk§	13 (2,55)	15 (6,50)	0.66
Weight at 10 years, kg	36.89 (10.08)	39.10 (9.99)	0.32
Weight SDS	−0.02 (1.44)	0.38 (1.16)	0.14
Height, cm	140.6 (8.8)	143.0 (6.8)	0.21
Height, SDS	−0.35 (1.24)	0.02 (0.85)	0.13
BMI, kg/m^2	18.4 (3.6)	18.9 (3.8)	0.44
BMI, SDS	0.25 (1.33)	0.49 (1.28)	0.35
Skin fold thicknesses, mm			
Subscapular	11.0 (6.5)	11.8 (6.8)	0.54
Triceps	13.5 (5.6)	14.1 (5.6)	0.48
Suprailiac	11.4 (7.4)	13.0 (8.4)	0.31
Biceps	8.5 (3.8)	9.3 (3.8)	0.23
Ln sum skin folds	3.68 (0.48)	3.74 (0.47)	0.48
Head circumference, cm	53.4 (2.2)	53.8 (1.8)	0.40
Head SDS	−0.68 (1.46)	−0.40 (1.29)	0.34
Waist circumference, cm	65.8 (10.0)	68.1 (9.2)	0.23
Mid-upper arm circumference, cm	22.8 (3.5)	23.5 (4.2)	0.36
Body composition from skin fold equations by Slaughter *et al*[17]			
Percentage FM	19.8 (6.9)	20.8 (6.6)	0.40
FM, kg	7.83 (4.71)	8.39 (4.31)	0.52
FFM, kg	29.06 (6.01)	29.90 (5.44)	0.65
FM index	3.85 (2.08)	4.05 (1.93)	0.57
FFM index	14.55 (1.76)	14.56 (1.62)	0.95
Body composition from bioelectrical impendence			
FFM, kg	27.2 (5.0)	28.5 (5.7)	0.38
FFM index	13.55 (1.41)	13.79 (1.70)	0.58
Body composition by deuterium dilution (total body water)			
FM, kg	10.7 (5.8)	11.6 (6.5)	0.35
FM index	5.3 (2.6)	5.6 (2.8)	0.56
FFM index	13.2 (1.4)	13.3 (1.3)	0.51
Blood pressure (BP)			
Systolic BP, mm Hg	105.8 (8.6)	107.7 (9.3)	0.32
Diastolic BP, mm Hg	60.8 (6.1)	63.1 (7.0)	0.07
Mean BP, mm Hg	78.0 (6.6)	80.1 (6.9)	0.13
Pulse, beats/min	74.7 (10.1)	75.6 (8.2)	0.50

*Three subjects did not complete the puberty questionnaire.
†Age of mother when child was born.
‡Parental anthropometry collected at time of follow-up.
§Proportion of enteral intake as human milk for those who received maternal breast milk.
BMI, body mass index; BP, blood pressure; FM, fat mass; FFM, fat free mass; SDS, SD score.

of age when data for boys and girls are combined. However, in planned subgroup analyses by gender, supplemented girls were heavier, taller, with greater head circumference, greater skin fold thicknesses and higher BP, even when adjusted for prerandomisation confounders. The observed effect on BP was no longer significant after adjusting for current weight, suggesting that the effect of LCPUFAs on later BP may be mediated at least in part by effects on body size.

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Table 4 Comparison of groups at follow-up according to randomised diet group and gender

	Boys (control), n=26	LCPUFA supplemented n=25	p Value	Girls (control), n=31	LCPUFA supplemented n=25	p Value
Age, years	10.9 (0.6)	10.9 (0.6)	0.99	10.8 (0.8)	10.9 (0.5)	0.69
Pubertal status, n (%)						
1	17 (65)	18 (75)		19 (66)	15 (60)	
2	6 (23)	5 (21)		8 (28)	8 (32)	
3	2 (8)	1 (4)		2 (7)	1 (4)	
4	1 (4)	0	0.33	0	1 (4)	0.45
Mother's age, years	29.8 (6.3)	31.0 (3.4)	0.40	26.3 (5.3)	29.2 (4.1)	0.03
Mother's weight, kg	66.23 (13.07)	68.54 (13.6)	0.54	72.7 (17.9)	72.0 (14.7)	0.88
Mother's height, cm	161.2 (10.4)	161.6 (5.8)	0.86	160.8 (6.8)	162.1 (5.1)	0.46
Mother's BMI, kg/m^2	25.5 (4.5)	26.2 (4.6)	0.58	27.9 (6.0)	27.2 (4.8)	0.67
Father's weight, kg	85.3 (18.3)	84.4 (14.6)	0.71	87.1 (16.9)	87.2 (17.1)	0.99
Father's height, cm	176.3 (7.3)	175.8 (6.2)	0.67	174.3 (5.4)	179.6 (11.5)	0.04
Father's BMI, kg/m^2	27.5 (5.0)	27.3 (4.3)	0.77	28.7 (5.1)	26.8 (4.3)	0.17
Social code 1 and 2, n (%)	4 (15)	11 (44)	0.03	3 (10)	7 (28)	0.08
Received human milk, n (%)	16 (62)	18 (72)	0.31	18 (58)	16 (64)	0.43
Percentage human milk*	13 (3,62)	9 (6,57)	0.92	13 (1,55)	15 (6,45)	0.78
Weight, kg	36.83 (10.97)	35.4 (9.59)	0.62	36.94 (9.46)	42.20 (9.61)	0.05
Weight SDS	−0.003 (1.58)	0.023 (1.16)	0.95	−0.027 (1.34)	0.70 (1.10)	0.03
Height, cm	140.3 (9.9)	140.3 (5.8)	0.99	140.9 (7.8)	144.6 (4.7)	0.03
Height SDS	−0.39 (1.33)	−0.25 (1.0)	0.66	−0.31 (1.18)	0.18 (0.65)	0.06
BMI, kg/m^2	18.4 (3.6)	1 7.8 (3.3)	0.56	18.4 (3.60)	20.10 (3.9)	0.10
BMI SDS	0.38 (1.26)	0.20 (1.23)	0.61	0.14 (1.29)	0.78 (1.31)	0.08
Head circumference, cm	53.9 (2.5)	53.4 (1.6)	0.37	52.9 (1.8)	54.0 (1.9)	0.03
Head SDS	−0.50 (1.50)	−0.80 (0.98)	0.40	−0.84 (1.45)	−0.06 (1.47)	0.05
Waist circumference, cm	66.3 (9.8)	65.7 (8.8)	0.83	65.4 (10.4)	70.3 (9.2)	0.06
Mid-upper arm circumference, cm	22.5 (3.8)	21.9 (3.6)	0.59	23.1 (3.3)	25.1 (4.3)	0.07
Skin fold thicknesses, mm						
Subscapular	10.4 (6.6)	9.2 (5.4)	0.52	11.5 (6.5)	14.7 (7.0)	0.09
Triceps	13.0 (5.9)	12.7 (5.6)	0.89	14.0 (5.4)	15.9 (5.1)	0.19
Suprailiac	10.7 (7.4)	9.6 (7.1)	0.57	12.0 (7.5)	16.7 (8.2)	0.03
Biceps	8.5 (4.2)	8.1 (3.9)	0.76	8.5 (3.6)	10.7 (3.3)	0.03
Ln sum skin folds	3.62 (0.52)	3.56 (0.46)	0.69	3.73 (0.45)	3.96 (0.40)	0.06
Percentage FM from Slaughter *et al*[17] equations	19.78 (8.0)	19.16 (7.5)	0.73	19.90 (5.9)	22.69 (4.91)	0.07
FM SI	7.91 (5.49)	7.42 (4.9)	0.74	7.77 (4.03)	9.48 (3.40)	0.11
FFM SI	28.92 (6.28)	28.0 (4.4)	0.55	29.17 (5.88)	31.26 (4.91)	0.17
FMI SI	3.88 (2.37)	3.65 (2.15)	0.73	3.83 (1.83)	4.53 (1.55)	0.15
FFMI SI	14.50 (1.51)	14.15 (1.41)	0.39	14.59 (1.97)	14.94 (1.79)	0.50
BIA						
FFM (Houtkooper *et al*[15])	27.13 (5.9)	27.49 (6.8)	0.72	27.19 (4.1)	29.49 (4.2)	0.06
FFMI (Houtkooper *et al*[15])	13.4 (1.3)	13.4 (1.8)	0.98	13.7 (1.5)	14.0 (1.6)	0.46
1/R*1000	0.13 (0.02)	0.13 (0.02)	0.80	0.14 (0.02)	0.14 (0.02)	0.81
Deuterium						
Fat, kg	9.9 (6.2)	8.9 (5.2)	0.57	16.5 (5.6)	19.8 (7.5)	0.07
FMI	4.9 (2.7)	4.4 (2.1)	0.48	8.2 (2.6)	9.4 (3.3)	0.13
FFMI	13.3 (1.3)	13.1 (0.97)	0.57	10.4 (2.0)	10.8 (1.5)	0.21
Blood pressure (BP)						
Systolic BP, mm Hg	105.7 (8.4)	103.8 (6.8)	0.41	105.9 (9.0)	111.4 (10.1)	0.04
Diastolic BP, mm Hg	60.4 (6.8)	61.5 (5.3)	0.53	61.1 (5.4)	64.8 (8.4)	0.05
Mean BP, mm Hg	77.9 (7.1)	77.9 (4.6)	0.98	78.1 (6.2)	82.2 (8.4)	0.04
Pulse, beats/min	71 (11)	74 (8)	0.25	78 (9)	78 (8)	0.97

*Proportion of enteral intake as human milk for those who received maternal breast milk.
BIA, bioelectric impedance; BMI, body mass index; FMI, fat mass index; FFMI, fat free mass index; LCPUFA, long-chain polyunsaturated fatty acid; SDS, SD scores.

Inconsistent gender differences in the response to LCPUFA supplementation have previously been noted. Supplemented boys from this cohort showed greater weight and length gain between birth and 9 months and significantly higher scores on the Bayley Mental Developmental Index (MDI) than unsupplemented infants at 18 months. Ryan *et al*[19] also reported slower growth and lower FFM in LCPUFA-supplemented preterm boys but not girls. In contrast, a recent trial of high dose DHA supplementation given to mothers expressing breast milk for their preterm infant found that supplemented infants

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Table 5 Regression analyses for girls unadjusted and adjusted for social code, maternal age and current weight

	Unadjusted difference (95% CI)	p Value	Difference adjusted for social code and maternal age	p Value	Difference adjusted for social code, maternal age and current weight of subject	p Value
Weight SDS	0.72 (0.06 to 1.39)	0.03	0.85 (0.13 to 1.58)	0.02		
Height, cm	3.7 (0.12 to 7.30)	0.04	4.2 (0.29 to 8.06)	0.04		
Head circumference, cm	1.09 (0.09 to 2.09)	0.03	1.15 (0.06 to 2.24)	0.04		
Biceps skin fold, mm	2.18 (0.27 to 4.08)	0.03	2.67 (0.61 to 4.73)	0.01		
Ln sum of skin folds	0.23 (−0.01 to 0.46)	0.06	0.27 (0.02 to 0.52)	0.04		
Systolic blood pressure, mm Hg	5.5 (0.25 to 10.73)	0.04	6.1 (0.45 to 11.7)	0.04	3.5 (−1.89 to 8.91)	0.2
Diastolic blood pressure, mm Hg	3.7 (−0.03 to 7.53)	0.05	4.5 (0.48 to 8.44)	0.03	3.85 (−0.32 to 8.02)	0.07
Mean arterial blood	4.1 (0.11 to 8.11)	0.04	4.6 (0.43 to 8.84)	0.03	3.04 (−1.13 to 7.20)	0.15

SDS, SD scores.

were longer at 18 months and that supplemented girls (but not boys) had higher MDI than control girls.[20] The reasons for different gender effects are not clear but could reflect the varied doses or sources of LCPUFA in different studies. Our study intervention used a relatively high level of DHA (0.5% DHA/100 g FA) from fish oil and for a long period (to 9 months post term), while the trial by Ryan *et al* also sourced DHA from fish oil but at a lower concentration (0.2%DHA/100 g FA) for the same duration. In contrast, the Makrides study compared infants receiving a 'standard' DHA intake (0.3%) with high dose DHA (1%).

Animal studies suggest that varying the ratio of n-3 and n-6 LCPUFAs can influence body composition, including the location of fatty depots and adipocyte size,[21 22] while site-specific alteration in gene regulation has been reported in adipose tissue from adult rats fed diets high in either eicosapentaenoic acid (EPA) or DHA or with no n-3.[21] In this study, we did not specifically attempt to examine fat distribution. However, there were significant differences between LCPUFA-supplemented and control girls in the sum of the four skin folds (predominantly an indicator of subcutaneous fat), while differences in total body FM measured by impedance or deuterium dilution were not significant. We suggest that future studies should investigate fat distribution as well as total fat mass.

A 6-year follow-up of an RCT in term children[10] reported that LCPUFA supplementation was associated with a presumably beneficial reduction in diastolic and mean BP. We found no evidence for such an effect in our preterm cohort at age 10 years. Our study involved a longer period of supplementation (9 months vs 4 months) using a formula with higher DHA concentration (0.5 g/100 g fat vs 0.2 g/100 g fat), albeit with a similar ratio of n-6: n-3 fatty acids (0.9:0.5 vs 0.3–0.4:0.15–0.25). However, the source of DHA differed in the two studies (tuna oil containing n-3 fatty acids in triglyceride form in our study; egg phospholipid in the Forsyth *et al* study) and this may contribute to the inconsistent findings.[3] Our study also used GLA (C18:3 n-6) sourced from borage oil as a precursor of AA. Plasma fatty acid profiles measured in a subgroup during the first month of the study showed supplemented infants had levels of GLA and AA that were 47% and 16% higher than control subjects, providing some evidence of biochemical efficacy.

The BP findings in our study are more comparable to those reported recently by Asserhøj *et al*[11] from an RCT of fish oil supplementation of lactating mothers, although in that study higher BP was seen in boys of supplemented mothers rather than girls. It is plausible that LCPUFAs (in particular n-3s) could influence BP by acting as structural components of cell membranes of the vascular endothelium, resulting in increased membrane fluidity. Alternatively, they may act as precursors for vasodilatory eicosanoids. However, these mechanisms would be unlikely to result in a long-term 'programming' effect given the rapid turnover of these fatty acids in cell membranes and eicosanoids. The effects of LCPUFAs on outcomes such as body composition and BP may be better explained by an impact on gene expression of transcription factors that have roles in fatty acid and triglyceride metabolism.[23 24]

Study limitations: the impact of cohort attrition

Cohort attrition and its effect on power and bias are important considerations given our 45% follow-up rate.[25] Our target sample size (64 subjects per group, requiring a 66% follow-up) would allow us to detect a 0.5 SD difference in outcome measures; in the event, we only had the power to detect a 0.6 SD difference between groups. LCPUFA supplementation was previously associated with a BP lowering of around 0.3 SD.[10] We may therefore have missed a small but biologically significant effect. However, we found that LCPUFA supplementation was associated with higher BP in girls, rather than with the expected decrease. Regarding bias, we identified and adjusted for characteristics that differed between groups at follow-up. It is difficult to estimate the impact of these characteristics on the generalisability of our findings to the whole cohort since some would be anticipated to indicate that the subjects seen at 10 years were a relatively higher risk population and some the opposite. Overall, we would suggest that our findings are generalisable to preterm infants born 10 years ago but, as with any follow-up study, the applicability to infants born today is less certain.

A relatively high proportion of our girl subjects had already entered puberty, and, although the distribution of pubertal stages did not differ between randomised groups, we cannot exclude this as an explanation for the observed differences in stature and adiposity between supplemented and unsupplemented girls. Further follow-up of this cohort in early adult life and follow-up of other similar trials are required to further investigate this issue.

Conclusions

Our findings suggest that LCPUFA supplementation of infant formula fed to preterm infants may result in gender-specific effects, as supplemented girls were significantly heavier, taller, with larger skin fold thicknesses and higher BP; even after adjustment for confounders. The observed effect on BP was partly mediated by the observed effect of LCPUFA on body

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weight. While taller stature might be regarded as a positive outcome, greater fatness and higher blood pressure, although generally within the normal range at present, may constitute risk factors for later overweight, obesity and hypertension, since these variables tend to track with time.

Our data suggest a need for a much broader approach to the exploration of long-term effects of LCPUFAs. As precursors to eicosanoids, LCPUFAs may have long-term effects on a wide range of outcomes, including immunity, vascular function, renal function, clotting, gene expression and body composition. Earlier trials that focused on growth and neurodevelopment were not broad enough to assess all aspects of LCPUFA function, but these outcomes should be incorporated in long-term follow-up of these cohorts.

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Competing interests MF, AL and AS have received research funding and performed advisory work for infant feeding manufacturers. LW is a member of the Infant and Toddler Forum, an educational charity funded by Danone. KK, SR and EI have no conflicts of interests to declare.

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Contributors KK provided the main draft of this article and supervised the project, SR collected and analysed the data, EI, AS and AL provided expert advice on the design and interpretation of data, LW was the local investigator in Glasgow and critically revised the article, MSF was the primary investigator who wrote the original protocol providing the original concept and design. All authors contributed to the drafting and revision of this manuscript.

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