



Original article

Effect of arachidonic and docosahexaenoic acid supplementation on quality of growth in preterm infants: A secondary analysis of a randomized controlled trial



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SUMMARY

Background & aims: A balanced supply of arachidonic acid (ARA) and docosahexaenoic acid (DHA) may be crucial for quality of growth in preterm infants. This secondary analysis of a randomized controlled trial aimed to determine the effect of enhanced ARA and DHA supplementation on growth and body composition in infants born before 29 weeks of gestation. Furthermore, we aimed to study associations between human milk feeding, growth patterns and body composition.

Methods: The ImNuT-trial randomized 121 infants to receive a daily supplement with medium chain triglycerides (control) or 100 mg/kg ARA and 50 mg/kg DHA (ARA:DHA group) from the second day of life until 36 weeks postmenstrual age. Growth and body composition were evaluated up to 3 months corrected age.

Results: The ARA:DHA group showed better linear growth from birth to term equivalent age compared to the control group; mean difference in z score change from birth for length was 0.74 ([95% CI, 0.17–1.3]; $p = 0.010$). There were no differences in growth and body composition outcomes at 3 months corrected age between the groups. An increase in z score for weight after 36 weeks postmenstrual age and breastfeeding at 3 months corrected age were the strongest positive predictors of fat mass% at 3 months corrected age (both, $p < 0.001$).

Conclusion: Early enhanced supplementation of ARA and DHA may be beneficial with respect to somatic growth in very preterm infants.

Clinical trial registration: The trial has been registered on www.clinicaltrials.gov, ID: NCT03555019.

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Abbreviations: ADP, Air Displacement Plethysmography; ARA, Arachidonic acid; CA, Corrected age; DHA, Docosahexaenoic acid; HC, Head circumference; ImNuT, Immature, Nutrition Therapy; ITT, Intention-to-treat; LCPUFA, Long-chain polyunsaturated fatty acid; MRI, Magnetic Resonance Imaging; PMA, Postmenstrual age; SGA, Small for gestational age; TEA, Term equivalent age.

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1. Introduction

Nutrition is a cornerstone of postnatal care in very preterm infants. The overarching goal is to support growth and development similar to the normal fetus [1,2]. Quality of growth, as indicated by appropriate accretion of fat-free and fat mass [1], may have important impact on neurodevelopment [3–5]. Altered growth patterns, including growth failure followed by rapid fat mass

accretion, are associated with adverse long-term metabolic health outcomes [6]. Postnatal linear growth and fat-free mass accretion may serve as prognostic markers for organ growth and development, particularly for the brain [7–11]. Of concern, very preterm infants often experience growth faltering due to illness and/or inadequate nutrition, frequently most evident for linear growth [9,12]. Previous studies have shown that preterm infants are commonly shorter and deficient in fat-free mass compared to term born peers [13]. Strategies to optimize quality of growth are needed in order to improve neurological outcomes without compromising metabolic health.

Some nutrients are particularly important modulators of organ growth and development, including the long-chain polyunsaturated fatty acids (LCPUFAs) arachidonic acid (ARA) and docosahexaenoic acid (DHA). ARA and DHA are structural components in cell membranes, but they also exhibit functional roles as precursors for bioactive mediators. Proposed mechanisms involved in growth include modulation of adipogenesis and bone metabolism through their metabolites (e.g. eicosanoids) and by regulation of gene expression [14,15]. The postnatal synthesis of ARA and DHA in very preterm infants is insufficient to meet their requirements during a crucial period of nutrient deposition [1]. Thus, very preterm infants rely on postnatal supplementation, but current nutritional practice commonly do not match intrauterine accretion rates [1,16,17]. Early, enhanced supply of ARA and DHA may improve neurological [18], visual [19] and respiratory outcomes [20] in preterm infants, but whether supplementation improves quality of growth is unclear [21].

The primary objective of the ImNuT-trial (Immature, Nutrition Therapy) was to assess the effect of early supplementation with ARA and DHA matching intrauterine accretion rates on brain maturation, neonatal morbidities and quality of growth in preterm infants born before 29 weeks of gestation [22]. The present study aimed to test the hypothesis that enhanced intake of ARA and DHA resulted in improved growth and body composition from birth up to 3 months corrected age (CA). Secondary aims were to explore how growth patterns and human milk feeding related to body composition at 3 months CA.

2. Materials and methods

2.1. Study design

The ImNuT-trial ([Clinicaltrials.gov](https://clinicaltrials.gov) ID: NCT03555019) was a single-center, double-blind, randomized controlled trial (RCT) conducted at the Department of Neonatal Intensive Care at Oslo University Hospital. A detailed description of the study protocol has previously been published [22]. The present study reports a pre-planned secondary analysis on growth and body composition from birth to 3 months CA [22].

2.2. Study participants

Infants born at Oslo University Hospital before 29 weeks of gestation were eligible for inclusion. Exclusion criteria were congenital malformations, chromosomal abnormalities or critical illness with short life expectancy. Participation required written informed, parental consent within 48 h after birth.

2.3. Randomization and blinding

Study investigators or the physician on call recruited infants to the trial. We used a computer-generated list of random numbers for allocation of the participants to the intervention or control group. The block size was randomly alternated between 4, 6 and 8. The

randomization was stratified by growth status at birth; small for gestational age (SGA) or not, with an allocation ratio of 1:1 within each block. In multiple births, siblings were allocated to the same randomization group. All caregivers, the medical staff and investigators, except for the study pharmacist, were blinded to the group allocation.

2.4. Trial intervention

The intervention group received an enteral supplement (Formulaid™, DSM Nutritional Products Inc.) consisting of ARA 100 mg/kg and DHA 50 mg/kg, while the control group received a supplement with medium chain triglycerides (MCT-oil™, Nutricia) ([Supplementary Appendix 1](#)). The dose was adjusted weekly based on the actual weight of the infant. Birthweight was used until birthweight was regained. The supplements were administered as a daily bolus via the naso-gastric feeding tube from the second day of life until 36 weeks postmenstrual age (PMA). All study participants followed a standardized nutritional protocol to accommodate the 2010 and 2018 European guidelines on preterm nutrition [2,23]. Information on formula and human milk composition for the intervention period are provided in [Supplementary Table 3](#). Details on the nutritional management and intakes have been published elsewhere [12]. The ImNuT-trial was approved by the Norwegian Regional Ethics Committee (2016-003700-31), and a Data Monitoring Committee with access to reported adverse effects and clinical outcomes reviewed the study for safety and efficacy.

2.5. Study outcomes

2.5.1. Growth

Body weight, length and head circumference (HC) were measured at 3 months CA by two trained investigators. Anthropometrics were measured regularly from birth to 36 weeks PMA. Weight, length and HC were assessed in infants eligible for Magnetic Resonance Imaging (MRI), or collected retrospectively, at term equivalent age (TEA). Z scores for weight, length and HC were derived from Niklasson 2008, a Swedish sex-specific growth chart [24]. Small for gestational age (SGA) was defined as z score for weight <−2.0 (<10th centile) [24]. We estimated changes in z scores from birth; a positive change in z score indicated increased growth velocity, i.e. upward centile crossing from the birth centile.

2.5.2. Body composition

Quality of growth was assessed at 3 months CA by a non-invasive Air Displacement Plethysmography (ADP) system, Pea Pod® (COSMED, Rome, Italy). The method uses whole body densitometry to determine fat mass and fat-free mass. Z scores were calculated according to sex and PMA specific body composition charts for term-born infants assessed with ADP [25]. In the ImNuT-trial, body composition was also assessed at 36 weeks PMA in infants hospitalized at Oslo University Hospital [12]. Assessments could not be performed in infants who required respiratory support at the time of measurement.

2.5.3. Type of milk feeding

The parents answered an interviewer-administered questionnaire on overall nutrition at TEA and 3 months CA. Type of milk feeding was classified into breast milk (volume of feeds >80% breast milk), formula (volume of feeds ≥80% formula) or mixed feeds (mix of breast milk and formula) [26].

2.6. Data analyses and statistics

The sample size calculation for the overall study was based on the primary outcome of the ImNuT-trial [22]; brain maturation assessed by MRI with diffusion tensor imaging at TEA.

We performed the Student *t* test for continuous variables and χ^2 test for categorical variables to compare growth and nutrition between the randomization groups. Repeated measures within groups were compared with the *Paired samples t-test*. To assess differences in growth patterns between birth and 3 months CA, we used mixed models for repeated measurements with a subject-specific random intercept. For comparisons of z scores for weight, length and HC, birth was used as a baseline and fixed effects in the models were the outcome measurements at baseline, treatment group, follow-up time (i.e. Day 28, 36 weeks PMA, TEA and 3 months CA) and the interaction between treatment group and follow-up time. For the comparisons of changes in z scores, randomization group, time (i.e. Day 28, 36 weeks PMA, TEA and 3 months CA) and the interaction between group and time were used as fixed effects. Analyses were performed on the intention-to-treat (ITT) population.

Previous studies have reported sex-specific effects of LCPUFA supplementation on growth [27–29]. Thus, we performed a subgroup linear regression analysis to test for any interactions between fatty acid supplementation and sex on growth (i.e. z scores and change in z scores) and body composition outcomes at 3 months CA.

Backward stepwise linear regression models were carried out to explore the influence of growth patterns and type of milk feeding, as well as for possible covariates, on body composition z scores. We included birth weight z score, sex, gestational age, postnatal steroid exposure, change in z score for weight, length and HC from Day 28 to 36 weeks PMA and from 36 weeks PMA to 3 months CA, type of milk feeding at TEA and at 3 months CA as possible predictor variables. A p-value threshold of 0.2 was set as the elimination criteria for variables included in the final model and missing values were excluded listwise. P-value <0.05 denoted statistical significance. Analyses were performed using IBM SPSS Statistics version 28 (SPSS Inc, Chicago, USA) and STATA statistical software Version 17.0.

3. Results

3.1. Study population

Between April 2018 and January 2021, 121 infants born before 29 weeks of gestation were randomized to the ImNuT-trial (Flow chart, Fig. 1). Sixty-one infants were allocated to the control group and 60 infants to the ARA:DHA group. One infant was later excluded due to a chromosomal abnormality, resulting in a total study cohort of 120 infants. During the study period, the ARA:DHA and control group received 94% and 93% of the prescribed fatty acid doses. Neonatal outcomes data were available in 110 study participants at 36 weeks PMA. Of these, 92 met for MRI scanning and growth evaluation at TEA. At 3 months CA, 97 infants were eligible for nutrition and

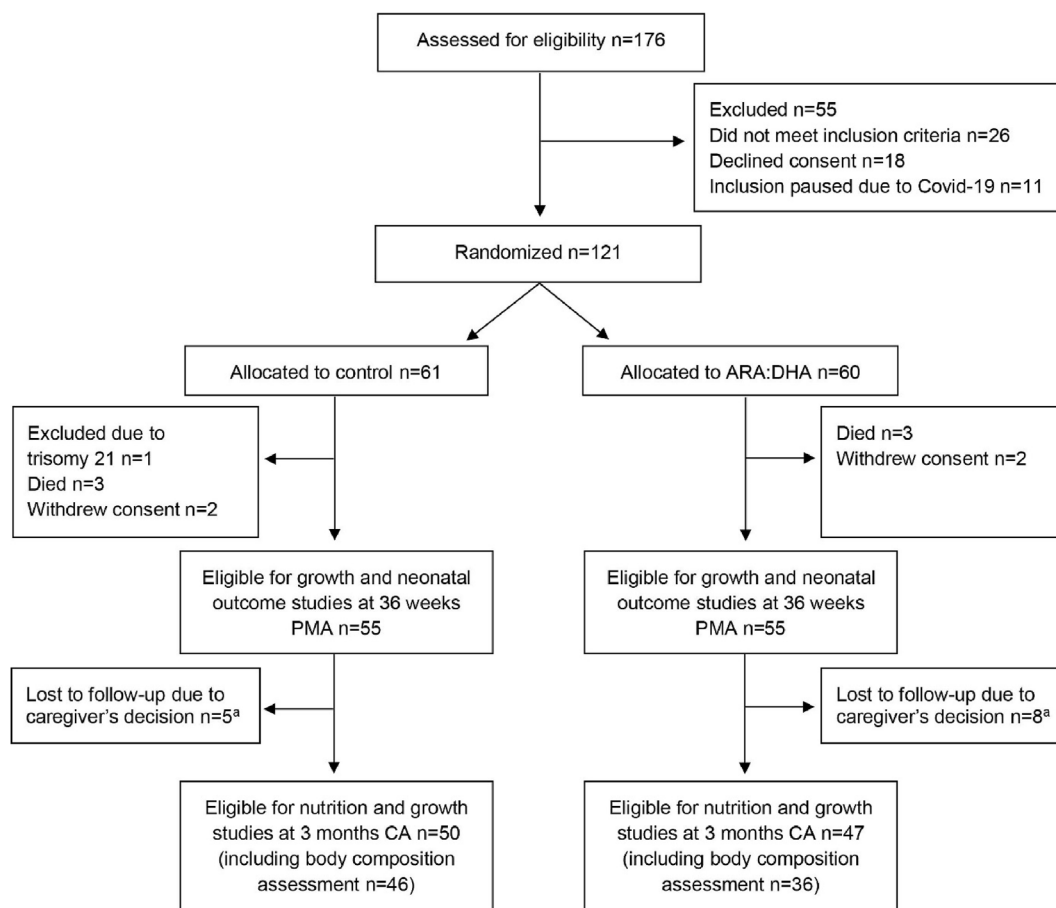


Fig. 1. Flowchart of study participants in the ImNuT-trial.

Abbreviations: ARA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; PMA, postmenstrual age.

^aThe primary reason for loss to follow-up was long travel distance.

growth studies, hereinafter referred to as the ImNuT follow-up cohort. Infant characteristics and neonatal clinical outcomes were similar between the ImNuT cohort and the ImNuT follow-up cohort (Table 1).

3.2. Anthropometry

Growth patterns from birth to 3 months CA are illustrated in Fig. 2. In both groups, z score for weight, length and HC dropped during the first four postnatal weeks and increased between Day 28 and TEA (Fig. 2a–c). The ARA:DHA group showed better linear growth from birth to TEA compared with the control group; mean difference in z score change from birth for length was 0.74 [95% CI, 0.17–1.3]; $p = 0.010$ (Fig. 2e). This corresponded to a difference in length of 0.9 cm. At 3 months CA, the mean change in z score from birth was positive for weight, length and HC in both groups (Fig. 2d–f), indicating upward centile crossing from birth centile. Weight, length and HC were below the 10th centile in 13 (13%), 12 (12%) and 6 (6%) of the 97 infants in the ImNuT follow-up cohort. Longitudinal growth patterns are presented with sex and PMA specific growth charts in Supplementary Fig. 1.

3.3. Body composition outcomes at 3 months corrected age

In total, 82 of the 97 infants in the ImNuT follow-up cohort underwent body composition assessment at 3 months CA (Table 2). Fifteen infants were not assessed: five due to device calibration error, three due to disrupted test (intense crying/caregiver's decision), one infant received respiratory support and one infant was colonized with multi-resistant bacteria. Finally, five consultations were performed by phone due to the Covid-19 pandemic or long travel distance. Infant characteristics and clinical outcomes were

similar between infants in the follow-up cohort with and without body composition measurements (Supplementary Table 1).

There were no differences in body composition outcomes between the randomization groups at 3 months CA (Table 2). Overall, mean z scores were positive for fat mass (both g and %) and negative for fat-free mass (g). The corresponding centiles are presented with sex and PMA specific growth charts in Supplementary Fig. 2. Regression models did not reveal any interaction effects between fatty acid supplementation and sex for body composition outcomes, z score or change in z score for weight, length and HC at 3 months CA (data not shown).

Body composition was assessed in 33 infants at both 36 weeks PMA and 3 months CA (Supplementary Table 2). There was a significant increase in z score for length and HC as well as for fat mass% between the visits; mean difference (95% CI) 1.3 (0.90, 1.6) z score for length, 0.72 (0.45, 1.0) z score for HC and 7.6% fat mass (5.5, 9.7) (all, $p < 0.001$).

3.4. Multivariable analyses between body composition and potential predictor variables

We used multiple linear regression models to explore associations between nutrition, growth patterns and body composition (Table 3). An increase in z score for weight, both before and after 36 weeks PMA, were associated with higher body composition z scores (all, $p \leq 0.003$). Formula feeding at 3 months CA was positively associated with fat-free mass z score ($p = 0.002$) and negatively associated with fat mass (absolute and %) z scores (both, $p < 0.001$). Exposure to postnatal steroids during hospitalization was negatively associated with absolute fat-free ($p < 0.001$) and fat mass ($p = 0.007$) z scores, but not related to fat mass% ($p = 0.263$). The adjusted R^2 suggests that the variables included in the

Table 1
Characteristics of the ImNuT cohort (ITT) and the ImNuT follow-up cohort.

	ImNuT cohort (ITT)		ImNuT follow-up cohort	
	Control (n = 60)	ARA:DHA (n = 60)	Control (n = 50)	ARA:DHA (n = 47)
Maternal characteristics				
Antenatal glucocorticoids any dose, n (%)	60 (100)	60 (100)	50 (100)	47 (100)
Cesarean delivery, n (%)	38 (63)	29 (48)	28 (56)	24 (51)
Infant characteristics				
Gestational age, weeks	26.2 (1.63)	26.6 (1.72)	26.3 (1.62)	26.5 (1.59)
Birth weight, g	833 (255)	879 (241)	866 (257)	869 (235)
Birth weight, z score	−1.25 (1.35)	−1.22 (1.46)	−1.01 (1.22)	−1.24 (1.57)
Birth length, cm ^a	33.5 (3.49)	33.7 (2.98)	33.8 (3.50)	33.7 (2.94)
Birth length, z score ^a	−1.66 (1.88)	−1.86 (1.73)	−1.46 (1.80)	−1.84 (1.83)
Birth HC, cm	23.9 (2.42)	24.4 (2.05)	24.1 (2.53)	24.3 (1.88)
Birth HC, z score	−0.68 (0.93)	−0.64 (0.95)	−0.60 (0.97)	−0.65 (1.04)
Male sex, n (%)	31 (52)	35 (58)	28 (56)	28 (60)
SGA, n (%)	12 (20)	11 (18)	7 (14)	10 (21)
Multiple birth, n (%)	13 (22)	16 (27)	10 (20)	9 (19)
Clinical characteristics^b				
Mechanical ventilation >7 days, n (%)	30 (55)	22 (37)	26 (52)	19 (40)
Postnatal steroids, n (%)	28 (51)	21 (35)	24 (48)	18 (38)
Major morbidity, n (%) ^c	32 (58)	26 (47)	29 (58)	23 (49)
Fatty acid intake^d				
ARA, mg/kg/d	24 (1.6)	111 (9.4)	24 (1.7)	110 (9.8)
DHA, mg/kg/d	19 (2.2)	63 (5.7)	18 (2.3)	63 (5.8)

Values are presented as mean (SD) and n (%) and statistical significant differences are highlighted in bold. Z scores were derived from Niklasson 2008 [24].

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; HC, head circumference; ITT, intention-to-treat; SGA, small for gestational age.

^a $n = 116$. Data on length were missing in two infants in both groups.

^b $n = 110$. Data were missing for patients who died or were withdrawn before 36 weeks postmenstrual age.

^c Major morbidity included bronchopulmonary dysplasia, severe brain injury (intraventricular hemorrhage grade III–IV or cystic periventricular leukomalacia) or retinopathy of prematurity stage ≥ 3 .

^d Mean daily intake from birth to 36 weeks postmenstrual age.

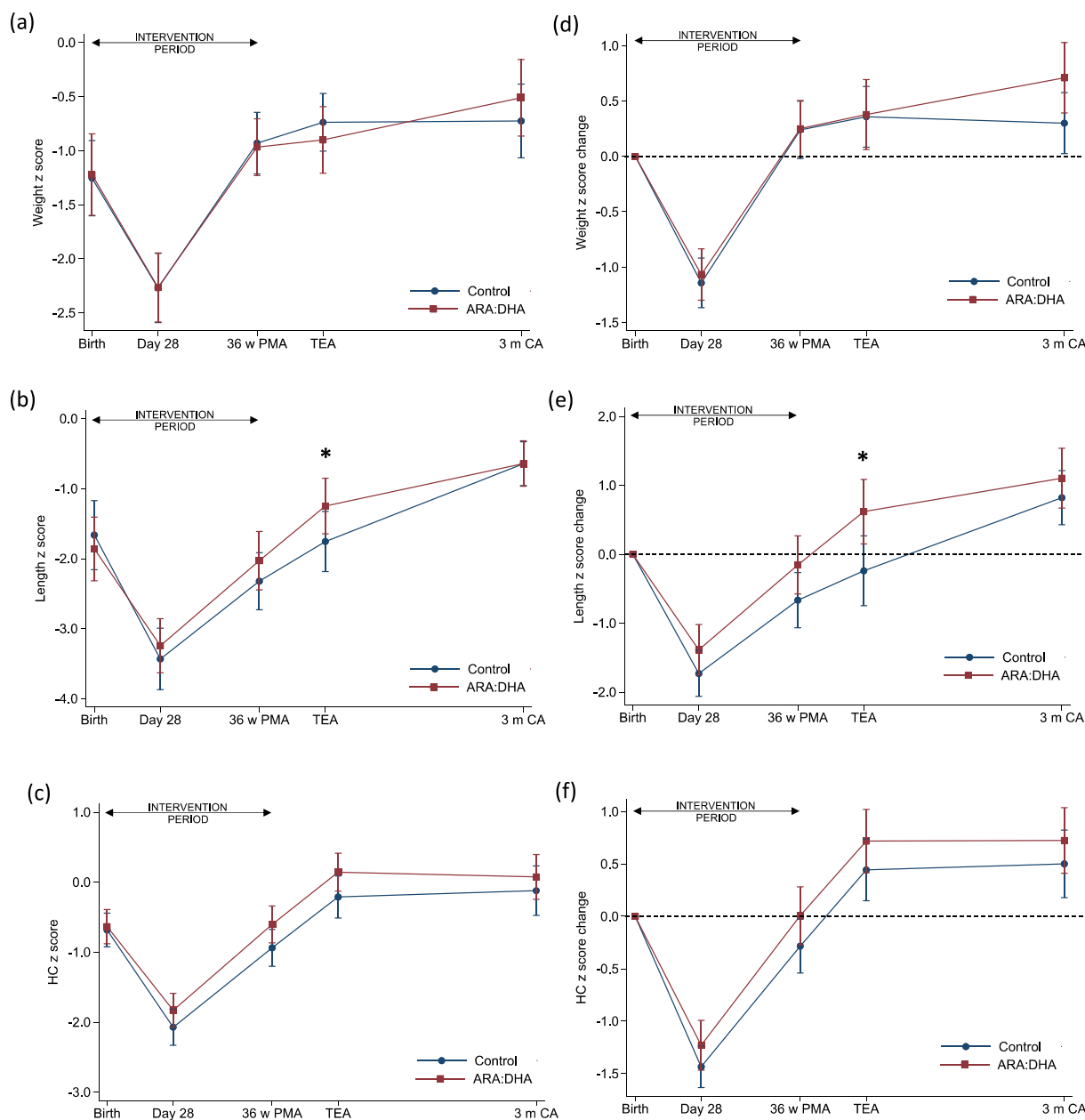


Fig. 2. Growth from birth to 3 months corrected age according to postnatal age and randomization group. Data are in means (95% CI) for each group at specific time points. Number of infants with growth data; Birth $n = 60$ vs. 60 , Day 28 $n = 55$ vs. 54 , 36 weeks PMA $n = 55$ vs. 55 , TEA $n = 46$ vs. 43 and 3 months CA $n = 50$ vs. 47 for the control and ARA:DHA group, respectively. Z scores were derived from Niklasson 2008 [24].

Abbreviations: ARA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; HC, head circumference; PMA, postmenstrual age; TEA, term equivalent age.

* $p < 0.05$.

regression models accounted for 70%, 66% and 45% of the variability in fat-free mass (g), fat mass (g) and fat mass (%) z scores, respectively (Table 3). Birth weight z score highly contributed to the variability in fat-free mass (beta = 0.767) and absolute fat-mass (beta = 0.529) z scores (both, $p < 0.001$). An increase in z score for weight after 36 weeks PMA and breastfeeding at 3 months CA were the strongest positive predictors of fat mass% z score (Table 3).

4. Discussion

In the present study, supplementation with ARA and DHA matching intrauterine accretion rates improved linear growth from birth to TEA in very preterm infants. Nevertheless, we did not find

any sustaining effect on growth or body composition at 3 months CA.

4.1. LCPUFA supplementation and quality of growth

Studies conducted in the 1990s showed that formula feeds supplemented with eicosapentaenoic acid and DHA, without concomitant ARA, led to suppressed serum levels of ARA and compromised growth in preterm infants [29–32]. Emphasizing that ARA and DHA are conditionally essential to preterm infants, these findings underlined the importance of providing both fatty acids to promote tissue accretion, although not consistently confirmed in later trials [21]. In a study of very preterm infants,

Table 2
Nutrition and growth outcomes at 3 months corrected age^a.

	All n = 97	Control n = 50	ARA:DHA n = 47	Mean diff. (95%CI) Control vs ARA:DHA	p
Anthropometry					
Weight, g	5889 (862)	5874 (843)	5905 (891)	-31 (-381 - 319)	0.861
z score	-0.62 (1.22)	-0.67 (1.22)	-0.58 (1.24)	-0.09 (-0.59 - 0.40)	0.712
Length, cm	60.0 (2.2)	60.1 (2.3)	59.9 (2.2)	0.23 (-0.68 - 1.1)	0.612
z score	-0.64 (-1.12)	-0.61 (1.13)	-0.68 (1.13)	0.07 (-0.38 - 0.53)	0.757
HC, cm	40.8 (1.5)	40.8 (1.6)	40.9 (1.5)	-0.16 (-0.78 - 0.47)	0.623
z score	-0.03 (1.19)	-0.10 (1.26)	0.05 (1.13)	-0.15 (-0.63 - 0.33)	0.533
Body composition n = 82					
Fat free mass, g	4255 (538)	4275 (576)	4229 (492)	46 (-194 - 285)	0.705
z score	-0.96 (1.21)	-0.90 (1.35)	-1.03 (1.03)	0.14 (-0.40 - 0.67)	0.598
Fat mass, g	1590 (417)	1582 (434)	1599 (399)	-17 (-202 - 169)	0.858
z score	0.06 (1.06)	0.03 (1.09)	0.09 (1.03)	-0.06 (-0.53 - 0.41)	0.804
Fat mass, %	26.9 (4.2)	26.7 (4.3)	27.2 (4.0)	-0.42 (-2.27 - 1.44)	0.656
z score	0.41 (0.97)	0.36 (1.00)	0.47 (0.93)	-0.11 (-0.54 - 0.32)	0.618
Age, PMA weeks	53.9 (1.5)	54.0 (1.5)	53.9 (1.6)	N/A	0.780
Nutrition					
Milk feed at TEA				N/A	0.365
Breast milk	38 (40)	18 (36)	20 (44)		
Mixed feeds	27 (28)	13 (26)	14 (31)		
Formula	30 (32)	19 (38)	11 (24)		
Milk feed at 3 months CA				N/A	0.551
Breast milk	24 (25)	11 (22)	13 (28)		
Mixed feeds	14 (14)	6 (12)	8 (17)		
Formula	59 (61)	33 (66)	26 (55)		

Values are presented as mean (SD) or n (%). Z scores were derived from Niklasson 2008 [24] and Norris 2019 [25]. Milk feed was defined as: Breast milk (volume of feeds >80% breast milk), formula (volume of feeds ≥80% formula) or mixed feeds (mix of breast milk and formula).

Abbreviations: ARA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; HC, head circumference; PMA, postmenstrual age; TEA, term equivalent age.

^a Infants eligible for analyses at 3 months CA; anthropometry and nutritional data n = 97 and body composition n = 82. Data on milk feed was missing in two infants in the ARA:DHA group at TEA n = 95.

Table 3
Associations between possible predictors and body composition z scores.

	Body composition z score (n = 81) ^a								
	Fat-free mass (g)			Fat mass (g)			Fat mass (%)		
	B	β	p	B	β	p	B	β	p
Birth weight z score	0.718	0.767	<0.001	0.432	0.529	<0.001	0.141	0.188	0.064
Sex	0.437	0.178	0.015	0.218	0.102	0.166			
Postnatal steroids	-0.690	-0.285	<0.001	-0.465	-0.220	0.007	-0.221	-0.114	0.263
Weight z score change, Day 28–36 weeks PMA	0.381	0.239	0.003	0.572	0.412	<0.001	0.503	0.395	<0.001
Length z score change, Day 28–36 weeks PMA	0.108	0.097	0.167				-0.115	-0.130	0.230
Weight z score change, 36 weeks PMA to 3 months CA	0.496	0.353	<0.001	0.705	0.576	<0.001	0.533	0.475	<0.001
Length z score change, 36 weeks PMA to 3 months CA				-0.124	-0.127	0.099	-0.190	-0.213	0.069
HC z score change, 36 weeks PMA to 3 months CA	0.295	0.210	0.004						
Milk feed at term equivalent age	-0.153	-0.108	0.166						
Milk feed at 3 months CA ^b	0.670	0.254	0.002	-0.726	-0.315	<0.001	-0.948	-0.449	<0.001
Adjusted R-square	0.700			0.662			0.453		

Data are presented as unstandardized (B) and standardized (β) coefficients with p-values. Birth weight z score, sex, gestational age, postnatal steroid exposure, change in z score for weight, length and HC from Day 28–36 weeks PMA and from 36 weeks PMA to 3 months CA, type of milk feed at TEA and at 3 months CA were included as possible predictor variables. For categorical variables; male sex, no postnatal steroid exposure and breast milk group were selected as reference categories.

Abbreviations: CA, corrected age; PMA, postmenstrual age.

^a Only infants with a complete dataset were included in the analyses. One infant was excluded due to hydrocephalus treated with a subgaleal shunt.

^b Infants with mixed feeds were grouped according to the proportion of breast milk in their diet; ≥50% (n = 4) to the breast milk group and <50% (n = 3) to the formula group. Two infants received 50:50 breast milk and formula and were randomly assigned to either the breast milk (n = 24) and formula (n = 58) group.

those fed formula supplemented with ARA and DHA had higher lean and less fat mass at one year CA compared to infants fed unsupplemented formula [33]. However, no difference in body composition was observed at four months CA, nor were there any differences in anthropometry. A meta-analysis of five RCTs showed increased weight and length at two months CA in preterm infants fed formula supplemented with ARA and DHA compared to unsupplemented control formula or with DHA only [21]. Similar to our study, the major effect on linear growth appeared after the period of supplementation, suggesting programming effects of ARA

and DHA on growth. Nevertheless, we did not find any sustaining effect on linear growth at 3 months CA. At TEA, most infants in the ARA:DHA group had regained their birth centile for length, and we speculate that these infants reached their growth potential at an earlier age compared to the control group. A secondary analysis of the ImNuT-trial suggest beneficial effects of ARA:DHA supplementation on short-term respiratory outcomes [20]. Although not significantly different, more infants in the control group received treatment with postnatal steroids and had longer duration of mechanical ventilation compared to infants in the ARA:DHA group.

This may have contributed to slower growth in the control group [12,34]. Consistent with previous observations in very preterm infants with slow postnatal linear growth [35,36], catch-up growth continued after TEA in the control group. At 3 months CA, anthropometric measurements were proportionate and similar to values of term born peers in both groups [13].

Differences in dose and ratio of supplemented ARA and DHA are some of the variables that may explain the inconsistent results regarding benefits of supplementation in preterm infants [1]. Earlier trials mainly focused on formula-fed infants, and the supplemented doses of ARA and DHA commonly corresponded to values observed in human milk, with full enteral feeds providing 15–30 mg/kg/day [1,16,17]. These levels are below the estimated intrauterine accretion rates [1,17], and may not meet the requirements for normal growth and functional development. Thus, recommended intakes of ARA and DHA were recently raised, and a balanced supply is encouraged to ensure adequate blood levels [1].

Supplementation of DHA that match intrauterine accretion rates (i.e. about 50 mg/kg/day), without concomitant enhanced ARA supply, has not shown any clear beneficial nor harmful effects on growth compared to standard feeding regimes [37–39]. To the best of our knowledge, the present study is the first to examine the effect of enhanced supplementation of both ARA and DHA on quality of growth in very preterm infants. A secondary analysis of the trial showed that the supplemented doses of 50 mg DHA/kg/day, combined with 100 mg/kg/day of ARA, did not suppress ARA levels in blood, but resulted in a modest increase in DHA concentrations [20]. Taken together, our findings suggest that intakes in line with the revised recommendations [1] ensured availability of both fatty acids, and may be beneficial with respect to somatic growth.

4.2. Body composition compared with current reference data

Compared to term born peers, preterm infants usually have less fat-free mass and a higher proportion of fat mass at TEA, but these differences become less apparent at 2–4 months CA [13]. In the present study, mean fat mass (1590 g and 26.9%) at 3 months CA were comparable to current reference data in term born infants (1570 g and 26%) [13]. The mean fat-free mass of 4255 g, corresponding to a z score of -0.96 , was similar to average preterm (4200 g), but not term (4450 g), reference values [13,25].

It should be noted that the majority of studies behind current reference data were based on growth measurements of infants born appropriate for gestational age [13,25]. We observed a strong positive association between birth weight z score and body composition, particularly for fat-free mass. Thus, excluding infants born SGA from our cohort analysis increased average values of fat-free mass (4355 g) closer to that of the term reference population [13]. Moreover, only two out of the 32 preterm cohorts assessed body composition in infants born before 28 weeks of gestation [13], suggesting that current reference data likely represent a healthier preterm population compared to the ImNuT cohort.

Our results show that very preterm infants have the potential to achieve growth similar to healthy term born peers at 3 months CA. Yet, the present findings suggest that anthropometric measures alone may overestimate fat-free mass.

4.3. Growth patterns and body composition

In a previous cohort of very preterm infants, weight gain between TEA and 5 months CA, but not between birth and TEA, was positively correlated with leptin levels at 5 months CA [35]. Leptin is a reliable marker of body fat mass [14]. In the present study, the incidence of growth faltering at 36 weeks PMA was low [12]. An increase in z score for weight, particularly after 36 weeks PMA, was

a strong predictor of higher fat mass%. Taken together, these findings suggest that early catch-up growth in weight may be more beneficial for the metabolic profile compared to accelerated weight gain after discharge.

4.4. Type of milk feeds and body composition

Several studies have examined the effect of human milk vs formula on quality of growth around TEA [40]. In contrast to our study suggesting a negative association between fat-free mass and breastfeeding at 3 months CA, previous data indicate that preterm infants fed with human milk show higher fat-free mass deposition around TEA compared to formula fed infants [40]. The majority of breastfed infants in our study changed to unfortified milk after discharge. A higher protein:energy ratio is associated with improved lean mass accretion in preterm infants before [41,42] and after [43,44] TEA. Since the protein:energy ratio is commonly higher in formula compared to unfortified mature breastmilk, this might explain the positive association between fat-free mass and formula feeds observed at 3 months CA. Also healthy breastfed term infants tend to have higher fat-mass compared to formula fed peers during infancy [13,45]. Nevertheless, our observations should be interpreted with caution as the feeding groups were dynamic; the proportion of infants who received any maternal milk dropped from 88% between birth and 36 weeks PMA [12] to 68% at TEA and 39% at 3 months CA. In comparison, 82% of Norwegian infants are breastfed at four months of age [46]. Considering the protective effects of human milk over formula on preterm outcomes, particularly in neurodevelopment [47], barriers and facilitators to breastfeeding after discharge should receive more attention.

4.5. Strengths and limitations

The major strengths of this study were the double-blind RCT design and longitudinal growth assessments. Body composition was assessed with ADP, which is considered to be an accurate method for body composition assessment in preterm infants [48]. As the differences in body composition between the study groups were minimal, our results are unlikely to be attributed to insufficient statistical power. More infants than anticipated were unavailable for body composition assessment at 36 weeks PMA due to early transfers to other hospitals. Because only 33 infants underwent repeated body composition assessments, we were not able to study longitudinal changes representative for the ImNuT cohort. Finally, the observational studies on associations between human milk feeding, growth patterns and body composition did not allow causal conclusions.

5. Conclusion

Supplementation with ARA and DHA similar to intrauterine accretion rates might have improved linear growth from birth to TEA, but did not affect growth or body composition at 3 months CA in very preterm infants. Our findings suggest that combined supplementation in line with recently revised recommendations may be beneficial with respect to somatic growth in very preterm infants.

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Author contributions

Rossholt: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; Bratlie: Conceptualization, Investigation, Writing – Review & Editing; Wendel: Investigation, Writing – Review & Editing; Aas: Investigation, Writing – Review & Editing; Gunnarsdottir: Investigation, Writing – Review & Editing; Fugelseth: Investigation, Supervision, Writing – Review & Editing; Pripp: Data Curation, Formal Analysis, Validation, Visualization, Writing – Review & Editing; Domellöf: Resources, Writing – Review & Editing; Størdal: Supervision, Writing – Review & Editing; Stiris: Investigation, Supervision, Writing – Review & Editing; Moltu: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Validation, Writing – Review & Editing.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2023.10.005>.

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