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Oral DHA supplementation and retinopathy of prematurity: the Joinville DHA Clinical Trial

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Abstract

Retinopathy of prematurity (ROP) is a leading cause of blindness in premature infants. The condition is associated with DHA deficiency. This study aimed to investigate the effect of DHA supplementation on the occurrence of ROP in infants receiving oral oil drops. It is part of the Joinville DHA study, a non-parallel-group cohort study conducted from March 2020 to January 2023 at a public maternity hospital in Brazil. Infants born before 33 weeks of gestational age or with a birth weight ≤ 1500 g were recruited. Among 155 infants, 81 did not receive and 74 received DHA supplementation until complete vascularisation of the peripheral retina. There was a higher incidence of infants with ROP in the unsupplemented group (58·6 %) compared with the DHA group (41·4 %), but this difference was NS ($P = 0{\cdot}22$). Unadjusted logistic regression analysis showed that patent ductus arteriosus and neonatal corticosteroids were significantly ($P < 0{\cdot}05$) associated with ROP in both groups. In the DHA group, surfactant use was also associated with ROP ($P = 0{\cdot}003$). After adjusting for important covariates, patent ductus arteriosus and neonatal corticosteroids continued to be significant for infants in the unsupplemented group (OR = 3·99; $P = 0{\cdot}022$ and OR = 5·64; $P = 0{\cdot}019$, respectively). In the DHA group, only surfactant use continued to be associated with ROP (OR = 4·84; $P = 0{\cdot}015$). In summary, DHA supplementation was not associated with ROP. Further studies are necessary to better understand the relationship between DHA supplementation, ROP and associated comorbidities.

Keywords: **Retinopathy of prematurity: Preterm infant: DHA: Surfactant: Patent ductus arteriosus**

Retinopathy of prematurity (ROP) is a vision-threatening disease characterised by abnormal vascular proliferation in premature infants. The prevalence of ROP varies across countries and studies. In Brazil, 27·5–68·3 % of premature children develop ROP[1–4].

ROP can regress spontaneously, with no sign of ocular alteration, or progress to a pathological ocular outcome that leads to blindness[5,6]. Despite its multifactorial aetiology, the main predictors of ROP are gestational age less than 33 weeks (32 weeks + 6 d) and birth weight ≤ 1500 g[7]. However, since the first description of ROP, numerous studies have investigated the use of different strategies to prevent or control the disease, such as controlled oxygen use, improved nutrition and, more recently, supplementation with DHA[8–12].

DHA is a *n*-3 long-chain PUFA that is deposited in the brain and in the retina during the third trimester of gestation[12–14]. This PUFA is important for adequate neural and retinal development[15]. It is present in the external segment of photoreceptors, which are responsible for transforming the light signal into an electrophysiological signal and for rhodopsin regeneration[15]. Since DHA is the main structural lipid of the retina and is found in high amounts in the outer segment disc membranes of photoreceptor cells, its deficiency can cause important visual problems[12–14]. Since intrauterine DHA accretion takes place in the third trimester of gestation, it is not completed in preterm infants, resulting in lower plasma concentrations and body reserves of DHA[16]. Within this context, DHA might be a supplementation option to prevent ROP and associated

Abbreviations: AA, arachidonic acid; ROP, retinopathy of prematurity.

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comorbidities. In fact, several researchers have explored the use of DHA for the prevention or control of ROP in preterm infants, with the main forms of administration being the parenteral and enteral routes[12,17–19].

A large number of preterm infants born < 1500 g require parenteral nutrition until full enteral feeds are established[20]. However, as soon as the preterm infant achieves full enteral feeding, the parenteral diet is stopped, a fact that limits the administration of DHA by this route[17]. On the other hand, if DHA is administered directly into the mouth using oral drops, treatment can be continued even after hospital discharge until retinal vascularisation is completed. Therefore, oral administration by a family member/guardian after hospital discharge could help improve retinal vascularisation and thus reduce or even prevent the onset of the disease.

Within this context, the aim of this study was to investigate the oral administration of DHA to reduce the incidence or prevent the development of ROP in Brazilian infants attending a public maternity hospital. Our hypothesis is that oral supplementation with DHA during hospitalisation and after hospital discharge will significantly reduce the incidence of ROP and the development of associated comorbidities. The results obtained in this study will contribute to better understand the relationship between DHA and ROP and to provide a new route of DHA administration in premature patients that can be treated in their own homes by parents/guardian.

Methods

Trial design

This was a non-parallel-group study conducted with infants from 1 March 2020 to 31 January 2023 at the Darcy Vargas Maternity Hospital in Joinville, Santa Catarina, Brazil. The hospital is the only public maternity hospital in Joinville and is responsible for 60 % of deliveries in the city. This study is part of a larger project named the Joinville DHA study (JoiDHA study, Brazil). The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Ethics Committee of the Hans Dieter Schmidt Regional Hospital (protocol no. 4.259.558/2020). Written informed consent was obtained from all subjects/patients. The study also followed the Consolidated Standards of Reporting Trials statement. The Brazilian Registry of Clinical Trials (Registro Brasileiro de Ensaios Clínicos, ReBEC) approved the trial protocol (protocol no. RBR-523wzcb; online Supplementary material 1).

Participants

The eligibility criteria included preterm neonates born before 33 weeks of gestational age (up to 32 weeks + 6 d) and/or with a birth weight ≤ 1500 g, whose mothers were older than 18 years and signed the informed consent.

The participants were recruited at two time points and divided as follows (Fig. 1): unsupplemented group (1 March 2020 to 8 June 2021), which did not receive DHA supplementation (n 81), and DHA group (9 June 2021 to 31 January 2023), which received DHA supplementation (n 74). Infants in the two groups with ocular malformations, who died before the first ophthalmological examination and who needed to be transferred to a hospital in another city, were excluded. We also excluded infants whose mothers had been supplemented with n-3 or had infectious diseases (toxoplasmosis, syphilis or coronavirus disease 2019). In the case of interhospital transfer, the Dr. Jeser Amarante Faria Children's Hospital was the referral institution since it is located in the same city (Fig. 1). Hospital transfer cases included infants who needed surgical intervention; children with congenital malformation who required immediate intervention; children with intestinal perforation, imperforate anus, oesophageal atresia and an intraventricular shunt due to hydrocephalus; and children undergoing laser treatment for ROP.

Data collection

Data of infants in the unsupplemented group were collected from the medical records at the maternity hospital. For infants in the DHA group, the parents/guardian received information about the study in a private room of the maternity hospital within 48 h after the child's birth. When both the mother and her child met the study criteria, they were invited to participate, and the mother signed the informed consent. Data collection included sociodemographic, biological, clinical and anthropometric measurements. The sociodemographic variables were the mother's age at delivery, educational level and marital status. Biological data included type of delivery, sex, gestational age at birth, birth weight, length and nutritional status at birth. The children's clinical variables were obtained from the hospital records and included the use of surfactant (poractant α intratracheal suspension; Curosurf™, Parma, Italy), presence of patent ductus arteriosus and necrotising enterocolitis and neonatal corticosteroid use.

All patients were routinely screened for the presence of ductus arteriosus by echocardiography on the 3rd day of life. When necessary, treatment consisting of paracetamol (15 mg/kg per dose) was administered every 6 h. The criterion for corticosteroid use (0·075 mg dexamethasone/kg per dose every 12 h for 3–5 d) was the presence of bronchopulmonary dysplasia.

The ophthalmological evaluations were conducted according to the Brazilian guidelines for screening and treatment of ROP[21]. Pupil dilation was performed with combined 2·5 % phenylephrine and 0·5 % tropicamide eye drops and was repeated according to the findings in the first examination every 1 or 2 weeks until retinal vascularisation was completed[21]. When necessary, an oral 25 % glucose solution was applied to relieve any discomfort caused by eye examination[22]. A blepharostat (Steel Inox®, Barraquer neonatal model, Engenheiro Coelho, Brazil) and a double-ended scleral depressor (Steel Inox®, Engenheiro Coelho, Brazil) were used for patients with palpebral oedema. Indirect ophthalmoscopy was performed with a halogen binocular indirect ophthalmoscope (Eyetec®, FCV-m2000, São Carlos, Brazil) and a 28-dioptre lens (Volk®). All examinations were

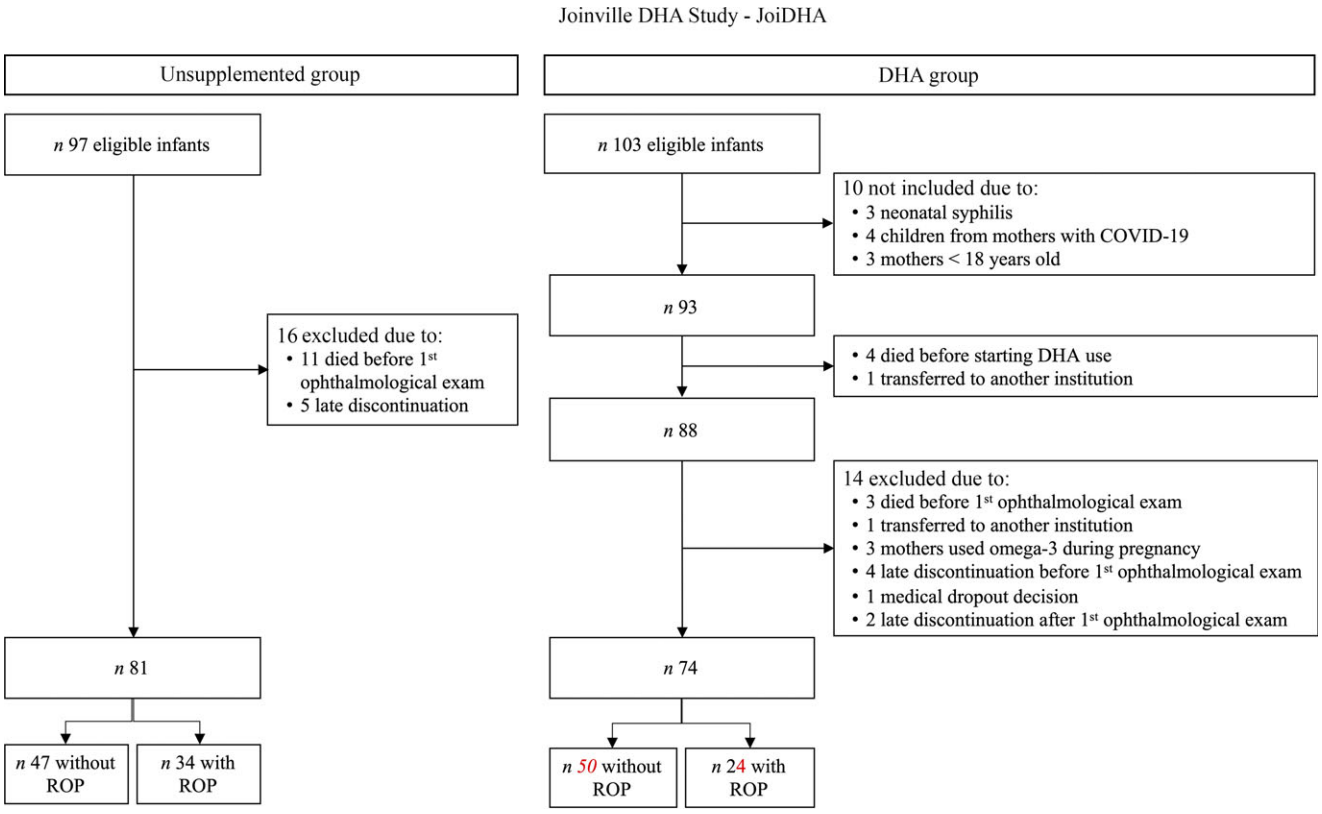

Fig. 1. Flow chart of participants through the recruitment process. Joinville DHA study, Brazil (2020–2023). ROP, retinopathy of prematurity; COVID-19, coronavirus disease 2019.

performed by the same ophthalmologist trained in ROP screening (unsupplemented and DHA groups).

The birth length was measured to the nearest 0·1 cm with a paediatric anthropometric ruler (WCS®, Wood, Curitiba, Brazil), and birth weight was measured to the nearest 1 g with a paediatric digital portable scale (Filizola®, BP Baby, São Paulo, Brazil) using the same equipment throughout the study. The nutritional status at birth was classified into three categories based on gestational age and sex according to the INTERGROWTH-21st standards[23]: < 10th, 10–50th and ≥ 50th percentile. However, in view of the small sample size, the nutritional status was classified into two categories (< 50th and ≥ 50th percentile) in the logistic regression analysis.

At the time of hospital discharge, the parents/guardian received one flask containing the same volume and concentration of DHA as used in the hospital (10 ml; 66·3 mg/ml) and were instructed to continue DHA administration at home until the next ophthalmological evaluation according to the infant's weight at hospital discharge, immediately before the regular diet. The infants had ophthalmological return visits every 15 d, when anthropometric measurements and indirect retinal ophthalmoscopy were performed. When DHA had finished, another 10 ml flask was provided to the parents/guardian. The use of DHA was interrupted when complete vascularisation of the peripheral retina was observed in the ophthalmological examination.

Intervention (DHA supplementation)

In this study, DHA was extracted from oil capsules of a commercial supplement composed of 57·0 % DHA, 13·5 % arachidonic acid (AA), 11·6 % eicosapentaenoic acid and 17·9 % other fatty acids. The oil of each capsule was transferred to a 10 ml flask (eye drop bottles). The oil content of each capsule weighed 890 mg and consisted of 507·3 mg (57·0 %) DHA. Each oil drop used weighed 116·3 mg and thus contained approximately 66·3 mg DHA. The oil manipulation procedure was performed by a compounding pharmacy contracted for the study. The 10 ml flasks containing DHA were stored at the maternity hospital for use during the study period and distributed free of charge to the families/guardian when the infants were discharged from the hospital.

DHA was administered according to the infant's weight considering 1 oil drop/kg: infant weight < 1000·0 g = 1 drop; 1000 to < 2000·0 g = 2 drops; 2000 to < 3000·0 g = 3 drops; 3000 to < 4000·0 g = 4 drops; 4000 to < 5000·0 g = 5 drops and 5000 to < 6000·0 g = 6 drops. Administration of DHA was started when the infant's daily feed volume reached 100 ml/kg, which occurred between the 4th and 6th day of life and was continued throughout the maternity stay. Before the daily feed volume was reached, SMOFlipid 20 % (Fresenius Kabi®) was offered as a parenteral lipid solution throughout the study. DHA was administrated daily, around 14.00 hours, by the attending nurse directly into the mouth, immediately before feeding. The

mother's own breast milk was preferentially used and the milk was extracted at the bedside in the neonatal intensive care unit. When the mother's own breast milk was not available, pasteurised donor breast milk from the breast milk bank of the same maternity hospital was used. Preterm formula was not used. The nurse opened the child's mouth and dripped the DHA drops equivalent to the child's weight. This procedure was maintained for all types of diet.

Outcome

The outcome was the occurrence of ROP at any stage (yes or no) according to DHA supplementation (yes or no). The occurrence and stage of ROP were classified according to the International Classification of ROP (Chiang *et al.*, 2021), recording the most advanced stage observed during the follow-up period.

Sample size

The sample size was calculated with the G*Power software (version 3.1.9.6) by proportion using Fisher's exact test, two tails and for two independent groups. Considering a prevalence of ROP of 40 %[3,24], a CI of 95 %, an alpha error of 5 %, a power of 80 % and losses of 10 %, the estimated number would be ninety participants in each group (unsupplemented and DHA).

Statistical analysis

Data were analysed using the IBM SPSS Statistics for Macintosh, version 29.0 (released 2022, IBM Corp.). Maternal and child characteristics are expressed as median and interquartile range for continuous variables and as absolute and relative frequencies for categorical variables. Continuous and categorical variables were compared using the Mann–Whitney *U* test and the χ^2 test for proportions, respectively (Table 1 and online Supplementary material 2).

To explore potential associations between the predictors and the outcome, we performed logistic regression analysis. The variables included in the unadjusted logistic regression model were chosen based on the association between each variable and the outcome (Tables 1 and 2). Variables with $P < 0{\cdot}1$ by the χ^2 test in the unsupplemented and DHA groups (Tables 1 and 2) were included in the adjusted models (surfactant, patent ductus arteriosus and neonatal corticosteroids; models 1 and 3, Table 3). Nutritional status at birth (which considers birth weight, sex and gestational age) was included in the adjusted analysis because of its biological importance. The 'Enter' method including each variable in the model was chosen to build the adjusted models (models 2 and 4, Table 3). Analyses adjusted for different covariates were performed until the best model that estimated the effect of different predictors on the outcome was obtained. The -2 log likelihood value was used to assess the goodness-of-fit of the models, with the lowest value indicating the best model. The results were considered statistically significant when $P < 0{\cdot}05$.

Results

Trial participants

Sixteen of the ninety-seven eligible infants in the unsupplemented group were excluded and eighty-one participated in the study. Among 103 eligible infants, 74 infants participated in the DHA group after application of the inclusion and exclusion criteria (Fig. 1).

Baseline data

The sociodemographic and biological characteristics of the study participants according to group and the presence of ROP are presented in Table 1. There was a higher prevalence of children with ROP who did not use DHA compared with children who did (*n* 34/81; 41·2 % *v.* *n* 24/74; 32·4 %, respectively). However, although the prevalence of ROP was about 27 % lower in the DHA group than in the unsupplemented group, this difference was not statistically significant ($P = 0{\cdot}22$).

Gestational age at birth and birth weight were associated ($P < 0{\cdot}05$) with the presence of ROP in infants of both the DHA group and the unsupplemented group. The nutritional status at birth was associated with ROP only in the unsupplemented group ($P < 0{\cdot}039$). In this group, there was a higher ($P < 0{\cdot}05$) prevalence of infants with gestational age at birth < 28 weeks, birth weight < 1000 g and nutritional status at birth < 10th percentile among those who developed ROP compared with those without ROP (75·0 % *v.* 25·0 %, 94·7 % *v.* 5·3 % and 58·8 % *v.* 41·2 %, respectively; Table 1). However, the opposite was observed for the same predictors in infants supplemented with DHA. There was a lower ($P < 0{\cdot}001$) prevalence of infants with gestational age at birth < 28 weeks and birth weight < 1000 g among those who developed ROP compared with infants in the same group who did not develop ROP (6·5 % *v.* 93·5 % and 73·9 % *v.* 26·1 %, respectively; Table 1).

Regarding clinical characteristics depicted in Table 2, patent ductus arteriosus and neonatal corticosteroids were associated ($P < 0{\cdot}01$) with the presence of ROP in infants of both the unsupplemented group and the DHA group. The use of surfactant was associated ($P = 0{\cdot}001$) with ROP in infants of the DHA group only. The prevalence of infants with patent ductus arteriosus was higher among those who developed ROP in both groups when compared with infants without ROP (71·4 % *v.* 28·6 % and 64·3 % *v.* 35·7 %, respectively; Table 2). A similar result was found for neonatal corticosteroid use (80·0 % *v.* 20·0 % in the unsupplemented group and 58·8 % *v.* 41·2 % in the DHA group for infants with and without ROP, respectively; Table 2). However, infants in the DHA group showed a lower prevalence of patent ductus arteriosus and neonatal corticosteroids than infants in the unsupplemented group (64·3 *v.* 71·4 and 58·8 *v.* 80·0, respectively; Table 2).

Outcome

Supplementary material 2 shows the comparison of biological variables between the unsupplemented and DHA groups. There was no significant ($P > 0{\cdot}05$) difference in the mother's age at delivery, gestational age at birth, birth weight, length, type of

Table 1. Sociodemographic and biological characteristics of the study participants according to DHA supplementation and presence of ROP (Joinville DHA study, Brazil, 2020–2023)
(Numbers and percentages)

		Unsupplemented group								DHA group						
		Without ROP (*n* 47)		With ROP (*n* 34)		Total (*n* 81)				Without ROP (*n* 50)		With ROP (*n* 24)		Total (*n* 74)		
Characteristic	*n*	*n*	%	*n*	%	*n*	%	*P**	*n*	*n*	%	*n*	%	*n*	%	*P**
Mothers																
Age at delivery (years)	81							0·320	74							0·872
<30		21	52·5	19	47·5	40	49·4			24	66·7	12	33·3	36	48·6	
≥ 30		26	63·4	15	36·6	41	50·6			26	68·4	12	31·6	38	51·4	
Education (years of schooling)	80†							0·565	73‡							0·077
≥ 12		23	62·2	14	37·8	37	46·3			26	60·5	17	39·5	43	58·1	
<12		24	55·8	19	44·2	43	53·7			24	80·0	6	20·0	30	40·5	
Marital status	79†							0·408	74							0·240
Married/consensual union		14	51·9	13	48·1	27	34·2			36	72·0	14	28·0	50	67·6	
Other		32	61·5	20	38·5	52	65·8			14	58·3	10	41·7	24	35·4	
Children																
Type of delivery	81							0·935	74							0·638
Normal		17	58·6	12	41·4	29	35·8			18	64·3	10	35·7	28	37·9	
Caesarean		30	57·7	22	42·3	52	64·2			32	69·6	14	30·4	46	62·1	
Sex	81							0·960	74							0·747
Male		26	57·8	19	42·2	45	55·6			27	69·2	12	30·8	39	52·7	
Female		21	58·3	15	41·7	36	44·4			23	65·7	12	34·3	35	47·3	
Gestational age at birth (weeks)	81							<0·001	74							<0·001
<28		7	25·0	21	75·0	28	34·6			43	93·5	3	6·5	46	62·2	
≥ 28		40	75·5	13	24·5	53	65·4			7	25·0	21	75·0	28	37·8	
Birth weight (g)	81							<0·001	74							<0·001
<1000		1	5·3	18	94·7	19	23·4			6	26·1	17	73·9	23	31·0	
≥ 1000		46	74·2	16	25·8	62	76·6			44	86·3	7	13·7	51	69·0	
Nutritional status at birth (percentile)§	81							0·039	74							0·894
<10th		7	41·2	10	58·8	17	21·0			11	68·8	5	31·3	16	21·6	
10th–50th		12	85·7	2	14·3	14	17·2			16	64·0	9	36·0	25	33·8	
≥ 50th		28	56·0	22	44·0	50	61·8			23	69·7	10	30·3	33	44·6	

ROP, retinopathy of prematurity.
* *P* value refers to the X^2 test (statistical significance at $P < 0·05$).
† Data not found in the medical records.
‡ One mother was unable to report her education.
§ INTERGROWTH-21st standards.

delivery or child sex between infants in the DHA and unsupplemented groups (online Supplementary material 2).

The relative frequency of children according to ROP stage and DHA supplementation is depicted in Fig. 2. The prevalence of infants without ROP (stage = 0) was higher in the DHA group than in the unsupplemented group (67·6 % *v.* 58·0 %). The opposite effect occurred in stages 1, 3 and 5, with a higher prevalence of ROP among infants who did not use DHA (37·0 % *v.* 31·1 %, 3·7 % *v.* 1·3 % and 1·2 % *v.* 0 %, respectively). ROP stages 2 and 4 were not observed in the two groups.

The models explaining the determinants of ROP in infants according to DHA supplementation are presented in Table 3. Unadjusted logistic regression analysis showed that patent ductus arteriosus and neonatal corticosteroids were significantly ($P < 0·05$) associated with ROP in infants of the unsupplemented and DHA groups (models 1 and 3, Table 3). In the DHA group, surfactant use was associated with ROP ($P = 0·003$). Additionally, for infants using neonatal corticosteroids, the odds of developing ROP were lower among infants in the DHA group than among those who did not receive DHA (OR = 4·39 *v.* OR = 8·00). The opposite effect was observed for surfactant use. Although surfactant use was not associated with ROP in the unsupplemented group (OR = 2·62; 95 % CI 0·95, 7·22; $P = 0·063$), the odds of infants who used surfactant developing ROP was greater in the DHA group (OR = 6·36 *v.* OR = 2·62, Table 3). However, after adjusting for important covariates (nutritional status at birth, patent ductus arteriosus and neonatal corticosteroids), patent ductus arteriosus and neonatal corticosteroids continued to be significant for infants in the unsupplemented group (OR = 3·99; 95 % CI 1·22, 13·05; $P = 0·022$ for patent ductus arteriosus and OR = 5·64; 95 % CI 1·33, 23·96; $P = 0·019$ for neonatal corticosteroids; model 2, Table 3). In the DHA group, only surfactant use continued to be associated with ROP, even after adjusting for nutritional status at birth, patent ductus arteriosus and neonatal corticosteroids (OR = 4·84; 95 % CI 1·36, 17·26; $P = 0·015$; model 4, Table 3).

Discussion

Despite the reduction in the incidence of ROP in the group of infants supplemented with DHA, our results showed that DHA supplementation was not associated with ROP. After adjusting for important covariates, the presence of patent ductus arteriosus

Table 2. Clinical characteristics of the study participants according to DHA supplementation and presence of ROP (Joinville DHA study, Brazil, 2020–2023) (Numbers and percentages)

		Unsupplemented group								DHA group						
		Without ROP (*n* 47)		With ROP (*n* 34)		Total (*n* 81)				Without ROP (*n* 50)		With ROP (*n* 24)		Total (*n* 74)		
Characteristic	*n*	*n*	%	*n*	%	*n*	%	*P**	*n*	*n*	%	*n*	%	*n*	%	*P**
Surfactant	81							0·059	74							0·001
No		19	73·1	7	26·9	26	32·0			28	87·5	4	12·5	32	43·2	
Yes		28	50·9	27	49·1	55	68·0			22	52·4	20	47·6	42	56·8	
Patent ductus arteriosus	81							0·001	74							0·005
No		41	68·3	19	31·7	60	74·0			45	75·0	15	25·0	60	81·0	
Yes		6	28·6	15	71·4	21	26·0			5	35·7	9	64·3	14	19·0	
Necrotising enterocolitis	81							0·063	74							0·746
No		45	61·6	28	38·4	73	90·1			45	68·2	21	31·8	66	89·2	
Yes		2	25·0	6	75·0	8	9·9			5	62·5	3	37·5	8	10·8	
Neonatal corticosteroids	81							0·001	74							0·008
No		44	66·7	22	33·3	66	81·5			43	75·4	14	24·6	57	77·0	
Yes		3	20·0	12	80·0	15	18·5			7	41·2	10	58·8	17	23·0	

ROP, retinopathy of prematurity.
* *P* value refers to the X^2 test (statistical significance at $P < 0·05$).

Table 3. Determinants of retinopathy of prematurity in Brazilian children according to DHA supplementation (Joinville DHA study, Brazil, 2020–2023) (OR and 95 % CI)

	Unsupplemented group (*n* 81)						DHA group (*n* 74)					
	Model 1			Model 2			Model 3			Model 4		
Characteristic	OR*	95 % CI	*P*†	OR*	95 % CI	*P*†	OR*	95 % CI	*P*†	OR*	95 % CI	*P*†
Nutritional status at birth (percentile)‡												
≥ 50th	Reference			Reference			Reference			Reference		
< 50th	0·80	0·32, 2·00	0·639	0·58	0·20;1·66	0·310	1·19	0·45, 3·19	0·726	1·41	0·46, 4·33	0·554
Patent ductus arteriosus												
No	Reference			Reference			Reference			Reference		
Yes	5·39	1·81, 16·08	0·002	3·99	1·22, 13·05	0·022	5·40	1·56, 18·65	0·008	3·45	0·88, 13·59	0·077
Neonatal corticosteroids												
No	Reference			Reference			Reference			Reference		
Yes	8·00	2·04, 31·31	0·003	5·64	1·33, 23·96	0·019	4·39	1·40, 13·70	0·011	2·40	0·67, 8·58	0·177
Surfactant												
No	Reference						Reference			Reference		
Yes	2·62	0·95, 7·22	0·063				6·36	1·89, 21·34	0·003	4·84	1·36, 17·26	0·015

* Logistic regression.
† *P* value refers to the Wald test in logistic regression. Models 1 and 3: unadjusted OR. Model 2: adjusted for nutritional status at birth, patent ductus arteriosus and neonatal corticosteroids. Model 4: adjusted for nutritional status at birth, surfactant, patent ductus arteriosus and neonatal corticosteroids.
‡ INTERGROWTH-21st standards.

and neonatal corticosteroid use were important predictors of ROP in infants who did not use DHA. Surprisingly, surfactant use was an important predictor of ROP only in infants who received DHA supplementation. We also observed an important reduction in the odds of ROP in infants who used neonatal corticosteroids and who were supplemented with DHA.

Our results agree with other studies conducted in Australia, New Zealand, Singapore, Sweden, Norway and Mexico, which found no relationship between DHA supplementation and ROP[12,17,25–27]. However, conflicting results regarding the effect of DHA specifically on reducing the incidence/severity of ROP have been reported in the scientific literature. Studies conducted in Poland, Mexico and Turkey revealed a 24–66 % reduction in the incidence and severity of ROP after DHA supplementation[12,18,28]. One important aspect to be addressed in studies on DHA supplementation is the requirement for AA in preterm infants[26,29–32]. Although some studies found no association between AA and DHA supplementation for preventing or reducing ROP[29–31], others suggested that the combined use of AA and DHA supplementation seems to prevent or reduce the severity of ROP[26,33]. Like DHA, an important quantity of AA is transferred during the third trimester of gestation to the fetus via the placenta in order to keep the physiological accretion process functioning properly[15,29,34]. Furthermore, AA has been

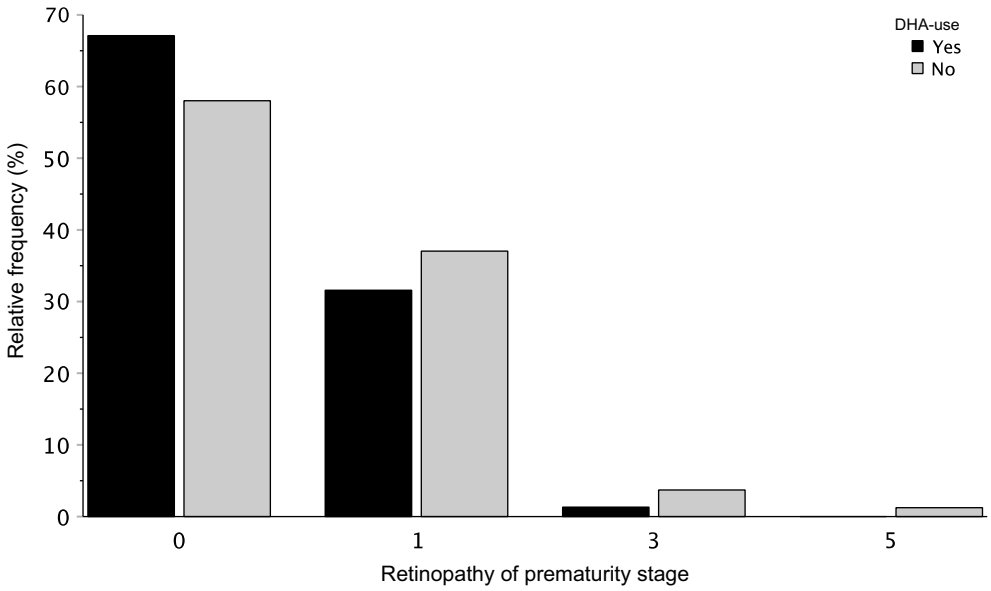

Fig. 2. Comparison of DHA supplementation according to the stage of retinopathy of prematurity.

associated with proinflammatory processes[15,34]. Thus, the combined use of AA and DHA supplementation should be viewed with caution, and new studies are needed to clarify the relationship between these two fatty acids and ROP. The other possible explanation for the divergence among studies is related to the study design, especially the route of DHA supplementation, whether enteral or parenteral. Some studies have shown that enteral and parenteral supplementation with DHA may prevent ROP at any stage as well as severe disease[18,28]. However, parenteral or enteral DHA administration is only possible while the infant is hospitalised. Parenteral nutrition for long periods of time has been suggested as a risk factor for ROP[35,36]. Furthermore, early and gradual enteral feeding promotes the development of villi and activates enzymes that improve absorption and motility of the child's gut function[37]. In the current study, we decided to use supplementation with oral DHA drops immediately before feeding with human milk because of the possible better absorption of DHA by an apparently immature gastrointestinal tract. In fact, the adoption of breast-feeding immediately after DHA supplementation may also induce endocrine and metabolic factors, stimulating the growth of bifidobacteria and *Lactobacillus* species and thus contributing to gastrointestinal absorption and motility[38].

The DHA dosage is another key factor in ROP, which depends on the route of supplementation. The divergences in the DHA dosage hamper the comparison between studies. In general, a DHA dosage of 40–120 mg/kg per d was offered to the infant[25–27,29]. In our study, we used a DHA dose of 66·3 mg/kg per d, similar to a study conducted in Mexico that revealed a positive effect of DHA on the reduction in ROP stages[12]. However, we used oral oil drops even after hospital discharge to continue DHA supplementation until the reduction in ROP severity and complete elimination of the disease. We believe that continuous oral supplementation ensures adequate DHA intake even after the end of parenteral or enteral feeding, consequently reducing pathological retinal angiogenesis[39].

Premature infants are susceptible to many specific conditions due to the incomplete development of physiological, metabolic and anatomical characteristics, among others[40]. Although we found no association between DHA supplementation and ROP, the presence of patent ductus arteriosus and the use of neonatal corticosteroids were significant predictors of ROP in the unsupplemented group, which may have been influenced by the premature condition of the infant. Prematurity is a proinflammatory condition characterised by high levels of circulating prostaglandins and nitric oxide, in which the ductal tissue does not respond adequately to constrictive factors when compared with non-preterm infants, leading to patent ductus arteriosus[41]. As a consequence, there is pulmonary oedema and congestion, conditions that make oxygen therapy even more necessary[42,43]. This reduced perfusion may result in a state of hypoxia and consequent retinal damage[9,44].

Regarding the use of neonatal corticosteroids, these drugs reduce the inflammatory process that occurs in prematurity, especially when respiratory distress syndrome is present[45]. This syndrome can lead to the interruption of normal epithelial lung development, which may be aggravated by life-saving medical interventions that elicit an exacerbated response to reactive oxygen species in preterm births[46,47].

The occurrence of an imbalance between pro- and anti-inflammatory processes may also impair retinal angiogenesis because retinal perfusion is compromised as a result of oxygen fluctuation, which is characteristic of very premature infants[45,48,49]. This process may increase the vulnerability of retina cells to developing ROP[45,48,49]. Furthermore, the use of corticosteroids in prematurity is controversial in the literature[50], and the relationship between corticosteroid use and DHA is complex and still not well understood[30]. Some studies demonstrated that corticosteroid use was associated with ROP[48,49,51], while others found no association between the use of corticosteroids and ROP[52,53].

In the present study, the fact that the effect of corticosteroid use on ROP was approximately 50 % lower in the group of infants supplemented with DHA compared with the unsupplemented group may be related to the anti-inflammatory activity of DHA[15]. Since corticosteroids and DHA exert anti-inflammatory effects, it is possible that DHA may have increased the anti-inflammatory effect of the corticosteroid and thus contributed to mitigating ROP. However, new studies are necessary to elucidate this relationship. Since corticosteroids are administered to prevent bronchopulmonary dysplasia, an increased risk of this disease in more mature infants supplemented with DHA has been reported[26,54]. However, other studies found no association between bronchopulmonary dysplasia and DHA[25,55]. Some authors also highlighted that DHA cannot be used for the prevention of bronchopulmonary dysplasia in infants born less than 29 weeks of gestational age[25,54]. We understand that more studies are needed to elucidate the relationship between bronchopulmonary dysplasia and DHA.

Regarding the need for surfactant use in preterm infants because of pulmonary immaturity, it is important to note that these patients have about ten times less endogenous surfactant than term infants[56,57]. Surfactants reduce the surface tension at the air–liquid interface in the alveolus, promoting better gas exchange[58]. These compounds are composed of 10 % proteins and 90 % lipids, with an important proportion (70–80 %) of phosphatidylcholine[57,58]. DHA also participates in the synthesis of phosphatidylcholine[59]. Despite the important function of surfactant in promoting a better gas exchange and thereby lung function in prematurity[60], we believe that DHA supplementation may have contributed to the endogenous surfactant synthesis[61], promoting hyperoxia and leading to damage in vision development, including ROP. In summary, the simple administration of DHA to the child does not prevent or minimise ROP. The duration of supplementation and concentration and the relationship of DHA with other substances administered at a time of extreme fragility due to prematurity also need to be considered, a fact that makes controlling the disease even more challenging.

To our knowledge, this is the first study that extended DHA supplementation after hospital discharge. The possibility of parents/guardian to continue DHA supplementation after hospital discharge at home for longer periods of time will improve the quality of treatment, with effects on reducing the incidence of any stage and of severe ROP.

This study has several strengths. First, continuing DHA supplementation after hospital discharge until peripheral retinal vascularisation was completed potentially increased the odds of mitigating ROP and even other diseases/comorbidities associated with prematurity. Second, the same team performed all procedures, thus reducing possible biases in the evaluation of patients. Finally, the oral supplementation used in our study is an interesting low-cost method easily applied to infants.

Some limitations of the study should also be mentioned. First, the relatively small sample size limited the multivariate analysis and the investigation of other important covariates. Second, differences between studies in the routes of DHA supplementation and concentrations used hampered the comparison of the results.

Conclusion

Our study showed that DHA supplementation with oral oil drops at a dose of 66·3 mg/kg per d was not associated with a reduction in the incidence or severity of ROP. Our results suggest the need for further studies with a larger number of patients to better understand the relationship between DHA supplementation, ROP and associated comorbidities.

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The authors' contributions are as follows: P. Z. C. designed the study, formulated the research question, collected and analysed the data and wrote the manuscript. V. R. F. H., E. L. R. K., M. C. M. C., Z. G. D. C., G. B., P. G. M. and G. B. L. collected and revised the manuscript. S. S. B. S. M. designed the study and revised the manuscript. M. F. M. organised and designed the study, formulated the research question, analysed the data and revised the manuscript.

The authors declare no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524001120.

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