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Parenteral MCT/ω-3 Polyunsaturated Fatty Acid–Enriched Intravenous Fat Emulsion Is Associated With Cytokine and Fatty Acid Profiles Consistent With Attenuated Inflammatory Response in Preterm Neonates: A Randomized, Double-Blind Clinical Trial

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Abstract

Background: Soybean oil–based intravenous fat emulsion (IVFE) administered to preterm neonates can induce oxidative stress and inflammatory response, which are associated with severe complications of prematurity. This study aimed to test the hypothesis that administration of medium-chain triglyceride (MCT)/ω-3 polyunsaturated fatty acid (PUFA)–enriched IVFE in preterm neonates is associated with a cytokine and fatty acid (FA) profile consistent with attenuated inflammatory response. *Patients/Methods:* In a double-blind randomized study, 60 preterm neonates (gestational age 26–32 weeks) were randomized to receive either MCT/ω-3 PUFA-enriched IVFE (intervention group) or soybean oil–based IVFE (control group). Serum biochemistry, tumor necrosis factor (TNF)–α, interleukin (IL)–6, IL-8, α-tocopherol, and FAs were assessed at baseline, on day of life 15, and day of life 30 or at the end of intervention. *Results:* All cytokine levels changed significantly across the 3 time points, whereas the type of IVFE had a significant effect on final IL-6 and IL-8 levels, which were lower in the intervention group. The difference in final IL-6 and IL-8 levels remained significant after controlling for bronchopulmonary dysplasia and/or infection. α-Tocopherol and FA values changed significantly over time. MCT/ω-3 PUFA-enriched IVFE administration was associated with significantly higher α-tocopherol, eicosapentaenoic acid, docosahexaenoic acid, and ω-3 PUFAs and lower linolenic acid, total PUFA, and ω-6/ω-3 PUFA values compared with soybean oil–based IVFE. Both IVFEs were well tolerated. *Conclusion:* Compared with the soybean oil–based IVFE, the MCT/ω-3 PUFA-enriched IVFE is associated with a more favorable cytokine and FA profile consistent with attenuated inflammatory response in preterm neonates. (*Nutr Clin Pract*.XXXX;xx:xx-xx)

Keywords

preterm neonates; parenteral nutrition; premature infant; total parenteral nutrition; intravenous fat emulsions; soybean oil; SMOFlipid; interleukins; fatty acids

Lipids are important source of calories in preterm and sick neonates requiring parenteral nutrition (PN). However, intravenous fat emulsions (IVFE) can induce oxidative stress and inflammatory response, which are associated with severe complications of prematurity, including cholestasis and bronchopulmonary dysplasia (BPD).[1–3] Soybean oil–based IVFE contains high concentrations of linoleic acid (LA), which is metabolized in arachidonic acid (AA),[4] a precursor of proinflammatory eicosanoids and can induce coagulation and proinflammatory cytokine production.[5,6] The concern about soybean oil–based IVFEs has led to the development of alternative IVFEs in which soybean oil–derived lipids have been partially substituted by coconut oil, olive oil, and fish oil. The medium-chain triglycerides (MCTs) derived from coconut oil are readily cleared from the circulation, easily oxidized, and resistant to peroxidation.[7] The monounsaturated fatty acid (FA) derived from olive oil (oleic acid) is also resistant to peroxidation and has no significant impact on immune function, inflammatory markers, and oxidative stress.[8–10] The very long-chain ω-3 polyunsaturated FAs (PUFAs)—namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—derived from fish oil are metabolized to the anti-inflammatory eicosanoids prostaglandin E_3, leukotriene B_5, and thromboxane A_3.[5,11,12] Experimental studies

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indicate that intravenous (IV) ω-3 PUFA-enriched IVFEs could prevent or attenuate the increase of inflammatory cytokines, prostaglandin E_2 and thromboxane B_2 in stressed animals.[13–16] Studies in adult surgery patients receiving fish oil–containing IVFEs indicated that they may have a beneficial effect on clinical outcomes (length of stay in intensive care unit [ICU] and hospital) and modulate immune function and production of inflammatory eicosanoids and cytokines, whereas no adverse effects were reported.[17–19]

In preterm neonates, the soybean oil–based IVFEs were mainly tested against either olive oil–derived IVFEs or MCT-enriched IVFEs,[20–24] whereas there are 4 published studies comparing the effect of an MCT/ω-3 PUFA-enriched IVFE vs a soybean oil–based IVFE on plasma biochemistry, FA profile, antioxidant status, and the incidence of BPD and cholestasis.[25–28] In addition, Larsen et al[29,30] reported on the effect of MCT-enriched IVFE on plasma levels of cytokines and inflammatory mediators in term neonates with congenital heart disease undergoing open-heart surgery. So far, there are no published data on the potential effect of MCT/ω-3 PUFA-enriched IVFEs on the cytokine levels of preterm neonates.

The aim of this study was to test the hypothesis that administration of an MCT/ω-3 PUFA-enriched IVFE in preterm neonates may be associated with decreased proinflammatory cytokine levels, reflecting attenuated inflammatory response and a more favorable lipid profile compared with the conventional soybean oil–based IVFE. The primary outcome was the profile of proinflammatory cytokines tumor necrosis factor (TNF)–α, interleukin (IL)–6, and IL-8, and the secondary outcomes were plasma α-tocopherol and FA profiles.

Patients and Methods

Study Design

This was a randomized, controlled, double-blind clinical trial with parallel design (2 arms).

Participants

Study sample consisted of neonates admitted to the neonatal ICU of "IASO" Maternity Hospital (September 2012 to September 2013) within 12 hours after birth. Inclusion criteria were (a) gestational age 26–32 weeks, (b) anticipated need for PN of >60% of total energy requirements for at least 15 days, and (c) parental consent for participation to the study. Each infant was followed up to day of life (DOL) 30 or until the PN-derived energy decreased to <40% of total daily energy requirements, whichever was earlier. Exclusion criteria were evidence of congenital infections, perinatal asphyxia, major congenital anomalies, and refusal of parental consent. Additional exclusion criteria following randomization included shorter than anticipated duration of PN (ie, if the PN-derived energy decreased to <60% of daily energy requirements by day 15 of life), inherited disorders of metabolism, and early death (within 2 weeks of age). No neonate received corticosteroids postnatally.

Definitions

BPD was defined as need for oxygen for at least 28 days. Infection was defined as possible in infants with clinical and laboratory evidence of infection but negative blood cultures and as proven when blood cultures were positive.

Bioethics

The study protocol was approved by the Scientific and Ethical Committee of "IASO" Maternity Hospital, and written consent was obtained from all parents before enrollment. This work was written according to the CONSORT statement (http://www.consort-statement.org).

Intervention Procedures

The preterm neonates were randomly assigned into the intervention group (IG) that received an IVFE enriched in MCT and ω-3 PUFAs, whereas the control group (CG) received a conventional soybean oil–based IVFE. For both groups, PN regimens were designed and automatically produced as described previously.[27,31] IVFE was added in the PN solution on the first or second DOL at a dose of 1 g/kg/d, which increased by 1 g/kg/d up to a maximum amount of 3 g/kg/d. Macronutrients were provided using the same products for glucose and amino acid solutions in both groups. Amino acids were derived from Vamin Infant (Fresenius Kabi HELLAS, Athens, Greece). The source of parenteral fat was different for each group; the IG was administered as SMOFlipid (Fresenius Kabi HELLAS), a formulation containing MCTs (30%), lipids from soybean oil (30%), olive oil (25%), fish oil (15%), and α-tocopherol (200 mg/L), while the CG was prescribed with the conventional soybean oil–based lipid formulation Intralipid 20% (Fresenius Kabi HELLAS), which contains 38 mg/L α-tocopherol. Enteral feedings were initiated as soon as possible with either maternal milk or DHA-enriched preterm formula. Neonates in both groups had been receiving PN solutions until oral feedings reached a minimum of 80% of total energy intake. Eligibility, based on the inclusion and exclusion criteria, was assessed by the neonatologists of the neonatal ICU.

Clinical and Laboratory Data

Collected data included gestational age, birth weight, perinatal history, neonatal problems, treatment, and outcome. Heart rate, blood pressure, and body temperature were continuously recorded, whereas weight, PN, and enteral feedings were recorded daily. White blood cell and platelet counts, hematocrit, and C-reactive protein (CRP) were assessed at least

weekly and when clinically indicated. Serum biochemistry and measurement of plasma cytokine, FA, and α-tocopherol levels were performed at 3 time points: T0 before intervention (baseline), DOL 15 (T1), and DOL 30 or at the end of intervention, whichever was earlier (T2). Blood samples were obtained after temporal cessation of parenteral IVFE for 4 hours and before oral feeding. Plasma FAs were assessed by gas liquid chromatography in the form of their methyl esters and were expressed as percent w/w of total FA measured as previously described.[32] Serum α-tocopherol levels were measured using high-pressure liquid chromatography (HPLC). An isocratic system was used, with an HPLC pump, injector, and a UV detector (AGILENT 1100; Agilent Technologies, Santa Clara, CA) using the appropriate reagent kits (RECIPE ClinRep, Munich, Germany) for each reaction. Flow rate for α-tocopherol was 1.5 mL/min. High-sensitivity Quantikine human enzyme-linked immunosorbent assay (ELISA) kits were used to measure TNF-α, IL-6, and IL-8 (R&D Systems, Abingdon, UK) with sensitivity to 0.191 pg/mL, 0.110 pg/mL, and 0.4 pg/mL, respectively. The intra-assay coefficients of variability were <7 % for all markers.

Sample Size and Power Calculation

A priori sample size calculation showed that to achieve a 0.80 power to detect a clinically meaningful difference in IL-8 levels between the study's arms, equal to 1 standard deviation (SD), at a $P < .05$ significance level of 2-sided hypotheses, 21 neonates were required for each arm. The recruited samples of 26 and 25 neonates in the 2 study arms led to an observed power of 87%, 36%, 10%, and 27% to detect a difference of 1 SD in IL-8, IL-6, TNF-α, and α-tocopherol levels, respectively.

Randomization, Sequence Generation, and Implementation

Simple randomization was based on a computer-generated randomization list. The list was given to the pharmacist, who prepared the different PN formulations in identical bags and assigned neonates in 1 of 2 groups. The pharmacist was not involved in neonates' care. All medical personnel and participants were blinded to treatment assignment during the whole study period.

Statistical Analysis

Statistical analysis was "per protocol" based and not "intent to treat" because no cases lost to follow-up were observed. Continuous variables were presented as medians (ranges) and mean (SD) depending on value distribution. Categorical variables were expressed as counts and percentages. Between-group associations were assessed using the Mann-Whitney *U* test and the Fisher exact test, as appropriate. Repeated-measures analysis of variance (ANOVA) and repeated-measures analysis of covariance (ANCOVA) were used for assessing the within-between subjects main effects across the 3 time points, before and after adjustment for BPD, infection, and both. For the within-groups paired comparisons among the 3 time points, paired sample *t* tests were applied, using the Bonferroni correction rule for the inflation of type I error. The threshold for significance in all tests was set at $P < .05$. Variables not normally distributed at one or more time points were transformed using the Box-Cox transformation before being used in the analysis. Time-by-group interactions were also evaluated and when significant (at $P < .05$) were reported. Statistical analysis was performed using SPSS software, version 21 (SPSS, Inc, an IBM Company, Chicago, IL) and the MedCalc version 13.1.2.0–64 bit (MedCalc software bvba, Ostend, Belgium).

Results

Of the 60 recruited neonates, 51 completed the study. Nine neonates (4 and 5 from the IG and CG, respectively) were excluded after randomization because their needs for PN on DOL 15 were <60% of total energy requirements (see study's flowchart, Figure 1).

Clinical, Hematology, and Biochemistry Data

The 2 groups did not differ significantly with regard to gestational age, birth weight, sex distribution, and perinatal/neonatal data (all *P* values >.10). However, the incidence of BPD in the IG was about half of that in the CG (odds ratio [OR], 0.368; 95% confidence interval [CI], 0.111–1.222; Table 1). The amounts of nutrient and fluid intake through enteral nutrition (EN) and PN as well as the amount of enteral feeding on day 15 and at the end of intervention did not differ significantly between the 2 treatment groups (Table 2). Values of all biochemistry parameters changed significantly across the 3 time points, whereas the type of IVFE had no significant effect (repeated-measures ANOVA; Table 3). Also, the 2 groups did not differ significantly regarding the T1 and T2 values of white blood cell counts, hematocrit, and platelets (data not shown).

Outcomes

Values of outcome parameters and the effect of time and treatment group are presented in Tables 3 and 4.

Cytokine levels. A significant main effect of time was observed on all cytokines studied ($P < .001$), revealing that they changed significantly across the 3 time points (Table 3). Specifically, IL-8 levels demonstrated a decreasing trend in the IG and increasing in the CG, whereas IL-6 levels showed a constant

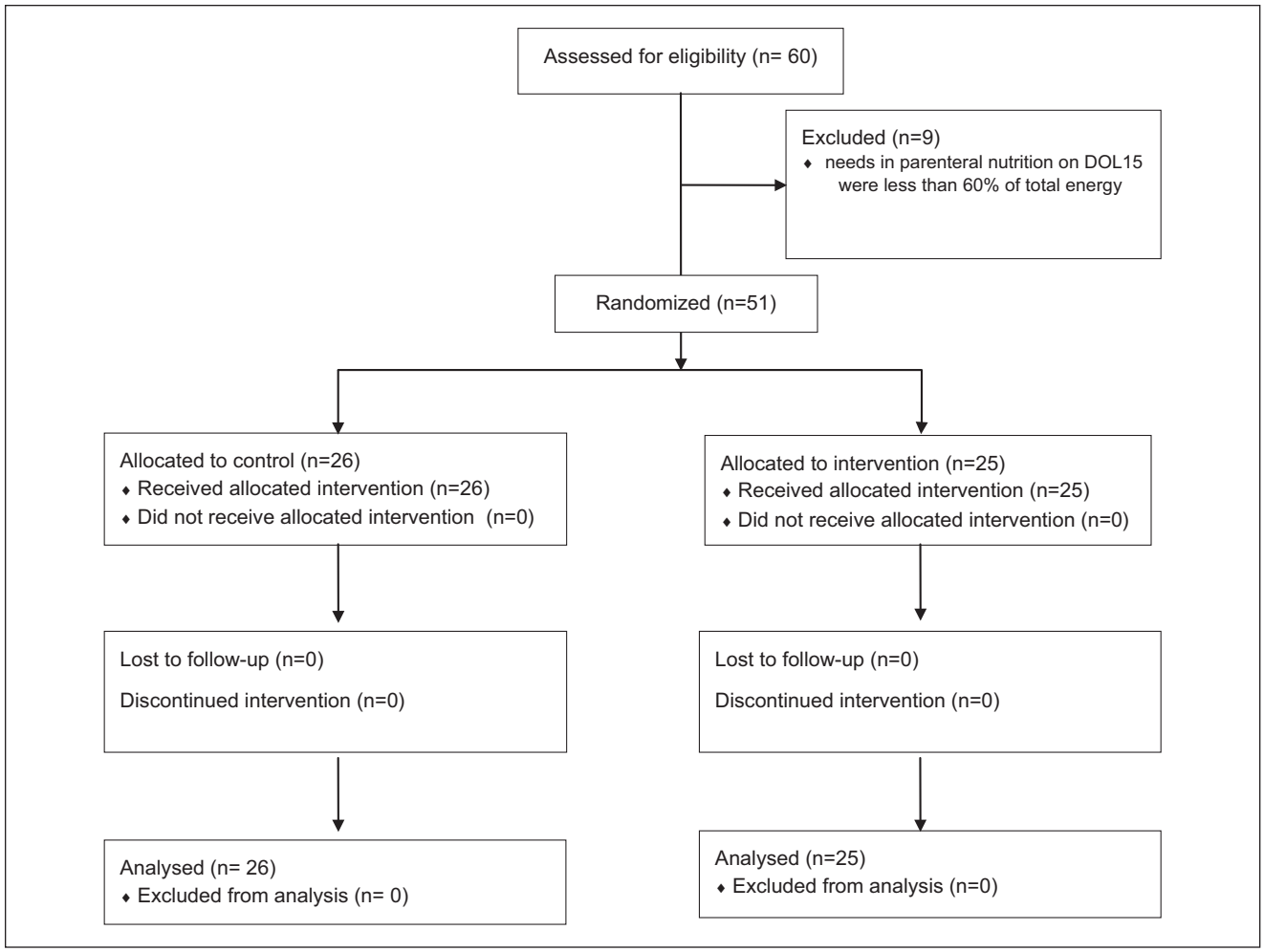

Figure 1. Study's flowchart (according to CONSORT statement). DOL, day of life.

decrease in the IG and a biphasic pattern in the CG (decreased up to T1 and then increased again up to the end of the intervention period; Figure 2). Serum TNF-α levels progressively increased in both study groups. The effect of time-by-group interaction was significant for IL-8 ($P = .024$). Analysis of the between-subject effect at the different time points showed that the type of IVFE had a significant effect on final levels of IL-6 (b-coefficient = 2.01, $p = .0\ 13$) and IL-8 (b-coefficient = 1.53, $P = .023$; Table 3). In the context of the possible effect of BPD and infection on cytokine levels, we further analyzed our results to investigate the effect of treatment group on the final levels of the 3 cytokines after adjustment for BPD, infection, or both. To this aim, we constructed models of repeated-measures ANCOVA with the dependent variable each cytokine level at the 3 time points and cofactors the treatment group along with BPD, infection, or both. We found that the treatment group had a significant independent effect on (a) final IL-6 levels after adjustment for BPD ($P = .011$), infection ($P = .030$), or both ($P = .049$) and (b) final levels of IL-8 after adjustment for infection ($P = .009$) or both BPD and infection ($P = .029$). In contrast, the type of IVFE had no significant effect on final TNF-α levels either before or after adjustment for BPD, infection, or both.

α-Tocopherol levels. α-Tocopherol levels increased significantly during the study period in both IVFE groups (Table 3). Repeated-measures ANOVA revealed that both the time and the group-by-time interaction had a significant main effect on α-tocopherol levels across the 3 time points ($P < .001$ and $P = .007$ for time and time-by-group interaction, respectively). The final levels were significantly higher in the IG (b-coefficient = –1.17, $P = .024$; Table 3).

Fatty acid levels. All FA values changed significantly across the 3 time points. The between-groups comparisons showed that the IG had significantly higher values of EPA, DHA, and ω-3 PUFAs and lower values of LA, total PUFAs, ω-6 PUFAs, and ω-6/ω-3 PUFA ratio (Table 4).

Table 1. Clinical Characteristics of the Groups Studied.[a]

Characteristic	Control (Soybean Oil–Based IVFE)	Intervention (MCT/ω-3 PUFA-Enriched IVFE)	*P* Value[b]	OR (95% CI)
n	26	25		
Birth weight, mean ± SD, g	1271 ± 199	1331 ± 290	NS	NA (–226 to 95)
Gestational age, mean ± SD, wk	29.1 ± 1.3	29.2 ± 1.6	NS	NA (–0.93 to 0.84)
Male sex	14 (54)	15 (60)	NS	1.0 (0.36 to 2.78)
Cholestasis	3 (11)	4 (16)	NS	1.46 (0.29 to 7.30)
RDS requiring surfactant	24 (92)	22 (88)	NS	0.61 (0.09 to 4.01)
BPD	12 (46)	6 (24)	NS	0.37 (0.11 to 1.22)
Total infections	10 (38)	10 (40)	NS	1.07 (0.35 to 3.28)
Possible sepsis	7 (26.9)	6 (24.0)	NS	0.86 (0.24 to 3.03)
Proven sepsis	3 (11.5)	4 (16.0)	NS	1.46 (0.29 to 7.30)
Maternal milk feeding	20 (77)	18 (72)	NS	0.77 (0.22 to 2.73)
Death before discharge	0	0		

BPD, bronchopulmonary dysplasia; CI, confidence interval; IVFE, intravenous fat emulsion; MCT, medium-chain triglyceride; NA, not applicable; NS, nonsignificant; OR, odds ratio; PUFA, polyunsaturated fatty acid; RDS, respiratory distress syndrome.
[a]Values are presented as number (%) unless otherwise indicated.
[b]*t* Test or Fisher exact test.

Table 2. Values of Nutrient, Energy, and Fluid Intake Through Parenteral and Enteral Nutrition on Day of Life 15 (T1) and on Day of Life 30 or at the End of the Intervention (T2).

	T1 Values, Mean (SD)			T2 Values, Mean (SD)		
Dietary Intake	Control (n = 26)	Intervention (n = 25)	*P* Value[a]	Control (n = 26)	Intervention (n = 25)	*P* Value[a]
Energy, kcal/kg/d	102 (12)	102 (10)	NS	112 (7)	116 (7)	NS
Protein, g/kg/d	3.6 (0.5)	3.6 (0.2)	NS	3.8 (0.1)	3.8 (0.2)	NS
Fat, g/kg/d	3.2 (0.3)	3.1 (0.4)	NS	3.6 (0.7)	3.5 (0.5)	NS
Carbohydrates, g/kg/d	15.6 (1.7)	15.7 (2.0)	NS	15.2 (1.2)	14.9 (10.0)	NS
Milk, mL/kg/d	38 (33)	33 (27)	NS	90 (37)	103 (32)	NS
Total fluids, mL/kg/d	159 (12)	157 (9)	NS	161 (7)	160 (8)	NS

NS, nonsignificant~~; T1, day of life 15; T2, day of life 30 or end of intervention~~.
[a]Mann-Whitney *U* test.

Tolerability and Safety

Both solutions were well tolerated, with serum triglyceride levels being within normal values for age, whereas no local reaction, thrombocytopenia that could be attributed to IVFE, or dropout related to any IVFE-associated adverse effect was observed.

Discussion

In this randomized, nutrition intervention clinical trial, premature neonates with gestational age <32 weeks were allocated to receive either a mixed IVFE containing soybean oil, coconut oil, olive oil, and fish oil or the conventional soybean oil–based IVFE starting on the first or second day after birth. It was found that treatment with MCT/ω-3 PUFA-enriched IVFE was associated with significantly lower serum IL-6 and IL-8 levels at the end of intervention compared with the soybean oil–based IVFE. This effect remained significant after controlling for the occurrence of BPD and/or infection. No significant effect of the type of IVFE on serum TNF-α levels was found. Furthermore, α-tocopherol levels were significantly higher in the IG compared with the CG and significantly increased over time. Concerning the effect of intervention on FA values, neonates in the IG had significantly lower ω-6 PUFAs and higher ω-3 PUFA levels, whereas all FA levels changed significantly over time in both groups.

Experimental studies showed that parenteral administration of ω-3 FAs in stressed animals exerts an anti-inflammatory effect expressed as an increased leukotriene B5/leukotriene B4 ratio,[33] decreased concentrations of IL-8 and IL-10, and attenuation of the stress-induced increase of IL-6.[13] In vitro studies on endothelial cells showed that the TNF-α–induced oxidative stress and inflammatory mediator release was blocked or

Table 3. Descriptive Statistics of Biochemical Parameters and Cytokines at the 3 Time Points in the 2 Groups of Neonates.

	Control (n = 26), Median (Range)			Intervention (n = 25), Median (Range)			Significance of Main Effects, *P* Value[a]	
Biochemistry	T0	T1	T2	T0	T1	T2	Between Groups	Within Groups
Direct bilirubin, mg/dL	0.4 (0.2–1.1)	0.6 (0.2–1.3)	0.4 (0.3–1.2)	0.4 (0.1–1.3)	0.6 (0.3–1.7)	0.7 (0.2–4.7)	NS	<.001
ALP, IU/L	232 (118–338)	281 (157–497)	347 (149–490)	189 (112–406)	275 (118–426)	278 (197–608)	NS	<.001
SGPT, IU/L	7 (3–24)	9 (6–25)	11 (6–28)	6 (3–28)	9 (4–28)	8 (5–39)	NS	<.001
SGOT, IU/L	41 (22–83)	21 (13–62)	24 (17–70)	41 (16–101)	22 (8–48)	24 (15–233)	NS	<.001
GGT, IU/L	93 (25–416)	60 (18–586)	102 (20–295)	99 (52–293)	58 (25–296)	106 (27–404)	NS	<.001
Triglycerides, mmol/L	0.9 (0.2–1.8)	1.1 (0.5–2.3)	1.4 (0.6–2.8)	0.7 (0.2–2.1)	1.6 (0.7–2.6)	1.6 (0.7–2.8)	NS	<.001
Cholesterol, mmol/L	2.5 (0.9–5.4)	3.2 (2.5–5.5)	3.0 (1.6–5.2)	2.3 (1.2–3.9)	3.3 (1.6–4.1)	3.0 (1.4–4.3)	NS	<.001
α-Tocopherol, µmol/L	4.2 (1.4–6.6)	6.0 (2.1–9.0)	5.6 (3.3–9.3)[b]	3.4 (1.9–7.7)	6.2 (2.3–11.4)	7.6 (2.6–16.8)	NS	<.001
IL-6, pg/mL	33.4 (7.8–63.0)	16.4 (1.5–44.0)	23.0 (5.4–56.6)[b]	35.5 (2.7–62.8)	10.8 (2.2–62.2)	16.0 (0.9–34.5)	NS	<.001
IL-8, pg/mL	13.8 (8.0–21.8)	16.2 (9.9–27.2)	21.0 (4.8–28.0)[b]	14.9 (9.2–25.4)	18.2 (9.5–27.4)	10.9 (7.2–24.7)	NS	<.001
TNF-α, pg/mL	12.3 (3.5–34.4)	12.1 (2.7–33.6)	16.8 (4.8–39.7)	13.1 (3.4–29.4)	10.3 (3.5–33.3)	18.5 (6.8–36.7)	NS	<.001
CRP, mg/L	3.4 (3.4–12)	3.4 (3.4–71)	3.4 (3.4–70)	3.4 (3.4–22)	3.4 (3.4–123)	3.4 (3.4–34)	NS	NS

ALP, alcaline phosphatase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; IL, interleukin; NS, nonsignificant; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; TNF-α, tumor necrosis factor–α; T0, baseline; T1, day of life 15; T2, day of life 30 or end of intervention.
[a]Within- and between-subject *P* values derived from repeated-measures analysis of variance.
[b]Significant differences ($P < .05$) between the 2 groups at the respective time points.

Table 4. Values of Fatty Acids (Percentage of the Weight of Total Plasma Fatty Acids) at the 3 Time Points in the 2 Groups of Neonates.

	Control (n = 26), Median (Range)			Intervention (n = 25), Median (Range)			Significance of Main Effects, *P* Value[a]	
Characteristic	T0	T1	T2	T0	T1	T2	Between Groups	Within Groups
Oleic acid (C18:1ω-9)	20.0 (15.6–25.3)	20.2 (17.2–25.6)[b]	20.1 (15.8–24.8)	18.7 (14.4–26.8)	22.9 (16.0–26.7)	20.8 (17.3–26.1)	NS	<.001
LA (C18:2ω-6)	6.8 (4.3–9.5)	16.5 (13.5–19.8)[b]	18.7 (12.8–26.3)[b]	6.8 (4.2–13.2)	15.0 (10.2–16.9)	17.0 (8.3–19.9)	<.001	<.001
ALA (C18:3ω-3)	0.11 (0.0–0.2)	0.47 (0.3–0.9)	0.35 (0.1–0.8)	0.13 (0.1–0.3)	0.41 (0.2–0.7)	0.43 (0.1–0.9)	NS	<.001
AA (C20:4ω-6)	11.0 (6.8–14.9)	7.3 (5.0–9.3)[b]	7.0 (5.8–8.7)	10.8 (4.72–15.5)	6.0 (4.2–7.3)	6.6 (4.0–11.6)	NS	<.001
EPA (C20:5ω-3)	0.13 (0.1–0.5)	0.43 (0.3–1.5)[b]	0.44 (0.2–0.8)[b]	0.14 (0.1–0.4)	1.58 (0.3–2.8)	0.55 (0.1–3.1)	<.001	<.001
DHA (C22:6ω-3)	2.5 (1.6–3.8)	1.9 (1.4–2.6)[b]	2.1 (1.4–2.7)[b]	2.6 (2.0–3.8)	2.6 (1.4–3.8)	2.6 (1.3–4.2)	<.001	<.001
Total PUFAs	24.2 (19.5–30.7)	31.1 (27.6–34.0)[b]	33.4 (27.8–39.0)[b]	25.2 (20.5–32.3)	28.5 (25.3–33.8)	30.7 (26.0–36.5)	<.001	.002
ω-6 PUFAs	20.3 (15.3–26.1)	26.8 (23.7–29.9)[b]	29.7 (23.9–35.1)[b]	20.5 (15.6–27.7)	23.2 (20.2–27.1)	25.8 (19.2–31.5)	<.001	<.001
ω-3 PUFAs	3.5 (2.6–5.2)	3.5 (2.9–5.8)[b]	3.8 (2.9–4.7)[b]	3.6 (2.7–5.1)	5.6 (2.7–8.3)	4.3 (3.1–9.4)	<.001	<.001
ω-6/ω-3 PUFA ratio	6.0 (4.5–7.5)	7.6 (4.4–9.9)[b]	7.6 (6.1–11.0)[b]	5.6 (4.5–7.7)	4.1 (3.1–9.0)	6.2 (2.6–8.3)	<.001	<.001

AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; NS, nonsignificant; PUFA, polyunsaturated fatty acid; T0, baseline; T1, day of life 15; T2, day of life 30 or end of intervention.
[a]Within- and between-subject *P* values derived from repeated-measures analysis of variance.
[b]Significant differences ($P < .05$) between the 2 groups at the respective time points.

reduced by α-linolenic acid (ALA) while amplified by LA.[34] These findings are in accordance with adult clinical studies reporting lower plasma IL-6 and TNF-α concentrations in patients who received a mix of MCT, soybean oil, and fish oil (Lipoplus; B. Braun Melsungen AG, Berlin, Germany)[17] or fish oil (Omegaven; Fresenius Kabi HELLAS, Athens, Greece) postoperatively.[35] However, other adult studies could not demonstrate significant differences in serum IL-6 related to the type of IVFE.[36]

In pediatric patients, clinical studies reported that MCT/ω-3 PUFA-enriched emulsion is safe and well tolerated and is associated with decreased plasma bilirubin levels and increased

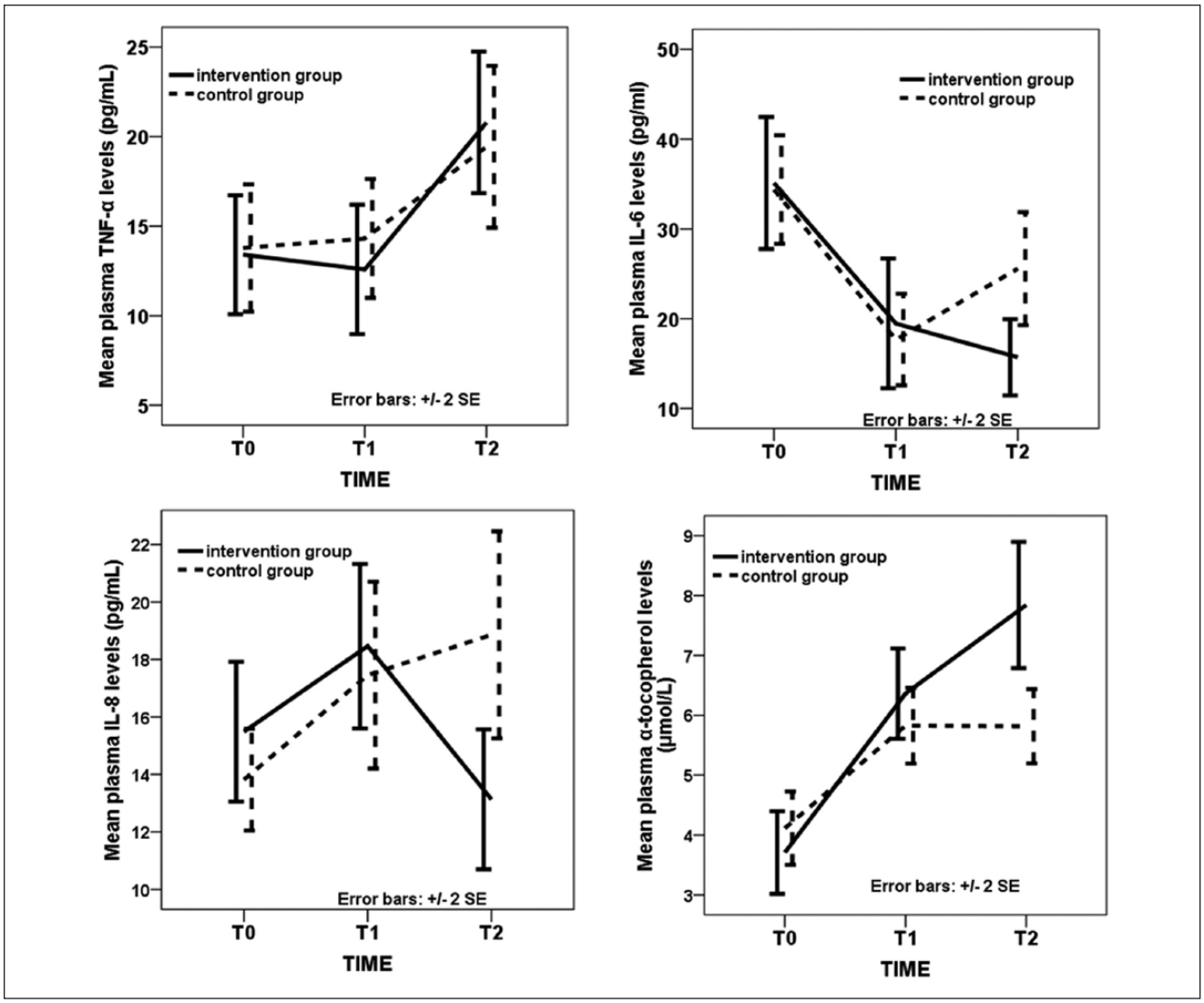

Figure 2. Mean ± SE plasma levels of cytokines and α-tocopherol. The changes across the 3 time points were significant for all parameters. The final levels of IL-6, IL-8, and α-tocopherol differed significantly between the 2 groups. IL, interleukin; T0, baseline; T1, day of life 15; T2, day of life 30 or end of intervention; TNF-α, tumor necrosis factor–α.

ω-3 FAs and plasma α-tocopherol levels.[37] In preterm neonates, there are several studies comparing the MCT/olive oil/fish oil–enriched IVFEs with that of the conventional soybean oil–based IVFEs in terms of safety, tolerability, and effects on FAs and antioxidant status.[25–28,38] These trials reported that MCT/ω-3 PUFA-enriched IVFEs are safe and associated with an increased ω-3/ω-6 ratio and α-tocopherol levels[25,26] and decreased oxidative stress.[27] Data on the effect of fish oil–containing IVFE on plasma cytokines in neonates are limited to term infants.[29,30]

Two recent studies by Larsen et al[29,30] report on the relation of MCT/ω-3–enriched IVFEs with inflammatory biomarkers in term infants with congenital heart disease undergoing open-heart surgery. The authors found that the time had a significant effect on plasma cytokine levels. Furthermore, levels of TNF-α, IL-1β, and IL-6 during the postoperative period were lower in infants treated with MCT/ω-3 PUFA-enriched IVFEs compared with those treated with soybean oil–based IVFEs. No difference was found regarding the levels of IL-8 and granulocyte-macrophage colony-stimulating factor. Results of the present study confirmed that cytokine levels changed significantly over time, while the main effect of IVFE type seemed to be nonsignificant. However, the between-groups comparison of cytokine levels at the different time points showed that the type of IVFE had a significant effect on final levels of IL-6 and IL-8 but not TNF-α. These data support the anti-inflammatory effect of the fish oil–containing IVFE, whereas the minor differences in the findings between our and published studies could be attributed to the different population studied (term vs preterm neonates with different morbidities) and different study design.[29]

When evaluating cytokine levels in preterm neonates receiving intensive care, certain morbidities should be considered, mainly the respiratory distress syndrome (RDS), sepsis, and BPD, which are associated with increased proinflammatory cytokine production.[6,39–41] Therefore, we further analyzed our results to control for BPD and infection. It was revealed that the type of IVFE significantly affected the IL-6 and IL-8 levels even after controlling for BPD and/or infection. Regarding the TNF-α levels, the type of IVFE had no significant effect either before or after adjustment for the occurrence of BPD and/or infection. These results partly confirm previously published data showing that treatment with fish oil–containing IVFEs had no significant effect in the absence of sepsis. However, TNF-α levels differed significantly between the 2 treatment groups when sepsis was present.[29] These difference between ours and previously reported findings could be explained by the different study design. The TNF-α is released within 30 minutes after the onset of sepsis and peaks in approximately 1.5 hours, and its half-time has been calculated to 70 minutes.[42] Therefore, increased plasma levels can be found only in blood samples obtained soon after the onset of infection, which was not part of our study design.

Although these results are suggestive of an attenuated inflammatory response in the neonates who received the MCT/ω-3 PUFA-enriched IVFE, it could be argued that the differences in cytokine levels were not as impressive as they would probably be expected. This may be due to the small sample size as well as to the fact that the MCT/ω-3 PUFA-enriched IVFE contains soybean oil, a source of ω-6 PUFAs, which may have blunted the effect of fish oil on inflammatory mediators.

The differences in cytokine levels between the 2 study groups are attributed mainly to the effect of the different IVFEs on FA profile. Comparison between the 2 groups showed lower LA, ω-6 PUFA, and ω-6/ω-3 PUFA ratio and higher EPA, DHA, and ω-3 PUFA in the IG. These differences in FAs are compatible with the observed differences in the inflammatory cytokines between the 2 groups. The total PUFA level was lower in the IG as previously reported.[26] A similar FA profile was reported by Larsen et al,[30] who found that the fish oil–containing IVFE was associated with increased EPA and ω-3, decreased ω-6/ω-3 ratio, and comparable AA levels. However, unlike our findings, Larsen et al could not demonstrate any significant difference in ALA and DHA levels between groups, whereas changes in ALA, AA, and DHA over time were not significant. The different population and study design may have contributed to these differences between the previous and the current study.

α-Tocopherol is an efficient inhibitor of lipid peroxidation that is important for the prevention of oxidation of tissue PUFAs.[43] Experimental studies demonstrated that increased PUFA consumption, especially in the form of the ω-3 long-chain PUFA EPA and DHA, increases lipid peroxidation of cell membranes and α-tocopherol requirements.[40–46] Therefore, α-tocopherol is added to fish oil–containing IVFEs. Previous studies in adults,[30,47] children,[37] and neonates[26] reported significantly higher levels of α-tocopherol in patients treated with fish oil–containing IVFEs. In line with previous reports, α-tocopherol levels were significantly higher in the IG at the end of intervention. Studies in children receiving PN showed that MCT/ω-3 PUFA-enriched IVFE containing increased α-tocopherol amounts increased the ω-3 PUFA without increasing lipid peroxidation.[37] Furthermore, a study in preterm infants showed enhanced antioxidant status in those receiving MCT/ω-3 PUFA/α-tocopherol-enriched IVFE compared with soybean oil–based IVFE.[27]

The oxidative stress and inflammatory response play a major role in the pathogenesis of BPD,[6,48] whereas early studies associated the soybean oil–based IVFE with lung injury in preterm infants.[41] The present study was not powered enough to demonstrate a significant difference in BPD incidence between the 2 groups, However, it should be noted that the incidence of BPD in the IG was almost half the incidence in the CG, although predisposing factors such as gestational age, birth weight, RDS, and infection did not differ significantly between the 2 groups. In the context of a lower ω-6/ω-3 PUFA ratio in the IG, it is possible that the anti-inflammatory properties of ω-3 PUFAs have contributed to the lower incidence of BPD in this group. The differential effect of different ω-6/ω-3 PUFA ratios on lung inflammation was demonstrated by in vitro studies on human alveolar cells that associated a decreased ω-3/ω-6 PUFA ratio (1:4 and 1:7) with increased release of inflammatory cytokines (TNF-α, IL-6, and IL-8). On the contrary, increased ratios of ω-3/ω-6 PUFAs (1:1 and 1:2) were associated with increased release of the anti-inflammatory cytokine IL-10.[49] Studies in neonates showed an inverse association between EPA and leukotriene B4 levels, supporting the anti-inflammatory properties of EPA and ω-3 PUFAs.[30] Furthermore, a cohort study by our research team in 282 preterm neonates reported a significantly lower incidence of BPD in preterm neonates receiving MCT/ω-3 PUFA-enriched IVFE compared with those receiving soybean-based IVFE.[28] Results of previous studies and the current study support a potential role of ω-3 PUFA- and α-tocopherol–enriched IVFE on the prevention of BPD.

Several studies have highlighted the role of fish oil–enriched IVFEs on the prevention and treatment of PN-associated liver disease in adults,[37,50–52] children, and preterm neonates.[4,25,26,37,53–55] Recent reviews reached the conclusion that the fish oil–enriched IVFEs appear to be safe and efficacious for the treatment of PN-related liver disease in children.[3,56,57] A previous cohort study of our research team reported a trend toward a lower incidence of cholestasis in preterm neonates receiving MCT/ω-3 PUFA-enriched IVFE compared with those receiving soybean oil–based IVFEs.[28] In the current study, we did not find any significant difference in the levels of direct bilirubin and liver enzymes at any time point or in the incidence of cholestasis, probably due to the short duration of IVFE administration (up to 4 weeks) and the low numbers of the neonates studied. In fact, previous pathology and clinical studies revealed the duration of PN as the main factor that was significantly associated with the development and severity of cholestasis in neonates.[28,58]

In terms of safety, both solutions were well tolerated as serum triglyceride levels did not exceed the suggested upper normal levels,[59] despite the high daily dose of lipids (3 g/kg) attained by the third day of PN. Furthermore, no local reaction or biochemistry and hematology abnormalities that could be attributed to the IVFE were observed.

Limitations

A limitation of the study is the small sample size that was determined by the low availability of preterm infants needing PN for longer than 15 days. The sample size, which was appropriate as calculated to illustrate a significant difference in IL-8 levels, was inadequate to detect a significant difference between the IG and CG regarding the IL-6 and TNF-α levels. Although assessment of more inflammatory mediators would probably strengthen our conclusions, this was not possible due to the limited amount of blood that could be obtained from preterm neonates. Future studies should address the effect of the MCT/ω-3 PUFA-enriched IVFE administered in a more selected population of preterm infants for longer than 30 days. In addition, further research could focus on the use of mixed IVFEs containing no or very small quantities of soybean oil and their effect on serum inflammatory mediators and FA profiles, as well as their association with nutrition status, potential FA deficiencies, morbidity, and long-term outcome of preterm infants.

The trial has not been included in a Clinical Trials database because the relevant database in Greece is reserved for phase III trials for novel treatments.

Conclusions

Results of the current study suggest that administration of MCT/ω-3 PUFA-enriched parenteral IVFE in preterm neonates may be associated with cytokine and FA profiles consistent with attenuated inflammatory response. From the clinical point of view, results of this study further support the use of MCT/fish oil– and α-tocopherol–enriched IVFEs for preterm neonates requiring PN who are at high risk of developing inflammation-related complications of prematurity, including BPD. Large multicenter studies and long-term data are needed to support the efficacy and safety of MCT/ω-3 PUFA-enriched parenteral IVFE for preterm neonates.

Statement of Authorship

M. Skouroliakou was responsible for study's concept and coordination. D. Konstantinou participated in study design and was responsible for patient recruitment and monitoring. C. Agakidis analyzed and interpreted data, reviewed literature and drafted the manuscript. N. Kalogeropoulos and A. Kaliora reviewed literature and carried out biochemical analyses. P. Massara performed the data acquisition and critically reviewed the manuscript. M. Antoniadi supervised the data monitoring. D. Panagiotakos thoroughly reviewed the statistical analysis. T. Karagiozoglou-Lampoudi is responsible for the study design, data interpretation and manuscript preparation.

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