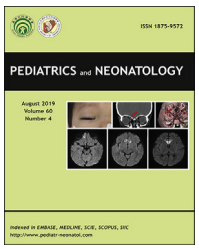
Pediatrics and Neonatology (2019) 60, 359–367

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



Original Article

Effects of two different lipid emulsions on antioxidant status, lipid peroxidation and parenteral nutrition- related cholestasis in premature babies, a randomized-controlled study

Hacer Yapicioglu Yildizdas [a,*], Burak Poyraz [a], Guluzar Atli [b], Yasar Sertdemir [c], Kurthan Mert [a], Ferda Ozlu [a], Mehmet Satar [a]

[a] *Cukurova University, Faculty of Medicine, Department of Paediatrics, Division of Neonatology, Turkey*
[b] *Cukurova University, Faculty of Medicine, Vocational School of Imamoglu, Adana, Turkey*
[c] *Cukurova University, Faculty of Medicine, Department of Biostatistics, Adana, Turkey*

Received Mar 9, 2018; received in revised form Jun 7, 2018; accepted Jul 27, 2018
Available online 2 August 2018

Keywords
cholestasis;
fish oil/MCT/olive oil/soy bean lipid;
olive oil/soybean lipid;
preterm

Background: Olive oil-soybean oil (OO/SO) based lipid emulsions (LE) lack ω-3 PUFAs eicosapentaenoic acid —EPA and docosahexaenoic acid- DHA, which have clinical benefits on inflammatory processes. Fish oil based LEs are good sources of DHA and EPA. Fish oil, MCT, Olive oil and Soya oil (FMOS) lipid is one of the fish oil containing LEs supplemented with high levels of α-tocopherol and lower levels of phytosterol compared to OO/SO lipid emulsions. We investigated the effects of OO/SO and FMOS lipid preparations on cholestasis, levels of antioxidant enzymes and lipid peroxidation.

Methods: Preterm neonates ≤32 gestational weeks age and/or ≤1500 g were randomly assigned to receive either FMOS or OO/SO in the first day of life. Catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and thiobarbituric acid reactive substances (TBARS) levels in the first day of life, 7th day of lipid use and 28th day of life were measured and cholestasis during parenteral nutrition was recorded.

Results: 34 and 33 patients were in FMOS and OO/SO lipid groups respectively. Although the TBARS levels were higher in the first day of life and 7th day of LEs in OO/SO lipid group ($p=0.014$ and $p=0.022$), on the 28th day of life TBARS level was similar and SOD level was higher ($p=0.014$) in OO/SO group. Cholestasis was significantly lower in FMOS lipid group (0% vs. 18.2%), ($p=0.011$) and neonates regained birth weight earlier ($p=0.006$). There was no significant difference in other morbidities.

* Corresponding author. Cukurova University, Faculty of Medicine, Department of Paediatrics, Division of Neonatology, 01330, Adana, Turkey. Fax: +90 322 338 66 10.
E-mail address: hyapicioglu@cu.edu.tr (H.Y. Yildizdas).

https://doi.org/10.1016/j.pedneo.2018.07.012

Conclusions: FMOS and OO/SO lipid emulsions have similar effects on lipid peroxidation on 28th day of life and on morbidities in short term period except for cholestasis.

Abbreviations

BPD	Bronchopulmonary dysplasia
CAT	Catalase
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FMOS	Fish oil, olive oil, MCT, soybean oil
GPx	Glutathione peroxidase
IVH	Intraventricular haemorrhage
LA	Linoleic acid
LE	Lipid emulsion
MCT	Medium chain triglycerides
NEC	Necrotizing enterocolitis
OO/SO	Olive oil-soybean oil
PDA	Patent ductus arteriosus
PN	Parenteral nutrition
PUFA	polyunsaturated fatty acid
ROP	Retinopathy of prematurity
SO	Soybean oil
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances

1. Introduction

Parenteral nutrition (PN) plays a crucial role in the care of premature neonates, especially for those with very low birth weight. Lipid emulsions (LE) are essential components of PN, providing a major source of energy, essential fatty acids and long-chain polyunsaturated fatty acids (PUFAs).

Pure soybean oil (SO) based LE has been the standard LEs for newborns for the last few decades. Pure SO LE contains small amounts of ω-3 PUFAs and high amounts of ω-6 PUFAs, mostly linoleic acid (LA). Soybean oil LEs may cause immune dysfunction and liver injury due to their high content PUFAs, high phytosterol and low α-tocopherol (vitamin E).[1] High LA in SO LEs leads to production of arachidonic acid, which is the substrate for proinflammatory factors (such as tumor necrosis factor- α, interleukin-6, platelet activating factor) and may have adverse effects on liver causing chronic inflammation.[2] Long chain fatty acids are prone to lipid peroxidation, and phytosterol may impair secretion of bile acids and bile.[3] Consequently, new generation LEs containing olive oil, fish oil and medium chain triglycerides (MCT) have recently become available.

Olive oil-soybean oil (OO/SO) based LEs have higher α-tocopherol level and are well tolerated in preterms compared to SO LEs.[4,5] OO/SO contains ω-9 and ω-6 PUFA but lacks ω-3 PUFAs eicosapentaenoic acid —EPA and docosahexaenoic acid- DHA, which are linked with clinical benefits relating to cardiovascular system, growth and development, inflammatory processes, mental and neurodegenerative diseases and retinal tissue.[6,7] Fish oil based LEs are good sources of DHA and EPA. EPA has been shown to modulate inflammatory pathways by decreasing the production of pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokine IL-10 by hepatic macrophage.[8,9] Both DHA and EPA act as precursors of inflammation resolving mediators such as resolvins and protectins.[10] Fish oil, MCT, olive oil, soybean oil (FMOS) lipid is one of the fish oil-containing LEs with a mixture of fish oil (15%), MCT (30%), OO (25%) and SO (30%) and is supplemented with high levels of α-tocopherol (200 mg/L vs.32 mg/L) and lower levels of phytosterol (47.6 mg/L vs. 274 mg/L) compared to OO/SO LEs. In animal models intravenous fish oil improves biliary flow, upregulates bile acid mechanisms and decreases cholestasis.[11]

There is no study aiming to analyze FMOS LE's effect on cholestasis compared with OO/SO LE. In this study we aimed to compare cholestasis rate in FMOS- and OO/SO- LE receiving premature infants with gestational age ≤32 weeks and/or birth weight ≤1500 g in a short-term period.

Increased oxygen radicals are considered to have important roles in oxidative injury of lipids, proteins and DNA. Serious morbidities such as intraventricular haemorrhage (IVH), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP) are related to oxygen radicals.[12] All aerobic organisms possess complex enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and non-enzymatic antioxidants like vitamin E and C, nitric oxide, iron and copper.[13] Unfortunately, premature infants are at risk of oxidant injury as their antioxidant defence mechanisms are deficient. In this study we also aimed to investigate the effects of these two LEs on antioxidant enzymes and lipid peroxidation of erythrocytes in these patients.

2. Material and methods

Cukurova University Balcalı Hospital has 42 Level III beds in the Neonatal Intensive Care Unit. Preterm neonates with gestational age ≤32 weeks and/or birth weight ≤1500 g who had LEs for at least 7 days for PN were enrolled to this prospective, randomized-controlled, non-blinded study during March 2015—August 2016. There was no blinding for the medical team; two of the investigators were not involved in the patient care (BP collected the data, GA performed the antioxidant and lipid peroxidation marker analysis). Infants with major congenital anomalies, congenital heart diseases, metabolic diseases, hydrops fetalis and those who had LE less for than 7 days were excluded from the study. Informed and written consents from parents were gained before randomization. The Ethics

Committee of the Medical Faculty of Cukurova University has reviewed and approved the study.

Sample size calculation: According to our previous research, the incidence of cholestasis was 27.9%.[14] We hypothesized that FMOS LEs might decrease the rate of cholestasis. A power analysis showed the setting error 0.05 with 80% power and an absolute reduction of the incidence of cholestasis from 28% to 5 or less, the total number needed to verify our hypothesis was 32 in each group. Infants were randomized to receive one of two LEs using sealed envelopes: OO/SO LE (ClinOleic, Baxter, Lessines, Belgium) as "OO/SO lipid'' group and FMOS LE (SMOFlipid, Fresenius Kabi, Pymble, Australia) as "FMOS lipid'' group.

Parenteral Nutrition Protocol: PN was started to all infants $\leq$1500 g in the first day of life in NICU. Aminoacid solution was administered as 2 g/kg/day in the first day and increased by 1 g/kg every day up to 4 g/kg/day. Lipid emulsions were infused at 1, 2, 3 g/kg/day in the first 3 days of life and then infused as 3.5 g/kg/day with an infusion rate of 0.25 g/kg/hr. The same trace elements and vitamins were used in both groups. Enteral feeding with breast milk or preterm formula (generally 8 $\times$ 1 ml/day for BW $\leq$ 1000 g and 8 $\times$ 2—5 ml/day for BW 1001—1500 g) is highly recommended in the first day of life in our unit. When enteral feeding reached the 75% of recommended total intake, LE was usually stopped. All infants were evaluated for primary (cholestasis) and secondary outcomes (patent ductus arteriosus- PDA, IVH, NEC, BPD, ROP, nosocomial infections, antioxidant and lipid peroxidation marker levels, days of PN and LEs, physical growth-days to regain birth weight and weekly weight gain in the first month of life, fluid intake in the first 14 days, PN associated complications such as elevated liver enzymes/cholestasis, days of ventilation, oxygen use and death during hospitalization in NICU).

All neonates were evaluated for IVH in the first week of life and then weekly by the same neonatologist with radiology training. IVH was classified according to Papile classification. NEC was staged according to Bell's classification; significant PDA was defined as internal ductal diameter of $\geq$1.5 mm and/or with a left atrium/aortic root ratio $\geq$1.5 requiring diuretics or ibuprofen/paracetamol/ligation. BPD was defined as oxygen dependency at 36 weeks postconceptional age for those with gestational age $\leq$32 weeks and at 56 days for those with gestational age $>$32 weeks. ROP was diagnosed by direct ophthalmoscopy as defined by the International Classification of ROP[15] and neonates were followed until full retinal vascularisation reached ora serrate and the most severe ROP stage was recorded. Nosocomial infection was defined as an infection after the first 72 h of hospital admission. Blood stream infection, meningitis and ventilator— associated pneumonia were defined as in our previous report.[16] Suspected clinical sepsis was a clinical deterioration without a positive culture. Cholestasis was defined as a direct bilirubin level higher than 1 mg/dl if total bilirubin level is less than 5 mg/dl or a value of $>$20% of total bilirubin if total bilirubin level is greater than 5 mg/dl during PN.[17]

Two cc of blood was drawn in to EDTA-containing tubes after birth before lipid infusion, on 7th day of lipid infusion

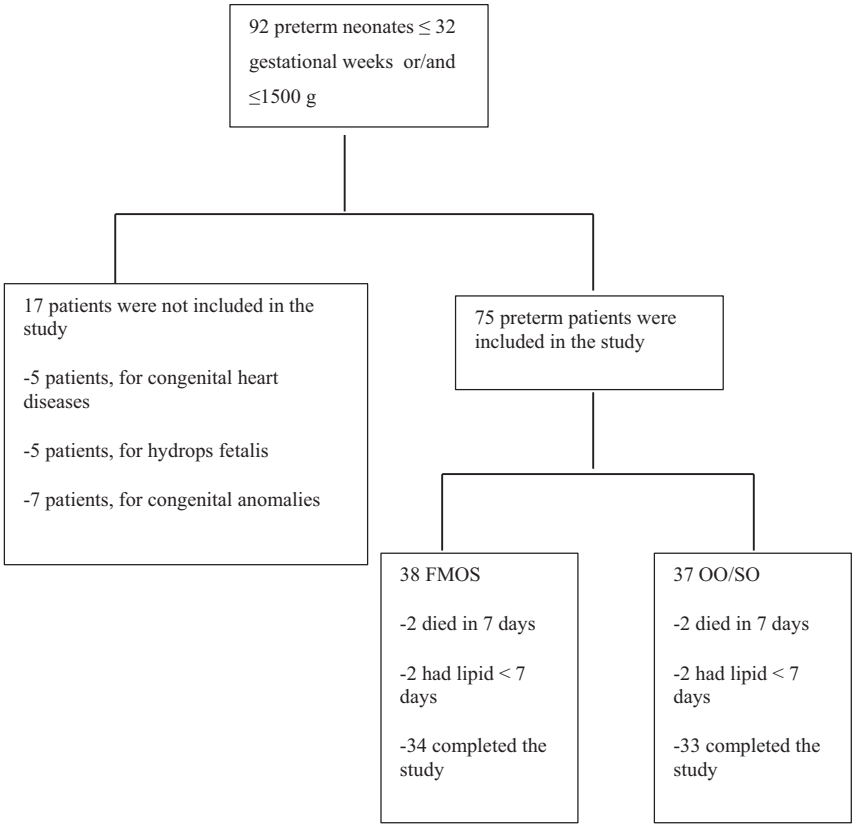


Figure 1 Flow diagram of the study. FMOS, Fish oil/MCT/olive oil/soy bean; OO/OS, olive oil/soybean oil.

Table 1 Characteristics of neonates in groups.

	FMOS Lipid group (n = 34)	OO/OS Lipid group (n = 33)	p
	Mean ± SD	Mean ± SD	
	Median (Min.– Max.)	Median (Min.– Max.)	
Birth weight (g)	1179 ± 440	1291 ± 446	0.284
	1075 (530–2200)	1285 (690–2530)	
Gestational age (weeks)	29.2 ± 2.3	29.3 ± 2.6	0.974
	29 (24–32)	30 (25–34)	
1. minute Apgar score	5.6 ± 2.0	5.0 ± 2.0	0.209
	5 (2–8)	5 (1–8)	
5. minute Apgar score	7.3 ± 1.6	6.9 ± 1.6	0.359
	8 (5–10)	7 (4–9)	
Duration of PN (days)	25.2 ± 20	27.4 ± 16	0.309
	18 (7–96)	22 (7–64)	
Protein intake of PN on the 7th day (g/kg/day)	3.3 ± 0.5	3.4 ± 0.4	0.351
	3.5 (1.3–4)	3.5 (2–4)	
Lipid intake of PN on the 7th day (g/kg/day)	2.9 ± 0.5	2.9 ± 0.5	0.995
	3 (1–3.5)	3 (2–3.5)	
Glucose intake of PN on the 7th day (g/kg/day)	6.9 ± 1.4	6.9 ± 1.9	0.742
	6.8 (4.6–10.5)	6.35 (3.5–14.9)	
Calorie intake of PN on the 7th day (kcal/kg/day)	81 ± 35	88 ± 30	0.292
	80 (32–178)	82 (43–165)	
Duration of lipid emulsion (days)	20.8 ± 13.7	22.1 ± 14.1	0.773
	16 (7–58)	18 (7–57)	
Day of first enteral feeding	4.0 ± 3.6	4.4 ± 4.0	0.685
	3 (1–14)	3 (1–19)	
Day of full enteral feeding	27.9 ± 21.1	28.6 ± 16.7	0.545
	21 (8–97)	23 (8–69)	
Day to regain birth weight	11.4 ± 4.9	15.5 ± 6.5	***0.006***
	10 (3–22)	14 (4–32)	
Days of oxygen in hood	14.1 ± 18.1	14.0 ± 13.9	0.437
	6 (1–79)	8 (1–45)	
Days of nCPAP treatment	7.1 ± 8.4	6.6 ± 7.3	0.914
	4 (1–35)	3 (1–33)	
Days of mechanical ventilation	18.2 ± 23.3	14.2 ± 16.2	0.399
	11 (1–102)	9 (0–48)	
Hospitalization days	58.5 ± 40.0	57.2 ± 32.1	0.792
	47 (18–196)	53 (10–145)	
	n (%)	**n (%)**	
Gender (female)	16 (47.1)	12 (37.5)	0.465
C/S delivery	31 (91.2)	28 (87.5)	0.705
Incidence of SGA	6 (17.6)	5 (16.1)	0.999
Need for surfactant	18 (52.9)	18 (54.5)	0.999

PN, parenteral nutrition, SGA, Small for gestational age.

and 28th day of life. Bloods were centrifuged at 3000 × g for 5 min (4 °C) to obtain the erythrocytes. Cells were washed three times with 0.09% NaCl and stored at −80 °C until analysis. SOD activity was measured by McCord and Fridovich method[18] according to the indirect method involving the inhibition of cytochrome C reduction at 550 nm for 1 min and calculated as U/mg prot. GPx activity was measured by Livingstone et al.'s method[19] based on the decrease of NADPH at 340 nm for 1 min and calculated as nmol/mg prot/min. CAT activity was measured at 240 nm according to the absorbance decrease for 1 min and given as μmol H_2O_2/mg prot./min.[20] For lipid peroxidation, thiobarbituric acid reactive substances (TBARS) were measured by Wills method[21] at 535 nm and calculated as nmol/mg prot. 1,1′,3,3′ tetramethoxypropane was used as a standard. Total protein concentrations of the samples were determined by the method of Lowry et al.[22] using bovine serum albumin as a standard.

2.1. Statistical analysis

All analyses were performed using SPSS 19.0 statistical software package (IBM SPSS Statistics). Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and

Table 2 Neonatal morbidities of neonates in groups.

	FMOS Lipid group (n = 34) %	OO/OS Lipid group (n = 33) %	p
Significant PDA	6 (17.6)	7 (21.2)	0.765
IVH in the first week	8/34 (23.5)	14/33 (42.4)	0.123
IVH > 28th day at discharge	4/31[a] (12.9)	9/33[a] (27.3)	0.392
NEC (all in Stage II)	2 (5.9)	2 (6.0)	0.999
BPD	10/31[a] (32.3)	8/31[a] (25.8)	0.780
ROP			0.582
Present/Total[a]	20/28 (71.4)	18/31 (58.1)	
Stage 1	12 (42.8)	11 (35.5)	
Stage 2	5 (17.9)	3 (9.7)	
Stage 3	0	4 (12.9)	
Stage 4	0	0	
Stage 5	0	0	
AP-ROP	3 (10.7)	0	
Laser for ROP	3/28[a] (10.7)	3/31[a] (9.7)	0.999
Cholestasis	0 (0.0)	6 (18.2)	***0.011***
Mortality	3 (8.8)	2 (6.1)	0.999

BPD, bronchopulmonary disease; IVH, intraventricular haemorrhage; NEC, necrotizing enterocolitis; PDA, Patent ductus arteriosus; ROP, retinopathy of prematurity; AP-ROP, Aggressive posterior ROP.
Bold represents significant values.
[a] Number of patients followed after discharge.

standard deviation and as median and minimum—maximum where appropriate. Chi-square test was used to compare categorical variables between the groups. The normality of distribution for continuous variables was confirmed with the Kolmogorov—Smirnov test. For comparison of continuous variables between two groups, the Student's t-test or Mann—Whitney U test was used depending on whether the statistical hypotheses were fulfilled or not. For comparison of two related (paired) continuous variables, paired samples t-test or Wilcoxon Signed Rank test was used depending on whether the statistical hypotheses were fulfilled or not. To evaluate the change over time of measurements obtained repeatedly, the Repeated Measurements Analysis was applied. The statistical level of significance for all tests was considered to be 0.05.

3. Results

Ninety-two patients were born during the study period; however, 17 patients were not included in the study (5 had congenital heart disease, 5 had hydrops fetalis, 7 had various congenital anomalies) and 8 were excluded (4 died in the first week of life, 4 had LE for less than 7 days) (Fig. 1). There were 34 patients in FMOS lipid group and 33 patients in OO/SO lipid group. Gestational age, birth weight, Apgar scores, gender, delivery type and surfactant use of the patients were similar ($p > 0.05$) (Table 1). There was no statistically significant difference between groups in terms of multiple pregnancy, maternal urinary tract infection, maternal diabetes, preeclampsia, preterm rupture of membranes, prolonged preterm rupture of membranes and antenatal steroid use ($p > 0.05$). Need for resuscitation in delivery room, first hour blood gas pH, base excess and HCO_3 values were also similar ($p > 0.05$).

Protein, lipid, glucose and calorie intake on the 7th, 14th, 21st and 28th days of life and fluid intake in 14 days of life of the groups were similar ($p > 0.05$). As seen in Table 1, there were no differences between groups in nutrition parameters, oxygen, ventilation and hospitalization days ($p > 0.05$). There was no difference in weight gain in the first month of life; however, neonates in FMOS lipid group regained their birth weight earlier ($p = 0.006$). Two groups did not differ in terms of antibiotic use for early neonatal sepsis and incidence of nosocomial infections ($p > 0.05$). Also there were no significant differences between groups in PDA, IVH, NEC, BPD and ROP, but the rate of cholestasis was higher in OO/SO lipid group (Table 2). Cholestasis was detected in 6 neonates and all of them were in OO/OS lipid group. One of 6 cholestatic patients was SGA. Birth weight and gestational age were lower ($p = 0.001$, $p = 0.018$), days of lipid and PN ($p = 0.001$, $p < 0.001$), days of full enteral feeding ($p = 0.005$) and days of hospitalization ($p = 0.024$) were longer, and rate of nosocomial clinical sepsis ($p = 0.016$), bacteraemia ($p = 0.003$) and BPD ($p = 0.043$) were higher in newborns who developed cholestasis compared to newborns who did not have cholestasis in OO/OS lipid group. The mean ± SD duration of PN and lipid were 47.5 ± 10.3 (40—64) days and 37.3 ± 9.2 (21—49) days in cholestatic newborns.

Cell blood count, biochemical values including blood urea nitrogen, creatinine, cholesterol, triglycerides, total and direct bilirubin, gamma glutamyl transferase and alanine aminotransferase were similar on day 0, day 7 and day 28 ($p > 0.05$). Three (8.8%) patients in FMOS lipid and two (6.1%) patients in OO/SO lipid group died ($p = 0.999$).

Catalase, SOD and GPx levels were similar in both groups in the first day of life ($p > 0.05$), but TBARS level was higher in OO/SO lipid group ($p = 0.014$). On the 7th day of LEs, CAT and TBARS levels were higher in OO/SO lipid group compared with FMOS lipid group ($p = 0.024$ and $p = 0.022$, respectively). On the 28th day of life, CAT, GPx and TBARS levels of groups were not different; however, SOD level of

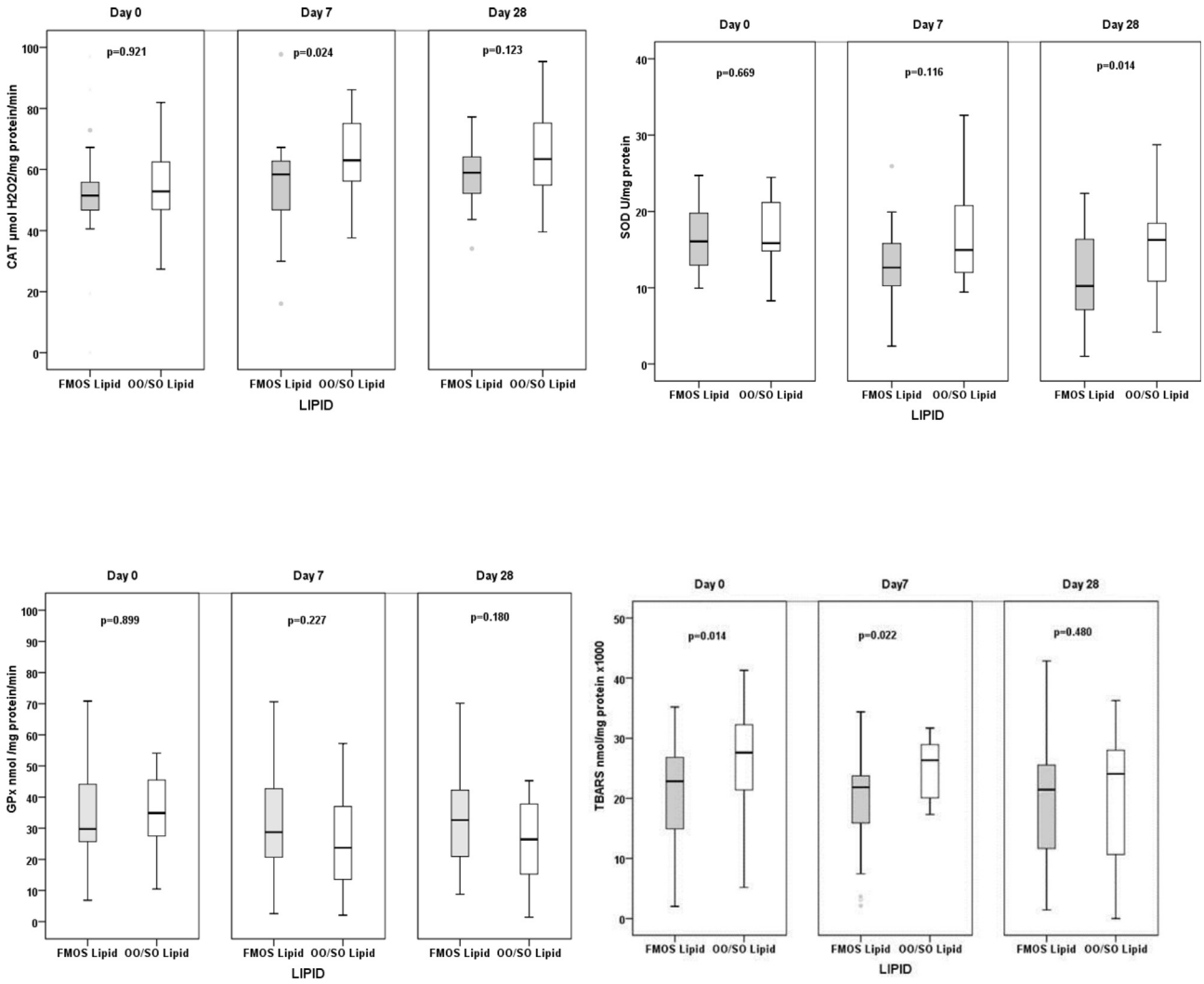


Figure 2 CAT, SOD, GPx and TBARS levels in neonates receiving parenteral nutrition with FMOS or OO/SO lipids at days 0, 7 and 28. CAT, Catalase; FMOS, Fish oil/MCT/olive oil/soy bean; GPx, Glutathione peroxidase; OO/OS, olive oil/soybean oil; SOD, Superoxide dismutase; TBARS, Thiobarbituric acid reactive substances. Boxplots show the 25th, median (50th) and 75th percentile of CAT, SOD, GPx and TBARS.

OO/SO lipid group was higher ($p = 0.014$). TBARS level significantly decreased ($p = 0.035$) while CAT, SOD and GPx levels did not change ($p > 0.05$) in OO/SO lipid group by time. No change was detected in CAT, GPx and TBARS in FMOS lipid group by time ($p > 0.05$); however, SOD levels decreased significantly ($p < 0.001$) (Fig. 2).

4. Discussion

In the present study, we compared the effects of FMOS and OO/SO LEs in preterm infants. The results showed that premature infants in FMOS lipid group reached their birth weight approximately 4 days earlier than those in OO/SO lipid group, although duration of parenteral nutrition and LEs, day of first enteral feeding and full enteral feeding, aminoacid/protein/glucose, calorie intake and weight gain on the 7th, 14th, 21th and 28th days, and fluid intake in the first two weeks of life were similar. We detected no difference in biochemical parameters of the patients in the first month of life. Also, sepsis, IVH, NEC, ROP and BPD rates were similar. However, cholestasis was higher in OO/SO lipid group.

To our knowledge only four studies compare FMOS LE with OO/SO LE in the literature.[23–26] In Deshpande et al.'s study,[23] 23–30 weeks preterm neonates were randomized to receive either OO/SO lipid or FMOS lipid. Similar to our study, they showed no differences in liver enzymes, conjugated bilirubin, BUN, creatinine, cell blood counts, blood culture positive sepsis, ventilation days, PDA, BPD and grade III or IV IVH. In Savini et al.'s study,[24] the authors investigated the effect of five different LEs on plasma phytosterols in preterm infants. There were 30 patients in all groups. They did not compare these two groups separately; however, there were no differences in terms of laboratory values (ALT, GGT, total bilirubin, conjugated bilirubin), BPD, PDA, NEC, sepsis, cholestasis and growth parameters between groups. Recently, Najm et al.[25] and Unal et al.[26] compared SMOFlipid and Clinoleic LEs and found no difference in morbidities (ROP, BPD, PDA) and growth.[25] In the

present study newborns in FMOS lipid group regained their birth weight earlier. In Cochrane metaanalysis,[27] 15 eligible studies comparing any of LEs with soybean LE were analysed. Seven of the studies compared FMOS lipid and soybean lipid.[24,28–33] In metaanalysis of these seven studies, there were no differences in weight gain, duration of ventilation, duration of supplemental oxygen, any sepsis, NEC, any PDA, significant PDA, grade III–IV IVH, cholestasis, hypertriglyceridemia, hyper/hypoglycaemia and death before discharge. However, there was a trend towards faster return to birth weight in soybean group compared to FMOS group. When all LEs were compared with soybean LEs, there was no statistically significant difference in terms of days to regain birth weight or weight gain.[27] Although the present study is an unblinded one, we do not think that this result is due to unblinding as neonates are weighed daily by nurses who are not involved in the study, but this result needs verification in further studies.

Fish oil is rich in DHA, a major structural lipid in sensory, vascular retina and brain. Neonates are prone to DHA deficiency, and for this reason premature babies may benefit from fish oil LEs. In Cochrane metaanalysis,[27] studies comparing FMOS and soybean LEs showed no difference in the incidence of ROP and IVH. In one of these studies, FMOS lipid or soybean lipid (Intralipid) was used in 80 preterm neonates born at $\leq$ 32 gestational weeks.[28] There was no difference in IVH, cholestasis, NEC and BPD incidence. However, ROP incidence was lower in FMOS lipid group (5% vs. 32.5%). The authors suggested that high content of DHA in FMOS lipid might be preventative for ROP development in premature neonates.[28] However, in Collins et al.'s study,[34] enteral DHA supplementation in infants born before 29 gestational weeks resulted in similar findings on IVH, BPD, ROP and death. In the present study, incidence of ROP was similar in both groups. We had three patients with A-P ROP in FMOS lipid group and four patients with Stage III ROP in OO/SO lipid group; laser photocoagulation need was similar in groups.

In our study, no cholestasis was detected in FMOS lipid group; however, there were 6 patients with cholestasis in OO/SO lipid group. Cholestasis is more evident in longer-term PN. In a study including 6543 neonates who received PN, the incidence of parenteral nutrition-associated liver disease was 21% for neonates receiving PN for 14–28 days, 43% for 29–56 days, 72% for 57–100 days and 85% for greater than 100 days.[17] In the present study, the median duration of PN in FMOS and OO/OS groups were 18 and 22 days respectively. In Unal et al.'s study,[26] cholestasis rate was lower compared to our study (1.2% vs.1.6% in FMOS vs. OO/SO LEs). However, duration of PN in Unal et al.'s study[26] is shorter compared to our study (median 7 days in both groups compared to 18 and 22 days in the present study). Duration of PN and LEs was even higher in cholestatic patients in our study. Phytosterols are steroid alcohols similar to cholesterol in mammals. Phytosterols have been reported to reduce bile acid secretion.[35] Phytosterol level is higher in OO/SO lipid and it may be a factor for cholestasis in OO/SO lipid group. Improved liver function, reversal of cholestasis and prevention of PN- associated liver disease were reported with the use of fish oil containing LEs.[36–39] However, in Savini et al.'s study,[24] although phytosterol serum levels were positively correlated with phytosterol intake, cholestasis was rare and there was no difference in liver function tests between groups. Although the results in our study showed higher cholestasis rate in OO/SO lipid group, our results should be carefully assessed, as the study was a short-term study and included a small number of patients. Also, cholestatic patients had significantly longer duration of PN, higher rates of BPD, nosocomial clinical sepsis, bacteraemia and lower birth weight and gestational age compared to other preterms in OO/SO lipid group. We conclude that studies evaluating effect of FMOS lipid in some risk groups such as preterms who need longer duration of PN and studies including more patients may give further information about its effect on cholestasis.

Preterm neonates are vulnerable to oxidative stress as they have limited antioxidant system. Also, they are exposed to more oxygen, ventilation and nosocomial infections. There are only two studies dealing lipid peroxidation with FMOS and OO/SO LEs.[23,26] In Deshpande et al.'s study,[23] lipid peroxidation marker, F2-isoprostane, red cell FAs and vitamin E levels were measured after seven days of LEs. F2-isoprostane levels were significantly reduced in FMOS lipid group after adjusting for baseline levels. EPA and vitamin E levels of FMOS lipid group were higher and DHA levels were similar despite higher DHA in FMOS lipid. Unal et al.[26] evaluated total antioxidant capacity, total oxidant status and oxidant stress index at baseline, at first week of PN and one week after PN discontinuation. They reported similar oxidant and antioxidant statuses in FMOS and OO/SO groups. Total antioxidant capacity, total oxidant status and oxidant stress index were lower than baseline after three weeks.[26] In the present study, TBARS levels on the first day of life, CAT level on the 7th day of LEs and SOD level on the 28th day of life were higher in OO/SO group. We could not explain the higher TBARs level in OO/SO group as there was no significant difference in terms of patient characteristics at baseline. However, there may be other contributing factors we have not assessed. Higher sustained levels of CAT and SOD may be a defence for increased lipid peroxidation at baseline in OO/SO LE group.

In conclusion, both FMOS and OO/SO LEs seem to have similar effects on morbidities in the early period except for cholestasis. Both showed similar results on lipid peroxidation on the 28th day of life. However, as the number of patients in this study is low and the study is not blinded, we need further research with more patients to evaluate the effectiveness of FMOS LEs compared with olive oil-soybean LEs in preterm infants.

Conflict of interest

The authors declare that there is no conflict of interest.
The study was funded by Çukurova University, Scientific Project Department, Project Code: TTU-2015-4910.

References

1. El Kasmi KC, Anderson AL, Devereaux MW, Vue PM, Zhang W, Setchell D, et al. Phytosterols promote liver injury and Kupffer cell activation in parenteral nutrition-associated liver disease. *Sci Transl Med* 2013;**5**:206ra137.
2. Hojsak I, Colomb V, Braegger C, Bronsky J, Campoy C, Domellöf M, et al. ESPGHAN committee on nutrition position paper. Intravenous lipid emulsions and risk of hepatotoxicity in infants and children: a systematic review and meta-analysis. *J Pediatr Gastroenterol Nutr* 2016;**62**:776–92.
3. Carter BA, Taylor OA, Prendergast DR, Zimmerman TL, Von Furstenberg R, Moore DD, et al. Stigmasterol, a soy lipid-derived phytosterol, is an antagonist of the bile acid nuclear receptor FXR. *Pediatr Res* 2007;**62**:301–6.
4. Göbel Y, Koletzko B, Böhles HJ, Engelsberger I, Forget D, Le Brun A, et al. Parenteral fat emulsions based on olive and soybean oils: a randomized clinical trial in preterm infants. *J Pediatr Gastroenterol Nutr* 2003;**37**:161–7.
5. Gawecka A, Michalkiewicz J, Kornacka MK, Luckiewicz B, Kubiszewska I. Immunologic properties differ in preterm infants fed olive oil vs soy-based lipid emulsions during parenteral nutrition. *JPEN J Parenter Enteral Nutr* 2008;**32**:448–53.
6. Seo T, Blaner WS, Deckelbaum RJ. Omega-3 fatty acids: molecular approaches to optimal biological outcomes. *Curr Opin Lipidol* 2005;**16**:11–8.
7. Calder PC, Yaqoob P. Omega-3 polyunsaturated fatty acids and human health outcomes. *Biofactors* 2009;**35**:266–72.
8. Fürst P, Kuhn KS. Fish oil emulsions: what benefits can they bring? *Clin Nutr* 2000;**19**:7–14.
9. Hao W, Wong OY, Liu X, Lee P, Chen Y, Wong KK. ω-3 fatty acids suppress inflammatory cytokine production by macrophages and hepatocytes. *J Pediatr Surg* 2010;**45**:2412–8.
10. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008;**8**:349–61.
11. Vlaardingerbroek H, Ng K, Stoll B, Benight N, Chacko S, Kluijtmans LA, et al. New generation lipid emulsions prevent PNALD in chronic parenterally fed preterm pigs. *J Lipid Res* 2014;**55**:466–77.
12. Saugstad OD. Update on oxygen radical disease in neonatology. *Curr Opin Obstet Gynecol* 2001;**13**:147–53.
13. de Zwart LL, Meerman JH, Commandeur JNM, Vermeulen NP. Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radic Biol Med* 1999;**26**:202–26.
14. Ozlü F, Yapıcıoğlu PH, Mer K, Satar M, Narlı N, Sertdemir Y. The effect of two different parenteral nutrition regimens on parenteral nutrition-associated cholestasis. *J Matern Fetal Neonatal Med* 2013;**26**:724–7.
15. International Committee for the Classification of Retinopathy of Prematurity. The international classification of retinopathy of prematurity revisited. *Arch Ophthalmol* 2005;**123**:991–9.
16. Yapicioglu H, Ozcan H, Sertdemir Y, Mutlu B, Satar M, Narli N, et al. Healthcare-associated infections in a neonatal intensive care unit in Turkey in 2008: incidence and risk factors, a prospective study. *J Trop Pediatr* 2011;**57**:157–64.
17. Christensen RD, Henry E, Wiedmeier SE, Burnett J, Lambert DK. Identifying patients on the first day of life, at high-risk of developing parenteral- nutrition associated liver disease. *J Perinatol* 2007;**27**:284–90.
18. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969;**244**:6049–55.
19. Livingstone DR, Lips F, Garcia Martinez P, Pipe K. Antioxidant enzymes in the digestive gland of the common mussel Mytilus edulis. *Mar Biol* 1992;**112**:265–76.
20. Lartillot S, Kedziora P, Athias A. Purification and characterization of a new fungal catalase. *Prep Biochem* 1988;**18**:241–6.
21. Wills ED, Wilkinson AE. Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release. *Biochem J* 1966;**99**:657–66.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951;**193**:265–75.
23. Deshpande G, Simmer K, Deshmukh M, Mori TA, Croft KD, Kristensen J. Fish oil (SMOFlipid) and olive oil lipid (Clinoleic) in very preterm neonates. *J Pediatr Gastroenterol Nutr* 2014;**58**:177–82.
24. Savini S, D'Ascenzo R, Biagetti C, Serpentini G, Pompilio A, Bartoli A, et al. The effect of 5 intravenous lipid emulsions on plasma phytosterols in preterm infants receiving parenteral nutrition: a randomized clinical trial. *Am J Clin Nutr* 2013;**98**:312–8.
25. Najm S, Löfqvist C, Hellgren G, Engström E, Lundgren P, Hård AL, et al. Effects of a lipid emulsion containing fish oil on polyunsaturated fatty acid profiles, growth and morbidities in extremely premature infants: a randomized controlled trial. *Clin Nutr ESPEN* 2017;**20**:17–23.
26. Unal S, Demirel N, Erol S, Isik DU, Kulali F, Iyigun F, et al. Effects of two different lipid emulsions on morbidities and oxidant stress statuses in preterm infants: an observational study. *J Matern Fetal Neonatal Med* 2018;**31**:850–6.
27. Kapoor V, Glover R, Malviya MN. Alternative lipid emulsions versus pure soy oil based lipid emulsions for parenterally fed preterm infants. *Cochrane Database Syst Rev* 2015;(12):CD009172.
28. Beken S, Dilli D, Fettah ND, Kabataş EU, Zenciroğlu A, Okumuş N. The influence of fish-oil lipid emulsions on retinopathy of prematurity in very low birth weight infants: a randomized controlled trial. *Early Hum Dev* 2014;**90**:27–31.
29. D'ascenzo R, Savini S, Biagetti C, Bellagamba MP, Marchionni P, Pompilio A, et al. Higher docosahexaenoic acid, lower arachidonic acid and reduced lipid tolerance with high doses of a lipid emulsion containing 15% fish oil: a randomized clinical trial. *Clin Nutr* 2014;**33**:1002–9.
30. Rayyan M, Devlieger H, Jochum F, Allegaert K. Short-term use of parenteral nutrition with a lipid emulsion containing a mixture of soybean oil, olive oil, medium-chain triglycerides and fish oil: a randomized double-blind study in preterm infants. *JPEN J Parenter Enteral Nutr* 2012;**36**:81S–94S.
31. Skouroliakou M, Konstantinou D, Koutri K, Kakavelaki C, Stathopoulou M, Antoniadi M, et al. A double-blind, randomized clinical trial of the effect of omega-3 fatty acids on the oxidative stress of preterm neonates fed through parenteral nutrition. *Eur J Clin Nutr* 2010;**64**:940–7.
32. Tomsits E, Pataki M, Tölgyesi A, Fekete G, Rischak K, Szollár L. Safety and efficacy of a lipid emulsion containing a mixture of soybean oil, medium-chain triglycerides, olive oil, and fish oil: a randomised, double-blind clinical trial in premature infants requiring parenteral nutrition. *J Pediatr Gastroenterol Nutr* 2010;**51**:514–21.
33. Vlaardingerbroek H, Vermeulen MJ, Carnielli VP, Vaz FM, van den Akker CH, van Goudoever JB. Growth and fatty acid profiles of VLBW infants receiving a multicomponent lipid emulsion from birth. *J Pediatr Gastroenterol Nutr* 2014;**58**:417–27.
34. Collins CT, Makrides M, McPhee AJ, Sullivan TR, Davis PG, Thio M, et al. Docosahexaenoic acid and bronchopulmonary dysplasia in preterm infants. *N Engl J Med* 2017;**376**:1245–55.
35. Iyer KR, Spitz L, Clayton P. BAPS prize lecture: new insights into mechanisms of parenteral nutrition-associated cholestasis: role of plant sterols. British Association of Paediatric Surgeons. *J Pediatr Surg* 1998;**33**:1–6.

36. Koletzko B, Goulet O. Fish oil containing intravenous lipid emulsions in parenteral nutrition-associated cholestatic liver disease. *Curr Opin Clin Nutr Metab Care* 2010;**13**:321—6.
37. Nandivada P, Carlson SJ, Cowan E, Chang MI, Gura KM, Puder M. Role of parenteral lipid emulsions in the preterm infant. *Early Hum Dev* 2013;**89**:S45—9.
38. Pawlik D, Lauterbach R, Turyk E. Fish-oil fat emulsion supplementation may reduce the risk of severe retinopathy in VLBW infants. *Pediatrics* 2011;**127**:223—8.
39. Puder M, Valim C, Meisel JA, Le HD, de Meijer VE, Robinson EM, et al. Parenteral fish oil improves outcomes in patients with parenteral nutrition-associated liver injury. *Ann Surg* 2009;**250**:395—402.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pedneo.2018.07.012.