



Randomized Control Trials

Intravenous fish oil containing lipid emulsion attenuates inflammatory cytokines and the development of bronchopulmonary dysplasia in very premature infants: A double-blind, randomized controlled trial



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SUMMARY

Background & aims: Preterm infants have lower levels of long-chain polyunsaturated fatty acids (LCPUFAs). Supplementing very premature infants with intravenous lipid emulsions that fish oil, which is rich in n-3 LC-PUFAs, may decrease bronchopulmonary dysplasia (BPD) by modulating inflammation and neonatal immune function.

Methods: Sixty very low birth weight (VLBW) premature infants requiring ventilator support were randomized in a double-blind manner to 2 groups and received total parenteral nutrition with fish oil containing LE (intervention group, n = 30) or soybean oil containing LE (control group, n = 30) for 7 days. Blood samples and bronchoalveolar lavage fluid (BALF) were obtained for assay on day 1 and 7 days after LE. The primary outcome was to compare the levels of interleukin (IL)-1 β and IL-6 in serum and BALF. Secondary outcomes were to compare mortality and co-morbidities.

Results: The levels of IL-1 β and IL-6 in serum and BALF were significantly lower in the intervention group at day 8 ($p < 0.05$). The incidence of BPD in the intervention group compared to the control group was 13.3% versus 36.7% ($p = 0.04$; odds ratio [OR], 0.36; 95% confidence interval [CI], 0.21–0.86). The duration of ventilator support and oxygen use was significantly less in the intervention group than in the control group ($p < 0.05$). The level of alanine aminotransferase was significantly lower in the intervention group on day 8 ($p = 0.031$).

Conclusions: In very premature infants, early administration of fish oil containing LE significantly decreased IL-1 β and IL-6 levels in serum and BALF and was associated with shorter duration of ventilator support and less bronchopulmonary dysplasia (BPD).

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1. Introduction

Preterm infants have significantly lower concentrations of long-chain polyunsaturated fatty acids (LCPUFAs) in the brain and other organs than full-term infants because they miss the major period of docosahexaenoic acid (DHA) and arachidonic acid (AA) accretion during the third trimester of gestation [1,2]. LCPUFAs play a crucial role in health related to growth, brain development, immune and

Abbreviations: AA, arachidonic acid; BPD, bronchopulmonary dysplasia; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, Fish oil; IL, interleukin; n-3 long-chain PUFA, omega-3 long-chain polyunsaturated fatty acids; LE, lipid emulsion; SO, soybean oil; TPN, total parenteral nutrition.

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allergic responses and nutrition-related chronic diseases [3,4]. The LCPUFAs are also known to modulate inflammation and immune cell functions at various levels including cell membrane properties, phagocytosis and production of bioactive substances such as cytokines [5]. The most common formulation for total parenteral nutrition (TPN) is a soybean oil-based lipid emulsion (LE), which is rich in n-6 long-chain PUFA and phytosterols that contribute to hepatotoxicity and production of proinflammatory cytokines (tumour necrosis factor α and interleukin-6) [6,7].

Many evidences strongly support a pathogenetic role of inflammation and oxidative stress on parenteral nutrition associated cholestasis [8] and bronchopulmonary dysplasia (BPD) [9,10]. Concerns about adverse reactions have led to the development of an alternative LE in which soybean-derived lipids have been partially substituted by n-3 long-chain PUFA (EPA and DHA) derived from fish oil [11]. Several studies in adult patients have demonstrated that fish oil containing LE can increase the plasma levels of EPA and DHA and upregulated the leukotriene B5 production in leukocytes [12,13]. In addition, recent studies demonstrated that oxidative stress and inflammatory response were significantly reduced in neonates receiving LE containing medium-chain triglycerides (MCTs) and n-3 long-chain PUFA [14,15].

Two randomized controlled trials (RCT) have demonstrated that parenteral infusion of LE containing fish oils was safe, well tolerated and showed a beneficial influence on cholestasis in premature infants [16,17]. Thus far, there have been limited trials of the effect of n-3 long-chain PUFA on the inflammatory cytokines and the incidence of BPD in VLBW infants. We hypothesised that the use of LE containing fish oil in VLBW infants may have a favourable effect on inflammation and oxidative stress-induced complications.

2. Methods

2.1. Participants

From March 1, 2012 to February 28, 2014, intubated premature infants who weighed below 1500 g and had a gestational age below 32 weeks in Changhua Christian Children's Hospital neonatal intensive care unit were randomized to an intervention or control group within 24 h and after informed consent. All the infants had an arterial line for blood sampling for at least 8 days. Infants were excluded from the study as follows: 1) lethal congenital abnormalities or chromosomal disorders; 2) congenital cyanotic heart disease; 3) gastrointestinal surgery, such as intestinal perforation, malrotation, volvulus and atresia; 4) confirmed or family history of hereditary metabolic disorder and 5) clinical sepsis at admission.

2.2. Sample size and randomization

The sample size calculation was based on the results of our previous pilot study. Effect size was defined as the mean difference between two groups divided by the pooled standard deviation for the data. The minimum sample size for estimating the difference in the mean change of serum IL-6 at 7 days between the intervention and control groups with an effect size of 0.8 (Mean: 89.5 and 49.6, SD: 55.8 and 42.7, $n = 10$ and 10 , respectively) was calculated. Using these parameters, a sample size of 26 subjects in each group was needed to have 80% power and 5% level of significance by independent t test. Using a predicted attrition rate of 10%, the minimum number of subjects required at baseline was calculated to be 29 per group. Simple randomization was done by a hospital pharmacist opening a sealed opaque envelope containing cards labelled SO (control group) or FO (intervention group) at a 1:1 ratio. The pharmacist prepared lipid emulsion syringes (without labels describing their contents) and which were identical in appearance

and colour. The physicians and nurses did not know to which group the patients had been allocated.

2.3. Interventions and treatment strategies

Within 24 h after birth, the intervention group received TPN containing a novel LE (SMOF lipid; containing 30% soybean oil, 30% MCT, 25% olive oil and 15% fish oil), providing 4.8 g/L of EPA and 3.5 g/L of DHA; the control group received TPN containing conventional soybean oil LE (Lipovenoes MCT 20% contain 50% soybean oil and 50% MCT). Both LEs were from Fresenius Kabi, Bad Homburg, Germany and approved for infant use by The Ministry of Health and Welfare in Taiwan. The study was approved by the Institutional Review Board at Changhua Christian Hospital and informed consent was obtained from the parents. The trial was retrospectively registered at Current Controlled Trials: ISRCTN11427103 after the study had finished.

Blood sample (1.5 mL) from the artery line was obtained within 24 h and at day 8. BALF, 0.5 mL from tracheal aspiration, was obtained within 24 h and day 8 if the patient was still intubated. The levels of IL-1 β and IL-6 in the serum and BALF were measured by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN) according to the manufacturer's instructions. Biochemical parameters were measured according to clinical routine care.

The dose of LE was 1 gm/kg/day on day 1 of TPN and increased to 2 gm/kg/day on day 2 and 3 gm/kg/day on day 3 to day 7. Glucose and amino acid solutions were using the same products in both groups. Enteral feedings were initiated as soon as possible. The premature infants were fed with breast milk or premature formula milk if breast milk was not sufficient. Infants in both groups continued the TPN schedule until oral feeding reached a minimum of 80% of total energy intake.

2.4. Primary and secondary outcomes

The primary outcome was comparing the immune effects of 2 different LEs, assessed during the first 24 h and day 8, including IL-1 β and IL-6 in serum and BALF. Secondary outcomes were mortality, length of hospital stay (LOS), ventilator use days, oxygen dependent days, weight gain rate, liver function, parenteral nutrition-associated cholestasis (PNAC), BPD, retinopathy of prematurity (ROP), necrotising enterocolitis (NEC), intraventricular haemorrhage (IVH), and late sepsis. The occurrence of PNAC was defined as 2 consecutive direct bilirubin measurements equal to or higher than 2 mg/dL (34.2 μ mol/L) and BPD severity was graded based on an assessment performed at 36 weeks of postmenstrual age. Mild BPD was defined as able to breathe room air; moderate BPD as requiring supplemental oxygen (but a fraction of inspired oxygen (FiO₂) of less than 0.30) and severe BPD as requiring an FiO₂ of 0.30 or greater, or requiring positive pressure ventilation. A diagnosis of NEC was made by clinical criteria according to Modified Bell's Staging, and IVH was diagnosed primarily through the use of brain-imaging studies, usually cranial ultrasonography.

2.5. Statistical analysis

We performed an intention-to-treat analysis. Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY). P-values < 0.05 were considered statistically significant. The 2 groups were compared with Student's t test for continuous data, and the chi-square test or Fisher's exact test for categorical data. Because cytokine data were not normally distributed, we used the non parametric Wilcoxon signed rank test to examine within-group changes at different time points. The Mann-Whitney U test was used to compare the median values of

cytokine between the groups. Continuous data are expressed as mean \pm standard deviation and dichotomous variables as counts and proportions. Cytokine data are expressed as median (range).

3. Results

3.1. Patient characteristics

Participants' distribution and grouping are presented in the flow diagram in Fig. 1. In total 70 VLBW infants were screened and 60 infants were enrolled; 30 received SMOF lipid (intervention group), and 30 received Lipovenoes (control group) following randomisation. The 2 treatment groups were similar with respect to demographic characteristics (Table 1) and enteral/parental nutrition (Fig. 2A and B). There are 25 and 22 infants still intubated on day 7 in control group and intervention group respectively.

3.2. Primary outcomes

Initial concentrations of IL-1 β and IL-6 in serum and BALF were similar in both groups (Figs. 3 and 4). Serum IL-1 β was significantly lower in the intervention group than in the control group on day 8 (135 (27–469) pg/mL versus 194 (92–519) pg/mL, $p = 0.01$). Serum IL-6 was significantly lower in the intervention group than in the control group on day 8 (27 (5–95) pg/mL versus 50 (10–154) pg/mL, $p = 0.02$). In BALF, IL-1 β was significantly lower in the intervention group than in the control group on day 8 (234 (54–773) pg/mL versus 367 (69–1017) pg/mL, $p = 0.03$), and IL-6 was significantly lower in the intervention group than in the control group on day 8 (489 (48–1684) pg/mL versus 823 (274–1554) pg/mL, $p = 0.02$). The decrease of BALF IL-1 β from day 1 to day 8 was significantly higher in the intervention group than in the control group (605 (–161–2391) pg/mL versus 414 (–244–1608) pg/mL,

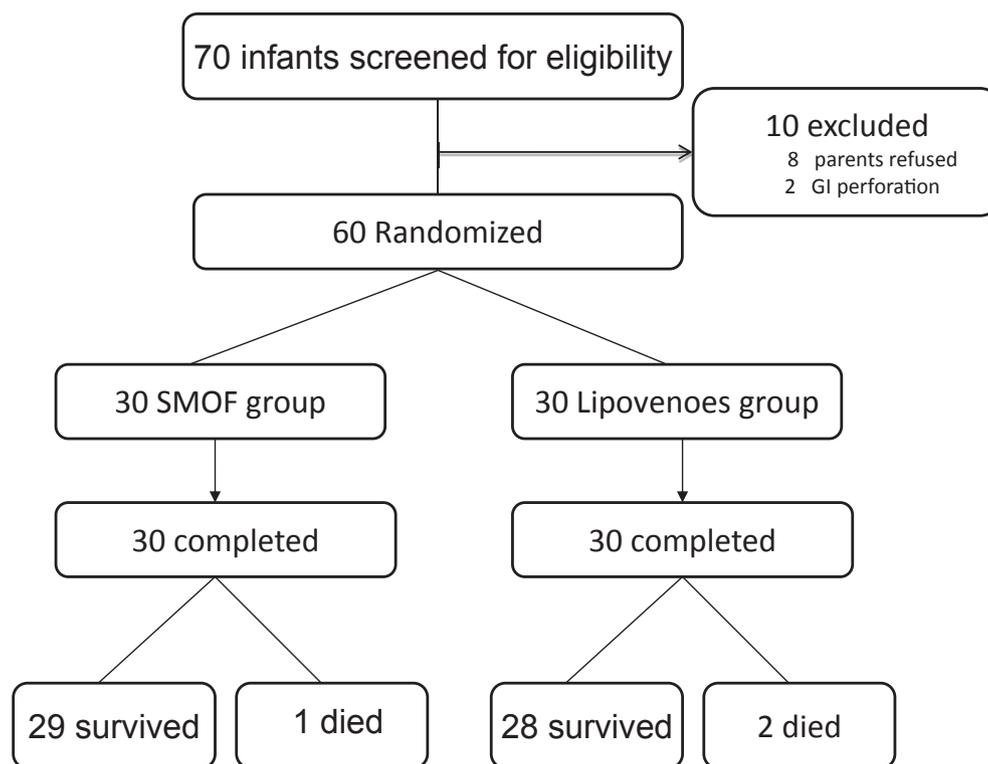


Fig. 1. Standard consort flow chart for study participant enrolment.

Table 1
Participant characteristics.

Perinatal data	Intervention group (n = 30)	Control group (n = 30)	P value
Male, n (%)	14 (47)	15 (50)	0.96
Gestational age, Mean (SD), weeks	28.5 (2.9)	28.3 (2.9)	0.79
Birth weight, Mean (SD), grams	1004 (265)	962 (194)	0.56
Caesarean delivery, n (%)	15 (50.0)	16 (53.3)	0.67
Antenatal steroid, n (%)	22 (73.3)	23 (76.6)	0.85
Surfactant use, n (%)	21 (70.0)	22 (73.3)	0.79
1-min Apgar score, median (interquartile range)	4 (2–6)	5 (3–7)	0.89
5-min Apgar score, median (interquartile range)	7 (6–8)	7 (6–8)	1.0
NTISS score, median (interquartile range)	14 (10–21)	15 (11–25)	0.92
Maximum OI, median (interquartile range)	24 (18–32)	22 (17–29)	0.62
Maximum PIP, median (interquartile range)	16 (13–18)	16 (12–20)	0.94
Vasopressor and inotropes, n (%)	12 (40.0)	14 (46.7)	0.75
Breast milk feeding, n (%)	19 (63.3)	20 (66.7)	0.89

There were no significant differences between the groups.

P values are from Student's *t* test, Chi-square test or Fisher's exact test when appropriate.

SD = standard deviation; NTISS = Neonatal therapeutic intervention scoring system; OI = oxygenation index; PIP = Peak inspiratory pressure; LE = Lipid emulsion.

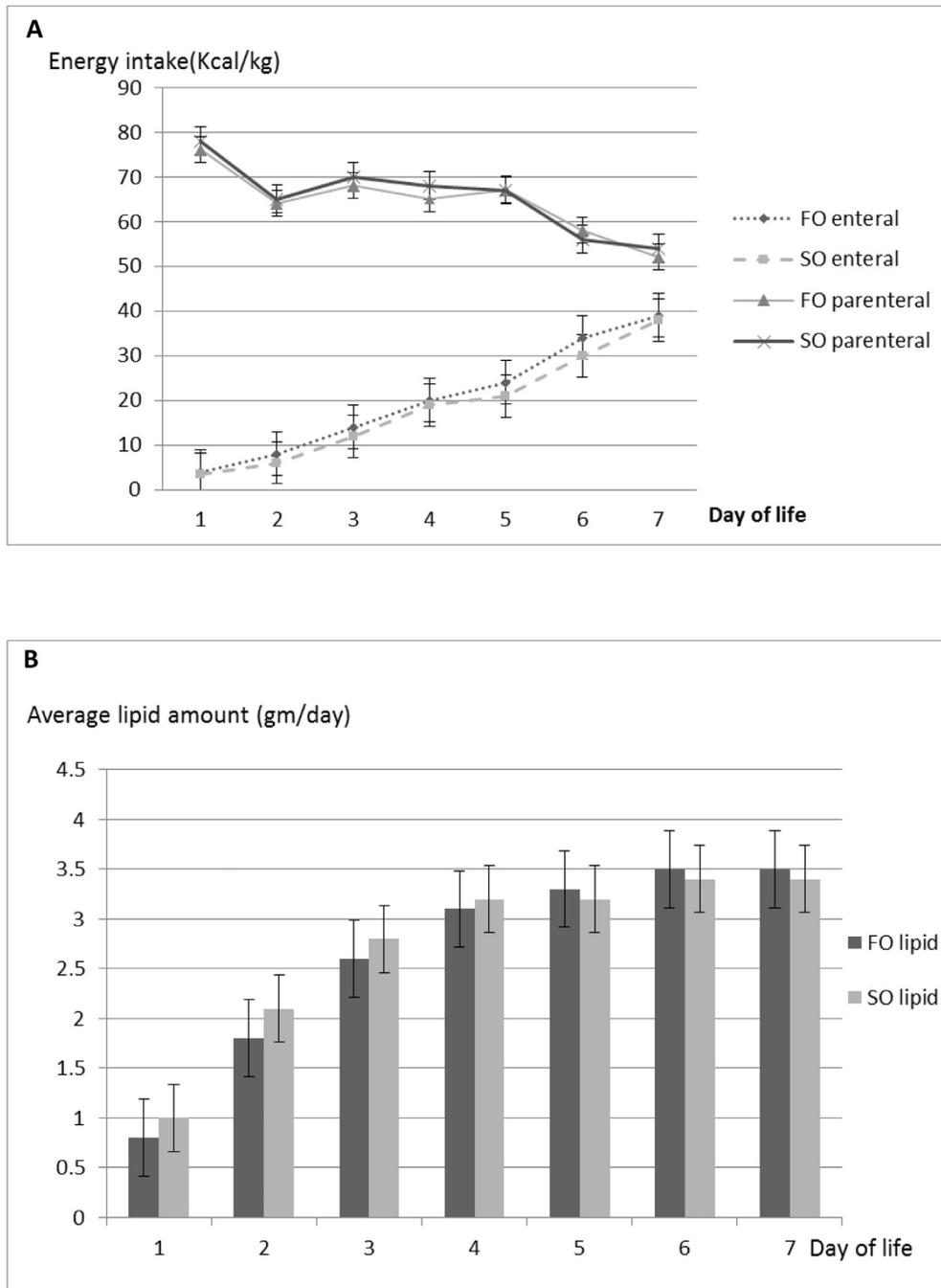


Fig. 2. A. Enteral/parenteral nutrition (mean \pm SD) during the first week. There were no significant differences of enteral/parenteral nutrition between the intervention group (FO) and control group (SO) during the first week of life. B. Average lipid emulsion amount (mean \pm SD) during the first week. There was no significant difference in amount of lipid emulsion between the intervention group (FO) and control group (SO) during the first week of life.

$p = 0.03$). The decrease of BALF IL-6 from day 1 to day 8 was significantly higher in the intervention group than in the control group (752 (125–1576) pg/mL versus 362 (–957–2171) pg/mL, $p = 0.04$). But, the decrease in serum IL-1 β and IL-6 concentrations from day 1 to day 8 were not significant difference between groups.

3.3. Secondary outcomes

White blood cell counts, platelet counts, aspartate aminotransferase, direct bilirubin, creatinine, C-reactive protein (CRP), glucose, triglyceride and cholesterol levels were not different at baseline and day 8 between the 2 groups ($p > 0.05$). Haemoglobin level decreased

and alkaline phosphatase and total bilirubin elevation were noted at day 8 in both groups, but there was no difference between the 2 groups. On day 8, alanine aminotransferase level showed the only significant difference between the control group and intervention group (63.7 ± 35.24 IU/L versus 24.8 ± 18.22 IU/L, $p = 0.031$).

Comorbidities of premature infants in these 2 groups are presented in Table 2. Ventilator days, oxygen use days and incidences of BPD (13.3% versus 36.7%, $p = 0.04$, odds ratio [OR], 0.36; 95% confidence interval [CI], 0.21–0.86) were significantly fewer in the intervention group than control group. After a multiple logistic regression analysis, BPD was significantly associated with a lower gestational age, fewer ICU days, a lower Apgar score at 1 min, and

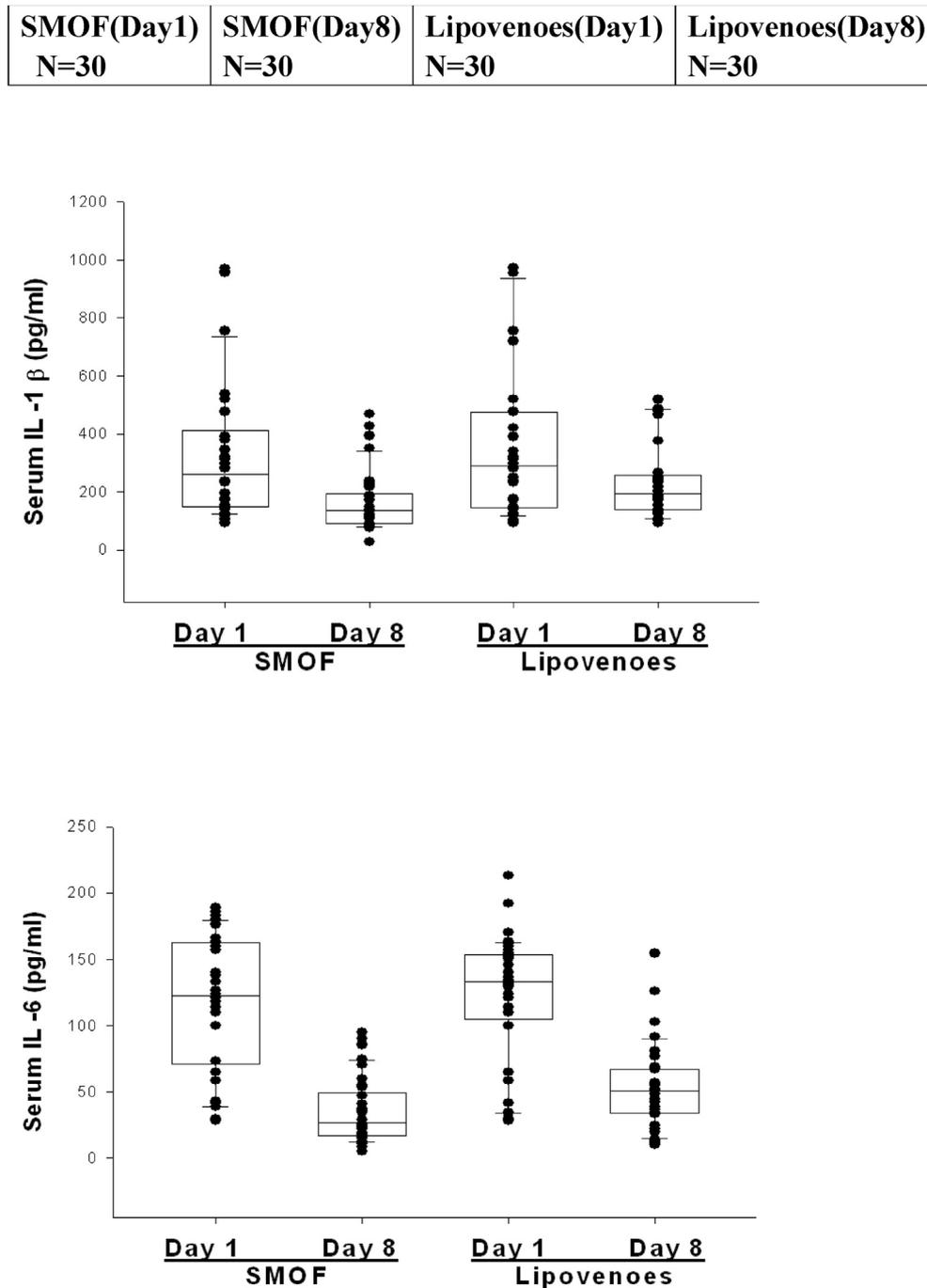


Fig. 3. Cytokine Parameters at Baseline and on Day 8 in serum. Serum IL-1 β was significantly lower in the intervention group than in the control group on day 8 [135.9 (27.8–469.7) pg/mL versus 194.2 (92.7–519.4) pg/mL, $p = 0.01$]. Serum IL-6 was significantly lower in the intervention group than in the control group on day 8 [27.2 (5.4–95.3) pg/mL versus 50.9 (10.0–154.7) pg/mL, $p = 0.02$].

the type of lipid emulsion. After controlling for these factors, the type of LE still demonstrated a significant effect on BPD. (Odds ratio = 0.033, 95% CI = 0.002–0.568, $p = 0.019$). Other comorbidities showed no significant difference between the groups. The incidence of PNAC (6.67%) in the intervention group was lower than the control group (13.33%), but the difference was not statistically significant ($p = 0.35$).

4. Discussion

To our knowledge, this is the first double-blind, randomized controlled trial in infants with VLBW that directly compared the

effect of TPN with fish oil or soybean oil on inflammatory cytokines in BALF. In the present study, infants with VLBW receiving fish oil containing LE had lower levels of IL-1 β and IL-6 in serum and BALF on day 8, a decreased incidence of BPD and lower serum levels of alanine aminotransferase. This is consistent with our hypothesis that premature infants receiving n-3 long-chain PUFA in parenteral nutrition may receive a beneficial effect on inflammation with a decrease in oxidative stress-induced complications, such as BPD and liver dysfunction.

The occurrence of BPD is the most common chronic lung disease in infancy with devastating short-term and long-term consequences, causing a large financial burden on society and families

SMOF(Day1) N=30	SMOF(Day8) N=22	Lipovenoes(Day1) N=30	Lipovenoes(Day8) N=25
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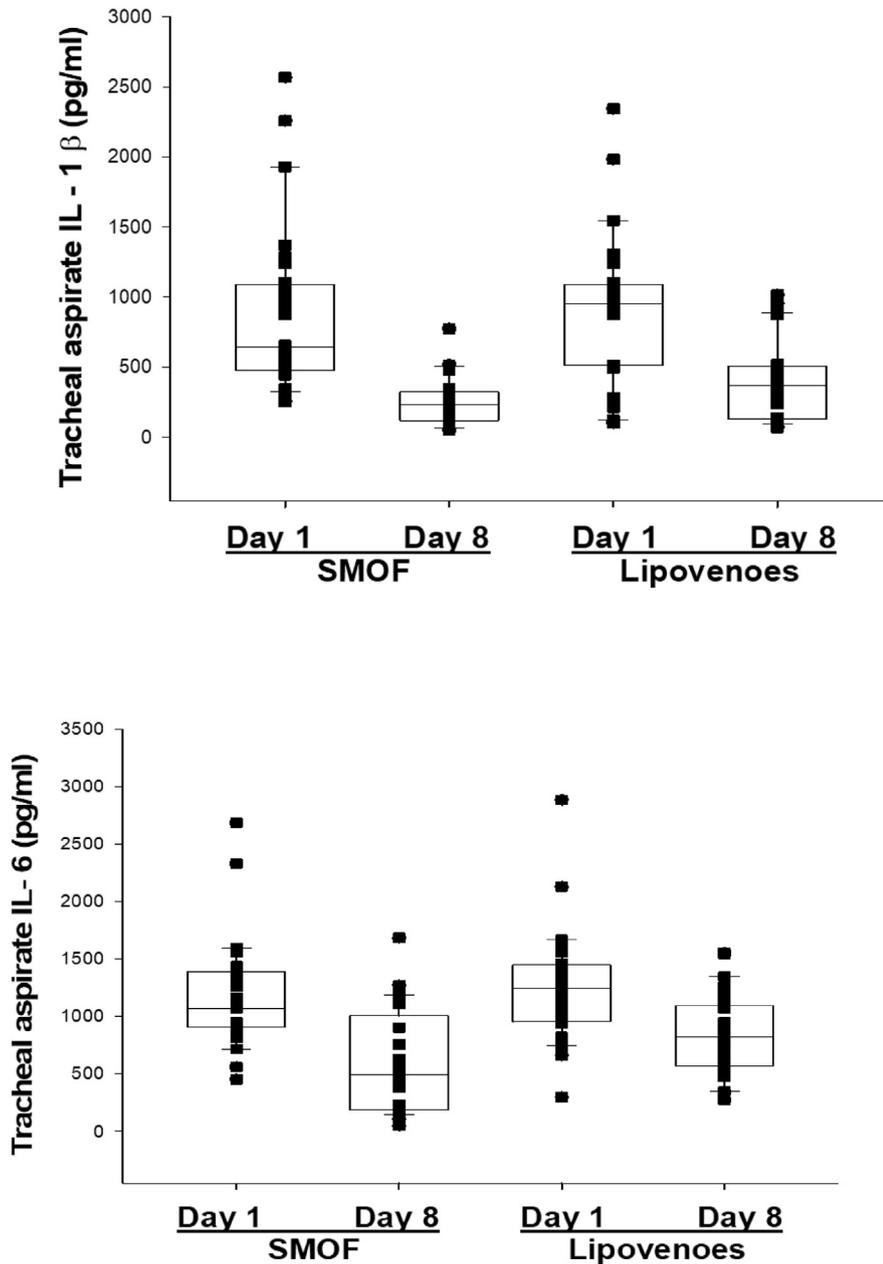


Fig. 4. Cytokine parameters at baseline and on day 8 in BALF. In bronchoalveolar lavage fluid (BALF), IL-1 β was significantly lower in the intervention group than in the control group on day 8 [234.4 (54.1–773.4) pg/mL versus 367.0 (69.8–1017.5) pg/mL, $p = 0.03$] and IL-6 was significantly lower in the intervention group than in the control group on day 8 [489.2 (48.9–1684.4) pg/mL versus 823.2 (274.9–1554.8) pg/mL, $p = 0.02$].

[18]. Developing drugs to prevent or treat BPD has been the major goal of neonatal care over the past 20 years, but few drugs have shown favourable results [19]. Our study demonstrated that after 7 days of parenteral nutrition with fish oil containing LE for infants with VLBW, IL-1 β and IL-6 levels in serum and BALF were significantly lower than in the Lipovenoes group and could be associated with the amelioration of BPD. Fish oil containing LE in TPN acts as an immunonutrient providing anti-inflammatory effects in premature infants. The pathogenesis of BPD is multifactorial, but all

triggers cause pulmonary inflammation. IL-1 β and IL-6 are the major proinflammatory cytokines mediating acute lung injury, exacerbating ventilator-associated lung injury, and modulating host defences, while also participating in normal lung growth [20]. Elevated IL-1 β and IL-6 were observed in tracheal aspirates and the serum of infants with respiratory distress syndrome who subsequently developed BPD [20–22]. Infants with VLBW with high postnatal serum concentrations of IL-1 β were associated with the development of BPD or death [23]. However, randomized

Table 2
Outcomes for premature infants.

Morbidity	Intervention group (n = 30)	Control group (n = 30)	P value
Sepsis, n (%)	3 (10)	5 (16.67)	0.45
PNAC, n (%)	2 (6.67)	4 (13.33)	0.39
BPD, n (%)	4 (13.33)	11 (36.67)	0.04
Mild, n (%)	1 (3.33)	2 (6.67)	
Moderate, n (%)	1 (3.33)	4 (13.33)	
Severe, n (%)	2 (6.67)	5 (16.67)	
NEC, n (%)	0 (0)	1 (3.33)	0.31
ROP, n (%)	6 (20)	7 (23.33)	0.75
IVH grade 1, 2, n (%)	6 (20)	4 (13.33)	0.49
IVH grade 3, 4, n (%)	1 (3.33)	2 (6.67)	0.55
TPN days, mean (SD)	32.59 (16.84)	31.62 (17.56)	0.94
Weight gain, mean (SD), grams/day	18.42 (7.21)	17.86 (6.57)	0.89
Death, n (%)	1 (3.33%)	2 (6.67%)	0.89
Ventilator days, mean (SD)	9.2 (3.5)	16.6 (7.2)	0.03
N-CPAP days, mean (SD)	20.3 (15.4)	23.4 (18.7)	0.76
O2 days, mean (SD)	35.6 (13.5)	49.4 (15.6)	0.04
LOS days, mean (SD)	72.62 (29.66)	75.97 (24.57)	0.85

P values by Student's *t* test, Chi-square test or Fisher's Exact Test when appropriate.

PNAC = parenteral nutrition associated cholestasis, BPD = bronchopulmonary dysplasia, NEC = necrotising enterocolitis, ROP = retinopathy of prematurity, IVH = intraventricular haemorrhage, N-CPAP = Nasal continuous positive airway pressure, LOS = length of hospital stay.

controlled studies about inflammatory cytokines and fish oil containing LE in premature infants are limited.

Compared with the Lipovenoes group, fish oils containing LE are rich sources of n-3 long-chain PUFA including DHA and EPA with associated anti-inflammatory properties [24,25]. The administration of n-3 long-chain PUFA, given parenterally, particularly in severely ill patients, is advantageous because the fatty acids are rapidly incorporated into cells and there are no losses from digestion and enteral absorption. In our study, we provided an early infusion of TPN with fish oil containing LE for infants with VLBW to prevent DHA and EPA deficiency. After a 7-day supply, we found IL-1 β and IL-6 levels in serum and BALF were significantly lower in the intervention group than the control group and consequently there was a lower incidence of BPD. Although the intervention group had a shorter duration of ventilation and O2 requirement, there was no shorter length of hospital stay. This may be due to our discharge criteria, which is mainly according to body weight reaching 2200 g, regardless of the O2 requirement. Manley et al. also revealed that DHA supplementation for preterm infants with a birth weight <1250 gm could significantly reduce the incidence of BPD [26]. Martin et al. demonstrated that a decreased DHA level was associated with an increased risk of BPD in infants born at <30 weeks of gestation (odds ratio: 2.5; 95% confidence interval: 1.3, 5.0). An increased linoleic acid/DHA ratio was associated with an increased risk of BPD (odds ratio: 8.6; 95% confidence interval: 1.4, 53.1) [27]. However, a recently published large RCT of DHA in preterm babies by Collins and colleagues contradicts these findings. They found that enteral DHA supplementation at a dose of 60 mg/kg/per day did not result in a lower risk of physiological bronchopulmonary dysplasia [28]. The different effects of enteral or parenteral supply of DHA in premature infants need further investigation.

We also found alanine aminotransferase was significantly lower in the intervention group than in the control group after a 7-day infusion. Although a trend toward less cholestasis was observed, it was not statistically significant. PNAC is one of the most challenging complications of prolonged parenteral nutrition in neonates. Recent studies suggested that the LE contain high concentrations of n-6 long-chain PUFA and phytosterols, which are associated with impaired biliary secretion and the authors hypothesised that this contributed to liver injury [29]. Downstream products of n-6 long-chain PUFA are the proinflammatory leukotrienes B4, C4 and E4 as well as prostaglandin E2, prostacyclin I2 and thromboxane A2. These eicosanoids are mediated by cyclooxygenase and 5-lipoxygenase

and thought to promote liver inflammation [30]. In contrast, fish oil containing LE includes n-3 long-chain PUFA and has shown lowered plasma concentrations of TNF- α and IL-6 and improved liver and pancreas function in adults [31]. Fish oil supplementation in TPN also showed a marked improvement in bile flow and cholestasis in new-born piglets [32] and rats [33]. Pscheidl et al. [34] reported on normalising splanchnic perfusion and enhance killing of translocated bacteria in a low-dose endotoxin rat model after administration of a parenteral diet supplemented with fish oil. All these results support our finding that the intervention group had less liver function impairment than the control group.

The limitations of our trial are the small population size and the low incidence of cholestasis in our study population, which limited the statistical significance of the differences between the subgroups. However, despite the small sample size, significant effects on BALF and serum cytokines levels and BPD incidence were observed. There is a paucity of head-to-head trials of different novel LEs evaluating short-term as well as long-term outcomes including ROP and neurodevelopment in premature infants. It is unclear whether there are any long-term benefits of introducing a costly novel lipid emulsion in infants with VLBW and other high-risk populations such as neonates with surgical conditions.

5. Conclusions

Early administration of fish oil containing LE decreased IL-1 β and IL-6 levels in serum and BALF which may be associated with less bronchopulmonary dysplasia in infants with VLBW. Favourable regulation of the immune response and improved balance of proinflammatory and anti-inflammatory mechanisms can prevent the development of clinical complications of prematurity, such as BPD and liver dysfunction. Despite the trend toward a less cholestasis in infants receiving fish oil containing LE, our data cannot adequately support a preventive effect of fish-oil containing LE for PNAC in preterm infants. Fish oil containing LE acts as a beneficial immunonutrient which maybe a new approach to BPD prevention in VLBW infants.

Ethics approval

The study was approved by the Institutional Review Board in Changhua Christian Hospital (CCH IRB No: 110909).

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Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

Contributors' statement page

Dr Hsiao conceptualised and designed the study, coordinated and supervised data collection, draughted the initial manuscript, and approved the final manuscript as submitted.

Drs Yang, Tsao, Lee, Chen and Lin carried out the initial analyses, reviewed and revised the manuscript and approved the final manuscript as submitted.

Dr Chang performed the statistical analysis.

Drs JY Chen and YG Tsai designed the data collection instruments, critically reviewed the manuscript, and approved the final manuscript as submitted.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.06.929>.

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