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**A DOUBLE-BLIND, RANDOMIZED CLINICAL TRIAL OF THE EFFECT
OF ω -3 FATTY ACIDS ON OXIDATIVE STRESS OF PRETERM NEONATES
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Running title: ω -3 effect on preterm neonates' oxidative stress

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ABSTRACT

Background/Objectives: The aim of this study was to research and draw conclusions about the effect of a parenteral nutrition (PN) fat emulsion, rich in ω -3 fatty acids, on the antioxidant markers of preterm infants, when compared with a standard fat emulsion. That was a double-blind, parallel-group study conducted in Athens, Greece, using an equal randomisation method.

Subjects/Methods: Thirty eight infants were selected using a double-blind method and one computer-generated randomization list. Both groups received PN based on the same protocols. Group A received SMOFlipid fat emulsion, while group B received the standard fat emulsion (Intralipid). Serum levels of vitamins A, E and total antioxidant potential (TAP) were measured on days 0, 7 and 14 of PN support. Clinical and biochemical data were collected on days 0, 14 and the day of discharge.

Results: Serum levels of vitamins E and A were significantly increased in group A, while only vitamin A was increased in group B, on the 14th day (Group A: vitamin E: p-value=0.002, vitamin A: p-value=0.000, Group B: vitamin E: p-value=0.065, vitamin A: p-value=0.000). TAP was increased only in the intervention group (Group A: p-value=0.000, Group B: p-value=0.287). Mild anaemia was developed in both groups, while no differences were detected in infection rate, days of hospitalization, days of ventilator support and days of phototherapy.

Conclusions: Oxidative stress was significantly reduced in those neonates fed with ω -3 fatty acids, while no effect was observed in the neonates fed with standard lipids. Intervention had no effect on infants' growth and clinical outcome.

Keywords: preterm neonates, oxidative stress, total antioxidant potential, parenteral nutrition, ω -3 fatty acids.

INTRODUCTION

Oxidative stress is caused due to the imbalance between the production of free radicals and the efficacy of body's antioxidant system (Maltepe & Saugstad, 2009). Free radicals are extremely unstable molecules, produced in the body during physiological metabolic processes. They tend to react with other molecules (polyunsaturated fatty acids of cell membranes, proteins, polysaccharides, and nucleic acids) causing structural alterations on molecular basis, functional abnormalities of the cell and tissue damage (Blackburn, 2005, Saugstad, 1996).

Overproduction of free radicals is possible to occur during perinatal period due to ischemia-reperfusion, arachidonic acid cascade, free iron, nitric oxide cascade, phagocyte activation, hypoxia, hyperoxia (Saugstad, 2001). Preterm neonates are more vulnerable to the effects of oxidative stress compared to term neonates. That is both due to their exposure to a highly hyperoxic environment and their immature antioxidant systems (Buonocore *et al.*, 2002, Thibeault, 2000). Oxidative stress in preterm neonates plays a significant role in a number of morbidity conditions including respiratory distress syndrome, chronic lung diseases, retinopathy, intracranial hemorrhage and necrotizing enterocolitis (Sharda, 2006).

Therefore, one of the basic goals in the treatment of preterm infants is the minimization of oxidative stress, either by reducing the production of free radicals or by augmenting antioxidant defense mechanisms (Perrone *et al.*, 2007). The use of certain ingredients in parenteral nutrition (PN) formulas has been suggested as a method of enhancing neonates' antioxidant defence mechanisms (Yeung, 2006, Perrone *et al.*, 2007). Although ω -3 polyunsaturated fatty acids have abilities of lowering the potential for in vivo generation of free radicals, their ability to modulate

oxidative stress in preterm infants through PN has not been assessed. (Perrone *et al.*, 2007). Their use in PN formulas for preterm infants has been regarded as safe and well tolerated (Calder *et al.*, 2009).

The aim of the present study was to assess the effect of an ω -3 enriched PN lipid emulsion, on the levels of antioxidant vitamins (A and E) and total antioxidant potential (TAP) in preterm infants. The study was conducted applying a randomized, double-blind method, according to which preterm infants treated with standard lipid emulsions were the control group (Gibson *et al.*, 2001, Uauy & Hoffman, 2000). Preterm infants treated with SMOFlipid emulsion were the intervention group, for which the hypothesis was that there would be a reduction in oxidative stress.

MATERIALS/ SUBJECTS AND METHODS

Study design

A double-blind, parallel-group study conducted in Athens, Greece, using an equal randomisation method.

Subjects and Settings

Infants born in “IASO” Maternity Hospital in Athens, and admitted in the neonates’ Intensive Care Unit (ICU) were candidates to be included in the study. Inclusion criteria were a) gestational age <32weeks, b) birth weight <1500g, c) admission in the ICU within 12 hrs after birth, d) >80% of total energy would be taken in through PN, during the first 8 days of life e) onset of fat administration through PN would be on the 2nd day of life the latest, with maximum lipid dose equal to 3g/kg/day, f) anticipated duration of PN would be >7days. Exclusion criteria included a) inherited metabolic diseases, b) major congenital malformations, c) severe malformations of visceral organs, kidneys, lungs or brain, d) participation in another drug research study, e) transfusion of packed red blood cells and/or fresh frozen plasma > 15ml/kg. All preterm neonates in IASO ICU were treated with ampicillin and gentamicin for the first 10 days and then with amikacin and cefotaxime for the prevention of severe infections.

The study protocol was approved by the Scientific and Ethical Committee of “IASO” Maternity Hospital and the parents of the infants signed a consent form before infants’ enrolment in the study.

Sample size

Sample size calculation showed that in order to have a 0.8 power to detect a significant difference ($P = 0.05$, two-sided) in the levels of antioxidant markers, 14 infants were required for each group.

Intervention

The preterm neonates were randomly divided into 2 groups, group A (intervention) and group B (control), and received the two different PN treatments starting from the day of birth (day 0).

Four protocols had been created according to gestational age, birth weight, body weight in the beginning of the parenteral support and clinical condition (Skouroliakou *et al.*, 2005). These were integrated into a software so that they could be used for automatic calculations, design and production of individualized PN regimens and were based on A.S.P.E.N guidelines (American Society of Parenteral and Enteral Nutrition) (ASPEN, 1993, National Advisory Group on Standards and Practice Guidelines for Parenteral Nutrition, 1997). For both subject groups, PN regimens were designed and automatically produced based on these protocols (figure 1). Lipid emulsion was added in the PN solution on the 1st or 2nd day of life, based on gestational age. Macronutrients were provided, using the same products for glucose and amino acid solutions in both groups. Amino acids derived from Vamin Infant (Fresenius Kabi HELLAS, Athens, Greece). The source of fat was different for each one of the two groups. Group A was administered SMOFlipid (Fresenius Kabi HELLAS, Athens, Greece), a formulation containing MCTs (30%), lipids from soya

bean oil (30%), olive oil (25%) and fish oil (15%), while group B was administered the standard lipid formulation with lipids from soya bean oil (Intralipid 20%, Fresenius Kabi HELLAS, Athens, Greece). Infants in both groups had been receiving the PN solutions for a trial period of at least 14 days, the end of which was when each infant started oral feedings.

Enteral feeding was initiated as soon as possible, since it is considered as extremely important for preterm neonates. However, the energy provided through oral feedings was <20% of total energy intake.

Data and sample collection

Eligibility, based on the inclusion and exclusion criteria, was assessed by the neonatologists of the ICU. A statistician used a computer-generated randomization list. That was given to the pharmacist, who was responsible for the placement of each infant in a group (intervention vs control) based on simple randomization procedures (the computer-generated randomization list) and the preparation of the different PN formulation in identical bags. The formulations were provided by the medical personnel. The statistician and the pharmacist did not participate in infants' care. All medical personnel and participants were blinded to treatment assignment during the whole course of study.

The primary aim, with respect to the effect of the different types of lipid emulsions, was the reduction of oxidative stress in the intervention group as measured from specific antioxidant markers (vitamins A, E, TAP). As second was the evaluation of

the effect of the different types of PN solutions on growth (body weight), blood count, clinical condition and length of hospitalization.

Collection and assessment of data was performed on day 0 (date of birth and initiation of PN), day 14 and at the time of discharge by the hospital. Collected data included anthropometry (weight, length and head circumference), biochemical tests, measurements of the levels of antioxidant vitamins and evaluation of TAP. TAP gives the total amount of radicals that can be cleared by an antioxidant or a biological sample, through the processes of radical scavenging, prevention of radical formation and activation of antioxidant enzymes (Griffiths *et al.*, 2002). Weight was measured using a digital baby scale (WM2300, Weigh South, Inc., Asheville, North Carolina, USA) with precision to 2g, crown-heel length was measured using a neonatometer (Harpenden neonatometer, Holtain Ltd, Crymych, UK), and head circumference was measured with a non-stretch tape. Blood tests were performed 6 hours after the cessation of enteral nutrition and included complete blood count [hematocrit levels (HCT), platelets, mean platelets volume (MPV), white blood cells (WBC)], c-reactive protein (CRP), bilirubin and creatinine levels. Vitamins E and A were measured in serum samples using HPLC techniques. An isocratic system was used, with HPLC pump, injector and UV-detector (AGILENT 1100, Agilent Technologies, Inc, Santa Clara, California, USA) using the appropriate reagent kits (Chromsystems Instruments & Chemicals GmbH, Germany) for each reaction. Flow rate for vitamins A and E was 1.5ml/min. TAP was estimated using Bioxytech AOP-490 kit (OxisResearch, OXIS Health Products, Inc., Portland, Oregon, USA). Vitamins E, A and TAP were assessed on day 0, 7 and 14 of PN.

Medical data concerning gestational age, diagnosis, clinical conditions and medications were collected from the medical records. Heart rate, blood pressure and

body temperature were daily monitored and recorded on days 0, 14 and at the time of discharge. Data concerning the clinical condition, infection rate, days of ventilation support and days of phototherapy were also collected. The number of days of PN support as well as the total number of the days of hospitalization were recorded (figure 1).

Statistical analysis

An independent t-test was used to compare the demographic characteristics of the two groups at baseline (group A vs group B). A 2x2 analysis of variance with repeated measures (RMANOVA) was used to assess the changes in the dependent variables between the two groups over time (day 0 to day 14 and day 0 to the day of discharge). The threshold for significance in all tests was set at $P=0.05$. Statistical analysis was performed with SPSS for Windows, version 16 (SPSS Inc, Chicago, Illinois, USA). All values are presented as mean \pm standard deviation.

RESULTS

Participants' distribution and grouping are presented in the flow diagram in figure 2. Recruitment was performed between November 2008 and April 2009. Neonates' characteristics at birth are presented in table 1. Gestational age was lower in neonates of group A compared to group B. Body weight was $<10^{\text{th}}$ centile for gestational age in 3 infants of group A (21%) and 4 infants of group B (22%). There were no differences between the two groups concerning birth weight, length and head circumference. All the other parameters were within normal ranges with the exception of blood pressure

that was low. Levels of vitamins A and E were below normal in both groups. Body temperature, platelet concentration and vitamin A levels were higher in group A compared to group B, while there were no differences between the two groups, concerning other parameters.

Based on neonates' gestational age, lipid emulsion was included in the PN plan on their 2nd day of life. There was no statistically significant difference between the two groups concerning the delivered constituents of PN (macro- and micronutrients) (table 2).

Three infants in group A and two in group B experienced an elevation of blood glucose up to a maximum of 200mg/dl. In such cases, the reduction of glucose delivery rate is recommended according to hospital's protocol, so that blood glucose levels switch to normal instantly. Therefore, glucose administration rate was decreased by 4 gr/kg/day for a maximum of 3 days, until blood glucose levels were normal and stable. Insulin was not administered in these cases of hyperglycemia.

The mean day of life for the onset of enteral nutrition (breast milk) was the same for both groups (Group A: 8.56±4.67 days, Group B: 9.46 ±5.12 days, p-value: 0.702).

The volume and the rate of administration was not different between the two groups (Group A: 31.78±17.19 ml, Group B: 21.03±12.15 ml, p-value: 0.208, Group A: 24.35±10.49 ml/kg, Group B: 16.76±9.52 ml/kg, p-value: 0.136). Also, enteral nutrition was <20% of total daily energy intake, for the first 14 days of feeding.

Levels of vitamins E and TAP were significantly increased in group A, while in group B only vitamin A was significantly increased (p-value<0.05, data not shown) on the 7th day of the intervention. Table 3 presents the results of the intervention on the 14th day. Body weight was not significantly altered in any group. Blood pressure and heart

rate were significantly increased in both groups and at the same rate. Hematocrit was decreased in both groups, again with no difference between them. Hematocrit values were below normal ranges. Platelet values increased in both groups (within normal ranges), while white blood cell count increased in group A, but still remained within normal ranges. Creatinine decreased significantly in group B (within normal ranges). Concerning vitamin levels, significant differences were observed between the two groups. Vitamin E increased only in group A, whereas vitamin A increased in both groups. However, there was no significant difference between the 2 groups concerning vitamin E values, at the end of the 14th day. On the other hand, vitamin A values were higher in group A compared to group B, at the end of the 14th day. This could not be completely attributed to the different interventions because there was a significant difference between vitamin A values in the beginning of PN. Also, the repeated measures analysis revealed no time*group effect for vitamins E and A. Interestingly, TAP was increased only in group A and the levels at the 14th day were significantly higher in group A compared to group B. Also, significant time*group effect was noticed regarding TAP levels (p-value=0.001).

Total days of PN (group A: 41.00±22.97, group B: 28.82±13.93, p-value=0.148) and total days of hospitalization (group A: 54.00±24.81, group B: 58.94±21.55, p-value=0.559) were the same for both groups. Table 4 presents information data for the neonates in the beginning of the PN and upon discharge. Body weight, length, head circumference, blood pressure and heart rate were significantly increased in both group, but with no statistical significance between them. Hematocrit and creatinine were decreased below normal ranges in both groups. There was no significant difference in the type, dose and duration of antibiotic therapy (Group A: 39.50±22.69 days, Group B: 45.94±19.17 days, p-value=0.398), the days of ventilation support

(Group A: 12.20±8.18 days, Group B: 9.11±6.19 days, p-value=0.371), maximum inspired oxygen fraction (Group A: 0.54±0.16%, Group B: 0.57±0.19% p-value=0.538) and the days of phototherapy between the two groups (Group A: 6.36±4.08 days, Group B: 6.36±3.10 days, p-value=1.000). None of the children, in any group, developed sepsis, and none manifested adverse effects attributed to the PN formulation.

DISCUSSION

The primary aim of this study was to find the effects of ω -3 polyunsaturated fatty acids on the antioxidant status of preterm infants, when those are administered through PN. Intervention enhanced the antioxidant defence mechanisms in the group that received the ω -3 lipid emulsion, compared to the group that received the standard lipid emulsion.

In our sample, serum levels of vitamins A and E were below normal ranges at birth. This is commonly observed in preterm neonates, probably due to the fact that their adipose tissue stores are limited, and so are (Gitto *et al.*, 2009, Debier, 2007, Orzalesi, 1987) alpha-tocopherol concentrations (alpha-tocopherol normally accumulates during the 3rd trimester of pregnancy) (Mactier & Weaver, 2005). Both vitamins are regarded as important biological antioxidants and their supplementation in preterm neonates was documented many years ago (Orzalesi, 1987). Vitamin E supplementation has been implicated in the prevention of bronchopulmonary dysplasia and elevations in hemoglobin levels in preterm neonates (Petrich *et al.*, 1976) while vitamin A has been shown to prevent chronic lung disease, bronchopulmonary dysplasia and sepsis (Atkinson, 2001, Tyson *et al.*, 1999, Darlow

& Graham, 2007). In the present study, both groups were supplemented with the same dose of fat soluble vitamins. However, only the group that received ω -3 fatty acids increased their vitamin E serum levels. ω -3 fatty acids may have enhanced the increase of vitamin E levels through the reduction of oxidative stress. Nevertheless, on the 14th day, levels of vitamin E were not different between the two groups, despite the fact that group A had lower vitamin E levels at baseline (not statistically significant). Therefore, it seems that the intervention had a possible modest effect on vitamin E, although the interpretation of the rise of vitamin E in group A is complex. On the contrary, vitamin A was increased in both groups, in the same pattern. Therefore, the intervention did not seem to have an effect on vitamin A status. Its increase could be attributed to the supplementation with fat soluble vitamins. The significant difference in vitamin A levels between the two groups, on the 14th day, probably lies on the fact that serum levels of vitamin A were higher in group A since birth.

The most interesting outcomes of the study were in reference with the changes in TAP levels. These were increased only in the group that received ω -3 fatty acids and at the 14th day they were significantly higher in this group compared to the group that received the standard lipid emulsion. The significant time*group effect on TAP levels confirms the antioxidant effect of ω -3 fatty acids. TAP is a strong indicator of body's antioxidant defense potential. When oxidative stress is reduced, the body does not consume its antioxidant molecules and therefore these are anticipated to accumulate in the blood. Thus, the increase in TAP levels verified the reduction of oxidative stress and since this was observed only in the intervention group, it could be assumed that ω -3 fatty acids were responsible for the reduction of oxidative stress.

We are not aware of any studies where ω -3 fatty acids have been used in lipid emulsions for PN, aiming at suppressing oxidative stress in preterm neonates. In accordance with our study, Antebi et al showed that the use of the ω -3 enriched fat emulsion SMOFlipid, in critically ill adult patients receiving PN, increased the levels of antioxidant vitamins (especially vitamin E) (Antebi *et al.*, 2004). Many randomized clinical trials have assessed the effect of ω -3 fatty acid supplementation in terms of visual and neural development in preterm infants and have shown that ω -3 have a positive effect on these parameters. However, their effect on oxidative stress had not been assessed (Gibsona *et al.*, 2001). These results may be generalized to other preterm infants population since the study sample included both sexes and a variety of clinical conditions. Also, the population derived from a large Maternity Hospital, where a significant proportion of preterm neonates from the whole country are born.

Growth was not affected by the intervention. Also, there was no significant difference between the two groups concerning clinical characteristics. Neonates in both groups developed mild anemia of prematurity, which is a common finding in preterm neonates (Salsbury, 2001). Finally, the intervention had no effect on their clinical outcome since there were no differences between the groups regarding infection rate, days of hospitalization, days of ventilation support and days of phototherapy.

A limitation of the study was the small sample studied. Due to the characteristics of the population and the severity of the exclusion criteria, the recruitment of a larger number of participants was extremely difficult. Another limitation was the assessment of a small number of biomarkers of oxidative stress, a fact that could limit results' interpretation. However, the amount of blood available for our research was limited due to the age of the sample studied.

In conclusion, administration of an ω -3 enriched PN fat emulsion resulted in significant increase in vitamin E and TAP levels in preterm neonates during a 14 day period of monitoring. Thus, the intervention resulted in decrease in the oxidative stress due to the antioxidant effect of ω -3 fatty acids. No effects of ω -3 fatty acids were observed concerning growth and clinical outcomes. These results have to be verified through other studies using a larger number of samples.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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TABLES

Table 1. Neonates' characteristics in the beginning of parenteral nutrition support (day 0).

Characteristics	Group A (n=14)	Group B (n=18)	p-value
	ω -3 emulsion mean \pm SD	Control emulsion mean \pm SD	
Gestational age (weeks)	28.21 \pm 2.89	30.39 \pm 1.58	0.011
Birth weight (kg)	1.14 \pm 0.26	1.21 \pm 0.17	0.378
Birth length (cm)	37.92 \pm 3.45	39.44 \pm 2.22	0.146
Head circumference (cm)	26.66 \pm 1.82	27.50 \pm 1.26	0.145
Diastolic blood pressure (mmHg)	63.46 \pm 11.95	61.56 \pm 10.16	0.636
Systolic blood pressure (mmHg)	40.38 \pm 9.45	41.39 \pm 9.75	0.776
Heart rate (beats/min)	132.07 \pm 14.87	136.33 \pm 13.27	0.409
Body temperature (°C)	36.60 \pm 0.09	36.40 \pm 0.21	0.004
Hematocrit (%)	50.53 \pm 6.70	48.70 \pm 6.63	0.456
Platelets (*10 ⁶ /l)	212.61 \pm 60.53	164.88 \pm 64.99	0.047

White blood cells (*10 ⁹ /L)	11.97±6.10	12.64±7.88	0.800
Bilirubin (mg/dl)	7.21±2.39	6.79±2.18	0.610
Creatinine (mg/dl)	0.76±0.26	0.95±0.31	0.083
C-reactive protein (mg/dl)	0.07±0.13	0.37±1.24	0.399
Vitamin E (mg/l)	2.04±0.63	2.47±0.78	0.106
Vitamin A (mg/l)	0.20±0.08	0.12±0.05	0.004
Total antioxidant potential (Mm uric acid)	0.71±0.18	0.73±0.24	0.799

Table 2. Constituents delivered through parenteral nutrition in the two groups.

Characteristics	Group A (n=14)	Group B (n=18)	p-value
	ω -3 emulsion mean \pm SD	Control emulsion mean \pm SD	
Energy (Kcal/day)	81.26 \pm 26.29	91.89 \pm 21.91	0.221
Energy (Kcal/kg/day)	72.21 \pm 8.95	72.93 \pm 9.66	0.832
Glucose (g/kg/day)	12.58 \pm 2.42	12.86 \pm 2.09	0.727
Amino acids (g/kg/day)	2.41 \pm 0.35	2.31 \pm 0.29	0.394
Fatty acids (g/kg/day)	2.31 \pm 0.59	2.29 \pm 0.41	0.894
Sodium (mEq/kg/day)	2.75 \pm 0.79	3.24 \pm 0.94	0.126
Potassium (mEq/kg/day)	1.76 \pm 0.18	1.80 \pm 0.25	0.681
Calcium (mEq/kg/day)	3.07 \pm 0.54	2.90 \pm 0.35	0.295
Magnesium (mEq/kg/day)	0.66 \pm 0.12	0.62 \pm 0.08	0.402
PO4 (mEq/kg/day)	1.38 \pm 0.22	1.34 \pm 0.15	0.566
Minerals (ml/kg)	0.58 \pm 0.14	0.62 \pm 0.23	0.571
Water soluble vitamins (ml/kg)	1.89 \pm 0.51	2.05 \pm 0.60	0.256

Lipid soluble vitamins (ml/kg)	4.74±1.14	5.20±1.23	0.448
Fluid volume (ml/kg/day)	142.48±20.29	139.10±18.34	0.625

Table 3. Neonates' characteristics in the beginning of parenteral nutrition support and at the 14th day of intervention.

Characteristics	Group A (n=14)		Group B (n=18)	
	ω-3 emulsion		Control emulsion	
	Day 0	Day 14	Day 0	Day 14
Body weight (kg)	1.14±0.26	1.13±0.29	1.21±0.17	1.22±0.19
Diastolic blood pressure (mmHg)	63.46±11.95	77.08±12.32*	61.56±10.16	79.56±5.09*
Systolic blood pressure (mmHg)	40.38±9.45	52.15±12.74*	41.39±9.75	52.00±8.26*
Heart rate (beats/min)	132.07±14.87	145.07±12.05*	136.33±13.27	148.38±10.36*
Body temperature (°C)	36.60±0.09	36.64±0.06	36.40±0.21	36.66±0.12*‡

Hematocrit (%)	50.53±6.70	38.03±4.73*	48.70±6.63	36.43±3.51*
Platelets (*10 ⁶ /l)	212.61±60.53	365.23±159.23*	164.88±64.99	316.27±89.75*
White blood cells (*10 ⁹ /l)	11.97±6.10	17.30±7.00*	12.64±7.88	14.95±5.21
Bilirubin (mg/dl)	7.21±2.39	5.85±2.61	6.79±2.18	6.13±2.72
Creatinine (mg/dl)	0.76±0.26	0.66±0.19	0.95±0.31	0.62±0.30*
C-reactive protein (mg/dl)	0.07±0.13	0.15±0.20	0.37±1.24	0.19±0.27
Vitamin E (mg/l)	2.04±0.63	2.55±0.71*	2.47±0.78	2.68±0.99
Vitamin A (mg/l)	0.20±0.08	0.26±0.06*	0.12±0.05	0.19±0.07*†
Total antioxidant potential (Mm uric acid)	0.71±0.18	1.086±0.29*	0.73±0.24	0.79±0.16†‡

* p-value<0.05 for significant changes from baseline within each group (day 0 vs day 14th between each group)

† p-value<0.05 for significant differences between values of the 2 groups at the end of the 14th day

‡ p-value<0.05 for time x group effect

Table 4. Neonates' characteristics in the beginning of parenteral nutrition support and at the discharge.

Characteristics	Group A (n=14)		Group B (n=18)	
	ω-3 emulsion		Control emulsion	
	Day 0	Discharge	Day 0	Discharge
Body weight (kg)	1.14±0.26	1.78±0.46*	1.21±0.17	2.01±0.36*
Body length (cm)	37.92±3.45	45.96±3.20*	39.44±2.22	46.80±3.05*
Head circumference (cm)	26.66±1.82	31.52±1.71*	27.50±1.26	31.85±1.67*
Diastolic blood pressure (mmHg)	63.46±11.95	78.69±8.05*	61.56±10.16	81.39±4.94*
Systolic blood pressure (mmHg)	40.38±9.45	53.62±7.50*	41.39±9.75	52.83±4.67*

Heart rate (beats/min)	132.07±14.87	143.38±6.13*	136.33±13.27	146.83±5.26*
Body temperature (°C)	36.60±0.09	36.60±0.07	36.40±0.21	36.62±0.06*‡
Hematocrit (%)	50.53±6.70	33.30±6.36*	48.70±6.63	30.92±4.96*
Platelets (*10 ⁶ /l)	212.61±60.53	386.30±143.11*	164.88±64.99	391.94±112.15*
White blood cells (*10 ⁹ /l)	11.97±6.10	13.24±3.16	12.64±7.88	11.90±3.26
Bilirubin (mg/dl)	7.21±2.39	4.40±3.00*	6.79±2.18	4.84±3.47
Creatinine (mg/dl)	0.76±0.26	0.48±0.35	0.95±0.31	0.34±0.11*†
C-reactive protein (mg/dl)	0.07±0.13	0.21±0.45	0.37±1.24	0.08±0.12

* p-value<0.05 for significant changes from baseline within each group (day 0 vs discharge day between each group)

† p-value<0.05 for the comparison within groups (discharge)

‡ p-value<0.05 for time x group effect

FIGURE LEGENDS

Figure 1. Study design

Figure 2. Participant flow diagram

