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ORIGINAL RESEARCH

Fatty acid profiles in blood and tissues of parenteral nutrition–fed neonatal piglets using a novel lipid emulsion containing choline

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Abstract

Background: For parenteral nutrition (PN)–dependent neonates, soybean oil intravenous lipid emulsions (SO-ILEs) and mixed emulsions (SO, medium-chain triglyceride [MCT], olive oil [OO], and fish oil [FO] ILEs) are likely not providing adequate amounts of key fatty acids (FAs) arachidonic acid (AA) and docosahexaenoic acid (DHA) and are devoid of choline. Current FO-containing ILEs provide excessive amounts of eicosapentaenoic acid (EPA). In neonatal piglets, we compared a novel lipid (NOV-C) with the addition of AA, DHA, and choline while sparing EPA with SO-ILE and SO,MCT,OO,FO-ILE.

Methods: We compared FA deposition in serum and tissues among groups of neonatal piglets fed exclusive PN based on SO-ILE ($n = 7$), SO,MCT,OO,FO-ILE ($n = 7$), or NOV-C ($n = 8$). On day 14, serum, liver, lung, brain, retina, and jejunum were collected and FAs in total phospholipid (PL) measured using gas liquid chromatography.

Results: In total PL, AA was higher for NOV-C compared with SO,MCT,OO,FO-ILE in serum ($P = 0.003$) and all tissues except brain ($P = 0.08$). DHA was higher for NOV-C and SO,MCT,OO,FO-ILE compared with SO-ILE in the liver ($P = 0.001$), jejunum ($P < 0.001$), and lung ($P < 0.001$). In the retina, DHA was higher for NOV-C compared with SO,MCT,OO,FO-ILE and SO-ILE ($P = 0.004$). EPA was higher for SO,MCT,OO,FO-ILE compared with SO-ILE and NOV-C in serum ($P = 0.002$) and all tissues except brain ($P = 0.48$).

[†]Abdelatif Elouahabi, who died during the peer-review process of this manuscript, made significant contributions to the study.

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Conclusion: Compared with SO-ILE and SO,MCT,OO,FO-ILE, a novel lipid designed to deliver optimal AA, DHA, EPA, and choline for neonates resulted in higher AA and DHA in blood and tissues. The impact on neonatal immune development and key organ functions needs further exploration.

KEYWORDS
life cycle, lipids, parenteral nutrition, pediatrics nutrition

INTRODUCTION

There have been many recent advances in parenteral intravenous lipid emulsion (ILE) composition. The first-generation ILEs, based on soybean oil (SO), were high in linoleic acid (LA) and ω-6 polyunsaturated fatty acids (PUFAs).[1] The most recent advance was the development of third-generation ILEs that were supplemented with fish oil (FO) providing higher ω-3 PUFAs, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). However, they were designed for critically ill adults and not for infants, a significant clinical population requiring parenteral nutrition (PN) because of prematurity and/or acute or chronic intestinal failure.[2] Growing infants, particularly preterm infants who do not receive the usual placental transfer of long-chain PUFAs in the third trimester of pregnancy, have unique lipid requirements.[2] Two key issues for consideration are the optimal PUFA content (both ω-3 and ω-6) and the provision of choline, currently devoid in all ILEs used for infants.

Postnatally, preterm infants rapidly develop a deficit of DHA and arachidonic acid (AA), a key ω-6 PUFA in the circulating blood.[3] Decreased DHA has been associated with an increased risk of chronic lung disease, and decreased AA has been associated with a risk of late-onset sepsis, and both are critically important for neurodevelopment.[3–6] Preterm infants' requirement for preformed AA and DHA is multifactorial, related to inadequate stores, increased demand, and decreased ability to synthesize from dietary fatty acid precursors.[2,7,8] The adequacy of conversion of LA and α-linolenic acid (ALA) into AA and DHA to meet preterm infant requirements is in question.[2,9] Hence, the optimal fatty acid profile for infants receiving PN remains unknown.[10,11] ILEs containing FO have addressed this concern by adding preformed EPA and DHA with some AA; however, concerning data are emerging showing that the concentration of AA declines in the blood of babies treated with these ILEs.[12–15] The very high EPA content in these FO-ILEs is likely to have a harmful role by inhibiting the synthesis of AA and its deposition in tissues.[2,16,17]

Choline is a dietary precursor for phosphatidylcholine (PC), a major component of all cell membranes, along with sphingomyelin predominant in nerve cells and lysophosphatidylchole important in cell signalling.[18–20] Choline is involved in lipid transport, lipid metabolism, cell membrane structure, and neurotransmission.[18] PC represents up to 40%–50% of all cellular membranes and 70%–95% of the phospholipids (PLs) in surfactant, bile, and lipoproteins.[21] Of note, PC is the major carrier of AA and DHA in plasma.[22] Choline, in the form of phosphocholine, binds to C-reactive protein to activate the classical complement pathway of host defence.[22] Although the role of choline in perinatal brain development has been well established, to date, ILEs used in infants have been devoid of choline.[23,24]

A novel ILE was developed to meet the needs of preterm and young infants by optimizing doses of DHA, AA, and choline without the excessive EPA seen in current FO-containing ILEs (Table S1). This study aimed to compare the fatty acid composition of serum and tissues in total PL and lipid subclasses (specifically PC) in neonatal piglets given exclusive PN (EPN) with this ILE compared with a traditional first-generation ILE and a third-generation ILE. In addition, given the importance of both fatty acids and choline for immune pathways, we measured tissue cytokines in the liver as a secondary outcome.

METHODS

All experiments were conducted in accordance with the guidelines of the Canadian Council of Animal Care (AUP00003707). Newborn male Duroc cross piglets were randomized (blocked by litter) to soy lipid (SO-ILE $n = 7$), mixed lipid emulsion (SO, medium-chain triglyceride, olive oil, and FO ILE [SO,MCT,OO,FO-ILE] $n = 7$) and novel ILE with choline (NOV-C $n = 8$) (for lipid compositions see Table S1). These piglets were compared with a reference range derived from litter-matched controls (CON; $n = 8$). In total, 30 of 37 piglets completed the study, two pigs were excluded because of sepsis (NOV-C and SO,MCT,OO,FO-ILE), four because of gastrointestinal infection (two SO,MCT,OO,FO-ILE, one NOV-C, and one SO-ILE), and one because of a protocol error (NOV-C).

Animal care

On day 0, under general anesthesia, 2- to 5-day-old piglets underwent placement of a central venous catheter for EPN delivery.[10] As previously described, prophylactic antibiotics were given preoperatively. Piglets were housed for 14 days in metabolic cages with a 12-h light cycle.[25] Piglets grow at approximately five times the rate of a human infant, leading to our dosing for ILEs at 10 g/kg/day that can translate to 2 g/kg/day for infants.[26] Nutrition commenced immediately postoperatively at the rates previously described.[27] Daily, the piglets' central lines were locked with a 4% tetrasodium ethylenediaminetetraacetic acid solution (KiteLock 4%; SterileCare)

with a dwell time of 2 h.[28] If piglets had suspected sepsis, aerobic and anaerobic blood cultures were taken and empiric antibiotics administered. On day 14, a laparotomy was performed and the entire small intestine from the ligament of Treitz to the ileocecal valve was measured. Following humane euthanasia, the intestine, liver, brain, lung, and retina were removed, weighed, and samples flash frozen in liquid nitrogen and stored at −80°C before further analysis.

Fatty acid analysis

As previously described,[10,27,29] using a modified Folch extraction PLs were separated on silica gel according to polarity.[30] Using automated gas liquid chromatography (Agilent GC model 7890a; Agilent Technologies), FAs within total PL and for each identified fraction (eg, PC, phosphatidylinositol [PI], and phosphatidylethanolamine) were measured.[30] At each tissue site, results are provided for the total PL (serum, liver, brain, lung, retina, and jejunum) and for the major fractions recovered (all tissues except retina and jejunum as fractionization was not achieved). The fatty acid profiles for other fractions recovered in smaller amounts in the tissues are available in Tables S2 and S3.

Cytokines

As an exploratory aim, we measured the protein expression of six key cytokines in liver tissue for any potential differences in inflammation between treatment groups and as compared with CON: interleukin 1-α (IL-1α), IL-1 receptor antagonist (IL-1ra), IL-6, IL-10, and tumor necrosis factor alpha. The protein concentration of each sample was quantified in duplicates by a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Analysis on normalized protein was performed using the Luminex 200 system (Luminex) by Eve Technologies Corp.

Statistical analysis

Piglet demographics and anthropometrics were normally distributed and analyzed by one-way analysis of variance (presented as mean and SD). Fatty acids and cytokines were analyzed via Kruskal-Wallis test (presented as median and IQR). Comparisons of fatty acid distribution in blood and tissues are made between lipid groups and CON data provided as a reference range. Samples were excluded from fatty acid analysis based on a predefined outlier definition of missing peaks or potential contamination via unusual peaks. All statistical analyses were performed in SPSS (version 29; SPSS Inc).

RESULTS

Piglet demographics and anthropometrics (Table 1)

There were no differences in baseline piglet age ($P = 0.82$) or weight ($P = 0.24$). There were no differences in weight gain ($P = 0.16$), termination weight ($P = 0.18$), whole brain weight ($P = 0.45$), whole brain weight adjusted for body weight ($P = 0.10$), or small bowel length ($P = 0.91$). Small bowel weight was lower for SO-ILE ($P = 0.007$), although it was not significant when adjusted for body weight ($P = 0.089$).

Fatty acids in serum

In total PL (Table 2), LA was lower for NOV-C than for SO-ILE and SO,MCT,OO,FO-ILE ($P < 0.001$). No significant differences between lipid groups were found for ALA ($P = 0.19$) or DHA ($P = 0.08$). AA was higher for NOV-C than for SO,MCT,OO,FO-ILE ($P = 0.003$). EPA was higher for SO,MCT,OO,FO-ILE than for SO-ILE and NOV-C, with SO-ILE and NOV-C not significantly different between each other ($P = 0.002$).

TABLE 1 Piglet demographics and anthropometrics.

	SO-ILE ($n = 7$), mean (SD)	SO,MCT,OO,FO-ILE ($n = 7$), mean (SD)	NOV-C lipid ($n = 8$), mean (SD)	CON ($n = 8$), range	*P* value
Age at day 0, days	3.6 (0.79)	3.6 (0.98)	3.9 (0.84)		0.82
Weight at day 0, kg	2.3 (0.17)	2.4 (0.35)	2.3 (0.24)		0.24
Weight gain, kg	2.6 (0.52)	3.1 (0.28)	3.0 (0.22)		0.16
Weight at day 14, kg	4.9 (0.61)	5.5 (0.58)	5.2 (0.20)	6.5–8.1	0.18
Small bowel weight, g	81 (13)[a]	108 (19)[b]	104 (8.6)[b]	199–245	0.007
Small bowel weight per body weight, g/kg	16 (2.8)	20 (2.9)	20 (1.4)	27–36	0.089
Whole brain weight,* g	41 (1.7)	38 (3.1)	39 (2.1)	42–43	0.45
Whole brain weight per body weight,* g/kg	7.9 (0.55)	7.1 (0.31)	7.6 (0.62)	5.8–6.7	0.10
Small bowel length, cm	645 (65)	637 (59)	630 (55)	939–1043	0.91

Note: Data are represented as mean (SD) for treatment groups and range for controls. Treatment groups that do not share a common superscript letter are significantly different as identified by a one-way analysis of variance followed by post hoc Tukey test, considered significant at $P < 0.05$.

Abbreviations: CON, control; SO-ILE, soybean oil intravenous lipid emulsion; SO,MCT,OO,FO-ILE, SO, medium-chain triglyceride, olive oil, and fish oil ILE.

*SO-ILE $n = 4$; SO,MCT,OO,FO-ILE $n = 5$; and CON $n = 5$.

TABLE 2 Total phospholipid fatty acid composition of serum, liver, brain, lung, retina, and jejunum.

	SO-ILE (n = 7), median (IQR)	SO,MCT,OO,FO-ILE (n = 7), median (IQR)	NOV-C (n = 8), median (IQR)	CON (n = 8), range	P value
Serum % wt/wt of total fatty acids					
Linoleic acid	12 (12)[a]	10 (3.0)[a]	6.7 (2.9)[b]	18–22	<0.001
α-Linolenic acid	0.35 (0.06)	0.30 (0.06)	0.35 (0.13)	0.72–1.2	0.19
Arachidonic acid	7.5 (4.9)[a,b]	5.4 (1.8)[a]	9.1 (2.9)[b]	6.7–9.4	0.003
Eicosapentaenoic acid	0.09 (0.02)[a]	0.77 (0.59)[b]	0.13 (0.14)[a]	0.25–0.45	0.002
Docosahexaenoic acid	1.8 (1.8)	2.9 (1.4)	2.3 (0.49)	1.5–2.8	0.08
Liver % wt/wt of total fatty acids					
Linoleic acid	18 (6.5)[a]	13 (1.0)[b]	6.2 (1.5)[c]	12–14	<0.001
α-Linolenic acid	0.35 (0.20)[a]	0.27 (0.08)[b]	0.23 (0.04)[b]	0.29–0.46	0.001
Arachidonic acid	14 (3.7)[a]	10 (0.89)[b]	23 (1.3)[c]	13–17	<0.001
Eicosapentaenoic acid	0.10 (0.07)[a]	1.6 (0.74)[b]	0.16 (0.17)[a]	0.23–0.52	<0.001
Docosahexaenoic acid	4.0 (0.37)[a]	7.5 (0.45)[b]	7.9 (2.1)[b]	4.3–6.6	0.001
Brain % wt/wt of total fatty acids					
Linoleic acid	1.5 (0.53)[a]	1.1 (0.28)[a,b]	0.81 (0.54)[b]	0.80–1.5	0.03
α-Linolenic acid	0.35 (0.07)	0.25 (0.26)	0.31 (0.43)	0.21–1.5	0.68
Arachidonic acid	10 (1.8)	10 (0.55)	9.8 (0.57)	8.8–13	0.08
Eicosapentaenoic acid	0.18 (0.12)	0.30 (0.38)	0.16 (0.18)	0.15–0.50	0.48
Docosahexaenoic acid	9.6 (3.1)	11 (1.9)	11 (3.7)	6.3–11	0.51
Lung % wt/wt of total fatty acids					
Linoleic acid*	19 (2.9)[a]	10 (0.98)[b]	5.8 (0.76)[c]	6.7–7.9	<0.001
α-Linolenic acid*	0.85 (0.16)[a]	0.57 (0.10)[b]	0.51 (0.05)[b]	0.19–0.69	<0.001
Arachidonic acid*	6.9 (1.1)[a]	5.5 (0.63)[b]	12 (0.48)[c]	8.0–10	<0.001
Eicosapentaenoic acid*	0.14 (0.08)[a]	2.5 (0.46)[b]	0.27 (0.05)[a]	0.29–0.53	<0.001
Docosahexaenoic acid*	0.62 (0.20)[a]	2.0 (0.42)[b]	3.3 (0.29)[c]	0.47–0.72	<0.001
Retina % wt/wt of total fatty acids					
Linoleic acid#	3.4 (0.80)[a]	1.9 (0.20)[a]	1.4 (0.12)[b]	2.2–3.6	<0.001
α-Linolenic acid#	0.30 (0.26)	0.20 (0.01)	0.21 (0.04)	0.19–0.38	0.10
Arachidonic acid#	8.7 (0.98)[a]	8.6 (0.39)[a]	9.4 (0.58)[b]	8.9–9.6	0.008
Eicosapentaenoic acid#	0.05 (0.01)[a]	0.25 (0.09)[b]	0.11 (0.03)[c]	0.05–0.09	<0.001
Docosahexaenoic acid#	13 (3.0)[a]	15 (2.0)[a]	17 (1.7)[b]	7.7–15	0.004
Jejunum % wt/wt of total fatty acids					
Linoleic acid	18 (3.8)[a]	13 (2.6)[b]	7.5 (1.7)[c]	13–24	<0.001
α-Linolenic acid	0.63 (0.32)[a]	0.43 (0.15)[b]	0.42 (0.25)[b]	0.96–1.4	0.011
Arachidonic acid	9.6 (2.4)[a]	9.2 (0.89)[a]	17 (1.4)[b]	6.9–8.8	<0.001
Eicosapentaenoic acid	0.18 (0.02)[a]	2.7 (0.42)[b]	0.63 (0.45)[c]	0.23–0.54	<0.001
Docosahexaenoic acid	2.0 (0.22)[a]	3.9 (1.1)[b]	5.0 (0.57)[b]	0.90–1.5	<0.001

Note: Data are represented as median (IQR) for treatment groups and range for controls. Treatment groups that do not share a common superscript letter are significantly different as identified by Kruskal-Wallis test, considered significant at $P < 0.05$.

Abbreviations: CON, control; SO-ILE, soybean oil intravenous lipid emulsion; SO,MCT,OO,FO-ILE, SO, medium-chain triglyceride, olive oil, and fish oil ILE; wt/wt, weight/weight.

*For analytical reasons, one sample was excluded from all analysis for NOV-C (n = 7).

#For analytical reasons, one sample was excluded from all analysis for SO-ILE (n = 6).

PC was the dominant fraction in serum for all groups (Table 3). In PC, LA was lower for NOV-C compared with SO-ILE and SO,MCT,OO,FO-ILE ($P < 0.001$). No significant differences between ILE groups were found for ALA ($P = 0.24$) or DHA ($P = 0.15$). AA was higher for SO-ILE and NOV-C than for SO,MCT,OO,FO-ILE ($P < 0.001$). EPA was higher for SO,MCT,OO,FO-ILE than for SO-ILE and NOV-C ($P = 0.001$).

Fatty acids in the liver

In total PL, LA was significantly lower for NOV-C and SO,MCT,OO,FO-ILE than for SO-ILE ($P < 0.001$). ALA was higher for SO-ILE compared with NOV-C and SO,MCT,OO,FO-ILE ($P = 0.001$). AA was higher for NOV-C than for SO-ILE and SO,MCT,OO,FO-ILE ($P < 0.001$), with SO-ILE greater than SO,MCT,OO,FO-ILE. EPA was higher for SO,MCT,OO,FO-ILE than NOV-C and SO-ILE ($P < 0.001$). DHA was higher for NOV-C and SO,MCT,OO,FO-ILE than SO-ILE ($P = 0.001$).

PC was the dominant fraction in liver tissue for all groups. In PC, LA was significantly lower for NOV-C compared with SO-ILE and SO,MCT,OO,FO-ILE ($P < 0.001$). ALA was higher for SO-ILE compared with SO,MCT,OO,FO-ILE and NOV-C ($P = 0.003$). AA was higher for NOV-C than SO-ILE and SO,MCT,OO,FO-ILE ($P < 0.001$). EPA was higher for SO,MCT,OO,FO-ILE than SO-ILE and NOV-C ($P < 0.001$). DHA was higher for NOV-C than SO-ILE and SO,MCT,OO,FO-ILE, with SO,MCT,OO,FO-ILE greater than SO-ILE ($P < 0.001$).

TABLE 3 Phosphatidylcholine fatty acid composition of serum, liver, brain, and lung.

	SO-ILE ($n = 7$)	SO,MCT,OO,FO-ILE ($n = 7$)	NOV-C ($n = 8$)	CON ($n = 8$)	*P* value
Serum % wt/wt of total fatty acids					
Linoleic acid*	13 (13)[a]	11 (1.6)[a]	7.1 (2.0)[b]	22–28	<0.001
α-Linolenic acid*	0.19 (0.05)	0.15 (0.04)	0.14 (0.07)	0.47–0.99	0.24
Arachidonic acid*	6.2 (3.0)[a]	4.7 (0.55)[b]	12 (5.5)[a]	5.6–9.2	<0.001
Eicosapentaenoic acid*	0.12 (0.06)[a]	0.57 (0.30)[b]	0.08 (0.03)[a]	0.15–0.31	0.001
Docosahexaenoic acid*	1.3 (0.85)	2.1 (0.73)	2.2 (2.4)	1.0–2.4	0.15
Liver % wt/wt of total fatty acids					
Linoleic acid	20 (5.8)[a]	16 (1.1)[a]	8.9 (1.5)[b]	16–17	<0.001
α-Linolenic acid	0.44 (0.12)[a]	0.29 (0.12)[b]	0.23 (0.11)[b]	0.32–0.55	0.003
Arachidonic acid	11 (1.9)[a]	7.1 (0.95)[b]	17 (1.1)[c]	9.3–12	<0.001
Eicosapentaenoic acid	0.10 (0.03)[a]	1.7 (0.33)[b]	0.20 (0.24)[a]	0.17–0.62	<0.001
Docosahexaenoic acid	4.0 (0.85)[a]	6.4 (0.61)[b]	8.5 (2.1)[c]	4.8–8.3	<0.001
Brain % wt/wt of total fatty acids					
Linoleic acid#	3.3 (1.5)[a]	1.6 (0.20)[a,b]	1.4 (0.36)[b]	1.9–2.7	<0.001
α-Linolenic acid#	1.1 (0.14)	1.0 (0.59)	0.99 (0.27)	0.92–2.1	0.73
Arachidonic acid#	5.5 (1.2)[a,b]	5.2 (1.2)[a]	6.5 (1.3)[b]	3.9–6.4	0.024
Eicosapentaenoic acid#	0.01 (0.01)[a]	0.05 (0.03)[b]	0.02 (0.007)[a]	0.01–0.04	0.001
Docosahexaenoic acid#	2.8 (0.61)[a]	3.1 (1.1)[a,b]	4.4 (1.9)[b]	1.7–7.0	0.035
Lung % wt/wt of total fatty acids					
Linoleic acid	22 (2.3)[a]	14 (0.92)[b]	9.3 (1.3)[c]	9.4–12	<0.001
α-Linolenic acid	1.2 (0.21)[a]	0.87 (0.08)[b]	0.68 (0.15)[c]	0.56–0.94	<0.001
Arachidonic acid*	3.1 (1.2)[a]	2.8 (1.0)[a]	7.3 (0.31)[b]	2.9–4.2	<0.001
Eicosapentaenoic acid*	0.08 (0.04)[a]	1.7 (1.1)[b]	0.23 (0.11)[c]	0.13–0.32	<0.001
Docosahexaenoic acid*	0.13 (0.09)[a]	0.61 (0.24)[b]	1.3 (0.63)[c]	0.11–0.26	<0.001

Note: Data are represented as median (IQR) for treatment groups and range for controls; Treatment groups that do not share a common superscript letter are significantly different as identified by Kruskal-Wallis test, considered significant at $P < 0.05$.

Abbreviations: CON, control; SO-ILE, soybean oil intravenous lipid emulsion; SO,MCT,OO,FO-ILE, SO, medium-chain triglyceride, olive oil, and fish oil ILE; wt/wt, weight/weight.

*For analytical reasons, one sample was excluded for SO,MCT,OO,FO-ILE ($n = 6$) and CON ($n = 7$) from all analysis.

#For analytical reasons, one sample was excluded for SO-ILE ($n = 6$) and SO,MCT,OO,FO-ILE ($n = 6$) from all analysis.

Fatty acids in brain

In total PL, LA was lower for NOV-C than for SO-ILE, whereas no significant difference was observed for SO,MCT,OO,FO-ILE ($P = 0.03$). There was no difference in ALA ($P = 0.68$), AA ($P = 0.08$), EPA ($P = 0.48$), or DHA ($P = 0.51$) between any of the lipid groups.

In the brain, PC and PI were the significantly recovered fractions. In the PC fraction, LA was significantly lower for NOV-C compared with SO-ILE, and SO,MCT,OO,FO-ILE not different to either ($P < 0.001$). There was no difference in ALA between lipid groups ($P = 0.73$). AA was higher for NOV-C compared with SO-ILE, and SO,MCT,OO,FO-ILE not different to either ($P = 0.024$). EPA was higher for SO,MCT,OO,FO-ILE than for the other groups ($P = 0.001$). DHA was higher for NOV-C than SO-ILE, whereas SO,MCT,OO,FO-ILE was not different ($P = 0.035$). In the PI fraction, LA was significantly lower for NOV-C compared with SO-ILE and SO,MCT,OO,FO-ILE ($P < 0.001$). ALA ($P = 0.54$), AA ($P = 0.39$), and DHA ($P = 0.18$) were not significantly different between any group. EPA was significantly higher for SO,MCT,OO,FO-ILE than NOV-C ($P < 0.001$).

Fatty acids in the lung

In the total PL, LA was significantly higher for SO-ILE than SO,MCT,OO,FO-ILE and NOV-C, with SO,MCT,OO,FO-ILE higher than NOV-C ($P < 0.001$). ALA was significantly higher for SO-ILE than for the other groups ($P < 0.001$). AA was significantly higher for NOV-C than for both other groups, with SO-ILE higher than SO,MCT,OO,FO-ILE ($P < 0.001$). EPA was significantly higher for SO,MCT,OO,FO-ILE than for both other groups ($P < 0.001$). DHA was higher for NOV-C than for both other groups, with SO,MCT,OO,FO-ILE higher than SO-ILE ($P < 0.001$).

PC was the dominant fraction in the lung. In the PC fraction, LA was significantly higher for SO-ILE than SO,MCT,OO,FO-ILE and NOV-C, with SO,MCT,OO,FO-ILE higher than NOV-C ($P < 0.001$). ALA was significantly higher for SO-ILE than SO,MCT,OO,FO-ILE and NOV-C, with SO,MCT,OO,FO-ILE higher than NOV-C ($P < 0.001$). AA was significantly higher for NOV-C than SO-ILE and SO,MCT,OO,FO-ILE ($P < 0.001$). EPA was significantly higher for SO,MCT,OO,FO-ILE than SO-ILE and NOV-C, with NOV-C higher than SO-ILE ($P < 0.001$). DHA was higher for NOV-C than SO-ILE and SO,MCT,OO,FO-ILE, with SO,MCT,OO,FO-ILE higher than SO-ILE ($P < 0.001$).

Fatty acids in retina

In the total PL, LA was significantly higher for SO-ILE and SO,MCT,OO,FO-ILE than for NOV-C ($P < 0.001$). AA was significantly higher for NOV-C than for both SO-ILE and SO,MCT,OO,FO-ILE ($P = 0.008$). EPA was higher for SO,MCT,OO,FO-ILE than for both other groups ($P < 0.001$). DHA was higher for NOV-C than for both other groups ($P = 0.004$).

Fatty acids in jejunum

In the total PL, LA was again lowest for NOV-C and highest for SO-ILE ($P < 0.001$). ALA was higher for SO-ILE than the other groups ($P = 0.011$). AA was significantly higher for NOV-C than for both other groups ($P < 0.001$). EPA was significantly higher for SO,MCT,OO,FO-ILE than for both other groups ($P < 0.001$). DHA was higher for NOV-C and SO,MCT,OO,FO-ILE than for SO-ILE ($P < 0.001$).

Cytokines in liver

As shown in Figure 1, in liver tissue IL-1ra was higher for NOV-C as compared with SO,MCT,OO,FO-ILE, whereas IL-10 was higher for NOV-C compared with SO-ILE. There were no other significant differences for the remaining cytokines (data shown in Figure 1).

DISCUSSION

As we have previously shown, fatty acid profiles in total PL differ in EPN piglets based on the ILE used and tissue site sampled. This study confirms those findings and adds new information on individual PL classes, including when using a novel ILE designed specifically for neonates. Across most tissues, the use of NOV-C was associated with lower levels of LA and ALA and higher levels of AA and DHA without excessive EPA in total PL and in PC of serum, liver, brain, lung, retina, and jejunum (see Tables 2 and 3).

As seen in the literature,[10,14,31] in total PL, the AA levels in serum and most tissues were lower for SO,MCT,OO,FO-ILE compared with SO-ILE, even though SO-ILE does not have added AA. This confirmed our previous findings that term EPN piglets receiving SO-ILE may convert adequate LA to AA to maintain tissue fatty acid status and that SO,MCT,OO,FO-ILE may have inadequate AA and a composition unfavorable to the adequate tissue deposition of AA. In total PL, levels for AA were lower for SO,MCT,OO,FO-ILE compared with NOV-C in all tissues except the brain. The 14-day duration is relatively short. It is possible that further increases in key fatty acids, like AA and DHA, could occur over a longer period of time, especially in the brain. The observed fatty acid tissue deposition likely reflects both the ILE fatty acid content as well as potential for enhanced AA and DHA tissue uptake with added choline. PC is the major transporter of AA and DHA in preterm infant plasma; therefore, a deficiency in choline could impact end-organ delivery of both in preterm infants.[32] In that regard, it is notable in the brain PC, AA was significantly lower for SO,MCT,OO,FO-ILE compared with NOV-C. In lung and liver PC, DHA was higher for NOV-C compared with both SO-ILE and SO,MCT,OO,FO-ILE. However, these differences can simply reflect better content of AA and DHA in NOV-C compared with the other ILEs. The specific role of the added choline in further improving tissue uptake could be explored in future experiments.

Consistently across most sites sampled, SO,MCT,OO,FO-ILE resulted in the highest amounts of EPA, with levels elevated well

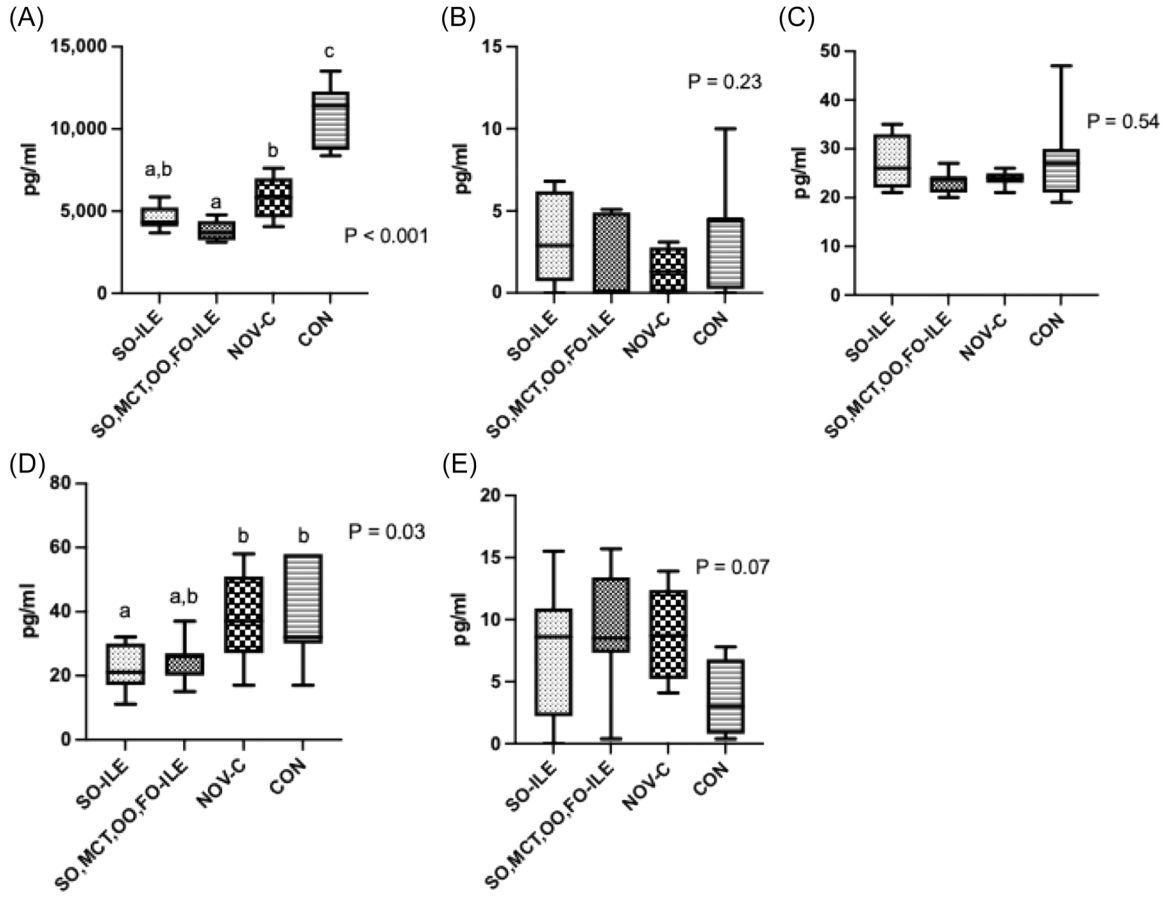

FIGURE 1 Liver tissue cytokines. (A) IL-1 receptor antagonist and SO-ILE ($n = 6$); SO,MCT,OO,FO-ILE ($n = 6$); NOV-C ($n = 8$); and CON ($n = 8$). (B) IL-1α and SO-ILE ($n = 7$); SO,MCT,OO,FO-ILE ($n = 7$); NOV-C ($n = 8$); and CON ($n = 8$). (C) IL-6 and SO-ILE ($n = 7$); SO,MCT,OO,FO-ILE ($n = 7$); NOV-C ($n = 8$); and CON ($n = 8$). (D) IL-10 and SO-ILE ($n = 6$); SO,MCT,OO,FO-ILE ($n = 7$); NOV-C ($n = 8$); and CON ($n = 8$). (E) Tumor necrosis factor alpha and SO-ILE ($n = 7$); SO,MCT,OO,FO-ILE ($n = 7$); NOV-C ($n = 8$); and CON ($n = 8$). Boxplot graphs are shown. The Kruskal-Wallis test was used. The lowercase "a," "b," and "c" represent post hoc differences. CON, control; IL, interleukin; SO-ILE, soybean oil intravenous lipid emulsion; SO,MCT,OO,FO-ILE, SO, medium-chain triglyceride, olive oil, and fish oil ILE.

outside the range of control in serum and all tissues except the brain. As stated previously, the amounts of EPA in FO-containing ILEs, like SO,MCT,OO,FO-ILE, are much higher than what would be present in human breastmilk, and elevated EPA is observed frequently when ILEs containing FO are used.[16] The estimated EPA delivered with FO-ILEs is approximately 10 times greater than that of a preterm infant receiving breastmilk and more than double what expert guidelines recommend for enteral nutrition.[16,33] An increase in the ratio of EPA/AA may be detrimental to both endogenous synthesis and tissue deposition of AA.[34] Preterm infants receiving FO showed lower AA in plasma and red blood cell membranes compared with traditional ILEs.[16,34]

The importance of AA in infant development cannot be understated,[35] with AA being the dominant fatty acid in vascular, immune, placental, and neural cells.[5,36] In human breastmilk, the concentration of DHA is lower and more variable than AA.[37] It has been suggested that AA in infant formula should be at least equal to that of DHA, recommended at approximately 0.5% of fatty acids.[38] In preterm infants, supplementation with AA and DHA has been shown to improve white matter maturation, reduce the number of days of respiratory support, and reduce the risk for retinopathy of prematurity.[39–41]

Although choline has importance for the tissue deposition of fatty acids like AA and DHA, it is also an important nutrient for neonates for immune health and brain and lung development. Serum free and PL bound choline concentrations increase throughout pregnancy, with newborns having higher plasma free choline and lower plasma PL bound choline than their mothers.[19,42] Infants receiving EPN have been shown to have lower free choline concentrations in plasma than infants receiving human milk or formula supplemented with choline.[43] Circulating free choline in extremely preterm infants receiving PN has been found to decline with increased PN volume.[44]

NOV-C in some piglets led to higher AA and DHA than the CON, specifically in liver and lung tissues. We recognize that this could be because of limitations in the diet fed to the sows that is predominantly soy based and without added long-chain PUFAs. However, we also recognize it will be important to determine if higher levels of AA and DHA in such key tissues are beneficial or could have adverse impact. Hence, in this study, as a preliminary step, we analyzed cytokine profiles in the liver to screen for any potential signal for pro-inflammatory or anti-inflammatory impact given the differences in

tissue fatty acid composition. In general, we did not see a cytokine profile suggestive of activation of inflammation. IL-10, considered a regular cytokine that decreases pro-inflammatory responses,[45] and IL-1ra, considered an anti-inflammatory cytokine, were both higher for NOV-C compared with the other ILEs.[46] We acknowledge this to be a limited assessment in one tissue, at one point in time, using indirect markers of active inflammation and in the absence of triggers for inflammation like sepsis or trauma.

Our sow diet is closer to SO- than FO-containing ILE diets, a limitation discussed previously that impacts the reference range of CON piglets.[10] Another limitation of this study is that we did not measure free choline in blood or tissues, providing a direct measure of the impact of adding choline in EPN. Arguably more importantly, we did measure the impact of choline as a precursor for PC fatty acid uptake and distribution. However, going forward an optimal study design would be to compare the NOV-C emulsion with and without the added choline to determine the additional benefit of choline over and above the favorable fatty acid composition alone with optimal AA, DHA, and EPA as discussed.

CONCLUSION

We present a novel ILE designed for infants with higher levels of AA and DHA, lower EPA, and added choline that led to significantly higher AA and DHA levels in blood and tissues. For the first time, we have shown that this is especially significant for PC bound AA and DHA across tissues in a 14-day study in piglets. Furthermore, similar to our prior studies in piglets and observations in infants, the use of SO,MCT,OO,FO-ILE led to lower concentrations of AA in blood and tissues, with consistently very high EPA levels.[10]

This preclinical study is a first step in the development of novel ILE with added choline specifically designed for preterm infants to increase tissue AA and DHA while not markedly increasing tissue EPA. Critically ill neonates warrant this attention, and the potential benefit to end-organ function (especially liver, lung, retina, and brain) of this novel ILE warrants further exploration.

AUTHOR CONTRIBUTIONS

Mirielle L. Pauline: Conceptualization; methodology; validation; formal analysis; investigation; writing—original draft; visualization. **Caitlin Huynh**: Investigation; writing—review and editing. **Rohan Persad**: Investigation; writing—review and editing. **Pamela R. Wizzard**: Investigation; project administration; writing—review and editing. **Patrick N. Nation**: Investigation; writing—review and editing. **Rajibur Rahman**: Investigation; writing—review and editing. **Benjamin P. Willing**: Methodology; validation; writing—review and editing; resources. **Catherine J. Field**: Methodology; validation; writing—review and editing; resources. **Abdelatif Elouahabi**: Conceptualization; methodology; validation; writing—review and editing. **Thibault Senterre**: Conceptualization; methodology; validation; writing—review and editing. **Paul W. Wales**: Conceptualization; methodology; validation; resources; writing—review and editing; Supervision. **Justine M. Turner**: Conceptualization; methodology; validation; formal analysis; writing—original draft; Resources; supervision. All authors drafted the manuscript, critically revised the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript. All coauthors vouch for their contribution.

CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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