The effect of lipid emulsions on free fatty acids and free bilirubin in premature newborns

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Background:
Recently published reports have renewed concerns that that lipid infusions might decrease bilirubin binding capacity (BBC) via competition for albumin binding sites from free fatty acids (FFA) such that free bilirubin (Bf) might rise to dangerous levels, particularly in newborns delivered at <28 weeks gestational age.[1,2] Most recently, Hegyi showed elevated levels of unbound free fatty acid (FFAu) and free (unbound) bilirubin (Bf) with lipid infusion rates ≥2.5 g/kg/day, while previously Amin showed that a similar dose response measuring total free fatty acids (FFAt), which is almost entirely bound to albumin.[1,3]

To date, the associations of lipid emulsions, FFA, and Bf, have only been studied with one soy-based lipid emulsion product, Intralipid. Soy based lipid emulsions are associated with cholestatic liver injury, particularly in premature newborns.[3] However, there is evidence that a newer lipid formulation based on a mixture of soy, medium chain triglycerides, olive oil and fish oil (SMOF), causes less liver injury.[4,5] It is believed that SMOF is more tolerable to the liver because of the reduced phytosterol components. [1] To date, neither FFA or Bf levels have been measured with SMOF or concurrently compared to levels with Intralipid, However, there is evidence that plasma triglyceride and other fat levels are higher with SMOF than Intralipid. D'Ascenzo conducted a small randomized trial comparing IL and SMOF in newborns infusing at 3.5g/kg/day and found that triglyceride (TG) levels were 39% higher in patients receiving with SMOF versus IL (231 vs 166 mg/dl).[6] Although FFA levels were not measured in the D'Ascenzo study, a previous study in premature newborns receiving Intralipid reported a strong positive correlation ($r = 0.89$, $P < 0.001$) between triglyceride and FFA levels.[7]

The primary research question for the present study is whether the observed difference in TG levels with IL versus SMOF might also be associated with a difference in FFA and Bf levels. Based on previous reports of higher triglyceride levels with SMOF versus Intralipid and a positive correlation between triglyceride and FFA levels, FFAu levels would be expected to be higher with SMOF than Intralipid. However, it is possible that the triglyceride – FFA correlation observed with Intralipid may not be observed with SMOF. In any case, clinicians should know whether FFA and Bf levels are increased, decreased, or equivalent with SMOF versus IL.

Hypothesis:
At a dose of 3 g/kg/day, patients receiving Intralipid will have lower mean concentrations of free bilirubin (primary outcome) and free fatty acids (secondary outcome) than patients receiving SMOF.

Study Population: 64 Premature newborns (<32 weeks gestation, stratified <28 weeks versus ≥28 weeks) <8 days of age and receiving lipid infusions ≤1 g/kg/day at enrollment and anticipated to be treated with 3 g/kg/day for a minimum of 48 hours.

Patients with direct hyperbilirubinemia >1.8 mg/dl, with suspected sepsis meeting SIRS criteria, undergoing treatment with a continuous infusion of morphine or positive blood cultures at the time of enrollment will be ineligible to enroll in the study. Patients on continuous infusion of pressors (dopamine, dobutamine, epinephrine, etc.) will be ineligible to enroll in the study if they have single lumen central access; patients with multiple lumen access will not be excluded.

Study Design: Randomized controlled trial, Intralipid versus SMOF lipid emulsion

Primary outcome measures:

- Bf serum levels during lipid infusion at 3 g/kg/day (first and second day of infusions at 3 g/kg/day)

Secondary outcomes:

- FFAu serum levels during lipid infusion at 3 g/kg/day (first and second day of infusions at 3 g/kg/day)

- FFAt serum levels during lipid infusion at 3 g/kg/day (second day of infusion at 3 g/kg/day)
- Direct bilirubin, proportion with peak >1.8 mg/dl
- Serum triglyceride level, mean and proportion with peak value >350 mg/dl
- Evaluation of growth at 28 days of life

Methods

Patients for whom informed consent has been obtained will be randomized to IL or SMOF using REDCap software. Treatment allocation per randomization (IL versus SMOF) will remain in effect through the intervention period: until the 2nd blood sample at 3 g/kg/day or 2 weeks of age, whichever comes first. Clinicians cannot be masked to the lipid emulsion – see safety precautions, below

Blood samples for the study will be obtained with blood draws for clinical measurement of bilirubin and/or triglyceride levels such that the study will not require additional interventions for blood sampling – see blood sampling schema below. When treated with 3 g/kg/day, blood will not be sampled if patients have received a mixture of SMOF and IL in the preceding 24 hours, or if SMOF or IL infusions were interrupted for ≥2 hours in the preceding 24 hours. Daily weights and serum bilirubin and triglyceride levels will be abstracted from the medical record.

This study will also include measurements that were not included in previous studies in order to validate measures and obtain a fuller picture cause and effect mechanisms. These measures include serum albumin, bilirubin binding capacity, reserve bilirubin binding capacity, and 2 methods for measuring free fatty acid – both bound and unbound free fatty acids. Serum for FFAu and Bf and albumin, will be stored at -80 C and shipped to Fluoresprobe Sciences.[8 9] Bilirubin binding capacity (BBC) and related measures will be measured with hematoflourometry within 12 hours of sampling.[10 11] Total FFA will be measured at UT Houston SOM.

Per usual care, lipids start at 1 g/kg/day and advance by 1 g/kg/day to a max of 3 g/kg/day as tolerated by the neonate. Blood samples will be collected when lipids are at the following amounts as listed below:

Blood Sampling Schema:

Lipid Dose (g/kg)	Total sample in (mL)
1	0.5
2	0.5
3	0.5
3	0.5
Total	2.0

Two samples will be obtained with the lipid emulsion at 3 g/kg/day in order to follow these values in a steady state at the maximum dosage.

Safety Precautions:

During the intervention period (until the 2nd blood sample at 3 g/kg/day or 2 weeks of age, whichever comes first), the lipid emulsion may be changed without violating the study protocol so long as the change follows usual standard of care practice. Subject to change in NICU guidelines, IL will be discontinued or changed to SMOF if direct bilirubin level is ≥2.0 mg/dl, and SMOF will be changed to IL while potentially incompatible medications are infusing via the same intravenous line This means that that both lipid emulsion infusions will be managed following usual standard of care practice (i.e., in the same way as in patients not enrolled in the study). After the intervention period, lipid emulsion may be changed at the discretion of the attending physician.

Data Analysis: Mean values will be compared using Student's t test, over all patients and within gestational age strata. Proportions will be compared using the chi square test. Dose responses will be analyzed and compared using multilevel regression to account for repeated measures within patients. Point estimates and 95% confidence levels will be reported for all outcomes.

Data Management: Data will be entered and stored in a secure University of Texas Health Sciences REDCap database.

Power and Sample Size:

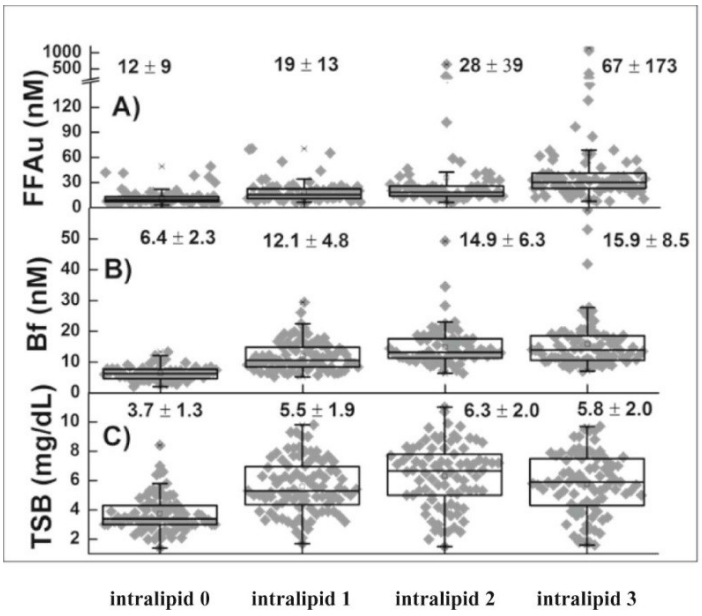
Based on the sample size calculation below, a feasible sample size of 64 patients (32 in 2 groups) would result in power of 0.81 with alpha at 0.05 to detect an 39% difference in mean Bf levels, SMOF versus IL infusing at 3 g/kg/day.

D'Ascenzo conducted a small randomized trial comparing IL and SMOF in newborns and infusion at 3.5g/kg/day and found that triglyceride (TG) levels were 39% higher with SMOF versus IL in patients receiving 3.5 g/kg/day (table below).[7]

Table	Intralipid	SMOF	delta	delta %
Triglyceride) mean +SD	166 ±47 mg/dl	231 ±59 mg/dl	65 mg/dl	39 %
Triglyceride levels Intralipid vs SMOF at 3.5 g/kg/day (D'Ascenzo et al)				

Absent other relevant data, we hypothesize that mean FFAu and mean Bf levels will also be 39% higher with SMOF versus IL (day 2 at 3 g/kg/day). Hegyi reported FFAu of 67 ± 173 and Bf levels of 15.9 ± 8.5 (mean,SD) with IL infusions of 3 g/kg/day (figure 1). For reasons of feasibility but also because Bf levels are clinically more important, we based sample size and power calculations on Bf rather than FFAu.

Figure Effects of increasing intralipid dose from 0 to 3 g/kg/d (Hegyi et al)



Given Bf levels (nM) of 15.9 ± 8.5 with IL versus 22.1 ± 8.5 with SMOF (delta = 39%), a sample size of 62 patients (31 in 2 groups) will result in power of 0.81 with alpha at 0.05. Likewise, power will be 0.81 to detect an 39% decrease (SMOF 9.7 ± 8.5).

Observational Outcomes :

Evaluation hematofluorometry's capacity to detect displacement of bilirubin from albumin by free fatty acids and of the correlation of Bf and the saturation index.

Unrelated to comparisons between Intralipid and SMOF, a number of analyses will address important questions. Hematofluorometry is the methodology employed to measure bilirubin binding capacity (BBC). With total serum bilirubin (TSB) and Bf, BBC is one of three measured components in the bilirubin binding panel proposed by Ahlfors.[10 11 12] Calculated components of the bilirubin binding panel include reserve bilirubin binding capacity (RBBC), calculated as the difference between BBC and bound bilirubin, and the bilirubin-albumin equilibrium dissociation constant (Kd). The clinical problem of unrecognized high Bf levels secondary to FFAs displacing bilirubin from albumin is a prime example of the potential utility of the bilirubin binding panel, and the limitations of relying on TSB alone. The question arises: should clinicians also measure BBC and Bf, and perhaps FFA, as they prescribe lipid emulsion during the first weeks after birth? The value of including BBC and RBBC in the bilirubin binding panel in addition to Bf is that the former measures should be give an earlier and more reliable danger signal. This is because Bf levels remain relatively stable until binding capacity is reached, then rises quadratically – such that the danger signal may only be appreciated too late. However, the capacity of hematofluorometry to detect FFAs effect on BBC in the clinical setting is uncertain. Specifically, there is a step in the measurement method that could, in theory, displace FFA from albumin such that the *in-vivo* effect on RBBC might be underestimated. In the current study, we can test the capacity of hematofluorometry to detect the effect of FFA on BBC in the following manor. To the extent that elevated Bf levels are caused by FFAs displacing bilirubin from albumin binding sites, measured BBC should decrease as Bf levels rise. It is believed that this phenomenon becomes when the molar ratios of FFA to albumin exceed ~4:1(Amin, Maisels, Watchko, 2017).[2] However, it is necessary to measure and adjust for concurrent changes in albumin concentrations because this also affects both Bf and BBC.

The hematofluorometry saturation index, calculated as bound bilirubin divided by RBBC, provides a second means by which the capacity of hematofluorometry to detect displacement of bilirubin from albumin by FFAs can be evaluated. The saturation index (SI) has been reported to have a tight correlation with Bf (figure 2) .[13] However, the correlation is dependent on accurate measurement of BBC and RBBC. Finding that the correlation of SI and Bf persists at high Bf levels despite high FFA levels would provide evidence that BBC and RBBC are measured accurately under these conditions. Conversely, finding that SI-Bf correlation deteriorates as FFA and Bf levels rise would provide evidence that hematofluorometry may not be suitable for guiding decisions regarding phototherapy and lipid infusions for premature newborns. It is acknowledged that these analyses are dependent on observing adequate variation in FFA and Bf levels, and that FFA reach levels that are high enough to displace bilirubin from albumin – all of which have been observed in previous similar studies.[1]

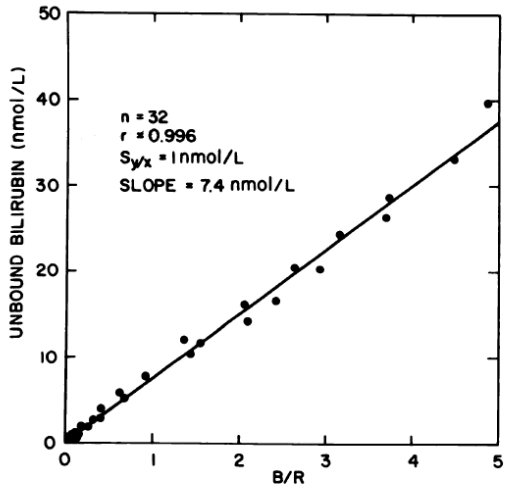


Fig. 1. A plot of the apparent unbound bilirubin concentration as determined by the "peroxidase" method (U) vs the ratio of albumin-bound bilirubin to reserve bilirubin binding capacity as determined with the hematofluorometer (B/R) for a series of specimens prepared by addition of bilirubin to blood obtained from a single adult donor

Figure 2

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