Bilirubin Neurotoxicity (BN) and Neurodevelopmental Impairment (NDI) in Extremely Preterm (EP) Infants: Avoidable by Reducing the Usual Intravenous Lipid (UL) Administration?

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Bilirubin Neurotoxicity (**BN**) and Neurodevelopmental Impairment (**NDI**) in Extremely Preterm (**EP**) Infants: Avoidable by Reducing the Usual Intravenous Lipid (**UL**) Administration?

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Rationale. Extremely premature infants have very limited caloric reserves**.** Because of the pressing need to at least meet maintenance caloric requirements, early aggressive parenteral nutrition with glucose, protein, and lipids has been recommended for preterm infants, particularly those born before 27-28 wks.[1] Intralipid, the most commonly used parenteral fat preparation, is commonly initiated starting 1-2 days after birth. However, the optimal age for administration is unclear as the most recent systematic review of the Cochrane Collaboration found no statistically significant benefits of "early" administration of lipids (≤5 days after birth) from later introduction.[2] Whether a relatively high caloric intake from lipids is better than a relatively low intake is also unclear. The dose of Intralipid is commonly increased from 1 g/kg/d on the day that it is started to 2 g/kg/d on the following day to 3 g/kg/d on the next day. The dose is usually limited to a maximum of 3 g/kg/d, partly to avoid potential dose-related adverse effects including chronic lung disease, increase in pulmonary vascular resistance, impaired pulmonary gas diffusion, sepsis, free radical stress and bilirubin neurotoxicity.

Free fatty acids (**FFA**) compete with bilirubin for albumin binding sites. There is a long-standing concern that infusion of Intralipid may increase FFA sufficiently to displace bilirubin from albumin and increase serum unbound bilirubin (**UB**) that passes into the brain and causes bilirubin neurotoxicity.[3] This concern was heightened by a 2017 report that Intralipid doses of 2-3 g/kg/d were associated with an increase in unbound free fatty acids (**uFFA**) and UB among infants <28 weeks gestation.[4] Whether these increases resulted in bilirubin neurotoxicity or neurodevelopmental impairment (**NDI**) was not determined. In the commentary[5] prompted by that report, Amin, Maisels, and Watchko, each a widely respected authority on neonatal hyperbilirubinemia concluded, "The current observations suggest that a more tempered approach to early lipid infusion in neonates ≤28 weeks gestation should be considered, until we have a better understanding of the risks and benefits."

Comparative effectiveness research is thus needed to compare usual with more reduced lipid administration in extremely premature infants during the first 1-2 weeks after birth when these infants are at risk for bilirubin neurotoxicity. A persisting problem in evaluating this issue has been the lack of a reliable, well validated, and commercially available method to assess UB. The study noted above involved a new method that avoids limitations of prior methods but is not yet commercially available. In the proposed study we will use that method to assess UB among extremely premature infants randomized to either our usual prescribed Intralipid dose (**UL**) or to a prescribed dose which is half that of the usual dose (reduced intralipid, **RL**). We will also measure uFFA profiles, brain stem auditory-evoked responses (**BAERs**) at 34-36 weeks postmenstrual age (**PMA**), and neurodevelopmental impairment (**NDI**) status at 22-26 months postnatal age. BAERs will be assessed because a prolonged wave V latency is a sensitive indicator of even transient bilirubin neurotoxicity.[6,7,8] It is unknown which summary UB measurement, either single UB peak level per patient or repeated measures per patient with a mean UB, area under the curve or proportion of values, is most predictive of NDI outcomes. In Hegyi et al[4], phototherapy was found to reduce both total serum bilirubin (**TSB**) and UB in all infants, but when evaluating the infants <28 weeks separately, TSB was reduced but UB was not, suggesting that UB may be a more preferred method to measure bilirubin toxicity.

We expect to see some difference between treatment groups in UB levels in the first 1-2 weeks after birth but not their BAERs at 34-36 weeks postmenstrual age (**PMA**) (the earliest PMA that BAERs are likely to be reliably assessed in a neonatal ICU setting) or in rates of NDI at 22-26 months postnatal age. This expectation is based in part on the absence of discernible BAER evidence of neurotoxicity with the usual Intralipid doses administered in our center (3 g/kg/d maximum) and the TSB levels reached during our recent trial of cycled versus continuous phototherapy (**PT**).[9] Nor did we identify evidence of bilirubin neurotoxicity in BAERs performed in our center using the same maximum Intralipid dose even with the relatively high TSB levels reached with more conservative use of PT in the prior NICHD Neonatal Research Network (**NRN**) Trial of aggressive vs. conservative PT.[10]

While bilirubin neurotoxicity with overt encephalopathy does occur, it is rare and currently unpredictable.[11] To improve our understanding of the relationship of UB to BAERs and NDI, we will request mothers who decline consent to randomize their infants to different Intralipid regimens to include their infants with those in the trial in an observational analysis relating their UB levels to their BAERs and neurodevelopmental findings (with and without adjustment for potential confounding variables).

We will also assess the clinical findings in all these infants in an effort to better understand their relationship to TSB and UB and the paradoxical findings reported by Oh and colleagues[12] that peak total bilirubin levels at 5 days age were inversely related to death or adverse neurodevelopmental outcomes in stable infants (a quite unexpected finding) although directly related to these outcomes in unstable infants (as expected). It is possible that because of bilirubin's antioxidant properties that higher TSB levels are protective in preventing death or from any cause providing the infants have not been exposed to factors that may predispose infants them to bilirubin neurotoxicity (e.g., sepsis, hypoxic ischemic episodes, severe acidosis, low albumin levels) and that the risk of bilirubin neurotoxicity with elevated TSB is over-estimated in these infants. Infants who were considered unstable in that study include all receiving mechanical ventilation at that age, and the need for mechanical ventilation at 5 days might simply be associated with factors that cause bilirubin neurotoxicity or that the need for mechanical ventilation is an effect of bilirubin neurotoxicity already present at that age. Informed consent will be obtained for each infant as approved by the IRB.

Hypotheses.

1. Among infants <27 weeks gestation or ≤750 g birth weight, the usual prescribed intralipid (**UL**) regimen and a reduced prescribed intralipid (**RL**) regimen (consisting of half the UL regimen) during the first two weeks after birth will result in somewhat higher mean unbound free fatty acids and mean UB values but similar BAERs, and in infants less than 27 weeks gestational age rates of death or impairment at 2 years postnatal age.

2. Any infants with clear evidence of bilirubin neurotoxicity on BAERs and NDI at 22-26 months (including all infants in observational analyses) will have A) moderate to increased mean and peak UB levels in combination with one or more other factors thought increase their susceptibility to bilirubin neurotoxicity; B) a lower peak TSB to UB level (as expected if albumin levels are low or bilirubin is displaced from albumin); C) will have a greater need for mechanical ventilation at 5 days than expected from baseline measures (a potential indicator of bilirubin neurotoxicity at 5 days).

Methods.

Population. Inborn infants <27 weeks' gestation by best obstetric estimate or ≤750 g birth weight who has no major congenital anomaly or overt nonbacterial infection who has not received Intralipid and whose mother has consented to inclusion of the infant for the NRN Cycled Phototherapy Trial (which warrants higher priority based partly on stipulations in joining the NRN). Mothers of infants <27 weeks gestational age who refuse consent for this randomization control lipid trial may also be approached for consent in the observational arm of this lipid study.

Possibility of inclusion of two other major centers. With the protected time and funding provided by a KL2 award, I hope to add two centers to increase the sample size and generalizability of this pilot study. These centers are 1) the University of Alabama which generally has the largest enrollment in NRN trials and is led by Wally Carlo, MD and 2) Stanford where Cody Arnold, MD is Visiting Professor and David Stevenson, MD, an international leader in studies of bilirubin and PT studies, are based. Both Drs. Carlo and Stevenson have expressed serious interest in joining the pilot.

Enrollment and Randomization. Patients will be approached for informed consent after informed consent has been given or refused for the NRN Cycled PT Trial. Infants for whom informed consent is obtained for this lipid study will be randomized using a variable block size stratified by study site and phototherapy use (continuous or cycled which may affect UB level) to either UL or RL using a web-based computerized program (REDCap). Lipid and phototherapy use are concurrent. Mothers who refuse consent infants for this lipid trial will be asked if they would consent for their infant to be included in the observational component of this study so that UB levels can be related to BAERs and 2-year outcomes. In the observational component Intralipid infusions will be at the attending physician's discretion and care would be altered only by collection of the small amount of blood (≤2 mL total) as for participants randomized to UL or RL.

Treatment Regimen.
UL regimen: Intralipid will be started at 1 g/kg/day and routinely advanced by 1 g/kg/day each day to a max of 3 g/kg/day as per usual clinical practice.
RL regimen: Intralipid will be started at 0.5 g/kg/day and routinely advanced by 0.5 g/kg/day each day to a max of 1.5 g/kg/day (half rate of usual clinical practice).

The assigned treatment regimen (UL or RL) will remain in effect for a minimum of 7 days and a maximum of 14. On postnatal day 8-14, the Intralipid preparation and dosage maybe changed at the discretion of the clinical team if phototherapy has been off for ≥48 hours (TSB <5 mg/dL). (After that point, the Intralipid preparation and dosage maybe changed at the discretion of the clinical team). Throughout the study period, the infusion rates for both regimens may be decreased, and advances may be delayed for reason of lipid intolerance providing the justification is documented in the daily progress note. (Each study site should strive to standardize criteria for lipid intolerance so as to minimize the likelihood of using different criteria for the treatment groups). Intralipid infusions will be routinely initiated on specified day after the birthday as per the routine in the study site; this will be the first day after the birthday in Houston. The dates and times of start, stop, and changes in infusion rates will be recorded.

Cointerventions. As appropriate for a pragmatic trial, the protein and glucose components of parenteral nutrition (**PN**) will be prescribed by the clinical team following usual care prescribing

guidelines and will likely differ between study groups. The quantities of macronutrients will be recorded during the intervention period (parenteral and enteral, g/kg/day).

Study Assessments. Blood samples for the study will be obtained with blood draws for clinical measurement of TSB levels such that the study will not require additional interventions for blood sampling. The first study blood sample will be obtained on the morning when the newborn is 4 days old (postnatal day 5). Age in days is defined as 0 days old at birth, and 1 day old at midnight on the birthday. Postnatal day 1 is until midnight of the birthday, and postnatal day 2 at midnight on the birthday, etc. Blood samples numbers 2-7 will be obtained on each subsequent morning through postnatal day 14 when a total bilirubin is obtained for clinical purposes as ordered by the clinical team. Bedside nurses collect blood using BD red-top microtainers at the same time that blood is sampled to measure total bilirubin for clinical purposes. A total of up to 6 study blood samples will be obtained within the first 2 weeks from birth, to not exceed total study blood volume of 2mL. (See sample schedule below).

Sample Number	Postnatal Day to Collect	Age (days)	Blood Volume*
*	1 (birthday)	0	0
*	2	1	0
*	3	2	0
1	4	3	0.25mL
2	5	4	0.25mL
3	6	5	0.25mL
4	7	6	0.25mL
5	8	7	0.25mL
6	9	8	0.25mL
	Total Blood Vol.		<2 mL

*All samples will be 0.25mL with the exception of the morning after the lipid infusion is advanced to the peak infusion rate (usual group 3 g/kg/day or reduced group 1.5g/kg/day). On this day ONLY, the blood sample volume will be 0.5mL.

Blood Volume and Measurements.
A 0.5 ml will be obtained on the morning after the lipid infusion has been advanced to the peak infusion rate (1.5 or 3.0 g/kg/d).
The priority for measurements for 0.5 ml sample will be:

1. UB [10ul serum] in duplicate [20 ul serum]
2. Albumin [10ul serum] in duplicate [20 ul serum]
3. Unbound FFA complete (60ul serum) in duplicate (120ul serum)
   This includes FFA blended estimate

On other days, 0.25 ml blood will be obtained each morning (up to 6 samples).
The priority for measurements for the 0.25 ml samples will be:

1. UB [10ul serum] in duplicate [20 ul serum]
2. Albumin in duplicate [10ul serum] in duplicate [20 ul serum]
3. Unbound FFA blended estimate [5ul serum] (#1)
4. Unbound FFA complete [60ul serum] (#1)
5. Unbound FFA blended estimate [5ul serum] (#2)
6. Unbound FFA complete [60ul serum] (#2)

The nurse or person obtaining blood for routine TSB levels will collect whole blood at the same time in study-provided red-top microtainer and immediately place the microtainer in a pre-

labeled amber plastic bag provided by the research team. The amber bag containing the sample will be placed in the specified location in the research refrigerator (4 degrees Celcius) as soon as feasible. A member of research team will pick up the specimen within 12 hours, transport the specimen to the UT-Houston McGovern Medical School. In the laboratory space at UT Houston McGovern Medical School, a centrifuge will be used to separate serum which will be labeled and frozen at minus 80 degrees Celsius until samples are shipped in batch to Fluoresprobe Sciences in San Diego, California.

Measurands in each whole blood sample will include concentrations of UB, albumin, total unbound free fatty acids (**uFFA**), and a uFFA profile, which includes concentrations of specific uFFA. These results will not be available with the bedside clinician and will not affect direct patient care. These will be linked with any of the following measurands from the same blood sample measured in the hospital laboratory: TSB, indirect bilirubin, direct bilirubin, albumin, triglyceride, glucose, serum bicarbonate. Additionally, if a blood gas is obtained within 4 hours of this blood draw, the pH from that blood gas will be linked to the sample, providing no intervening ventilator changes were made prior to the blood draw. Other data reflecting general status and severity of illness and/or potentially influencing UB levels will be abstracted from the medical record, including baseline variables such as sex, gestation, anthropometrics, multiple gestation, delivery information, Apgar scores, SNAP-II scores, Z scores at birth, 28 days of life, and 36 weeks postmenstrual age, etc.

Data Management. Data will be entered and stored in a secure UTH REDCap database. Paper logs of data will be secured in a locked cabinet or room.

Outcome Measures. To be compared by treatment group:
Primary outcomes:

- Area under the UB curb with UB concentration >40 nM/L
- BAER wave V latency at 34-36 weeks post menstrual age (or as soon as feasible if the infants if an artifact free BAER cannot be obtained at 34-36 weeks)

Secondary outcomes:

- Mean UB concentration and proportion of measurements >40 nM/L, peak UB
- Peak UB concentration
- Area under the total unbound FFA (**uFFA**) concentration – time curve; mean FFAu and peak uFFA
- Peak total serum bilirubin (TSB)
- Direct bilirubin > 1.5 mg/dL before discharge
- Macronutrients prescribed (parenteral and enteral protein, fat and carbohydrate, g/kg/day)
- Weight change (g/kg/day) and Z score change from birth through day 28 and at 36 weeks postnatal age
- Relevant data abstracted from the medical record and stored in the existing databases:
    - Serial FiO_2 and ventilator settings (routinely collected generic data)
    - Bronchopulmonary dysplasia (oxygen requirement at 36 weeks PMA)
    - Perinatal and hospital acquired sepsis episodes
    - Other clinical findings potentially related to signs of or susceptibility to bilirubin neurotoxicity
    - Death or NDI as assessed at 24 months PMA
    - Death or hearing loss at 24 months PMA
    - Death or cerebral palsy at 24 months PMA

Cohort analyses (across study groups and any infants in the observational arm) Associations of interest will include:

- UB and death or NDI, unadjusted and adjusted for potential confounders
- UB and wave V latency, unadjusted and adjusted for potential confounders
- Wave V latency and death or NDI, unadjusted and adjusted for potential confounders
- uFFA measured by single probe and total uFFA measured as the sum of individual uFFA
- uFFA and UB
- Total uFFA and individual uFFA(s)

Power and Sample Size. We plan to study sufficient infants at UTH to assess BAERs in 40 infants in each treatment group and have more than 80% power at $P < 0.05$ to identify a difference between groups in wave V latency longer than 0.3 millisecond (SD = 0.5 millisecond). This sample size would also afford sufficient power to assess a clinically important difference in UB level.

I have the opportunity to enroll more patients within the limitations of my funding period, scheduled to end June 2024. I have been successful at enrolling patients at an accelerated rate from the minimum needed for adequate power. My current enrollment rate is 4-5 patients per month, and my target is 3 patients per month. The greater than anticipated enrollment rate has occurred because of the significant and unanticipated increase in the census of tiny and premature babies. By increasing our sample size, this will allow me the opportunity to ensure adequate power for an important trial, not likely to ever be repeated, and provide a more narrow confidence interval for more precise results. If differences are appreciated this would provide pilot data to motivate a larger and multicenter trial to investigate an earlier measure for neurodevelopmental impairment, allowing for earlier interventions and treatment. I propose the largest feasible enrollment for the remainder of my funding period or n=175 infants (Enroll an average of 5 infants per month for 11 months remaining in funding).

Data Analysis. Intention-to-treat analyses will be performed under the supervision of Claudia Pedroza. Generalized linear mixed models will be performed to analyze all outcomes and will include treatment group, PT type (continuous and cycled), as covariates with random effects for study site. Interaction will be assessed for treatment group and PT type. Bayesian analyses will be performed in evaluating differences in treatment effects overall and in different patient subgroups and centers. For the primary outcome and any other continuous outcome, a linear regression model will be used. Binary outcomes will be analyzed with a logistic regression and count data with a Poisson or negative binomial regression model. All priors will be neutral. For binary outcomes, all priors will be centered at RR of 1.0 (indicative of no difference between treatment groups with a 95% prior interval of 0.3-3.0 (in the log RR scale a Normal distribution with mean of 0 and variance of 0.50). A Normal $(0,10^2)$ prior will be used for the intercept term and Normal (0,1) for all other variables in the model. For the standard deviation of the center random effect, a half-Normal (0,1) prior will be used. Similar neutral informative priors (based on previous NRN PT trial[9] where possible) will be used for non-binary outcomes. We will report posterior medians and 95% credible intervals for group differences and relative risks. We will also report posterior probability of benefit/harm for each outcome.

Similar analyses will be conducted for the cohort analyses (including infants in the observational arm).

Data Interpretation. If UB is higher and BAER wave V latency is longer in the UL than the RL group, the findings would likely prompt changes to more conservative use of Intralipid use in the

first 1-2 weeks after birth in extremely preterm infants. If values for UB and BAER wave V latency in the UL group are no higher than the RL group, the findings would support continue use of the UL regimen to provide somewhat greater caloric intake. If UB is higher with no discernible effect on wave V latency, the findings would suggest that the UB level were not high enough to cause bilirubin neurotoxicity with effects lasting to 34-36 weeks. However, findings at 2 years would need to be scrutinized for evidence of adverse neurodevelopmental effects.

Data Safety and Monitoring. Claudia Pedroza, PhD, the study statistician, will conduct an interim analysis of mortality when half the planned sample size has been discharged from the neonatal ICU. If there is a Bayesian posterior probability >99% (assuming a neutral prior probability), the study statistician (Claudia Pedroza, PhD), will notify the Dr. Amir Khan, Medical Director of the CMHH Neonatal ICU and the Director of the Neonatology Division. Unless there are important chance differences between treatment groups in baseline risk factors that are known to influence outcomes of extremely premature infants (birth weight, gestational age, sex, multiple births (twins, triplets), antenatal steroids, high ventilator settings) and that could account for differences in outcome, she would contact Amir Khan, MD. They would decide whether to halt enrollment in the study.

Predischarge outcome date will of course be entered into the study database for each infant once he/she is discharged from the NICU. The rationale for the stopping guideline is that impairment is likely to be a persisting problem and that other neonatal outcomes short of death (IVH, NEC, BPD) are short term outcomes less important than impairment or death. Were we performing frequentist analysis, the stopping guideline would be based on a $p<0.01$ (two-tailed).

References

[1] Hay WW. Aggressive nutrition of the preterm infant. Curr Pediatr Rep 2013;1:doi:10.1007/s40124-013-0026-4.

[2] Simmer K, Rao SC. Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database Syst Rev. 2005 Apr 18;(2):CD005256.

[3] Effect of free fatty acids on bilirubin-albumin binding affinity and unbound bilirubin in premature infants. Amin SB.JPEN J Parenter Enteral Nutr. 2010 Jul-Aug;34(4):414-20

[4] Hegyi T, Kleinfeld A, Huber A, Weinberger B, Memon N, Shih WJ, Carayannopoulos M, Oh W Effects of Soybean Lipid Infusion on Unbound Free Fatty Acids and Unbound Bilirubin in Preterm Infants. J Pediatr. 2017 May;184:45-50

[5] Amin SB, Maisels MJ, Watchko JF Early Lipid Infusions and Unbound Bilirubin in Preterm Neonates: A Cause for Concern?.J Pediatr. 2017 May;184:6-7

[6] Shapiro SM, Popelka GR. Auditory impairment in infants at risk for bilirubin-induced neurologic dysfunction. Semin Perinatol. 2011;35(3):162-170.

[7] Wennberg RP, Ahlfors CE, Bickers R, McMurtry CA, Shetter JL. Abnormal auditory brainstem response in a newborn infant with hyperbilirubinemia: improvement with exchange transfusion.J Pediatr. 1982;100(4):624-626.

[8] Kuriyama M, Tomiwa K, Konishi Y, Mikawa H. Improvement in auditory brainstem response of hyperbilirubinemic infants after exchange transfusions. Pediatr Neurol. 1986;2(3):127-132.

[9] Arnold C, Tyson JE, Pedroza C, et al Cycled Phototherapy Dose-Finding Study for Extremely Low-Birth-Weight Infants: A Randomized Clinical Trial. JAMA Pediatr. 2020 Apr 27:e200559

[10] Lasky RE, Church MW, Orlando MS, et al The effects of aggressive vs. conservative phototherapy on the brainstem auditory evoked responses of extremely-low-birth-weight infants..Pediatr Res. 2012 Jan;71(1):77-84.

[11] Watchko JF, Maisels MJ. The enigma of low bilirubin kernicterus in premature infants: why does it still occur, and is it preventable? Semin Perinatol. 2014;38(7):397-406

[12] Oh W, Stevenson DK, Tyson JE et al.Influence of clinical status on the association between plasma total and unbound bilirubin and death or adverse neurodevelopmental outcomes in extremely low birth weight infants. Acta Paediatr. 2010 May;99(5):673-8.