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Intravenous Lipid and Bilirubin-Albumin Binding Variables in Premature Infants

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Abstract

BACKGROUND—The lipid intake at which a significant bilirubin-displacing effect occurs as a function of gestational age (GA) is unclear.

OBJECTIVE—To determine the effect of gradual increase in IL intake from 1.5 to 3 g/kg per day on bilirubin-albumin binding variables as a function of GA in premature infants with indirect hyperbilirubinemia.

METHODS—Infants of 24 to 33 weeks' gestation at birth who received IL (20% Intralipid [Fresenius Kabi, Uppsala, Sweden]) doses of 1.5, 2, 2.5, and 3 g/kg per day over 4 consecutive days were prospectively evaluated. The blood samples were drawn twice at least 8 hours apart on each IL intake to measure total serum bilirubin and free bilirubin by the peroxidase test. The highest free bilirubin on each IL intake, the corresponding total serum bilirubin, and serum albumin were used to calculate the bilirubin/albumin binding constant or binding affinity.

RESULTS—Sixty-two infants (median GA: 28 weeks) were studied during the first 10 days of life. None of the subjects had culture-proven sepsis, had triglyceride levels of >2.05 mmol/L, or were receiving steroids. Infants were grouped in 2-week GA intervals. The cumulative frequency of elevated free bilirubin concentration (90th percentile or $B_f \geq 32$ nmol/L) as a function of IL intake was inversely related to GA and was significantly different among 2-week GA groups. There was significant decrease in binding affinity and increase in free bilirubin concentration with higher IL intake for ≤ 28 week but not for >28 week GA groups.

CONCLUSIONS—The IL intake may be associated with a significant fall in the binding affinity of bilirubin for plasma protein and a concomitant increase in free bilirubin concentration in premature infants. The lipid intake at which this occurs depends on GA.

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Keywords

free bilirubin; bilirubin-albumin binding; intravenous lipid emulsion; premature infants

Intravenous lipid emulsion (IL) provides a concentrated isotonic source of calories in neonates. Clearance of free fatty acids (FFAs) is slower in premature infants than in term infants, and IL intake in premature infants may result in elevated FFA.^{1–3} The elevated FFA concentration may displace bilirubin from albumin-binding sites, increasing free or unbound bilirubin (B_f , bilirubin not bound to albumin) concentration.^{1,4–6} B_f crosses the intact blood-brain barrier and may cause brain injury, according to the current theory on the pathogenesis of bilirubin-induced neurotoxicity.^{7–9}

There is limited knowledge on the effect of increasing IL intake in premature infants on B_f concentrations.^{10–12} Current literature suggests that the bilirubin-displacing effect may be seen at FFA to albumin molar (F/A) ratios of $>4:1$.¹⁰ Previous studies have shown that an F/A ratio of $>4:1$ may occur with IL intake of 2 to 3 g/kg per day in infants of 30 weeks' gestational age (GA).¹⁰ Because of the potential for the bilirubin-displacing effect at higher IL doses, IL intake is usually limited to <2 g/kg per day in the presence of indirect hyperbilirubinemia in premature infants, irrespective of the degree of prematurity.^{13,14}

However, the evidence to support the practice of restricting the amount of IL intake during hyperbilirubinemia has been based on indirect estimation of B_f using fluorometer or saturation index tests in a small number of premature infants.^{10,11} Recently, the Food and Drug Administration approved an unbound bilirubin analyzer, a semiautomated spectrophotometer, for B_f measurement using the peroxidase method.^{15,16} The peroxidase method was subsequently modified using 2 concentrations of peroxidase to avoid underestimation of B_f .¹⁷ There is little information on the effect of a gradual increase in IL intake from 1.5 to 3 g/kg per day on bilirubin-albumin binding variables, as measured by the modified peroxidase test using an unbound bilirubin analyzer.¹² It is also unclear if the effect of IL intake on bilirubin-albumin binding variables, specifically bilirubin binding affinity and B_f concentration, varies as a function of GA.

METHODS

We performed a prospective nested study to determine the effect of gradually increasing IL intake from 1.5 to 3 g/kg per day on bilirubin-albumin binding variables, as a function of GA, in premature infants with indirect hyperbilirubinemia by using the approved modified peroxidase test. The study was approved by the institutional research review board at the University of Rochester School of Medicine. Informed consent was obtained from the parents.

Study Population

Premature infants of 33 weeks' GA delivered at the University of Rochester Medical Center between March 2006 and December 2007 and enrolled in the National Institutes of Health-funded study "Bilirubin-Induced Auditory Neuropathy" were eligible for this study. GA was assessed by obstetrical dating criteria or, when obstetrical data were inadequate, by

Ballard examination. Infants who received IL (20% Intralipid [Fresenius Kabi, Uppsala, Sweden]) doses of 1.5, 2, and 2.5 g/kg per day over 3 consecutive days or 1.5, 2, 2.5, and 3 g/kg per day over 4 consecutive days were studied to evaluate the effect of IL intake on bilirubin-albumin binding variables. Infants with major congenital malformations, TORCH (toxoplasmosis, other infections, rubella, cytomegalovirus infection, and herpes simplex) infections, or a family history of hearing loss were excluded. In addition, premature infants who did not receive a gradual increase in IL intake up to 2.5 g/kg per day during the first 10 days after birth were excluded.

The NICU policy at the Golisano Children's Hospital is to administer IL over 16 to 20 hours at a rate of <0.15 g/kg per hour. The IL intake is gradually increased from 0.5 to 3 g/kg per day during the first week at the discretion of the attending neonatologist.¹⁴ The major component fatty acids in Intralipid are linoleic (44%–62%), oleic (19%–30%), palmitic (7%–14%), linolenic (4%–11%), and stearic (1.4%–5.5%). The triglyceride level is measured at least once for each subject during the first postnatal week while gradually advancing IL intake from 1.5 to 3 g/kg per day. In the presence of hypertriglyceridemia (triglycerides of >2.05 mmol/L or 180 mg/dL), the IL intake is reduced or discontinued, depending on these verity of hypertriglyceridemia. The IL intake is often limited to 2 g/kg per day in the presence of hyperbilirubinemia. The total serum bilirubin (TSB) concentrations at which IL is restricted is highly variable and dependent on physician bias. The IL is usually infused via central venous catheters in infants of <30 weeks' GA. The heparin is usually administered with central venous catheters at a dose of 0.5 U/mL. Phototherapy is used according to the institutional guidelines for the management of hyperbilirubinemia in premature infants that is currently based on TSB concentrations, birth weight, and presence of clinical factors that increases the risk for bilirubin encephalopathy.

Outcome Variables: Bilirubin-Albumin Binding Variables

Blood samples were drawn twice at least 8 hours apart on each IL intake to measure TSB ($\mu\text{mol/L}$) and B_f (nmol/L) by the peroxidase test (UB analyzer, Arrows Company, Osaka, Japan). Serum albumin levels were measured on the second and third postnatal days by the Bromocresol green method.¹⁸ Because the half-life of serum albumin is ~3 weeks, albumin concentration was assumed to be constant for the duration of the study. The modified peroxidase test was performed at 2 peroxidase concentrations with the stock peroxidase diluted 1:42 or 1:83, and the steady state B_f at each peroxidase concentration used to calculate the equilibrium-free bilirubin.¹⁷ The highest equilibrium-free bilirubin on each IL intake, the corresponding TSB, and serum albumin were used to calculate the bilirubin/albumin equilibrium association binding constant (K) ($\text{L}/\mu\text{mol}$), a measure of binding affinity, on each IL intake by using the following equation:

$$K = (\text{TSB} - B_f) / [B_f(\text{albumin} - \text{TSB} + B_f)].$$

Covariables

Data on serum triglyceride levels, culture-proven sepsis, and exposure to postnatal steroids during the study period were collected prospectively on each subject. Ibuprofen, a potential bilirubin displacer, was not used during the study period.

Statistical Analyses

Statistical analyses were performed with SAS 9.1 (SAS Institute, Inc, Cary, NC). Continuous variables were analyzed by using the Student's *t* test if normally distributed; otherwise, the Mann-Whitney test was used. Categorical variables were analyzed by using the χ^2 test or Fisher's exact test. To examine the effects of IL doses on the TSB, B_f , and *K*, longitudinal data analyses were performed using mixed models with random subject effects to account for within-subject correlation across different IL dosages.¹⁹ Autoregressive heterogeneous variance covariance structure was chosen by using Akaike information criterion, a tool for model selection, which helps determine which hypothesized structure provides the best fit to the observed data structure.²⁰ To evaluate the differences of TSB, B_f , and *K* among doses at different GAs, each analysis was repeated including the GA group indicator and the interaction term with IL doses as independent variables in the model.

To determine cumulative frequency of elevated B_f , we used the 90th percentile (1.87 $\mu\text{g/dL}$ or 32 nmol/L) of all measured B_f concentrations as a cutoff and defined B_f of ≥ 32 nmol/L as elevated B_f . The cumulative frequency of elevated B_f (subjects with elevated B_f at a given IL dose plus at a lower IL dose) with increasing IL intake was determined for each GA group and then compared between GA groups at each IL dose.

RESULTS

A total of 210 infants were eligible for the primary bilirubin-induced auditory neuropathy study between March 2006 and December 2007. Eight infants (6 with major congenital malformations and 2 with TORCH infections) were excluded. Nine infants died within the first 36 hours and were not enrolled. A total of 140 infants ≥ 33 weeks' GA were enrolled after obtaining consent. Of the 140 infants enrolled, 62 infants were identified to have received IL doses of 1.5, 2, and 2.5 g/kg per day over 3 consecutive days during the first 10 postnatal days. Of these, 37 infants received IL doses up to 3 g/kg per day over 4 consecutive days during the first 10 postnatal days.

The mean postnatal age for all 62 subjects at 1.5 g/kg per day IL intake was 3.7 days (median: 4 days [range: 3–5 days]). The mean GA at birth of the 62 infants was 28.4 weeks (median: 28 weeks [range: 24–33 weeks]). The mean birth weight of these infants was 1156 g (range: 450–1840 g). Thirty-seven of 62 (60%) infants were male and 41 of 62 (66%) infants were white. Three infants (5%) were small for GA. Forty-six infants had triglyceride levels measured during the study period (within 3 days of IL doses of 1.5–3 g) and were normal (<2.05 mmol/L). None of the subjects had active infection or were receiving postnatal steroids.

To analyze the results as a function of GA, we categorized subjects ($n = 62$) into 2 strata on the basis of the median GA of study subjects: (1) infants ≤ 28 weeks' GA ($n=32$) and (2) infants > 28 weeks' GA ($n = 30$).

Binding Variables As a Function of IL Intake Within Each GA Group

For the >28 -week-GA group, TSB, K , and B_f did not change significantly as the IL dose increased to 2.5 or 3 g/kg per day (Fig 1). Although there was no statistically significant difference in TSB between different IL intake for the 24- to 28-week-GA group, K was significantly ($P = .01$) lower at the highest IL dose (2.5 or 3 g/kg per day) and was associated with a concomitant significant increase in B_f ($P = .01$). Findings for TSB, K , and B_f were similar when analyses were restricted to 16 infants in the >28 -week-GA group, and 21 infants in the 24- to 28-week-GA group who actually received up to 3 g/kg per day IL (not shown).

Binding Variables As a Function of GA at Each IL Intake

The binding variables were compared at each IL dose between 2 GA groups (Fig 1). The median difference in B_f (9.74 vs 8.37 nmol/L) and K (65 vs 74 L/ μ mol) between the 2 groups on IL intake of 1 g/kg per day (baseline) was not statistically significant. However, at each IL dose of >1.5 g/kg per day, there was statistically significant lower K in infants ≤ 28 weeks' GA compared with infants >28 weeks' GA (Fig 1). There was also significantly higher B_f concentration, despite significantly lower TSB concentration, at each IL dose ≥ 1.5 g/kg per day in ≤ 28 -week-GA infants compared with >28 -week-GA infants.

The mean (SD) birth weights in each 2-week GA subgroup were: (1) 690 (117) g for 24 to 26 weeks' GA; (2) 1005 (178) g for 26¹/₇ to 28 weeks' GA; (3) 1266 (194) g for 28¹/₇ to 30 weeks' GA; (4) 1415 (204) g for 30¹/₇ to 32 weeks' GA; and (5) 1686 (129) g for 32¹/₇ - to 33-week-GA infants. The cumulative frequency of elevated B_f concentration as a function of IL intake was inversely related to GA and was significantly different between GA groups (Table 1). IL intake of ≥ 1.5 g/kg per day may be associated with elevated B_f concentration in ≤ 28 -week-GA infants. None of the infants of >28 weeks' GA had elevated B_f , despite gradual increase in IL intake from 1.5 to 2.5 or 3 g/kg per day.

DISCUSSION

Indirect hyperbilirubinemia is extremely common in premature infants during the first week and may lead to bilirubin-induced neurotoxicity. Current literature suggests that B_f may be a better predictor of bilirubin-induced neurotoxicity than TSB.²¹⁻²⁴ IL provides a concentrated source of calories, and a sustained higher IL intake is essential for optimum growth of premature infants; however, because of the potential bilirubin-displacing effect of FFA generated from IL, IL intake is often limited in premature infants with indirect hyperbilirubinemia. IL intake at which significant bilirubin-displacing effect occurs, as a function of GA, is unclear. Our findings suggest that the amount of IL intake that is tolerated without decreasing bilirubin binding affinity and secondary increase in B_f increases with GA.

Bilirubin bound to proteins, specifically albumin, can be displaced by various anions, including FFA, releasing B_f at a lower concentration of TSB.⁸ The relationship between F/A ratio and the binding of bilirubin to albumin has been evaluated by several investigators.^{4,6,25,26} Spear et al¹⁰ reported linear correlation between B_f concentration and F/A ratio, with infants who had an F/A ratio of >4:1 having the largest increase in B_f . Because FFA was not measured in our study, we were not able to evaluate the relationship between F/A ratio and bilirubin-albumin binding variables as a function of IL intake and GA.

Our findings suggest that IL intake up to 2.5g/kg per day when infused slowly in infants of >28 weeks' GA with indirect hyperbilirubinemia is not associated with a bilirubin-displacing effect and elevated B_f , and may be administered safely. These data complement that of Spear et al¹⁰ who reported an F/A ratio of <3:1 with IL intake of 2 to 3 g/kg per day in infants of >30 weeks' GA. Spear et al also reported that IL intake of 2 to 3 g/kg per day in premature infants of <30 weeks' GA may be associated with an F/A ratio of >4:1 and possible displacement of bilirubin from binding sites, leading to elevated B_f levels. However, because there were only 7 subjects in the group of <30 weeks' GA, additional GA-specific information was unavailable from their study. Brans et al¹² subsequently reported no effect of IL on B_f in 27- to 34-week-GA infants when using a single concentration of peroxidase.

Our findings differ from previous studies by providing information on IL intake that may be associated with elevated B_f concentration and changes in binding affinity in infants of 28 weeks' GA. The critical B_f concentration that may be associated with neurotoxicity in premature infants using the modified peroxidase method is not known. Previous studies that reported $B_f > 1 \mu\text{g/dL}$ to be associated with neurotoxicity in premature infants used an older peroxidase method that vastly underestimates B_f compared with the modified peroxidase method that was used in our study.^{22,27,28} We chose a 90th percentile B_f value as elevated B_f for statistical reasons. Our findings suggest that IL intake of 1.5 and 2 g/kg per day may be associated with elevated B_f and decrease in binding affinity in 24- to 26-week-GA and 26- to 28-week-GA infants, respectively.

Our study also differs from previous studies in the methodology of B_f measurement used and measurement of binding affinity. Previous studies have either involved indirect estimation of B_f by using a fluorometer or saturation index test, or a direct estimation of B_f by using a peroxidase test with a single concentration of peroxidase.¹⁰⁻¹² Although the peroxidase test involving 1:41 dilution of serum provide B_f values that are valid and proportional to true B_f values, it can underestimate bilirubin-displacing effect and true B_f values.^{29,30} Dilution may reduce the free concentration of the bilirubin competitor, attenuating its displacing effect.²⁹ However, a recent study suggests that there is no change in FFA concentration with sample dilution.³¹ To minimize underestimation of B_f , we used a modified peroxidase test.^{17,32} Recent reports suggest that measuring B_f at lesser sample dilution by using peroxidase may further improve accuracy of B_f measurement by decreasing the effect of dilution on intrinsic albumin binding of many ligands, including bilirubin.^{17,33}

A limitation of our findings is that they are based on observational data. There may be other factors besides IL intake, such as sepsis and exposure to steroids, although excluded in the present study, that may have increased FFA and contributed to the bilirubin-displacing effect.^{34–36} Bilirubin displacers other than FFA may also explain some of our findings.⁸ However, potential bilirubin displacers such as sulfonamides, ibuprofen, and ceftriaxone were not used during the study period. The weak displacers when used in combination may also contribute to the bilirubin-displacing effects; however, these weak displacers are usually used at the same dose over several days and, therefore, are unlikely to solely explain the findings. It is also possible that the binding changes may be due to some allosteric changes in albumin or other binding proteins secondary to the worsening of the clinical status of the infant.³⁷ Our findings may only apply to the intravenous lipid emulsion used in this study. Lipid emulsions with a different FFA profile may not have the same effect on bilirubin binding variables.

Although it is difficult to draw conclusions regarding a cause and effect relationship between IL intake and bilirubin-displacing effect in the absence of FFA measurement, the finding of a decrease in binding affinity and a simultaneous increase in B_f despite a similar TSB concentration, with incremental increase in IL intake in infants of 28 weeks' GA, is strongly suggestive of a bilirubin-displacing effect from higher IL intake. Our findings of a difference in cumulative frequency of elevated B_f between different GA groups, as a function of IL intake, also strongly suggest that the bilirubin-displacing effect is from the increase in IL intake and is modified by the degree of prematurity. Increase in TSB concentration may also cause increase in B_f by saturating the binding capacity of albumin. However, TSB concentrations were similar between IL intake groups or lower at higher IL intake. Similarly, TSB concentrations were lower in infants of 28 weeks' GA compared with infants of >28 weeks' GA, and therefore unlikely to solely explain the increase in B_f concentrations. Furthermore, increase in TSB should not decrease the binding affinity. Despite limitations that may be associated with any observational study, the finding of an absence of bilirubin-displacing effect and secondary increase in B_f concentration to a clinically significant concentration in response to a specific IL dose for infants of >28 weeks' GA is most reliable and applicable for clinical use.

CONCLUSIONS

The threshold IL intake at which bilirubin-displacing effect and clinically significant increase in B_f occur is GA dependent. A prospective, well designed, randomized trial with inclusion of FFA measurement is warranted to confirm our findings. The affinity of FFAs seems to depend on the chain length of FFAs; long chain FFAs have higher affinity for albumin-binding sites compared with medium chain FFAs.¹¹ Future randomized studies should also evaluate the effect of IL with different FFA profiles on bilirubin-albumin binding variables by using the modified peroxidase method.

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ABBREVIATIONS

TSB	total serum bilirubin
B_f	free or unbound bilirubin
K	bilirubin/albumin equilibrium association or affinity binding constant
F/A	free fatty acid to albumin molar
GA	gestational age
IL	intravenous lipid emulsion
FFA	free fatty acid

References

1. Andrew G, Chan G, Schiff D. Lipid metabolism in the neonate. II. The effect of Intralipid on bilirubin binding in vitro and in vivo. *J Pediatr*. 1976; 88(2):279–284. [PubMed: 814226]
2. Shennan AT, Bryan MH, Angel A. The effect of gestational age on Intralipid tolerance in newborn infants. *J Pediatr*. 1977; 91(1):134–137. [PubMed: 327047]
3. Ruben S, Kleinfeld AM, Richeiri GV, Hiatt M, Hegyi T. Serum levels of unbound free fatty acids. II: The effect of Intralipid administration in premature infants. *J Am Coll Nutr*. 1997; 16(1):85–87. [PubMed: 9013439]
4. Odell GB, Cukier JO, Ostrea EM Jr, Maglalang AC, Poland RL. The influence of fatty acids on the binding of bilirubin to albumin. *J Lab Clin Med*. 1977; 89(2):295–307. [PubMed: 833468]
5. Thiessen H, Jacobsen J, Brodersen R. Displacement of albumin-bound bilirubin by fatty acids. *Acta Paediatr Scand*. 1972; 61(3):285–288. [PubMed: 5021449]
6. Whittington PF, Burckart GJ, Gross SR, Korones SB, Helms RA. Alterations in reserve bilirubin binding capacity of albumin by free fatty acids. II. In vitro and in vivo studies using difference spectroscopy. *J Pediatr Gastroenterol Nutr*. 1982; 1(4):495–501. [PubMed: 7186064]
7. Wennberg RP, Ahlfors CE, Bhutani VK, Johnson LH, Shapiro SM. Toward understanding kernicterus: a challenge to improve the management of jaundiced newborns [published correction appears in *Pediatrics*. 2006;117(4):1467]. *Pediatrics*. 2006; 117(2):474–485. [PubMed: 16452368]
8. Amin SB. Clinical assessment of bilirubin-induced neurotoxicity in premature infants. *Semin Perinatol*. 2004; 28(5):340–347. [PubMed: 15686265]
9. Bratlid D. How bilirubin gets into the brain. *Clin Perinatol*. 1990; 17(2):449–465. [PubMed: 2196140]
10. Spear ML, Stahl GE, Paul MH, Egler JM, Pereira GR, Polin RA. The effect of 15-hour fat infusions of varying dosage on bilirubin binding to albumin. *JPEN J Parenter Enteral Nutr*. 1985; 9(2):144–147. [PubMed: 4039374]
11. Rubin M, Harell D, Naor N, et al. Lipid infusion with different triglyceride cores (long-chain vs medium-chain/long-chain triglycerides): effect on plasma lipids and bilirubin binding in premature infants. *JPEN J Parenter Enteral Nutr*. 1991; 15(6):642–646. [PubMed: 1766054]
12. Brans YW, Ritter DA, Kenny JD, Andrew DS, Dutton EB, Carrillo DW. Influence of intravenous fat emulsion on serum bilirubin in very low birthweight neonates. *Arch Dis Child*. 1987; 62(2):156–160. [PubMed: 3103546]
13. Kerner JA Jr, Poole RL. The use of IV fat in neonates. *Nutr Clin Pract*. 2006; 21(4):374–380. [PubMed: 16870805]
14. American Academy of Pediatrics, Committee on Nutrition. Use of intravenous fat emulsions in pediatric patients. *Pediatrics*. 1981; 68(5):738–743. [PubMed: 6796933]

15. Shimabuku R, Nakamura H. Total and unbound bilirubin determination using an automated peroxidase micromethod. *Kobe J Med Sci.* 1982; 28(2):91–104. [PubMed: 6285074]
16. Nakamura H, Lee Y. Microdetermination of unbound bilirubin in icteric newborn sera: an enzymatic method employing peroxidase and glucose oxidase. *Clin Chim Acta.* 1977; 79(2):411–417. [PubMed: 890977]
17. Ahlfors CE, Vreman HJ, Wong RJ, et al. Effects of sample dilution, peroxidase concentration, and chloride ion on the measurement of unbound bilirubin in premature newborns. *Clin Biochem.* 2007; 40(3–4):261–267. [PubMed: 17069786]
18. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta.* 1971; 31(1):87–96. [PubMed: 5544065]
19. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics.* 1982; 38(4):963–974. [PubMed: 7168798]
20. Akaike H. Data analysis by statistical models [in Japanese]. *No To Hattatsu.* 1992; 24(2):127–133. [PubMed: 1567644]
21. Calligaris SD, Bellarosa C, Giraudi P, Wennberg RP, Ostrow JD, Tiribelli C. Cytotoxicity is predicted by unbound and not total bilirubin concentration. *Pediatr Res.* 2007; 62(5):576–580. [PubMed: 18049372]
22. Amin SB, Ahlfors C, Orlando MS, Dalzell LE, Merle KS, Guillet R. Bilirubin and serial auditory brainstem responses in premature infants. *Pediatrics.* 2001; 107(4):664–670. [PubMed: 11335741]
23. Amin SB, Charafeddine L, Guillet R. Transient bilirubin encephalopathy and apnea of prematurity in 28 to 32 weeks gestational age infants. *J Perinatol.* 2005; 25(6):386–390. [PubMed: 15843815]
24. Nakamura H, Takada S, Shimabuku R, Matsuo M, Matsuo T, Negishi H. Auditory nerve and brainstem responses in newborn infants with hyperbilirubinemia. *Pediatrics.* 1985; 75(4):703–708. [PubMed: 3982902]
25. Berde CB, Hudson BS, Simoni RD, Sklar LA. Human serum albumin. Spectroscopic studies of binding and proximity relationships for fatty acids and bilirubin. *J Biol Chem.* 1979; 254(2):391–400. [PubMed: 216673]
26. Brodersen R. Binding of bilirubin to albumin. *CRC Crit Rev Clin Lab Sci.* 1980; 11(4):305–399. [PubMed: 6985857]
27. Nakamura H, Yonetani M, Uetani Y, Funato M, Lee Y. Determination of serum unbound bilirubin for prediction of kernicterus in low birthweight infants. *Acta Paediatr Jpn.* 1992; 34(6):642–647. [PubMed: 1285512]
28. Ahlfors CE. Criteria for exchange transfusion in jaundiced newborns. *Pediatrics.* 1994; 93(3):488–494. [PubMed: 8115210]
29. Ahlfors CE. Measurement of plasma unbound unconjugated bilirubin. *Anal Biochem.* 2000; 279(2):130–135. [PubMed: 10706781]
30. Jacobsen J, Wennberg RP. Determination of unbound bilirubin in the serum of newborns. *Clin Chem.* 1974; 20(7):783. [PubMed: 4835230]
31. Huber AH, Kampf JP, Kwan T, Zhu B, Kleinfeld AM. Fatty acid-specific fluorescent probes and their use in resolving mixtures of unbound free fatty acids in equilibrium with albumin. *Biochemistry.* 2006; 45(48):14263–14274. [PubMed: 17128966]
32. Roca L, Calligaris S, Wennberg RP, et al. Factors affecting the binding of bilirubin to serum albumins: validation and application of the peroxidase method. *Pediatr Res.* 2006; 60(6):724–728. [PubMed: 17065581]
33. Boobis SW, Chignell CF. Effect of protein concentration on the binding of drugs to human serum albumin: I. Sulfadiazine, salicylate and phenylbutazone. *Biochem Pharmacol.* 1979; 28(6):751–756. [PubMed: 454474]
34. Amin SB, Sinkin RA, McDermott MP, Kendig JW. Lipid intolerance in neonates receiving dexamethasone for bronchopulmonary dysplasia. *Arch Pediatr Adolesc Med.* 1999; 153(8):795–800. [PubMed: 10437750]
35. Dahn MS, Kirkpatrick JR, Blasier R. Alterations in the metabolism of exogenous lipid associated with sepsis. *JPEN J Parenter Enteral Nutr.* 1984; 8(2):169–173. [PubMed: 6425520]

36. Zaidan H, Dhanireddy R, Hamosh M, Pramanik AK, Chowdhry P, Hamosh P. Effect of continuous heparin administration on Intralipid clearing in very low-birth-weight infants. *J Pediatr.* 1982; 101(4):599–602. [PubMed: 6811713]
37. Goessling W, Zucker SD. Role of apolipoprotein D in the transport of bilirubin in plasma. *Am J Physiol Gastrointest Liver Physiol.* 2000; 279(2):G356–G365. [PubMed: 10915645]

WHAT'S KNOWN ON THIS SUBJECT

IL intake may elevate plasma fatty acid to levels sufficient to compete with bilirubin for albumin-binding sites in neonates.

WHAT THIS STUDY ADDS

IL intake at which bilirubin-displacing effect and clinically significant increase in B_f concentration occur depends on GA.

FIGURE 1.

B_f (nmol/L) (A), binding affinity or constant K ($L/\mu\text{mol}$) (B), and TSB concentration ($\mu\text{mol/L}$) (C) as a function of IL intake and GA ($n = 62$). Analyses were performed using 2-way repeated measure analysis of variance. The symbols “a” and “b” on top of the lines signify the significance of the test comparing GA groups at each level of IL intake (^a $P < .05$; ^b $P < .01$). The Δ on the right signifies the significance of the test comparing the IL dosages within the GA group; $P < .01$.

TABLE 1
Cumulative Frequency of Elevated Free Bilirubin Level (B_f 90th Percentile) as a Function of IL Intake in Premature Infants

	24 to 26 wk GA (N = 11)	26 to 28 wk GA (N = 21)	28 to 30 wk GA (N = 11)	30 to 32 wk GA (N = 11)	32 to 33 wk GA (N = 8)	<i>p</i> ^a
Subjects with EFB at IL 1.5 g/kg per d, n (%)	4 (36)	1 (4)	0 (0)	0 (0)	0 (0)	.015
Subjects with EFB at IL 2 g/kg per d, n (%)	5 (45)	5 (24)	0 (0)	0 (0)	0 (0)	.009
Subjects with EFB at IL 2.5 g/kg per d, n (%)	6 (54)	7 (33)	0 (0)	0 (0)	0 (0)	.001
Subjects with EFB at IL 3 g/kg per d, n (%) ^b	5 (63)	10 (76)	0 (0)	0 (0)	0 (0)	.001

EFB indicates elevated B_f.

^aP values were obtained by Fisher's exact test.

^bFor calculation of percentage of subjects, only subjects who received 3 g/kg per day were included.