2-1-2021

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HHS Public Access

Author manuscript

J Perinatol. Author manuscript; available in PMC 2021 February 18.

Published in final edited form as:

J Perinatol. 2021 February ; 41(2): 247–254. doi:10.1038/s41372-020-00799-5.

Blood *myo*-inositol concentrations in preterm and term infants

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Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information The online version of this article (https://doi.org/10.1038/s41372-020-00799-5) contains supplementary material, which is available to authorized users.

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Abstract

Objective—To describe relationship between cord blood (representing fetal) *myo*-inositol concentrations and gestational age (GA) and to determine trends of blood concentrations in enterally and parenterally fed infants from birth to 70 days of age.

Design/Methods—Samples were collected in 281 fed or unfed infants born in 2005 and 2006. *Myo*-inositol concentrations were displayed in scatter plots and analyzed with linear regression models of natural log-transformed values.

Results—In 441 samples obtained from 281 infants, *myo*-inositol concentrations varied from nondetectable to 1494 μmol/L. Cord *myo*-inositol concentrations decreased an estimated 11.9% per week increase in GA. Postnatal *myo*-inositol concentrations decreased an estimated 14.3% per week increase in postmenstrual age (PMA) and were higher for enterally fed infants compared to unfed infants (51% increase for fed vs. unfed infants).

Conclusions—Fetal *myo*-inositol concentrations decreased with increasing GA. Postnatal concentrations decreased with increasing PMA and were higher among enterally fed than unfed infants.

Introduction

Inositol is present throughout the body and particularly in the central nervous system as an intracellular free sugar alcohol [1]. At least 90% is *myo*-inositol, the major stereoisomer in the body. *Myo*-inositol is additionally a constituent of a number of inositol-phosphates, glycolipids, glycoproteins, and particularly phosphoinositides. The most common phosphoinositide, phosphatidylinositol, is a structural component of various membranous structures and a component of lung surfactant phospholipids. Cord blood concentrations of *myo*-inositol are high in the early gestation and decrease with increasing gestation [2, 3]. Postnatal blood concentrations of *myo*-inositol increase through day 2 and then decrease with increasing postnatal age [3].

Preterm colostrum has higher *myo*-inositol concentrations than mature preterm milk (average 2.24 mmol/L [40.36 mg/dL] vs. 1.34 mmol/L, respectively, in one study and 4.23 mmol/L vs. 1.86 mmol/L in another study) [4–6]. A minimum of 0.96 mg/100 kJ (4 mg/100 kcal) *myo*-inositol was recommended by the American Academy of Pediatrics Committee on Nutrition in 1976 for milk-based formula [7]. A maximum of 9.56 mg/100 kJ (40 mg/100 kcal) was recommended by the American Academy of Pediatrics in 2004 following a recommendation from the Life Sciences Research Organization [8]. Over the ensuing years, increasing amounts of *myo*-inositol have been added to formulas and to breast milk fortifiers; however, no intravenous product has been developed. Two studies conducted in the 1980s have shown higher postnatal concentrations of *myo*-inositol in enterally fed preterm infants than in those receiving parenteral nutrition exclusively [4, 6].

In preparation for a large multicenter trial of *myo*-inositol to prevent retinopathy of prematurity to be conducted by the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network [9], a series of pilot studies [10, 11] were planned to select an appropriate dose and increase understanding of the pharmacokinetics of this endogenous sugar alcohol in the very low birth weight infant. This preparatory observational study was designed [1] to describe the relationship between cord blood (representing fetal) concentrations and gestational age (GA) and [2] to determine trends of blood *myo*inositol concentrations in enterally and parenterally fed infants from birth to 70 days of age, which was the planned duration of *myo*-inositol administration in the trial.

Methods

This was an observational study to describe the concentrations of *myo*-inositol in cord and postnatal blood at various ranges of GA and postmenstrual age (PMA) without *myo*-inositol supplementation and how they are affected by oral feeding. Residual serum or plasma samples from infants born at GA of 24–42 weeks and postnatal age of 0–70 days were scavenged from clinical laboratories and linked to deidentified demographic (gender, GA, size for GA, postnatal age) and feeding data (formula, human milk [HM], supplements, intravenous fluids, all without *myo*-inositol). Characteristics of the sample were recorded (source, type of anticoagulant [none, ethylenediamine-tetra-acetic acid {EDTA}, lithium heparin], time from collection to storage), and a unique code was assigned. Samples of serum/plasma were batched, frozen, and sent to the Pediatric Pharmacology Research Unit at the University of Utah. *Myo*-inositol was measured by high pressure liquid chromatography in 50 μL of serum or plasma; this method analyzes total free *myo*-inositol. This assay is linear from 0 to 1000 μmol/L with a correlation coefficient of 0.997 and a lower limit of detection of 50 μmol/L [12]. Concentrations ranging 50–99 μmol/L were reported as below quantifiable limit (BQL). The chromatographic peak of *myo*-inositol immediately follows that of glucose; however, the chromatographic curve returns to baseline except for very high glucose concentrations, thereby yielding accurate and precise measurements of *myo*-inositol using the peak area ratio of inositol to internal standard. The day-to-day coefficient of variation was 13% for 50 μmol/L, 7% for 100 μmol/L, and <3% for 300 and 700 μmol/L. Intraday coefficient of variation was <2% for values 100–700 μmol/L. The lowest quantified reported value in this study was 100 μmol/L. *Myo*-inositol concentrations are stable when stored at room temperature for up to 1week and are unaffected by anticoagulation with lithium heparin, or EDTA [12].

A target of seven samples was sought for each of four GA groups, at five postnatal age ranges. In addition, for the youngest two GA groups, a similar target sample was sought in the four feeding categories explained in Table 1. Nothing per os (NPO) was defined as no enteral feeding for the previous 72 h. The half-life of *myo*-inositol in preterm infants has been estimated as 5.22 h for single intravenous administration data and 7.90 h for multiple intravenous administration data [10, 11]. We are not aware of any pharmacokinetic study of *myo*-inositol administered enterally in neonates. In adults, the serum concentration of *myo*-inositol after a single enteral dose peaks at 3 h and is back to baseline after 25 h [13]. Thus,

after 72 h NPO (i.e., 6 half-lives beyond 25 h), the majority of *myo*-inositol administered enterally is likely to have been eliminated.

Data analyses included descriptive statistics for *myo*-inositol concentration, including frequencies of results where *myo*-inositol was not detected (ND), BQL and quantifiable as well as first quartile, median, and third quartile values for the quantifiable values. Nonparametric locally weighted scatter-plot smoother (LOESS) curves and associated 95% confidence intervals were plotted over scatter plots to descriptively assess the profile of *myo*-inositol concentrations by GA for cord samples and by PMA for postnatal samples. For cord and postnatal samples separately, the natural log-transformed concentration was modeled employing left-censoring techniques to account for values that were not quantifiable. The left censoring assumes that the nonquantified values are below 100 μmol/L but are not assumed to be a specific value. For cord samples, natural log-transformed inositol concentration was modeled as a function of GA. For postnatal samples, natural log-transformed inositol concentration was modeled as a function of GA, PMA, and feeding category (fed vs. NPO) with a random effect for infant to account for correlation between observations within infant. The final model was selected to fit available data best as determined by the Akaike information criterion (AIC). Results include the parameter estimate and its standard error (SE) and the percentage change per week or for feeding vs. NPO. Predictive curves resulting from the model were plotted over scatter plots to descriptively assess the profile of *myo*-inositol concentrations by GA for cord samples and by PMA for postnatal samples. Sensitivity analyses using linear regression models with imputed values of log-transformed postnatal concentrations instead of left censoring were also performed that explored quadratic and cubic terms of age and size for GA. Statistical analysis was conducted using SAS version 14.2; in particular PROC NLMIXED for the left-censoring models (SAS Institute, Inc., Cary, NC).

The study was reviewed and approved by the IRB at each participating center. Approval with waiver of consent was granted for six of the seven centers, whereas written consent was obtained in one center.

Results

Demographics

The study recruited 281 infants in calendar years 2005–2006. The range of GA at birth was 24–42 weeks with a median GA of 29 weeks. Among the infants sampled, 47% were female. Information on race and ethnicity were incomplete and thus not reported. Among the 441 samples, 1 sample was obtained in 193, 2 samples in 52, 3 samples in 18, and 4–8 samples in 18 infants. The range of PMA for sample collection extended from 22 to 45 weeks (Table 2).

Myo-inositol concentrations

Myo-inositol was measured in serum (18% of the samples) or plasma (74% heparin, 8% EDTA) obtained from 34 cord samples and 407 infant samples (Table 2). *Myo*-inositol was ND in 84 (19%) samples while concentrations were BQL in 63 (14%) samples. The overall

distribution of natural log-transformed *myo*-inositol concentrations is approximately consistent with a normal distribution that is left-censored at 100 μmol/L (Supplementary Fig. 1).

A total of 34 cord samples were obtained and analyzed with *myo*-inositol concentrations appearing to decrease as gestation advanced from 24 to 42 weeks; the relationship was not linear, with progressive flattening of the curve when plotted on a linear scale (Supplementary Table 1, Fig. 1). The parameter estimate for GA was −0.13 (SE = 0.04), corresponding to a 11.9% decrease in cord *myo*-inositol concentrations for each 1 week increase in GA (*P* value = 0.003) (Table 3).

A total of 407 postnatal samples were analyzed, with a general trend of *myo*-inositol concentrations decreasing as PMA increased; the relationship was not linear, with progressive flattening of the curve when plotted on a linear scale (Supplementary Table 1, Fig. 2). The adjusted geometric mean for *myo*-inositol concentration of samples obtained from enterally fed infants are estimated to be increased by 50% compared to samples from the NPO infants.

The parameter estimate for PMA was −0.15 (SE = 0.02) corresponding to a 14.3% decrease in postnatal *myo*-inositol concentrations for each 1 week increase in PMA. The adjusted geometric means for *myo*-inositol concentration (μmol/L) for samples obtained from fed infants were increased by ~50% compared to samples from the NPO infants.

Sensitivity analyses using imputed values of postnatal *myo*-inositol concentrations provided consistent results with respect to significance of parameter estimates and did not show any evidence of an effect of size for GA nor of a cubic or quadratic relationship between age and natural log-transformed *myo*-inositol concentration. As a note, only six samples were from small for GA infants, among which three were BQL, one was ND, and two were quantified.

Discussion

In this study, we observed a large range of mixed cord blood *myo*-inositol concentrations, which decreased with increasing GA. Postnatal *myo*-inositol concentrations decreased after 3 weeks of postnatal age. A linear model of the natural log-transformed concentration with left censoring of nonquantified values fit available data best as determined by AIC criterion (not shown). To our knowledge, this study reports results from the largest dataset of postnatal *myo*-inositol concentrations in newborn infants. Postnatal concentrations decreased with increasing PMA. Concentrations were higher among infants who were feeding compared to those who remained NPO. Preliminary results of this study were used in establishing the dosing of *myo*-inositol in the randomized controlled trial that was later conducted by the NICHD Neonatal Research Network [9].

These data are in agreement with previous publications, which showed a progressive decrease in serum *myo*-inositol concentration with increasing GA and postnatal age and higher concentrations in patients receiving enteral feedings compared to those receiving parenteral nutrition exclusively [2, 4, 14]. No significant relationship was found between *myo*-inositol cord blood concentrations and size for age, possibly due to the small sample

size for growth restricted infants and the high proportion of those values that were not measurable. Intrauterine growth restriction is associated with increased *myo*-inositol concentrations in sheep and human placenta and neonatal human urine [15–19].

Myo-inositol is synthesized in many tissues by cyclisation of glucose-6-phosphate into *myo*-inositol-1-phosphate, which is dephosphorylated by inositol-1-phosphatase to produce inositol [20]. Most inositol is located intracellularly as the free *myo*-inositol stereoisomer. In addition, it is present as a component of *myo*-inositol-containing physiological molecules [20–22]. Since *myo*-inositol has been identified as a second messenger of insulin, it has been proposed as a dietary supplement for women with gestational diabetes [23]. A systematic review of randomized trials found that the only benefit of maternal *myo*-inositol supplementation was a reduced risk for neonatal hypoglycemia [23]. The Na^+/*myo*-inositol cotransporter (SMIT1) has a critical role in developing neural control system, peripheral nerve function and osteogenesis [24–26]. Brain *myo*-inositol depletion in mice missing the Na^+/*myo*-inositol cotransporter leads to central apnea that is prevented by *myo*-inositol supplementation [24, 27].

There is no significant correlation between fetal and maternal serum *myo*-inositol concentrations. Maternal concentrations are stable in pregnancy, while fetal concentrations are five times as high as maternal concentrations in the first trimester and decrease during gestation. Umbilical cord concentrations are higher in the artery than in the vein, consistent with fetal synthesis [3, 28, 29]. Quirk et al. found activity of glucose-6-phosphate: inositol-1-phosphate cyclase, a putative regulatory enzyme in *myo*-inositol synthesis, in human fetal liver and lung and in placenta [29]. High concentrations of fetal *myo*-inositol in early pregnancy may help maintain redox potential in low-oxygen environment in the first trimester and prevent neural tube defects [30, 31]. *Myo*-inositol affects surfactant composition by increasing phosphatidylinositol and the ratio of diphosphatidyl-choline to sphingomyelin while decreasing phosphatidyl-glycerol [32–34]. Sex-related differences in composition of surfactant, including a higher proportion of phosphatidylinositol and GA-dependent progressive increase in ratio of phosphatidyl-choline to sphingomyelin in male preterm infants, may contribute to the sex-related difference in respiratory distress syndrome [35]. Phosphatidylinositol specific phospholipase C, coupled to diacylglycerol lipase action, could provide a mechanism for the release of arachidonic acid for prostaglandin biosynthesis during parturition [36].

The rate of endogenous production of *myo*-inositol in 23–29 week preterm infants at 0.44 ± 1.15 weeks of age (0.36 mmol × kg^{-1} × d^{-1}) is in the range of the amount ingested when receiving full enteral feeds (average in two studies 0.36 and 0.68 mmol × kg^{-1} × d^{-1} in colostrum and 0.21 and 0.30 mmol × kg^{-1} × d^{-1} in HM) [5, 6, 10]. In contrast, the rate of endogenous production of *myo*-inositol in term and late preterm infants (1.52 mmol × kg^{-1} × d^{-1}) far exceeds the amount a breast-fed infant typically ingests [4–6, 37].

Myo-inositol clearance by the kidney involves both glomerular filtration and catabolism in the parenchyma by *myo*-inositol oxygenase and aldehyde reductase [38–40]. Urinary *myo*-inositol excretion, large at birth, decreases progressively in parallel with decreasing serum concentrations and with maturation of aldehyde reductase in the proximal tubule [14]. The

premature fetus likely secretes *myo*-inositol-rich urine into the amniotic fluid that is swallowed and recycled back into the fetus [1]. It is not known whether prolonged rupture of the fetal membranes or some other pregnancy complication leads to *myo*-inositol depletion at very premature birth. In the present study, 20% of infants born before 30 weeks of gestation had cord blood concentrations that were not quantifiable (i.e., BQL μmol/L). In a randomized trial, intravenous *myo*-inositol supplementation starting at 4 to 12 h after birth resulted in a remarkable increase in serum *myo*-inositol and decrease in severity of respiratory distress [31]. Since most of the infants born with a mean gestation of 28 weeks in this early trial received neither antenatal steroids nor surfactant, these results are not replicated at present.

Previous studies have shown an inverse relationship between serum concentrations of *myo*-inositol and severe retinopathy of prematurity [41]. This led to a series of pilot trials and then a large randomized trial of *myo*-inositol supplementation, starting from a mean age of 2.8 days and continuing up to 10 weeks, which eventually did not show a benefit [9–11].

Strengths of this study include large sample size, rigorous methods designed to find the best model to fit the data, multivariate analysis including GA, PMA, and feeding category, and multiple centers.

One limitation of the study is that samples were measured in 2006 using an HPLC method with overlap between BQL threshold (100 μmol/L) and expected blood concentration (~30–250 μmol/L [3, 4]), resulting in a high proportion of values that were ND or BQL. This may have resulted in a bias with higher levels of median concentration in samples that were quantified. A more recent technique has been described, in which *myo*-inositol is separated from glucose and other hexose monosaccharides using a lead-form resin-based column and measurement using liquid chromatography-double mass spectrometry allows accurate and precise measurements of low *myo*-inositol concentrations below 50 μmol/L [42]. Additional limitations of the study include a small number of cord blood samples, a lack of separation of umbilical arterial and venous blood, lack of prospective data collection at rigorous time points, missing samples, lack of measurement of *myo*-inositol in HM or formula, lack of assessing changes in *myo*-inositol concentration over time, and lack of analysis by race/ethnicity. Thus, it is possible that we may have missed significant differences between groups.

In summary, in the absence of *myo*-inositol supplementation, cord *myo*-inositol concentrations decreased with increasing GA. Postnatal concentrations decreased with increasing PMA and were higher among infants who were feeding compared to those who remained NPO. Present and previous data [8, 31] suggest that in some cases serum concentrations of *myo*-inositol at birth are low.

Data availability

Data reported in this paper may be requested through a data use agreement. Further details are available at https://neonatal.rti.org/index.cfm?fuseaction=DataRequest.Home.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The National Institutes of Health and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), through the Neonatal Research Network and the Pediatric Pharmacology Research Units Network, and the National Eye Institute provided grant support for the Inositol Cross-Sectional Blood study. The study was planned in 2004; recruitment was in 2005–2006. While NICHD staff did have input into the study design, conduct, analysis, and manuscript drafting, the content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Data collected at participating sites of the NICHD Neonatal Research Network (NRN) were transmitted to RTI International, the data coordinating center (DCC) for the network, which stored, managed, and analyzed the data for this study. On behalf of the NRN, Dr. Abhik Das (DCC Principal Investigator) and Ms. Lisa Wrage (DCC Statistician) had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. The NICHD Pediatric Pharmacology Research Unit (PPRU) network provided support and advice in designing the PK study (JVA, JVDA, AAV, SEK, RMW), analyzing the data (SEK), and conducting the Inositol Assay (RDL): Jacob V. Aranda, MD PhD FRCPC, Wayne State University (U10 HD37261); John van der Anker, MD, Children's National Medical Center (U10 HD45993); Steven E. Kern, PhD, College of Pharmacy, Robert M. Ward, MD, Department of Pediatrics, University of Utah Medical Center (U10 HD45986); Alexander A. Vinks, PharmD PhD FCP, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati (U10 HD37249); Richard D. Leff, PharmD FCCP, Texas Tech University Health Sciences Center, Pediatric Pharmacology Research & Development Center (U10 HD46000). We are indebted to our medical and nursing colleagues and the infants and their parents who took part in this study.

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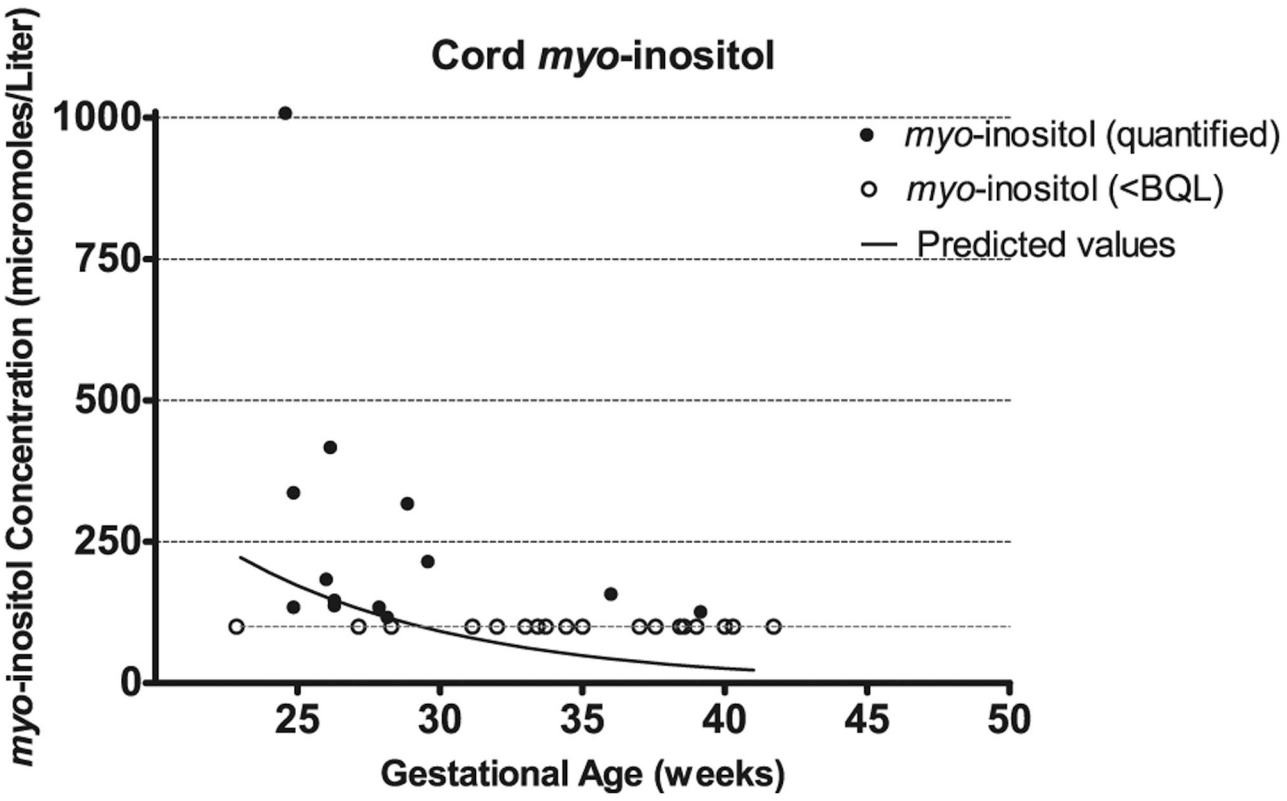

Fig. 1. Cord *myo*-inositol concentration with prediction curves resulting linear regression models of natural log-transformed *myo*inositol concentration with left censoring by gestational age at birth (weeks).

Left-censored includes BQL, below quantifiable limit (<100 μmol/L), and not detected, 0–50 μmol/L.

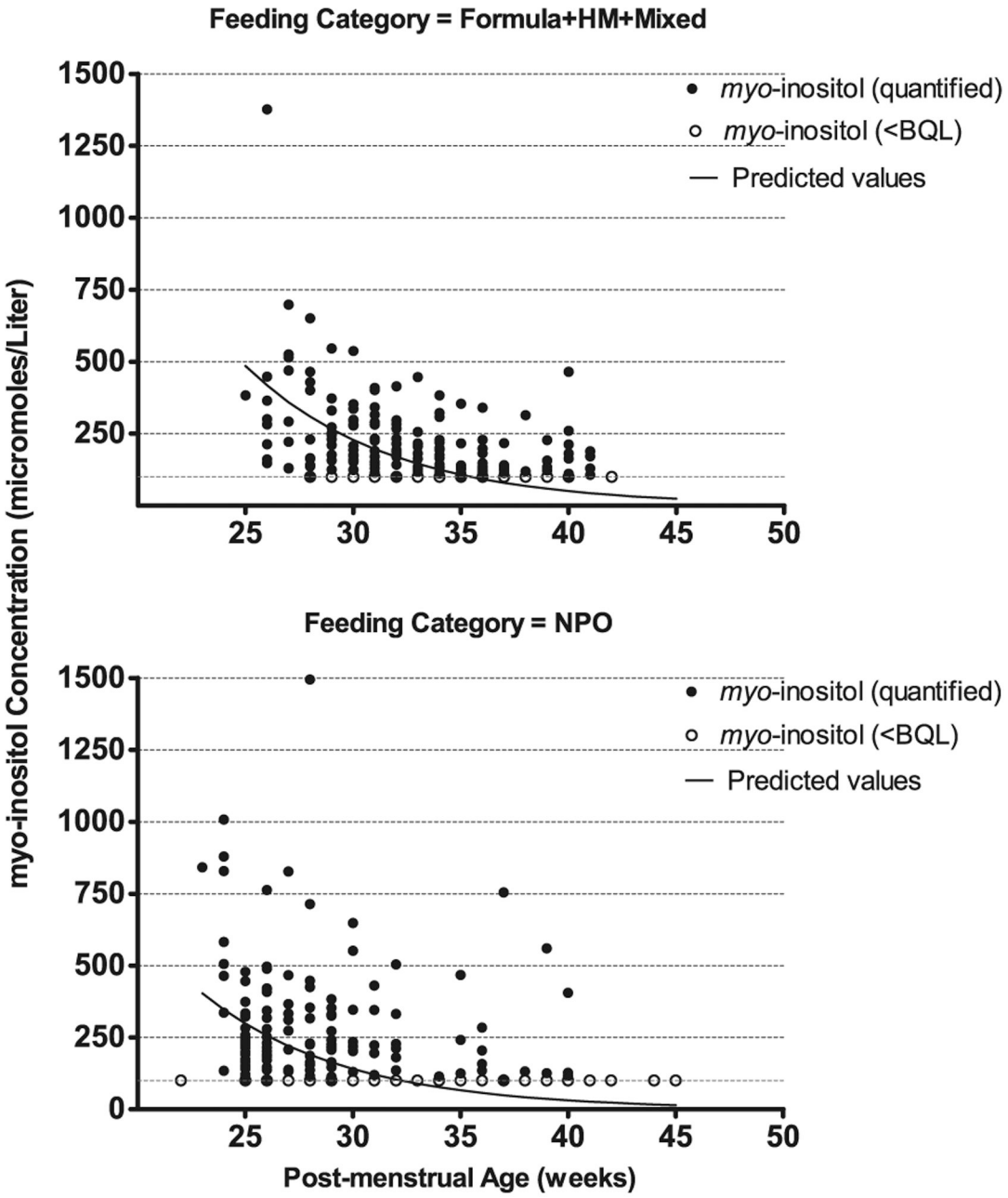

Fig. 2. Serial *myo*-inositol concentration with prediction curves resulting linear regression models of natural log-transformed *myo*inositol concentration with left censoring by PMA (weeks) and feeding category (enterally fed, upper panel; NPO, lower panel).

Left-censored includes BQL, below quantifiable limit (<100 μmol/L), and not detected, 0–50 μmol/L. For the linear model, a random effect for infant was included to account for correlation between observations within infant.

Table 1

Number of blood samples in predefined gestational age and postnatal age groups obtained for inositol concentrations.

Gestational age (weeks)	Postnatal age					Total
	Cord	1–7 days	8–21 days	22–42 days	43–70 days	
24–26	8	53	36	35	30	162
27–29	7	28	35	33	20	123
30–35	8	27	31	17	14	97
36–42	11	25	18	3	2	59
Total	34	133	120	88	66	441

A target of seven samples was sought for each of the four gestational age groups, at five postnatal age ranges. In addition, for the youngest two GA groups, a similar target sample was sought in the four feeding categories. The feeding categories were defined, based on intake in the last 72 h prior to sampling, as exclusive intravenous (IV) feeding (nothing per os, NPO), >80% formula, >80% human milk [HM], or mixed IV/HM/formula).

Table 2

Demographics and sample description.

Infant characteristic	**Statistic/category**	**All infants (*N* = 281)**
Gestational age (weeks)	Mean (SD)	30.4 (4.9)
	Median (Q1, Q3)	29.0 (26.3, 33.7)
Size	SGA	33 (12%)
	AGA	244 (87%)
	LGA	4 (1%)
Gender	Female	131 (47%)
	Male	150 (53%)
Number of samples	Median (Min, Max)	1 [1, 8]
Sample characteristic	**Statistic/category**	**All samples (N = 441)**
Postmenstrual age (weeks)	<27	74 (17%)
	27–29	91 (21%)
	30–35	177 (40%)
	36–45	99 (22%)
Anticoagulant use	Serum	80 (18%)
	Plasma-EDTA	33 (7%)
	Plasma-heparin	328 (74%)
Sample type	Cord	34 (8%)
	Infant	407 (92%)

Q1 first quartile, *Q3* third quartile, *SGA* small for gestational age, *AGA* appropriate for gestational age, *LGA* large for gestational age, *EDTA* ethylenediamine-tetraacetic acid.

Table 3

Modeling results for natural log-transformed inositol concentration.

Model by type of sample	Factor	Parameter estimate (SE)	Derived estimate	Derived estimate	Type 3 *P* values
Model 1: Cord	GA (Week)	−0.13 (0.04)	% Change for 1-week change in GA	−11.9%	0.003
Model 2: Postnatal	GA (Week)	0.09 (0.02)	% Change for 1-week change in GA	9.2%	<0.001
	PMA (Week)	−0.15 (0.02)	% Change for 1-week change in PMA	−14.3%	<0.001
	Feeding vs. NPO	0.41 (0.09)	% Increase in geometric mean for Feeding vs. NPO	51.0%	<0.001

Feeding includes formula only, human milk only, or mixed intake. Natural log-transformed inositol concentration was modeled using linear regression models with left censoring to account for concentrations that were not detected or below quantifiable limits. Separate models were used for cord and postnatal samples. For postnatal/serial samples, a random effect for infant was included to account for correlation between observations within infant. The derived estimate converts the Parameter Estimate to a percent change per week in inositol concentration.

SE standard error, *GA* gestational age, *PMA* postmenstrual age, *NPO* nothing per os.

J Perinatol. Author manuscript; available in PMC 2021 February 18.