Gestational Age and Age at Sampling Influence Metabolic Profiles in Premature Infants

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acylcarnitine, amino acids, newborn screening, and nutrition

ABBREVIATIONS
ANOVA—analysis of variance
EGA—estimated gestational age

Dr Clark has contributed to the conception and design of the study. The article from first to final draft was written and edited by Dr Clark with the help of his co-authors who offered suggestions for improvement; Mrs Kelleher has contributed to the drafting and revising of the article for important intellectual content; Dr Chace has contributed to the conception and design of the study analysis and interpretation of data; Dr Spitzer has contributed to the conception and design of the study, analysis and interpretation of data, and critical revisions for important intellectual content; and all authors approved the final manuscript as submitted.

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WHAT'S KNOWN ON THIS SUBJECT: Prematurely born infants commonly have abnormal metabolic screens.

WHAT THIS STUDY ADDS: Both gestational and chronological age influence metabolic profiles used to screen for inborn errors of metabolism.

abstract

OBJECTIVE: To describe the influence that gestational age and chronological age have on amino acid and acylcarnitine profiles in an at-risk population of premature infants.

METHODS: Metabolic profiles (15 amino acids and 35 acylcarnitines) were obtained by using standard newborn techniques on infants born between 23 and 31 completed weeks of gestation. The profiles were drawn within the first 24 hours after birth and on approximately days 7, 28, and 42 of life or at discharge. A single, central, contract laboratory analyzed and managed the samples.

RESULTS: We studied 995 patients; none was subsequently diagnosed with an inborn error of metabolism. Of the 3579 samples, there were 257 (7.2%) amino acid or acylcarnitine alerts reported in 214 infants (21.5% of infants studied). Both gestational age and postbirth chronological age significantly influenced the metabolic profile. Twenty-nine percent of infants at 23 to 26 weeks' gestational age had an abnormal metabolic profile compared with 17% of infants at 29 to 31 weeks' gestational age ($P < .01$). On the day of birth, 12% of the profiles were abnormal compared with 2% on day 28 ($P < .01$). The highest rate of abnormal values occurred on day 7 in the infants 23 to 26 weeks' gestational age (21%).

CONCLUSIONS: These results demonstrate the complexity of understanding the impact of immaturity and disease on metabolic profiles used to screen for inborn errors of metabolism. Our data provide reference values for studies aimed at better understanding metabolism in preterm infants. *Pediatrics* 2014;134:e37–e46

We previously reported that the amount of amino acids provided in parenteral nutrition influences blood amino acid and acylcarnitine values. Other investigators have reported similar results.[1–5] Establishing a consensus regarding what represents a normal metabolic profile in premature infants would allow investigators to identify infants with abnormal values and to evaluate the impact of those abnormal values on important health outcomes (liver injury, growth, and neurodevelopmental outcomes). Failure to establish normal values undermines our understanding of amino acid and fatty acid metabolism in premature infants. The purpose of this study is to describe the influence that gestational age and postbirth chronological age have upon amino acid and acylcarnitine profiles, to better understand what represents "normal" for this stressed and at-risk population.

METHODS

Study Subjects

We included inborn infants between the gestational ages of 23 weeks and 0/7 days and 31 weeks and 0/7 days and excluded infants if they had major congenital anomalies. Parental consent was obtained for each subject. The protocol was approved by the Western Institutional Review Board and/or each site's local institutional review board.

Nutritional Support

Fluid management and nutritional guidelines were used as per site specific protocol. Data on precisely what was received by the study subject and growth parameters were collected on postnatal days 1, 7, 28, and 42.

Metabolic Profiles

An experienced health care professional used newborn screening consensus protocols for obtaining study and state screening blood samples. There was no charge for study samples, and the samples were drawn when other laboratory tests were done. Study filter papers were provided at the site initiation visit, and the precise procedure for collection of blood samples was reviewed. If scavenged blood was used, the sample had to be obtained from a line that did not contain amino acids, glucose, or lipids to prevent contamination. The date and time of the sample was recorded on the case report form and the filter paper. Cord blood was not used for the initial sample. Filter papers were not batched; they were mailed to the central laboratory within 24 hours of collection and analyzed within 48 hours of arrival.

Metabolic profiles were collected within the first 24 hours after birth (median age in hours = 16 hours), on approximately day 7 (7–8), on day 28 (27–29), and on day 42 (41–43) of life or at discharge, whichever came first. All patients were followed until the time of discharge to collect data on morbidity and mortality. Investigators were instructed to obtain the initial metabolic profile sample before the start of parenteral nutrition. This approach proved difficult to accomplish because all sites included some amino acids in the initial intravenous fluids (range, 1–3 g/100 mL).

A central, contract laboratory (PerkinElmer Genetics, Bridgeville, PA) analyzed the study samples by using tandem mass spectrometry. Alert values were set as defined by Chace et al.[6,7] The reference values for healthy newborn term infants were defined as per our previous study.[8] Investigators were notified if a sample value fell outside of the reference range for normal term newborns and a plan for follow-up was discussed. We collected data on all abnormal results reported by the state and study laboratories.

Data Collection

An electronic system was used to assign unique study codes and collect data on each subject. Clinical Research Associates monitored each site for adherence to the protocol and data accuracy.

Statistical Analysis

Categorical variables were evaluated by 2-tailed χ^2 and Fisher's exact test. Continuous variables were compared by using a 2-tailed analysis of variance (ANOVA) for parametrically distributed data and Kruskal-Wallis ANOVA for nonparametrically distributed data. ANOVA for repeated measures was used to evaluate the changes in laboratory and nutritional values over time, and within each estimated gestational age (EGA) group. A comparison for all possible pairs was done by using Tukey–Kramer tests. To summarize and compare the values obtained for each analyte, we calculated a z score for each patient by using the mean and SD values from a normal term infant population sample reported in our previous work.[8] The z score for each value was calculated: observed value minus the mean value of a normal term infant/divided by the SD from the normal term infant sample. A median z score of >1 or less than -1 means that 50% of the patients had a value that was >1 SD away from the mean value seen in term infants (Figs 1 and 2).

RESULTS

There were 1002 unique subjects enrolled from 23 sites in 17 states. Six patients had anomalies that were found after they were enrolled; these patients were excluded (2 with Trisomy 21, 1 bowel atresia, 1 chromosomal abnormality not defined, 1 Nager Syndrome/type II, and 1 chromosome 22Q duplication). One subject had a duplicate study code generated; leaving 995 subjects for analyses. On follow-up, no study subject was diagnosed with an inborn error of metabolism.

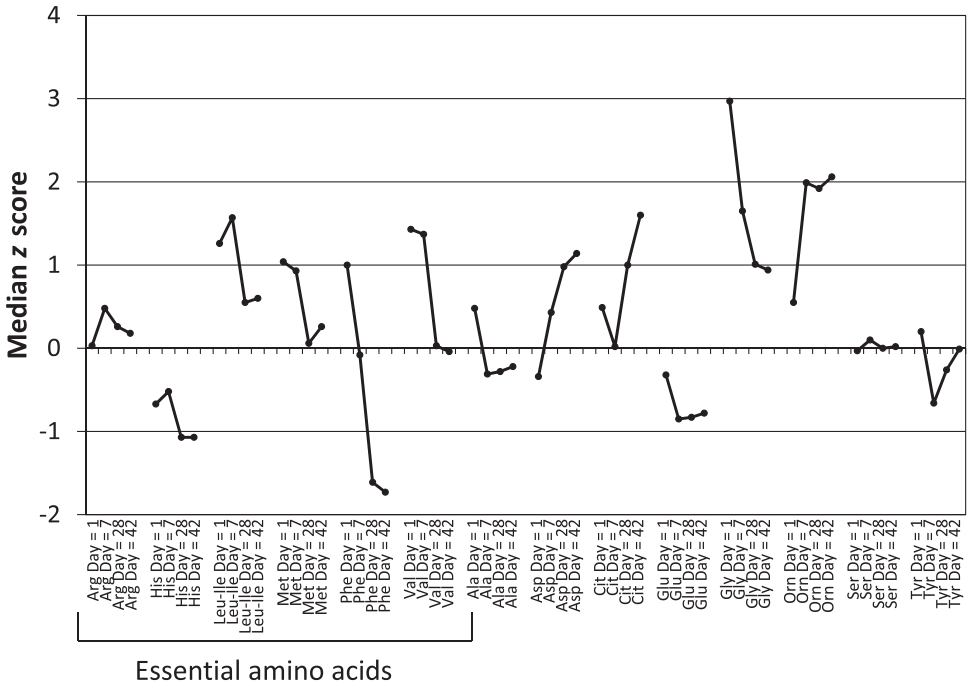

FIGURE 1
Median z score values for amino acid analytes.

Population Characteristics

Characteristics are described as median (10–90th percentile): birth weight 1057 (644–1514) grams, EGA was 28 (24–30) weeks; Apgar at 1 minute 6 (1–8); Apgar at 5 minutes 8 (5–9; Table 1).

Before analysis, the 995 infants were subdivided into 3 EGA groups; 23 to 26 weeks (n = 293); 27 to 28 weeks (n = 277); and 29 to 31 weeks (n = 425). These subgroups were selected based on sample size (goal >200) and degree of prematurity. Each EGA group is distinctive; the more immature infants are smaller, had lower Apgar scores, less often were white, and were more often treated with steroids, surfactant, and insulin. The most immature infants (23 to 26 weeks) had more morbidity and it took them longer to reach full feeds (Table1).

Nutrition

Parenteral nutrition was started at a median of 2 (1 to 25) hours; the median amount of glucose given on day 1 was 7 (5–9) g/kg per day. Lipids were started at a median of 27 hours (9–48) at 2 (1–3) g/kg per day. In 390 (39%) infants, carnitine was added to the parenteral nutrition and the proportion of infants given carnitine increased with decreasing gestational age. The majority of early nutrition (≤7 days) was provided by intravenous glucose, amino acids, and fatty acids. Most (78%) infants did not receive enteral feedings during the 24 hours after birth. Infants who were fed received <30 cc/kg per day (Table 2). Infants in the 23 to 26 EGA group were exposed to higher volumes of intravenous fluids, were on parenteral nutrition longer, and took more time to get to full feedings (Tables 1 and 2). The peak use of intravenous lipids and amino acids was on day 7. Over time there was a shift from parenteral to enteral nutrition. By day 42, all 3 groups approached 120 calories/kg per day and most infants (84%) were receiving strictly enteral feedings. Growth velocities for each EGA group were similar, but the highest growth velocity was in the more mature infants (29 to 31 weeks) and during the enteral phase of nutrition.

Abnormal Newborn Screens

Differences Across State Newborn Screen

We requested that each site provide their newborn state screen panel and alert values. We received 15 of the 17 state references. Although the panels and the alert limits were similar, none were exactly the same. What is measured, units of measurement, alert levels, and analytes used as primary and secondary markers for identification of disease were different for each state. (The National Newborn Screening and Genetics Resource Center Web site http://genes-r-us.uthscsa.edu/sites/genes-r-us/files/nbsdisorders.pdf).

State Newborn Screen Results

There is an important limitation to our observations. Each state has its own panel and recommended procedures

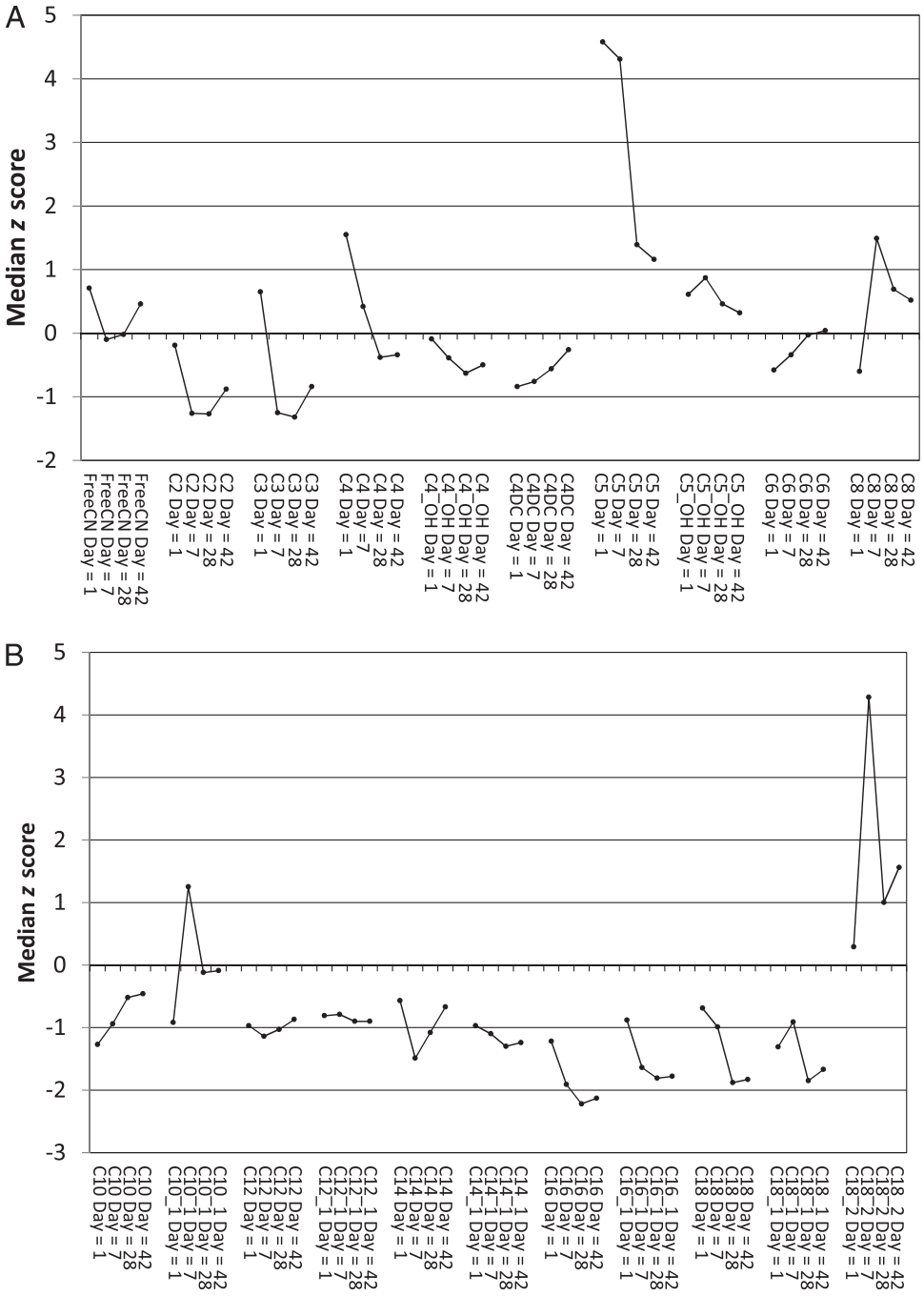

FIGURE 2
Median *z* score values for acylcarnitine analytes (A and B).

for identifying infants with inborn errors of metabolism and there are no accepted standards that are universally applied.[9–11]

The initial state screening test was abnormal and a repeat study was requested in 461 (46%) of the 995 infants studied. The more immature infants most often had an abnormal state screen (Table 1; 64% vs 36%, $P < .01$). The most common abnormalities reported by the state laboratory were as follows: low thyroxine or high thyroid-stimulating hormone (n = 263, 26.4%); elevated 17-hydroxyprogestrone (n = 78, 7.8%); abnormal amino-acid profile (n = 89, 8.9%), and abnormal acylcarnitine profile (n = 26, 2.6%). The most common abnormal amino acids were elevations of methionine (n = 43, 4.3%), tyrosine (n = 26, 2.6%), and/or leucine (n = 16, 1.6%). The most common acylcarnitine elevation was isovalerylcarnitine (C5; n = 14, 1.4%).

TABLE 1 Population Characteristics

	23–26	27–28	29–31	Group Differences	All Enrolled
Number of study subjects	293	277	425	—	995
Demographics					
Cesarean section, *n* (%)	200 (68.3)	200 (72.2)	325 (76.5)	.05	725 (72.9)
Antenatal steroids, *n* (%)	253 (86.3)	243 (87.7)	383 (90.1)	.4	879 (88.3)
Maternal metabolic disease, *n* (%)					
Hyperthyroidism	3 (1)	1 (0.4)	0 (0)	.4	4 (0.4)
Maternal thyroxine use	10 (3.4)	13 (4.7)	18 (4.2)	.7	41 (4.1)
Multiple gestations, *n* (%)	73 (25)	58 (21)	148 (35)	<.001	279 (28)
Boy, *n* (%)	154 (52.6)	121 (43.7)	231 (54.4)	.01	506 (50.9)
Race, *n* (%)					
American Indian	6 (2)	9 (3.2)	6 (1.4)	—	21 (2.1)
Asian	8 (2.7)	10 (3.6)	9 (2.1)	—	27 (2.7)
African American	99 (33.8)	72 (26)	80 (18.8)	<.001	251 (25.2)
Hispanic	33 (11.3)	26 (9.4)	41 (9.6)	—	100 (10.1)
Other	1 (0.3)	2 (0.7)	7 (1.6)	—	10 (1)
Pacific Islander	2 (0.7)	1 (0.4)	0 (0)	—	3 (0.3)
White	144 (49.1)	156 (56.3)	282 (66.4)	<.001	582 (58.5)
Apgar 1 min, median (10–90th)	4 (1–7)	5 (1–8)	7 (3–8)	<.001	6 (1–8)
Apgar 5 min, median (10–90th)	7 (3–9)	8 (6–9)	8 (6–9)	<.001	8 (5–9)
EGA (wk), median (10–90th)	25 (23–26)	28 (27–28)	30 (29–30)	<.001	28 (24–30)
Birth weight (g), median (10–90th)	750 (522–988)	1040 (732–1279)	1290 (981–1670)	<.001	1057 (644–1514)
Nutrition data					
Type of amino acids, *n* (%)					
Aminosyn	19 (6.5)	22 (7.9)	36 (8.5)	.5	77 (7.7)
Premasol	123 (42)	104 (37.5)	149 (35.1)	.5	376 (37.8)
Trophamine	150 (51.2)	147 (53.1)	230 (54.1)	.5	527 (53)
Intravenous nutrition fluids covered, *n* (%)	91 (31.1)	108 (39)	153 (36)	.1	352 (35.4)
Carnitine added to intravenous nutrition, *n* (%)	158 (53.9)	105 (37.9)	127 (29.9)	<.001	390 (39.2)
Age amino acids started (h), median (10–90th)	2 (1–23)	2 (0–25)	2 (1–27)	.6	2 (1–25)
Age lipids started (h), median (10–90th)	27 (4–53)	28 (11–44)	27 (10–45)	.2	27 (9–48)
Total days of amino acids, median (10–90th)	23 (9–42)	17 (8–37)	11 (6–25)	<.001	15 (7–37)
Age first enteral feed (day), median (10–90th)	2 (1–8)	2 (1–5)	1 (1–4)	<.001	2 (1–5)
Age full feeds (days), median (10–90th)	25 (13–45)	17 (10–31)	11 (7–22)	<.001	15 (8–35)
Medications					
IV postnatal steroids in first 42 d, *n* (%)	101 (34.5)	23 (8.3)	9 (2.1)	<.001	133 (13.4)
Both	12 (4.1)	0 (0)	0 (0)	<.001	12 (1.2)
Dexamethasone	21 (7.2)	9 (3.2)	1 (0.2)	<.001	31 (3.1)
Hydrocortisone	68 (23.2)	14 (5.1)	8 (1.9)	<.001	90 (9)
Age first dose steroids, d, median (10–90th)	10 (0–36)	19 (0.4–46.6)	20 (1–43)	.1	14 (0–37)
Insulin used within 42 d of birth, *n* (%)	64 (21.8)	14 (5.1)	6 (1.4)	<.001	84 (8.4)
Age insulin first used, d, median (10–90th)	2 (0.5–13)	2.5 (0.5–10)	2.5 (1–28)	.9	2 (1–11)
Surfactant, *n* (%)	287 (98)	231 (83.4)	234 (55.1)	<.001	752 (75.6)
Outcomes					
State screen abnormal, repeat requested, *n* (%)	188 (64)	119 (43)	154 (36)	<.001	461 (46.3)
Study screen abnormal, *n* (%)[a]	85 (29)	57 (21)	72 (17)	<.001	214 (21.5)
Severe intraventricular hemorrhage, *n* (%)	47 (16)	10 (3.6)	4 (0.9)	<.001	61 (6.1)
Cystic PVL, *n* (%)	25 (8.5)	6 (2.2)	7 (1.6)	<.001	38 (3.8)
Acquired bowel disease, *n* (%)	50 (17.1)	21 (7.6)	23 (5.4)	<.001	94 (9.4)
Isolated intestinal perforation	13 (4.4)	5 (1.8)	0 (0)	.01	18 (1.8)
Necrotizing enterocolitis treated medical	20 (6.8)	11 (4)	19 (4.5)	.01	50 (5)
Necrotizing enterocolitis treated surgical	17 (5.8)	5 (1.8)	4 (0.9)	.01	26 (2.6)
Age bowel disease first reported, d, median (10–90th)	18 (7–56.8)	19 (2.2–32.4)	13 (8–33.3)	<.001	17 (5.4–41.8)
Retinopathy of the premature, *n* (%)					
ROP 2	41 (14)	15 (5.4)	9 (2.1)	<.001	65 (6.5)
ROP 3	9 (3.1)	2 (0.7)	0 (0)	<.01	11 (1.1)
ROP surgery	30 (10.2)	3 (1.1)	0 (0)	<.001	33 (3.3)
Transfused, *n* (%)	275 (93.9)	178 (64.3)	117 (27.5)	<.001	570 (57.3)
Age first transfuse (d), median (10–90th)	2 (0–10)	9 (1–26)	15 (1.8–38.2)	<.001	5 (0–25)
Cholestasis study definition, *n* (%)	35 (11.9)	8 (2.9)	6 (1.4)	<.001	49 (4.9)
Cholestasis reported as an adverse event, *n* (%)	43 (14.7)	10 (3.6)	11 (2.6)	<.001	64 (6.4)
Cholestasis treated, *n* (%)	36 (12.3)	11 (4)	7 (1.6)	<.001	54 (5.4)
Phenobarbital	9 (3.1)	1 (0.4)	1 (0.2)	—	11 (1.1)

TABLE 1 Continued

	23–26	27–28	29–31	Group Differences	All Enrolled
Phenobarbital and ursodeoxycholic acid (ursodiol)	8 (2.7)	4 (1.4)	1 (0.2)	—	13 (1.3)
Ursodeoxycholic acid (ursodiol)	18 (6.1)	6 (2.2)	5 (1.2)	—	29 (2.9)
Ursodeoxycholic acid (ursodiol) & cholestyramine	1 (0.3)	0 (0)	0 (0)	—	1 (0.1)
Died, *n* (%)	54 (18.4)	10 (3.6)	4 (0.9)	<.001	68 (6.8)
Age died (d), median (10–90th)	10 (2–73)	12 (4–126.6)	10.5 (1–29)	.9	10 (2–63.4)
Age study exit, median (10–90th)	86 (9–140)	63 (37–97.8)	45 (30–71)	.001	56 (26–111)

[a] Does not have thyroid panel.

Central Laboratory Alerts Based on Chace et al Values[6]

Of 3579 samples, there were 257 (7.2%) amino acid or acylcarnitine alerts reported in 214 infants (21.5% of infants studied). We did not include thyroid screening as a part of our study alerts. Similar to the state screening results, the most common alerts were in the most immature infants and most often occurred on days 1 and 7 (Table 1). Twenty-nine percent of infants who were 23 to 26 weeks' EGA had an abnormal metabolic profile compared with 17% of infants 29 to 31 weeks' EGA ($P < .01$). On the day of birth, 12% of the profiles were abnormal compared with 2% on day 28 ($P < .01$); the highest rate of abnormal values occurred on day 7 in the infants 23 to 26 weeks' EGA (21%).

Of the 257 alerts, the most common reports were as follows: elevated methionine ($n = 89$); generalized elevation of short-chain and medium chain acylcarnitine possibly consistent with L-carnitine supplementation ($n = 69$); mild elevation of multiple amino acids including phenylalanine, leucine, and methionine ($n = 22$); elevation in concentrations of propionylcarnitine (C3) and other indices, such as the relative ratios of propionylcarnitine (C3) to acetylcarnitine (C2) or propionylcarnitine (C3) to palmitoylcarnitine (C16; $n = 15$); elevation in tyrosine ($n = 15$); elevation in methionine and tyrosine ($n = 7$); and moderate elevation of multiple amino acids including phenylalanine, leucine, and methionine ($n = 5$).

RESULTS OF METABOLIC PROFILES

Comparison of Preterm to Term Controls

With the exception of arginine, serine, free carnitine, malonylcarnitine (C3-DC)

TABLE 2 Growth and Nutrition

	EGA Group	Day 1	Day 7	Day 28	Day 42
Weight (kg)	23–26	750 (522–988)	730 (540–928)	1020 (795–1354)	1290 (960–1754)
	27–28	1040 (732–1279)	1005 (752–1234)	1410 (1053–1711)	1771 (1311–2165)
	29–31	1290 (981–1670)	1250 (970–1590)	1785 (1410–2259)	2180 (1767–2669)
Head circumference (cm)	23–26	23 (20.5–25)	22.5 (20.5–24.5)	24.5 (22.5–27)	27 (24–29)
	27–28	25.5 (23–27)	25 (23–27)	27.5 (25.5–29.4)	29.5 (27–31.5)
	29–31	27.5 (25.5–29.5)	27 (25.2–29)	30 (28–32)	31.5 (30–33)
Length (cm)	23–26	32 (28.5–35.5)	33 (29–36.5)	35 (32–39.4)	37.5 (33.5–41)
	27–28	36 (32–39)	37 (32.5–39.7)	39.4 (35–42.5)	41.5 (38–45)
	29–31	39 (35–42)	39 (36–42)	42 (38.5–45.7)	44 (40.5–47.5)
Total intravenous fluids (mL/kg per d)	23–26	102 (75–144)	139 (105–180)	23 (0–135)	0 (0–107)
	27–28	90 (70–118)	123 (68–156)	0 (0–117)	0 (0–63)
	29–31	82 (69–99)	92 (0–146)	0 (0–32)	0 (0–0)
Intravenous amino acids (g/kg per d)	23–26	1.7 (1–2.6)	3.1 (2–3.8)	0 (0–3.5)	0 (0–3.1)
	27–28	1.9 (1.2–2.9)	3 (1.4–3.9)	0 (0–2.8)	0 (0–1.9)
	29–31	1.8 (0.5–2.5)	2.4 (0–3.8)	0 (0–0.7)	0 (0–0)
Intravenous lipids (g/kg per d)	23–26	0 (0–0.6)	2 (0.6–3.1)	0 (0–2.9)	0 (0–2.3)
	27–28	0 (0–0.7)	2.4 (0.7–3.2)	0 (0–1.9)	0 (0–1.4)
	29–31	0 (0–0.7)	2 (0–3.1)	0 (0–0)	0 (0–0)
Intravenous glucose (g/kg per d)	23–26	6.6 (4.5–9)	10 (6.4–13.4)	1.9 (0–13.2)	0 (0–10.7)
	27–28	7.3 (5.2–9.6)	10.8 (5.8–14.8)	0 (0–11.7)	0 (0–6.4)
	29–31	7.4 (5.5–9.1)	8.5 (0–13.9)	0 (0–2.1)	0 (0–0)
Total enteral feedings (mL/kg per d)	23–26	0 (0–1)	8 (0–46)	118 (0–157)	140 (13–161)
	27–28	0 (0–4)	23 (0–87)	148 (10–162)	151 (71–169)
	29–31	0 (0–7)	60 (9–138)	151 (106–167)	157 (129–188)
Total calories (cal/kg per d)	23–26	31 (22–43)	76 (54–100)	107 (71–128)	116 (82–136)
	27–28	35 (26–46)	92 (69–115)	120 (87–134)	122 (94–140)
	29–31	35 (26–43)	100 (77–119)	120 (102–136)	120 (99–144)

Data include 0 value if no fluids or feedings were being given; data presented as median (10–90th percentile).

+3-hydroxyoctanoylcarnitine (C8-OH), hydroxybutyrylcarnitine (C4OH), methylmalonylcarnitine (C4-DC), 3-hydroxyisovalerylcarnitine (C5-OH), glutarylcarnitine (C5-DC)+3-hydroxydecanoylcarnitine (C10-OH), hexanoylcarnitine (C6), methylglutarylcarnitine (C6DC), hydroxyhexanoylcarnitine (C6OH), dodecenoylcarnitine (C12:1), 3-hydroxydodecanoylcarnitine (C12-OH), hydroxytetradecadienylcarnitine (C14OH), hydroxypalmitoylcarnitine (C16OH), 3-hydroxystearoylcarnitine (C18-OH), and 3-hydroxyoleoylcarnitine (C18:1-OH), all analytes were significantly different from term controls (Supplemental Table 3) and changed over time (Figs 1 and 2). Every possible pattern of change was observed (up over time, down over time, up then down, down then up). The time points most consistently found to be different from the other time points were days 1 and 7 (peak of intravenous nutrition was day 7; the peak of enteral nutrition was day 42).

Comparison of EGA Groups Over Time

Amino Acid Profile

Effect of EGA

Across EGA groups, there were significant differences in alanine, aspartate, citrulline, glutamine, glutamate, glycine, phenylalanine, serine, and valine (Supplemental Table 3). Alanine, glutamate and serine were lower in the 23- to 26-week EGA group compared with the other 2 groups. Aspartate, phenylalanine, and valine were higher in the 23 to 26 EGA group compared with the other 2 groups. Citrulline and glycine were significantly higher in the 29 to 31 EGA group compared with the other 2 groups.

Effect of Time

With the exception of serine, all the amino acids changed over time, and every possible pattern of change was observed. The time points most consistently found to be different from the other time points were days 1 and 7. With the exception of citrulline, glutamine, and tyrosine, there were no differences between the amino acid profiles obtained on days 28 and 42; citrulline, glutamine, and tyrosine were slightly higher on day 42 compared with day 28 (Supplemental Table 3).

Interaction Between EGA and Time

The pattern of change over time for each EGA group was similar, but the degree of change over time was significantly different for histidine, phenylalanine, aspartate, citrulline, glutamate, glutamine, glycine, and tyrosine. The most distinctive changes were in citrulline (increase over time especially in the 29 to 31 EGA group), leucine-isoleucine (increased on day 7 and then decrease with time for all EGA groups), and tyrosine (decreased on day 7 and then increase with time especially in the 23 to 26 EGA group; Supplemental Table 3). There was a weak correlation between leucine-isoleucine values and the dose of amino acids (g/kg per day; $R^2 = 0.1$, $P < .01$) that was independent of EGA group and age at when the blood sample was done. On day 7, the type of amino acids also influenced the amino acid profile (data not shown).

Acylcarnitines

Impact of EGA

Across all EGA groups, there were significant differences between the acylcarnitine levels except for hydroxybutyrylcarnitine (C4OH), hydroxyhexanoylcarnitine (C6OH), dicarboxydodecanoylcarnitine (C12DC), and tetradecenoylcarnitine (C14:1; Supplemental Table 3). The most distinctive group was the 23 to 26 EGA group. With the exception of the 4 acylcarnitines listed above, the 23 to 26 EGA group was significantly different from the other 2 EGA groups. The 2 more mature EGA groups were similar to each other for all acylcarnitine analytes.

Impact of Time

Every acylcarnitine changed over time except hydroxytetradecadienylcarnitine (C14OH). Every possible pattern of change was observed (up over time, down over time, up then down, down then up). The time points most consistently found to be different from the other time points were day 1 and day 7. Although the acylcarnitine values obtained on days 28 and 42 were similar, free carnitine, acetylcarnitine (C2), propionylcarnitine (C3), methylmalonylcarnitine (C4-DC), dodecanoylcarnitine (C12), and tetradecenoylcarnitine (C14) were statistically higher on day 42 than on day 28. The acylcarnitine with the most striking increase from day 1 to 7 followed by a decrease was linoleoylcarnitine (C18:2; Fig 2). Linolenic acid is one of the primary fatty acids in intralipids. Linoleoylcarnitine returned to lower levels on days 28 and 42 when the primary nutrition was enteral feedings and use of intravenous intralipids was less common. There was a direct correlation between the dose of intralipids and the linoleoylcarnitine (C18:2) values that was independent of EGA and age at sample ($R^2 = 0.3$, $P < .001$).

Interaction Between EGA and Time

With the exception of dodecanoylcarnitine (C12), hydroxytetradecadienylcarnitine (C14OH), tetradecenoylcarnitine (C14:1), hydroxypalmitoylcarnitine (C16OH), 3-hydroxystearoylcarnitine (C18-OH), and 3-hydroxyoleoylcarnitine (C18:1-OH), there was an interaction between EGA and time for all the acylcarnitines. The pattern of change for all the acylcarnitines over time for each EGA group was similar, but the degree of change was significantly different for all acylcarnitines except those listed above. The most significant changes over time and within

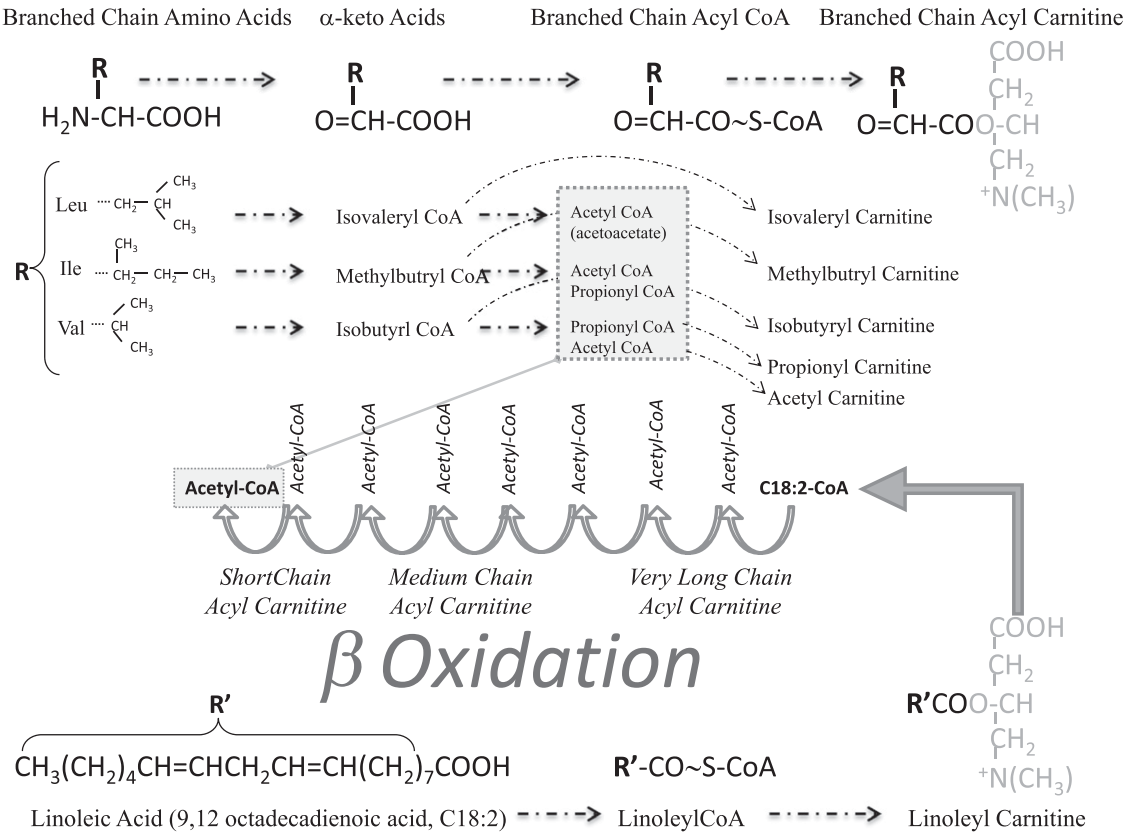

FIGURE 3
Metabolic pathways that explain our observations.

EGA group were for isovalerylcarnitine + methylbutyrylcarnitine (C5), octanoylcarnitine (C8), and linoleoylcarnitine (C18:2; Fig 2). All 3 of these acylcarnitines increased from day 1 to 7 and then progressively decreased for days 28 and 42. The most immature infants (23–26 weeks) were the most distinctive and were significantly different from the other 2 groups.

Summary Observations for All Analytes

Age at sampling was a more important factor associated with changes in metabolic profiles than EGA group. The difference in the median value for any specific analyte was smaller between EGA groups than between time groups.

DISCUSSION

Our study reveals a high rate of newborn screening alerts in premature infants. Twenty-one percent of the infants had values above the prespecified cutoff values used to identify infants with inborn errors of metabolism. None of the abnormal values could be explained by contamination of the blood sample, or by inappropriate collection methods. These results confirm what is generally observed in practice, that the use of cutoff values derived from healthy term infants results in a high rate of "abnormal" values (false-positives) in premature infants.[4,8,12] Figures 1, 2, and 3 graphically demonstrate the problem and reveal that the values for commonly measured analytes are often >1 SD above or below the mean value for healthy term infants.

Both EGA and time confound the ability to define normal. The most premature infants appear to be metabolically different from more mature premature infants and the metabolic profile changes over time for most (96%) of the measured analytes. This variability makes it impossible to define normative values without correcting for time and EGA. Based on the literature and the patterns we describe above, we must also correct for degree of illness and the type of nutritional support at the time of sampling.[4,6,8,12]

Nutrition is a dynamic interaction between what is provided (glucose/carbohydrates, protein/amino acids, lipids/fatty-acids/carnitine), how it is provided (intravenous or enteral), how it is used for energy and growth, and how each nutritional component is metabolized. Just as there are adverse events associated with drug–drug interactions that relate to metabolism, the potential for adverse side effects related to nutritional–nutritional interactions is real.

We believe our data reveal precisely this type of problem in the most immature infants (23–26 EGA group; Figure 3). Both leucine-isoleucine (primary component of intravenous amino-acid solutions) and linoleoylcarnitine (linoleic and linolenic acids are key components of intralipids) are elevated on day 7 when the doses of amino acids and intralipids are highest. At the same time, isovalerylcarnitine + methylbutyrylcarnitine and octanoylcarnitine are elevated. The accumulation of these carnitines may lead to the formation of toxic organic acid metabolites and/or liver injury.

CONCLUSIONS

Our results reveal the complexity of understanding the impact of immaturity and postnatal age on metabolic profiles. The inherent metabolic instability manifested by the premature infant makes an abnormal result likely, even though few of these infants will ever demonstrate an inherited metabolic abnormality. Just as both high and low values of glucose can cause injury, high and low values of amino acids and acylcarnitines may cause long-term morbidity. Investigations are needed to define what values are safe in premature infants. New reference standards that include adjustments for gestational age, age at sampling, and nutritional support may better identify infants who have inborn errors of metabolism and allow us to better understand the metabolic consequences of premature birth.

PEDIATRIX-OBSTETRIX CENTER FOR RESEARCH, EDUCATION AND QUALITY

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