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The influence of maternal disease on metabolites measured as part of newborn screening

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Abstract

Objective—Measurements of neonatal metabolites are commonly used in newborn screening programs to detect inborn errors of metabolism. Variation in these metabolites, particularly in infants born preterm (<37 weeks gestation), can result from multiple etiologies. We sought to evaluate the impact of maternal complications of pregnancy and environmental stressors on newborn screening metabolites.

Methods—We examined 49 metabolic biomarkers obtained from routine newborn screening in 452 infants born preterm for association with maternal environmental stressors and complications of pregnancy.

Results—Neonatal free carnitine (C0, $P=1.4\times10^{-7}$), acetylcarnitine (C2, $P=2.7\times10^{-7}$), octenotylcarnitine (C8:1, $P=5.2\times10^{-11}$) and linoleoylcarnitine (C18:2, $P=9.1\times10^{-7}$) were elevated in infants born to preeclamptic mothers. Similar elevations were observed in small for gestational age infants and in infants where labor was not initiated prior to delivery. When accounting for all three factors associations remained strongest between acylcarnitines and preeclampsia.

Conclusion—We observed that maternal conditions, particularly preeclampsia, influence newborn screening biomarkers. This is important for identifying maternal conditions that influence metabolites measured during routine newborn screening that are also markers of fetal growth and overall health.

Keywords

preeclampsia; newborn screening; metabolic profiles

Introduction

Newborn metabolic profiles serve as critical biomarkers for the detection of rare congenital conditions that, if identified early by newborn screening (NBS) programs, can be treated. In addition to NBS, neonatal metabolite measurements serve as markers for fetal growth and

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Declaration of Interest
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overall health [1–3]. Infants born preterm have varied metabolic profiles compared to infants born at term [4]. This has largely been attributed to fetal stress and sickness due to the early birth; however, the metabolic profiles of "healthy" preterm newborns also differ compared to their term counterparts [4].

Maternal and cord blood amino acid and acylcarnitine levels have been examined in relation to preeclampsia and intrauterine growth restriction (IUGR) [5–9]. Higher levels of several metabolites were identified in infants of women with preeclampsia [8]. In one study, amino acid levels were highest in small for gestational age infants of women with preeclampsia [8]. These studies shed interesting insights on the complexity of preeclampsia and demonstrates that maternal and pregnancy conditions influence neonatal metabolites as well as fetal growth. While previous studies have examined cord blood we set to determine if similar profiles could be detected from dried blood spots used for routine neonatal metabolic screening. In addition to preeclampsia we also investigated the impact of maternal environmental stressors and other pregnancy complications.

Materials and Methods

This is a retrospective analysis of data collected as part of a prospective cohort for studying the epidemiology and genetics of preterm birth [10,11]. Study samples were collected at the University of Iowa Hospitals and Clinics in Iowa City, IA after approval by the University of Iowa Institutional Review Board (IRB200506792). Signed informed consent was obtained from all families for enrollment. Existing data collected by an interview with the mother, medical chart review or both were mined for 30 maternal clinical and demographic factors; completeness of the data varied across subjects (Supplemental Table I). Clinical diagnoses were made for preeclampsia, gestational diabetes and other maternal conditions and these data were abstracted from the medical record for use in this study. Infants were included in data analysis if they were born preterm (delivery at 23–36 weeks gestation), had not received a blood transfusion at the time of sample collection, and had their sample collected between 24 and 72 hours after birth. Gestational age and birth weight were obtained from the medical record, if available; otherwise information was obtained from the newborn screening requisition card. Data on analyte measurements for 13 amino acids, 36 acylcarnitines and 22 metabolite ratios were obtained from tandem mass spectrometry (MS/MS) by the State Hygienic Laboratory (Supplemental Table II) and linked to the clinical medical record data.

Initial analysis was performed with 689 preterm infants, on all 30 clinical variables and 71 metabolites. While all significant results can be found in supplemental table IV we chose to present in the main tables and results only variables and metabolites with <10% missing data to avoid potential bias in the interpretation of the data. We performed analysis of variance (ANOVA) adjusting for year of sample collection, assay lot changes, gestational age and birth weight. Year of sample collection and assay lot were included in the final model because there were independently significantly ($P<0.001$) associated with more than 85% of the analytes examined. Birth weight and gestational age were independently significantly associated with more than 68% of the analytes and there is extensive literature on the influences of birth weight and gestational age on these analytes [4,12]. Multiple statistical modeling methods were compared including one or more of these covariates with each of the 30 tested variables and few differences were identified (Supplemental Table III), none of which impact our main findings. For the weight for gestational age (WGA) variable adjustment in the final model was only performed with year of sample collection and assay lot changes to avoid potential over fitting with birth weight and gestational age. Feeding method may also impact the associations we are examining. We obtained this variable from the newborn requisition card and defined feeding method as bottle fed, breast fed, bottle and

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breast fed, and nothing by mouth (NPO). We individually tested each analyte adjusted for birth weight and gestational age with feeding method and found only a few significant ($P<0.01$) differences including PHE/TYR ($P=2.2\times10^{-4}$), TYR/PHE ($P=6.4\times10^{-4}$), TYR ($P=6.8\times10^{-3}$) and C8/C10 ($P=8.9\times10^{-3}$). With the exception of TYR, we did not include this variable in the final model with the rest of the analytes. For the final presented model we excluded multiple gestations (N=130), congenital anomalies (N=44), infants on total parenteral nutrition (N=51) and infants with abnormal (high or low) initial analyte values (N=12) on the metabolic screen to avoid potential confounding, leaving 452 infants for the final analysis. Standardized residuals were examined for outliers and measurements that were < -3.5 or > 3.5 were removed. Initial analysis included 1,470 ANOVA models (30 clinical variables x 49 analyte and ratio measurements) and a Bonferonni significance threshold [13] of $P<3\times10^{-5}$ (i.e. P=0.05/1,470 tests) was used to correct for multiple testing. Stata, version 12.0 (Stata Corporation, College Station, Tx) was used for all analyses. Subsequent evaluation significant associations including more than one of the clinical variables of interest are uncorrected for multiple testing.

Results

The strongest associations, after correction for multiple testing using the Bonferonni significance threshold of $P<3\times10^{-5}$, were observed for preeclampsia; newborn concentrations of alanine (ALA), free carnitine (C0), acetylcarnitine (C2), octenoylcarnitine (C8:1) and linoleoylcarnitine (C18:2) were significantly elevated in infants born to preeclamptic mothers (Table I). Neonatal tyrosine (TYR) was decreased in infants born to preeclamptic mothers (Table 1). Adding feeding method to the model did not affect the associations with TYR nor did adding infant gender to any of the significant associations in Table 1. Associations, significant after correction for multiple testing, similar to those in preeclampsia were observed for ALA, C0, C2 and C8:1 in infants where labor was induced or did not occur prior to delivery (Supplemental Table IV). Also significant after correction were elevations in ALA, C0, C2, C18:2 and a decrease in TYR were also observed in infants small for gestational age (SGA) (Supplemental Table IV). Other notable and significant associations, after correction for multiple testing, included elevations in dodecanoylcarnitine (C12), tetradecanoylcarnitine (C14), tetradecenoylcarnitine (C14:1), palmitoylcarnitine (C16) and palmitoleylcarnitine (C16:1) in large for gestational age infants (LGA) (Supplemental Table IV).

As expected, fetal growth, gestational age and type of labor and delivery differed between women with and without preeclampsia (Table II). We examined preeclampsia, weight for gestational age (i.e. LGA, SGA and AGA) and labor type together and found that the elevations with C0, C2 and C18:2 remained significant for both preeclampsia and SGA; C8:1 was still significant (uncorrected for multiple testing) with preeclampsia ($P=1.9\times10^{-4}$) but not SGA (P=0.48) (Table III). There were no strong interaction effects between preeclampsia and weight for gestational age (Table IV).

Discussion

Expanded newborn screening including tandem mass spectrometry provides a basic metabolic profile for the newborn and represents a panel of biomarkers for fetal growth and overall health [1–3]. Many factors both fetal and maternal may impact the metabolites captured through newborn screening; however, other than gestational age and birth weight, there is limited knowledge on the underlying etiologies that influence these measurements at birth. Few studies, if any, have examined metabolites obtained from expanded newborn screening with maternal conditions associated with preterm birth. We identified higher concentrations of acylcarnitines, adjusted for birth weight and gestational age, in newborns

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of preeclamptic women. These results are consistent with previous studies that observe similar patterns of elevated newborn amino acid and acylcarnitine values in cord blood and maternal plasma from preeclamptic mothers [8,9,14–17].

The reasons for observed acylcarnitine changes are likely complex and may be influenced by maternal, fetal, and placental factors. It was proposed that preeclamptic women may have higher plasma acylcarntine levels due to abnormalities of fatty acid oxidation, renal function, dyslipidemia, hemoconcentration and oxidative stress [9]. It is possible that elevations of acetylcarnitine (C2), medium- and long-chain acylcarnitines in maternal blood samples indicate activation of the fatty acid oxidation pathway due to stress of preeclampsia. Elevated acylcarnitines in the maternal circulation can then be transferred to the fetal circulation via the placenta and detected by the newborn screen soon after birth. Alternatively, preeclampsia could be a stress factor for the neonate, thus triggering activation of the fatty acid oxidation pathway, a non-specific marker of the catabolic state.

One weakness of our study is the inability to examine maternal-fetal correlations of acylcarnitine concentrations as we only obtained samples of the infant. Future studies that focus on collecting measurements from both the mother and newborn will be important in elucidating the mechanisms of these findings. Additionally, studies that examine metabolites at multiple time points during pregnancy and can examine the changes of these metabolites through the course of a pregnancy complication such as preeclampsia and its treatment will be important. While the metabolite changes associated with preeclampsia are small and well within the limits for a normal newborn screening test designed to detect rare inborn errors of metabolism, one cannot reject the possibility that they may still be markers for anticipating suboptimal biochemical responses of neonates resulting in clinically relevant outcomes, as has been shown with other biomarkers such as thyroid hormones [18].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Illsinger S, Schmidt KH, Lucke T, Vaske B, Bohnhorst B, Das AM. Plasma and urine amino acid pattern in preterm infants on enteral nutrition: impact of gestational age. Amino Acids. 2009
2. Rakheja D, Bennett MJ, Foster BM, Domiati-Saad R, Rogers BB. Evidence for fatty acid oxidation in human placenta, and the relationship of fatty acid oxidation enzyme activities with gestational age. Placenta. 2002; 23:447–450. [PubMed: 12061861]
3. Shekhawat P, Bennett MJ, Sadovsky Y, Nelson DM, Rakheja D, Strauss AW. Human placenta metabolizes fatty acids: implications for fetal fatty acid oxidation disorders and maternal liver diseases. Am J Physiol Endocrinol Metab. 2003; 284:E1098–105. [PubMed: 12582009]

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4. Oladipo OO, Weindel AL, Saunders AN, Dietzen DJ. Impact of premature birth and critical illness on neonatal range of plasma amino acid concentrations determined by LC-MS/MS. Mol Genet Metab. 2011; 104:476–479. [PubMed: 21908220]
5. Cetin I, Corbetta C, Sereni LP, Marconi AM, Bozzetti P, Pardi G, Battaglia FC. Umbilical amino acid concentrations in normal and growth-retarded fetuses sampled in utero by cordocentesis. Am J Obstet Gynecol. 1990; 162:253–261. [PubMed: 2301500]
6. Cetin I, Ronzoni S, Marconi AM, Perugino G, Corbetta C, Battaglia FC, Pardi G. Maternal concentrations and fetal-maternal concentration differences of plasma amino acids in normal and intrauterine growth-restricted pregnancies. Am J Obstet Gynecol. 1996; 174:1575–1583. [PubMed: 9065132]
7. Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Evans MI. Plasma amino acids in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol. 1989; 161:1219–1227. [PubMed: 2589443]
8. Evans RW, Powers RW, Ness RB, Cropcho LJ, Daftary AR, Harger GF, Vergona R, Finegold DN. Maternal and fetal amino acid concentrations and fetal outcomes during pre-eclampsia. Reproduction. 2003; 125:785–790. [PubMed: 12773100]
9. Thiele IG, Niezen-Koning KE, van Gennip AH, Aarnoudse JG. Increased plasma carnitine concentrations in preeclampsia. Obstet Gynecol. 2004; 103:876–880. [PubMed: 15121560]
10. Steffen KM, Cooper ME, Shi M, Caprau D, Simhan HN, Dagle JM, Marazita ML, Murray JC. Maternal and fetal variation in genes of cholesterol metabolism is associated with preterm delivery. J Perinatol. 2007; 27:672–680. [PubMed: 17855807]
11. Ehn NL, Cooper ME, Orr K, Shi M, Johnson MK, Caprau D, Dagle J, Steffen K, Johnson K, Marazita ML, Merrill D, Murray JC. Evaluation of fetal and maternal genetic variation in the progesterone receptor gene for contributions to preterm birth. Pediatr Res. 2007; 62:630–635. [PubMed: 17805208]
12. Slaughter JL, Meinzen-Derr J, Rose SR, Leslie ND, Chandrasekar R, Linard SM, Akinbi HT. The effects of gestational age and birth weight on false-positive newborn-screening rates. Pediatrics. 2010; 126:910–916. [PubMed: 20974783]
13. Dunn OJ. Multiple Comparisons Among Means. Journal of the American Statistical Association. 56:52–64.
14. Kenny LC, Broadhurst DI, Dunn W, Brown M, North RA, McCowan L, Roberts C, Cooper GJ, Kell DB, Baker PN. Screening for Pregnancy Endpoints Consortium. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension. 2010; 56:741–749. [PubMed: 20837882]
15. Kenny LC, Broadhurst D, Brown M, Dunn WB, Redman CW, Kell DB, Baker PN. Detection and identification of novel metabolomic biomarkers in preeclampsia. Reprod Sci. 2008; 15:591–597. [PubMed: 18492697]
16. Li J, Sun J, Lu H. Determination of serum free amino acids and ammonia in pregnant women with severe pregnancy induced hypertension. Zhonghua Fu Chan Ke Za Zhi. 1996; 31:468–470. [PubMed: 9275438]
17. Cockburn F, Blagden A, Michie EA, Forfar JO. The influence of pre-eclampsia and diabetes mellitus on plasma free amino acids in maternal, umbilical vein and infant blood. J Obstet Gynaecol Br Commonw. 1971; 78:215–231. [PubMed: 5558838]
18. Roef G, Lapauw B, Goemaere S, Zmierczak H, Fiers T, Kaufman JM, Taes Y. Thyroid hormone status within the physiological range affects bone mass and density in healthy men at the age of peak bone mass. Eur J Endocrinol. 2011; 164:1027–1034. [PubMed: 21393448]

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Table I

Significant associations with preeclampsia

	[1]Non Preeclamptic (N=320)	Preeclamptic (N=103)	P-value
Alanine (ALA)	163.4±80.8	210.4±102.3	3.8×10^{-5}
Tyrosine (TYR)	80.1±54.8	64.6±44.6	7.9×10^{-5}
Free carnitine (C0)*	23.3±9.3	32.1±12.1	1.4×10^{-7}
Acetylcarnitine (C2)*	25.1±9.7	32.8±11.0	2.7×10^{-7}
Octenoylcarnitine (C8:1)*	0.07±0.04	0.11±0.07	5.2×10^{-11}
Linoleoylcarnitine (C18:2)*	0.22±0.15	0.31±0.20	9.1×10^{-7}

Mean (μmol/L) and standard deviations by preeclampsia.

*indicates significant after correction for multiple testing ($P<3\times10^{-5}$).

[1]The comparison is preterm infants born to mothers with (Preeclamptic) and without (Non Preeclamptic) preeclampsia

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Table II

Demographic characteristics of cohort by preeclampsia

Trait	[1] Non Preeclamptic (N=320)	Preeclamptic (N=103)	P-value
Gestational age (weeks)	31.7±3.3	30.5±3.0	3.6×10^{-4}
Birth weight (grams)	1,916.0±751.1	1,427.4±625.6	3.1×10^{-9}
SGA	18 (5.6%)	29 (28.2%)	2.7×10^{-10}
Infant gender (male)	193 (60.3%)	57 (55.3%)	0.37
Race			0.54
White	268 (85.4%)	90 (90.0%)	
African-American	13 (4.1%)	3 (3.0%)	
Asian	4 (1.3%)	0 (0%)	
Other	29 (9.2%)	7 (7.0%)	
Ethnicity - (Non-Hispanic)	285 (90.8%)	93 (94.9%)	0.19
Delivery method (C-section)	131 (40.9%)	78 (75.7%)	8.1×10^{-10}
Type of labor			4.8×10^{-39}
no labor	38 (12.2%)	60 (58.8%)	
induction	28 (9.0%)	37 (36.3%)	
spontaneous	246 (78.9%)	5 (4.9%)	

SGA: small for gestational age

[1] The comparison is preterm infants born to mothers with (Preeclamptic) and without (Non Preeclamptic) preeclampsia

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Table III

Multivariate models of significant associations with preeclampsia.

		Preeclampsia	WGA	Labor	Assay Lot	Year
Variable	R^2	P-value	P-value	P-value	P-value	P-value
ALA	0.42	0.05	0.02	0.03	9.1×10^{-4}	4.7×10^{-14}
TYR	0.13	0.10	0.07	0.13	0.29	0.13
C0	0.23	3.6×10^{-6}	2.6×10^{-3}	1.9×10^{-3}	0.58	1.5×10^{-3}
C2	0.19	1.6×10^{-5}	1.9×10^{-3}	0.14	0.22	0.69
C8:1	0.11	1.9×10^{-4}	0.48	0.47	0.72	0.39
C18:2	0.18	1.3×10^{-4}	2.3×10^{-3}	0.60	0.27	0.03

ALA: alanine; TYR: tyrosine; C0: free carnitine; C2: acetylcarnitine; C8:1: octenoylcarnitine; C18:2: linoleoylcarnitine. Weight for gestational age (WGA) id defined by three groups: small for gestational age, average for gestational age and large for gestational age. These p-values are not corrected for multiple testing.

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Table IV

Multivariate models of the interaction between WGA and preeclampsia.

Variable	R^2	Preeclampsia P-value	WGA P-value	Interaction[1] P-value	Assay Lot P-value	Year P-value
C0	0.21	0.04	1.9×10^{-4}	0.35	0.52	2.6×10^{-3}
C2	0.19	0.11	4.9×10^{-3}	0.16	0.19	0.67
C18:2	0.20	0.23	1.1×10^{-4}	0.01	0.30	0.04

[1] Interaction between preeclampsia and weight for gestational age (WGA), defined by three groups: small for gestational age, average for gestational age and large for gestational age. Interactions were only tested for C0: free carnitine; C2: acetylcarnitine; C18:2: linoleoylcarnitine where there were significant main effects for both preeclampsia and WGA in the multivariate model (Table III). These p-values are not corrected for multiple testing.