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*NOT an intervention
*Association study → leads to intervention studies

Acylcarnitine Profiles in HIV-Exposed, Uninfected Neonates in the United States

1) Identify what this is!
*This is an enzyme (translocase) which transports carnitine and carnitine fatty acids across the inner mitochondrial membrane.
*KEY STEP IN FATTY ACID OXIDATION → β-oxidation breaks down fatty acids to generate acetyl-CoA to enter the Krebs cycle.

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Abstract

We sought to determine the prevalence of abnormal acylcarnitine profiles (ACP) in HIV-exposed uninfected (HEU) newborns and to explore the association of abnormal ACP with clinical laboratory outcomes and antiretroviral drug exposures. Clinically, ACP are used to assess for fatty acid oxidation (FAO) dysfunction and normal FAO is necessary for optimal fetal/neonatal growth and development. We analyzed serum ACP in 522 HEU neonates enrolled in the Surveillance Monitoring for ART Toxicities (SMARTT) study of the Pediatric HIV/AIDS Cohort Study (PHACS) and evaluated the associations of abnormal ACP with *in utero* exposure to combination antiretroviral therapy (cART) in logistic regression models, adjusting for maternal demographic, disease, and behavioral characteristics. We evaluated the associations of abnormal ACP with laboratory parameters and measures of neurodevelopment and growth. Of 522 neonates, 89 (17%) had abnormal ACP. In adjusted analyses, *in utero* exposure to a protease inhibitor (PI) was associated with higher odds of having an abnormal ACP [adjusted odds ratio (aOR) = 2.35, 95% CI: 0.96, 5.76, $p = 0.06$] with marginal significance while exposure to a nonnucleoside reverse transcriptase inhibitor (NNRTI) was associated with lower odds (aOR = 0.23, 95% CI: 0.07, 0.80, $p = 0.02$). Mean ALT levels were slightly higher in those with abnormal ACP, but no differences in lactate, glucose, or CPK were observed. ACP status was not associated with neurodevelopment at 1 year or growth at 2 and 3 years of age. Abnormal ACP in HEU neonates are associated with exposure to PI-containing as opposed to NNRTI-containing antiretroviral (ARV) regimens but are not associated with serious postnatal clinical problems. Further studies are needed to determine the long-term health implications of abnormal acylcarnitine metabolism at birth in HEU children.

Question ↔ ACP → Clinical outcomes? → Drug effect?

blood samples
study
These are potential confounding variables

ALT = alanine aminotransferase → ↑ = liver damage | CPK = creatinine phosphokinase → muscle injury

Introduction

ANTIRETROVIRAL DRUGS (ARV) are indispensible for preventing maternal-to-child transmission of HIV, yet there are concerns about their long-term effects on exposed fetuses and newborns.[1] Of particular concern are the clinical effects of nucleoside reverse-transcriptase inhibitors (NRTIs) on mitochondrial oxidative phosphorylation (OXPHOS)[2] and HIV protease inhibitors (PIs) on lipid and glucose metabolism.[3–5]

Fatty acid oxidation (FAO) is an intramitochondrial metabolic pathway situated at the crux of mitochondrial OXPHOS, lipid catabolism, and glucose utilization. FAO is the major source of energy for the heart and plays an important role in fetal–placental health.[6,7] Severe inherited disorders of FAO manifest as hypoglycemia, myopathy (skeletal and cardiac), liver dysfunction, neurodevelopmental delay, as well as preterm birth and fetal growth restriction.[8] Even mild dysfunction in the pathway, which is also heritable, has been associated with chronic diseases such as type 2 diabetes and cardiovascular disease in adults.[9–11]

Newborn screening (NBS) is a state-sponsored public health program that tests almost all neonates (~4 million/

Basically they're wondering if HIV drugs cause these too!

Q: What molecule is produced by FAO?

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A: acetyl-coA

NOTES about abstract

Taken by HIV+ mom

Question: Do HIV-exposed neonates with an abnormal ACP have different clinical outcomes or effects from drugs than HIV-exposed neonates who have normal ACP?

(AKA: does ACP impact clinical outcomes/drug effect)

protease inhibitor: drug to prevent viral replication

↑PI = ↑ACP

non-nucleoside reverse transcriptase inhibitor

Binds to reverse transcriptase enzymes which prevent the conversion of RNA → DNA

↑ACP = ↑ALT (liver damage) = − Lactate, glucose, or CPK ≠ neuro imparaments

Why observe this? > This is to help pin point where in the metabolic pathway will be affected with abnormal ACP levels.

PI drugs = ↑ACP in neonates
NNRTI = −ACP in neonates
⇒ no serious clinical outcomes

mcat style Q

Question: If a pregnant mother were to take a cocktail of anti-HIV drugs which prevented viral replication, could researchers anticipate this to affect the neonate?

✓ a. Yes, protease inhibitors increase ACP to an abnormal levels.

~~b~~. Yes, NNRTI drugs increase ACP to abnormal levels.

~~c~~. No, there were not any clinical changes observed in HEV neonates.

d. No, the placenta blood barrier prevents transmission from mother to fetus.

↑ logical leap

year) in the United States for inherited disorders of FAO by measuring acylcarnitine levels in blood.[12] Acylcarnitine profiles (ACP) exploit the fact that dysfunctional FAO results in the accumulation of esterified fatty acid metabolites (acylcarnitines) in biofluids, that these metabolites can be reliably quantified by their unique mass-to-charge ratio, and that certain patterns of acylcarnitine elevations reflect dysfunction at unique points in the pathway.[13] In the clinical management of patients with inherited disorders of FAO, acylcarnitine elevations are used to diagnose specific FAO disorders as well as to clinically follow patients for metabolic decompensation and response to treatment.[14] Beyond being biomarkers of FAO dysfunction and compromised energy metabolism, acylcarnitines themselves are thought to be cytotoxic, particularly to myocytes and hepatocytes.[15,16]

To date little is known about the effects of HIV or ARV on fatty acid and acylcarnitine metabolism in neonates, although we recently reported a higher proportion of abnormal ACP in HIV-exposed infants compared to the general population of newborns.[1] In this study we sought to determine the prevalence of abnormal ACP in a large cohort of HIV-exposed, uninfected (HEU) newborns and explore the association of abnormal ACP with clinical laboratory outcomes and *in utero* ARV exposures.

Materials and Methods

Study population

We analyzed data from HIV-infected pregnant women and their newborns enrolled into the Dynamic Cohort of the Surveillance Monitoring for ART Toxicities (SMARTT) study, a component of the Pediatric HIV/AIDS Cohort Study (PHACS). In 2007, the Dynamic Cohort began enrolling pregnant women and their infants between 22 weeks of gestation and 1 week after birth into an ongoing surveillance study at 22 sites in the United States including Puerto Rico. The study protocol was reviewed and approved by the institutional review board of each participating site and at the Harvard T.H. Chan School of Public Health. Written informed consent was obtained from all women. Infants are followed prospectively with annual study visits. Our sample was limited to infants born from 2007 to 2011, with accessible serum specimens within 7 days from birth, as of July 1, 2011.

Acylcarnitine profiles

ACP were analyzed and interpreted at Mayo Medical Laboratories as previously described.[18] Briefly, previously collected serum was deproteinized and derivatized with *N*-butanol. Butyl-esters of acylcarnitine species were measured on an ABSciex3000 tandem mass spectrometer in precursor scan mode (–85 amu). Quantitation was performed using stable isotopically labeled standards and compared against a reference range of age-matched controls (0–7 days of age). An abnormal ACP was defined according to published guidelines from the American College of Medical Genetics.[13] Succinctly, an abnormal acylcarnitine profile was any profile that contained at least one flagged (outside of the age-matched Mayo Biochemical Genetics Laboratory's reference range), clinically relevant acylcarnitine and had an overall pattern that was interpreted and characterized by a board-certified clinical biochemical geneticist (Hofherr) as abnormal. Abnormal ACP results were further categorized into four types (generalized elevations, short-chain AC elevations, medium-chain elevations, and long-chain elevations) based on the pattern of elevated acylcarnitines.[19]

Maternal ARV use and pregnancy characteristics

Start and stop dates for all ARVs taken during pregnancy were obtained via chart abstraction to classify trimester(s) and duration of exposure to each agent.[20] Combination antiretroviral therapy (cART) was defined as any regimen containing at least three ARVs from two or more drug classes. Data on income, education, smoking, and alcohol use during pregnancy were collected by interview. Passive smoking was defined as any regular exposure to people smoking either inside or outside the home. Self-reported data on illicit drugs were validated in a subset of women based on results of meconium testing.[21] HIV RNA levels and $CD4^+$ lymphocyte counts as well as percentages during pregnancy were abstracted from medical records for two time points during pregnancy: first available and latest available prior to delivery.

Infant clinical, neurodevelopmental, language, and laboratory outcomes

Gestational age was based on obstetrician estimate. Nonfasting glucose, alanine transaminase (ALT), creatine phosphokinase (CPK), and point-of-care (POC) lactate levels were also considered if they were performed within 7 days of the date when the serum for ACP was drawn; these clinical measures were chosen because FAO dysfunction is associated with fluctuations in blood glucose levels, liver dysfunction, muscle injury, and generalized mitochondrial dysfunction. Anthropometric measurements at age 2 and 3 visits were performed for those who reached those ages by January 1, 2013. *Z*-scores for anthropometric measures were calculated based on CDC 2000 growth standards for full-term infants, and adjusting for gestational age for preterm infants.[22] Neurodevelopmental functioning was assessed at age 1 year using the Bayley Scales of Infant and Toddler Development—third edition, providing standardized measures of Cognitive, Language, Motor, Social-Emotional, and Adaptive Behavior[23] in infants, as well as the MacArthur–Bates Communicative Development Inventory (CDI) language assessment, providing percentile scores in four domains: Phrases Understood, Vocabulary Comprehension, Word Production, and Total Gestures[24] (Supplementary Tables S1 and S2; Supplementary Data are available online at www.liebertpub.com/aid), Late language emergence (LLE) was defined as a score ≤10th percentile in any of the four domains. Only language/neurological assessments performed by age 18 months were included.

Statistical analysis

Infant and maternal characteristics were summarized overall and compared between those with normal versus abnormal ACP using Fisher's exact tests or Wilcoxon rank sum tests. Covariates including demographics and maternal and infant characteristics were first identified using logistic regression models. All covariates with p-values <0.3 were included in a multivariable model, which was then reduced to a final core model based on stepwise selection to include only covariates with $p < 0.15$. Associations between ARV exposures during pregnancy and ACP abnormality were then

Statistical analysis

ARV Regimin

1. cART + NNRTI
   - a. with PI 7% = ↓
   - b. without PI 4% = ↓
2. cART + PI without NNRTI 19% = ↑
3. cART + 3 NNRTI
   - 73% zidovudine
   - 76% lamivudine ↓ exposure = ↑ ACP

Question:

Which treatment of cART, NNRTI, and PI would be preferable in an HIV+ pregnant mother to avoid abnormal ACP?

a. cART and zidovidine with no PI
b. cART and PI without NNRTI
c. cART and NNRTI without PI
d. cART and NNRTI with PI

assessed in logistic regression models both unadjusted and adjusted for covariates in the core model. ARV regimen was evaluated by a composite classification: cART with NNRTI (with or without PI), cART with PI (no NNRTI), and others (other cART, three or more NNRTI, mono- or dual-ART therapy). Drug classes (NNRTI, PI) and two NRTIs (zidovudine, lamivudine) were also studied separately. ARV exposure by trimester was analyzed. Because preterm birth may be on the causal pathway between *in utero* ARV exposure and abnormal FAO, sensitivity analyses were conducted excluding gestational age. Additional sensitivity analyses using mixed effect models were conducted to adjust for research site and repeated births by the same mother.

Medians of nonfasting glucose, ALT, CPK, and POC lactate levels[25] were compared between infants with normal and abnormal ACP using both numerical values by Wilcoxon rank sum test and adverse event grades (Department of AIDS Adverse Events Table Version 1.0) by Fisher's exact test. If $p < 0.1$ from either test, then generalized linear models were fit adjusting for ARV exposure and covariates in the previous core model. Language and neurocognitive measures at age 1 year and anthropometric measures at ages 2 and 3 years were summarized and compared between children with and without ACP abnormality using the above approach. Analyses were conducted using SAS statistical software (v. 9.2). All *p*-values were two-sided. Because SMARTT is a safety study, no adjustment was made for multiple comparisons in order to minimize the chance of missing true associations.

Results

Study population

Of 1,202 infants in the SMARTT dynamic cohort, 522 newborns had sufficient serum sample available for ACP evaluation within 7 days after birth. Compared to infants not included in this study, included newborns were less frequently black, more frequently Hispanic/Latino, less frequently exposed to maternal smoking *in utero*, less frequently born preterm, and had lower median head circumference *z*-scores at birth. Those included were more frequently exposed *in utero* to lamivudine and longer durations of zidovudine, lamivudine, and PI-containing regimens.

Prevalence, distribution, and patterns of abnormal ACP

Eighty-nine infants (17.1%, 95% CI: 13.8%, 20.3%) had an abnormal ACP (Fig. 1). Table 1 summarizes the characteristics of infants and mothers by ACP status. Infants with *in utero* exposure to alcohol and smoking had a significantly higher prevalence of abnormal ACP than those without such exposures (34% vs. 15% and 21% vs. 14%, respectively). Preterm infants were more likely to have abnormal ACP. Infants with abnormal ACP had lower length and tended to have lower birth weight *z*-scores; however, no differences in weight-for-length or head circumference *z*-scores were observed. Almost all (99%) had maternal ARV exposure during gestation, with the majority exposed to cART. Infants exposed to cART with NNRTI had a lower prevalence of abnormal ACP (7% with and 4% without coexposure to PI) than infants exposed to cART with PI but not NNRTI (19%). All ARV exposures included at least one NRTI, most commonly zidovudine (73%) or lamivudine (76%). Infants with abnormal ACP had a shorter average exposure to lamivudine (median 107 vs. 136 days).

Association of maternal and demographic risk factors with abnormal ACP

The adjusted associations of each covariate with abnormal ACP are displayed in Table 2. In the adjusted model, lower gestational age at birth, maternal alcohol use, and active or passive smoking (marginal) were associated with greater odds of abnormal ACP. Maternal age at delivery >37 years was marginally associated with lower odds of abnormal ACP. Although low maternal CD4% early in pregnancy was associated with higher odds of abnormal ACP, viral load above 1,000 cp/ml early in pregnancy was associated with lower odds of abnormal ACP relative to those with less than 1000 cp/ml. Sensitivity analyses not adjusting for gestational age did not substantially alter the core model; the same significant covariates were identified and the largest change in OR estimates was within 7%.

Association of in utero *ARV exposures with abnormal ACP*

In adjusted models (Table 3), *in utero* exposure to cART with NNRTI, without or with PI, was associated with decreased odds of ACP abnormality as compared to a regimen of cART with PI but without NNRTI, while exposure to other combination regimens (85% of which were three or more NRTIs) had no statistically significant association with ACP abnormality. Most NNRTI exposure was either efavirenz (44%) or nevirapine (51%), and most PI exposure was ritonavir with atazanavir (28%) or with other PI (59%), or nelfinavir with or without ritonavir (17%). There was no exposures to indinavir or the newer PI, tipranavir.

With respect to individual NRTIs, longer exposure to lamivudine was associated with reduced odds of abnormal ACP, but any lamivudine exposure vs. none showed no statistically significant association with abnormal ACP. Exposure to other NRTIs known to be associated with mitochondrial toxicity was rare: among those exposed to NRTI, only ~1% were exposed to stavudine (d4T) and ~2% were exposed to didanosine (ddI).

ARV exposure increased over trimesters (54%, 92%, and 99% for first, second, and third trimester, respectively). There was a consistently lower odds of ACP abnormality for infants exposed *in utero* to cART with NNRTI (with and without PI) relative to cART with PI (without NNRTI) over all three trimesters, although not statistically significant (Table 4). However, exposure (versus no exposure) to NNRTI-containing regimens was consistently associated with about a 70% decreased adjusted odds of abnormal ACP, and exposure to PI-containing regimens was associated with increasing odds of abnormal ACP; no significant effect in the first and second trimesters was observed but aOR was more than doubled at the third trimester. The association between lower aOR of abnormal ACP with longer exposure to lamivudine was reflected in the 51% lower odds in exposure versus no exposure during the first trimester. Sensitivity analyses accounting for repeat pregnancies and site yielded similar estimates.

Association of abnormal ACP with clinical laboratory measures

Of 522 infants 472 had valid laboratory tests results, and 365 (77%) of them were from the same day as the serum

figure to show pathway

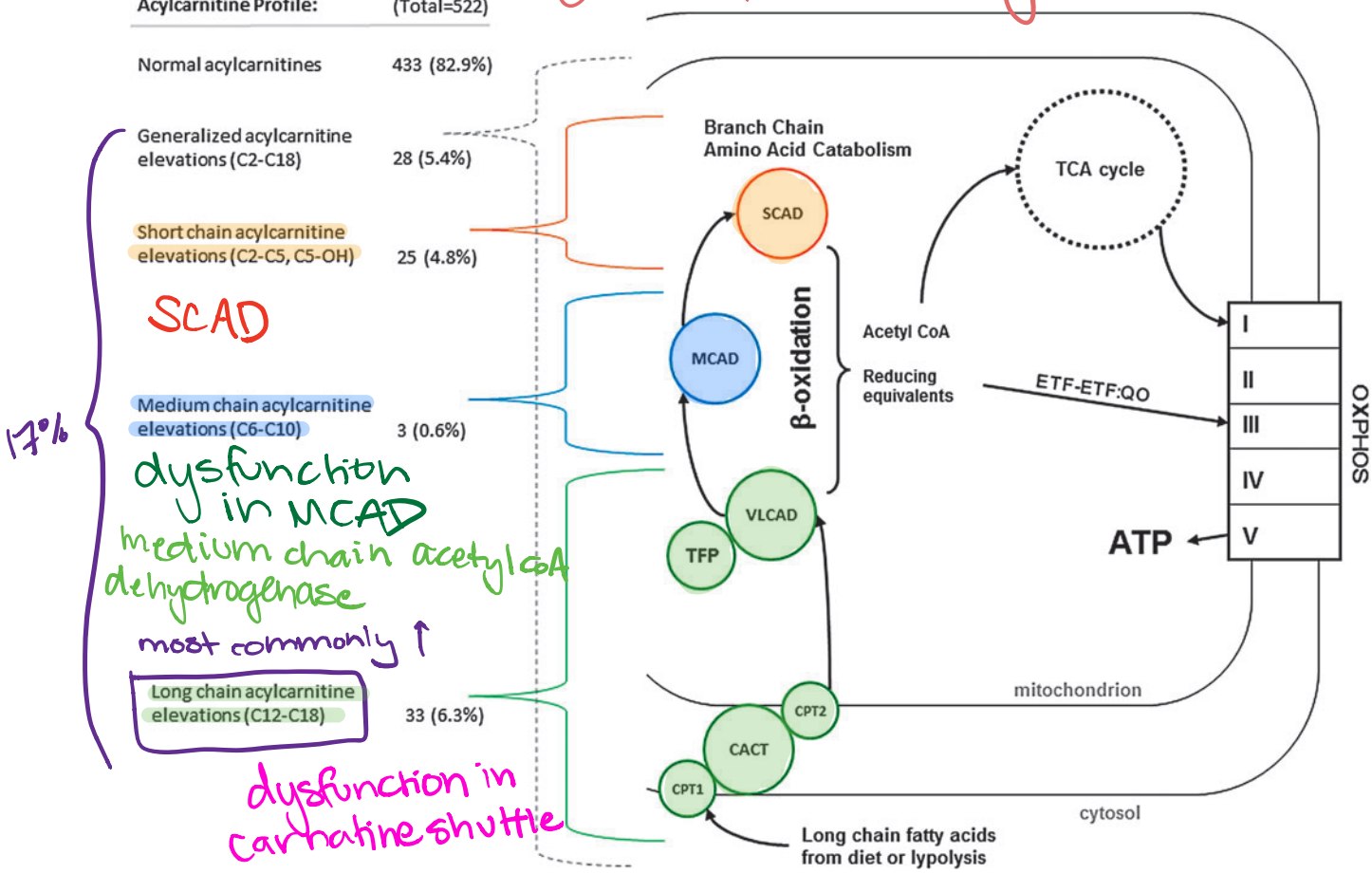

FIG. 1. Proportions and patterns of abnormal ACP in HEU newborns. The pattern of acylcarnitine elevations can point to the level of biochemical dysfunction in FAO. Long-chain acylcarnitine elevations (C12–C18, where the number corresponds to the number of carbons chain length of fatty acid) indicate dysfunction in the carnitine shuttle (CPT1, CACT, CPT2), which transports long-chain fatty acids into the mitochondria, or the first steps (TFP, VLCAD) of beta-oxidation, which convert long-chain fatty acids into medium-chain fatty acids. Elevations of medium-chain acylcarnitines (C6–C10) indicate dysfunction in MCAD. Short-chain acylcarnitine elevations can indicate dysfunction in SCAD (C4) or in pathways of organic acid catabolism (C3, C5, C5-OH). Generalized acylcarnitine elevations can be seen in ETF-ETF:QO dysfunction or in primary dysfunction of oxidative phosphorylation. ACP, acylcarnitine profiles; ATP, adenosine triphosphate; HEU, HIV-exposed uninfected; FAO, fatty acid oxidation; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid cycle; CPT, carnitine palmitoyltransferase; CACT, carnitine-acylcarnitine translocase; TFP, trifunctional protein; VLCAD, very-long-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase deficiency; SCAD, short-chain acyl-CoA dehydrogenase deficiency; ETF, electron transport flavoprotein; ETF:QO, electron transport flavoprotein oxidoreductase. Color images available online at www.liebertpub.com/aid

} define

↑ALT= ↑ACP

used for the ACP. In this subset, 51/365 (14.0%) had an abnormal ACP. Although only three infants had elevated alanine aminotransferase (ALT) by DAIDS AE grades 1 and 2, all three had abnormal ACP, which in turn was associated with higher ALT levels. Unadjusted (least square) geometric mean ALT was 19 IU/liter in those with an abnormal ACP and 14.6 IU/liter in those with a normal ACP ($p < 0.01$). Adjusted, the geometric mean ALT levels were 5.21 IU/liter higher in infants with abnormal ACP compared to infants with normal ACP. There were three infants with abnormal ACP who had ALT above the reference range; none of the infants with normal ACP had ALT above the reference range. No associations of abnormal ACP with lactate, glucose, or CPK were observed. Sensitivity analyses including all 472 infants yielded similar results.

Abnormal ACP and neurodevelopmental, growth measures

There were no significant differences in composite scores between children with normal and abnormal ACP in any of the four domains of the 1-year MacArthur–Bates CDI assessments (Supplementary Table S1), in the proportion with LLE (22% versus 20%) among 346 children with data available, or in any of the five domains of the 1-year Bayley scores for 256 children with data available, or in anthropometric measures at age 2 years (334 children) and age 3 years

summarizing data

TABLE 1. SUMMARY OF MATERNAL AND INFANT CHARACTERISTICS BY ACYLCARNITINE PROFILE

Characteristic[a]	*Acylcarnitine profile*			
	Normal (N = *433*)	*Abnormal* (N = *89*)	*Total* (N = *522*)	p-*Value*[b]
Infant age (days) at time of specimen draw				
0	147 (34%)	7 (8%)	154 (30%)	<0.001 *
1	108 (25%)	42 (47%)	150 (29%)	
2–3	96 (22%)	26 (29%)	122 (23%)	
4–7	82 (19%)	14 (16%)	96 (18%)	
Infant gender–female	217 (50%)	40 (45%)	257 (49%)	0.42
Infant race				
Black	241 (56%)	44 (49%)	285 (55%)	0.50
White	154 (36%)	35 (39%)	189 (36%)	
Other	4 (1%)	0 (0%)	4 (1%)	
Not known/not reported	34 (8%)	10 (11%)	44 (8%)	
Hispanic or Latino	195 (45%)	45 (51%)	240 (46%)	0.46
Maternal age at delivery (years)				
21–37 years	326 (75%)	73 (84%)	399 (77%)	0.25
<21 years	47 (11%)	7 (8%)	54 (10%)	
>37 years	59 (14%)	7 (8%)	66 (13%)	
Mother not high school graduate	164 (38%)	31 (35%)	195 (38%)	0.72
Annual household income <$20,000	306 (76%)	64 (76%)	370 (76%)	1.00
Maternal substance use during pregnancy				
Alcohol	29 (7%)	15 (17%)	44 (8%)	0.005 *
Illicit drugs	39 (9%)	9 (10%)	48 (9%)	0.69
Maternal exposure to smoking: active or passive	161 (38%)	44 (52%)	205 (40%)	0.022 *
Gestational Age				
Mean (SD)	38.3 (1.9)	37.6 (2.0)	38.2 (1.9)	<0.001 *
Preterm (<37 weeks)	64 (15%)	20 (22%)	84 (16%)	0.018 *
Growth *z*-scores at birth [mean (SD)]				
Weight	−0.54 (0.87)	−0.68 (0.97)	−0.56 (0.89)	0.082
Length	−0.13 (1.04)	−0.37 (0.99)	−0.17 (1.03)	0.014 *
Weight for length	−0.53 (1.21)	−0.54 (1.37)	−0.53 (1.24)	0.94
Head circumference	−0.61 (1.13)	−0.72 (0.85)	−0.63 (1.08)	0.57
Maternal health measures during pregnancy				
First CD4% <25%	170 (40%)	40 (46%)	210 (41%)	0.28
First RNA ≥1,000 copies/ml	224 (52%)	39 (45%)	263 (51%)	0.29
Last CD4% <25%	120 (28%)	27 (31%)	147 (29%)	0.60
Last RNA ≥1,000 copies	54 (13%)	12 (14%)	66 (13%)	0.72
ARV regimen during pregnancy				
cARV with NNRTI	23 (5%)	1 (1%)	24 (5%)	0.087
cARV with NNRTI and PI	28 (6%)	2 (2%)	30 (6%)	
cARV with PI	331 (76%)	76 (87%)	407 (78%)	
cARV with RAL (no PI or NNRTI)	6 (1%)	0 (0%)	6 (1%)	
Other[c]	42 (10%)	6 (7%)	48 (9%)	
No ARV	3 (1%)	2 (2%)	5 (1%)	
Specific ARV exposures				
NNRTI	51 (12%)	3 (3%)	54 (10%)	0.020 *
PI	359 (83%)	78 (90%)	437 (84%)	0.15
ZDV	313 (72%)	61 (70%)	374 (72%)	0.70
3TC	332 (77%)	59 (68%)	391 (75%)	0.10
Duration of ARV exposure during pregnancy (median days (Q1, Q3)				
ZDV	115.5 (0, 177)	105 (0, 150)	111 (0, 170)	0.11
3TC	136 (21, 186)	107 (0, 155)	131 (8, 182)	0.003 *

[a]Some characteristics were not available for all subjects, including ethnicity (1), maternal age at delivery (3), maternal education (3), income (37), substance use (2), smoking (15), gestational age (4), weight for length (36), head circumference (3), CD4% (7), RNA (5), ARV exposure (2), and duration of ARV (3).

[b]*p*-value by Fisher's exact test for categorical characteristics and by Wilcoxon rank sum test for continuous measures.

[c]Other includes 46 3+ NRTI and 2 mono-/dual-ARV.

cARV, combination antiretroviral drug; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RAL, raltegravir; ZDV, zidovudine; 3TC, lamivudine.

statistical significance

$\alpha = .05$ → <5% chance of being due to random chance

Table 2. Adjusted Associations for Abnormal Acylcarnitine Profile by Maternal and Demographic Risk Factors in 498 SMARTT Infants

	Adjusted odds ratio	*95% confidence interval*	p-*value*
Demographic characteristics			
Gestational age (per week lower)	1.21	(1.07,1.36)	0.002
Maternal age at delivery (years)			0.080
<21 vs. 21–37	0.71	(0.30, 1.70)	0.72
>37 vs. 21–37	0.36	(0.14, 0.91)	0.090
Maternal exposures and risk behaviors during pregnancy			
Alcohol use	2.55	(1.21, 5.37)	0.014
Smoking (active or passive)	1.65	(0.98, 2.77)	0.058
Maternal health status during pregnancy			
Earliest available CD4% <25%	1.64	(0.98, 2.75)	0.058
Earliest available RNA ≥1,000 cp/ml	0.61	(0.36, 1.01)	0.056

(236 children) for those who had reached those ages (Supplementary Table S2).

Discussion

In this cohort of HEU newborns, abnormal ACP are more common than expected (17%). ACP abnormalities in this cohort were not in the range that would suggest a classic inborn error of FAO, but they suggest a subgroup of HEU newborns with biochemical evidence of dysfunctional FAO. While we did not detect any serious clinical problems in those infants with abnormal ACP, we did find that newborns with evidence of dysfunctional FAO were more likely to have been exposed *in utero* to alcohol, smoking, and protease inhibitors, as well as to have been born preterm and, as a group, have slightly higher ALT levels.

Although we used interpretation guidelines similar to NBS, 17% is higher than the proportion of HEU with abnormal ACP (1.6%) observed in our previous population-based study.[26] The higher prevalence of abnormal ACP here may be due to bias in the subset of SMARTT subjects who had available serum (studied subjects had longer duration of exposure to NRTIs and PIs), differences in the sample type (serum here versus dried blood spots in NBS), as well as our not having employed second tier tests (secondary analytes, analyte ratios, postanalytic filtering tools) that are commonly used in state NBS laboratories to decrease false-positive rates and refine the population of newborns who are reported as having abnormal ACP to pediatricians. Because the parent study, PHACS SMARTT, focuses on evaluating the safety of perinatal ARV exposure, we sought to capture the full range of acylcarnitine abnormalities in HEU neonates and our aim was not to diagnose bona fide NBS disorders. Admittedly, a major limitation of our study is the lack of an appropriate control group for comparison, but samples from such a group were not available through the parent study, which does not enroll HIV-unexposed newborns, nor the testing laboratory where ACP are analyzed primarily for clinical indications such as critical illness and follow-up of an abnormal state NBS.

The most common pattern of abnormal ACP (6.3%) was characterized by elevations in long-chain acylcarnitines, localizing dysfunction to either the enzymes that transport long-chain fatty acids into the mitochondria (''the carnitine shuttle'') or the intramitochondrial enzymes that initiate long-chain FAO.[27] The next most common pattern of abnormal ACP (5.4%) comprised several elevated acylcarnitine species of various fatty acid chain lengths (short, medium, and long) suggesting generalized dysfunction in the pathway. This generalized pattern can be seen in primary OXPHOS

Table 3. Associations Between Abnormal Acylcarnitine Profiles and Maternal Antiretroviral Regimens Adjusted for Other Risk Factors in 494 SMARTT Infants

ARV exposure	*Adjusted odds ratio*	*95% confidence interval*	p-*value*
Combination ARV regimen		—	0.024
cARV with NNRTI (w/ and w/o PI)	0.22	(0.06,0.75)	0.015
Other regimen	0.49	(0.18,1.32)	0.16
cARV with PI (no NNRTI)	1.00	(ref)	
ARV exposures by individual class or drug			
NNRTI	0.23	(0.07,0.80)	0.020
PI	2.35	(0.96,5.76)	0.062
ZDV	0.88	(0.51,1.53)	0.66
Duration of ZDV exposure (weeks)	0.98	(0.96,1.00)	0.12
3TC	0.67	(0.39,1.15)	0.15
Duration of 3TC exposure (weeks)	0.97	(0.95,0.99)	0.011

Each row represents a separate logistic regression model on one single *in utero* ARV exposure variable adjusted for gestational age, maternal age, alcohol use, smoking exposure, earliest CD4% <25%, and earliest viral load ≥1,000 copies/ml during pregnancy.

cARV, combination antiretroviral drug; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; ZDV, zidovudine; 3TC, lamivudine.

TABLE 4. ASSOCIATIONS BETWEEN ABNORMAL ACYLCARNITINE PROFILES AND ANTIRETROVIRAL REGIMENS BY PREGNANCY TRIMESTER

	First trimester (N = 494)			*Second trimester (N = 494)*			*Third trimester (N = 491)*		
ARV exposure	*Adjusted odds ratio*	*95% confidence interval*	p-*value*	*Adjusted odds ratio*	*95% confidence interval*	p-*value*	*Adjusted odds ratio*	*95% confidence interval*	p-*value*
Combination ARV regimen		—	0.32		—	0.36		—	0.10
cARV with NNRTI (w/ and w/o PI)	0.31	(0.09,1.09)	0.069	0.28	(0.06,1.22)	0.090	0.28	(0.06,1.26)	0.097
Other regimen	1.03	(0.34,3.11)	0.96	0.76	(0.32,1.83)	0.54	0.52	(0.21,1.29)	0.16
No ARV	1.02	(0.59,1.78)	0.93	0.80	(0.26,2.46)	0.70	—	—	—
cARV with PI (no NNRTI)	1.00	(ref)		1.00	(ref)		1.00	(ref)	
Exposure to Individual ARV drug or class									
NNRTI	0.30	(0.09,1.05)	0.061	0.29	(0.07,1.27)	0.10	0.30	(0.07,1.34)	0.12
PI	0.97	(0.58,1.61)	0.90	1.40	(0.72,2.72)	0.32	2.23	(0.97,5.17)	0.060
ZDV	0.62	(0.34,1.12)	0.11	1.09	(0.65,1.85)	0.74	1.01	(0.58,1.76)	0.98
3TC	0.49	(0.27,0.89)	0.018	0.85	(0.51,1.44)	0.56	0.80	(0.46,1.40)	0.44

cARV, combination antiretroviral drug; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; ZDV, zidovudine; 3TC, lamivudine.

dysfunction as well as disruption of electron transport flavoprotein, the physical connection between FAO and OXPHOS.[28] Additionally, 4.8% of the cohort had ACP characterized by abnormal elevations of short-chain acylcarnitines, which can indicate perturbed short-chain FAO or branched-chain amino acid catabolism.

We found that *in utero* exposure to PIs was associated with a greater odds of abnormal ACP of any pattern. There are several potential mechanisms by which PIs might perturb FAO. First, PIs are associated with abnormal lipid metabolism[29] and may directly suppress FAO through inhibition of CD36, the fatty acid translocase.[30] There is *in vitro* and clinical evidence that PIs can cause mitochondrial dysfunction through the generation of reactive oxygen species and by triggering pathways of mitophagy.[31,32] Importantly, PIs can also cause insulin resistance. FAO is perturbed in patients with insulin resistance, resulting in abnormal acylcarnitine levels[33] and excessive acylcarnitines may even play a role in the signaling pathways that lead to the development of insulin resistance.[15] Taken together, the effects of PIs on FAO may be multifactorial and include direct FAO suppression, mitochondrial dysfunction, and insulin resistance.

Conversely, NNRTI use was strongly associated with lower odds of abnormal ACP. NNRTIs, as a class, are not clinically associated with abnormal mitochondrial or lipid metabolism in children, although they are associated with hepatotoxicity in some.[34] The protective effect of NNRTI in our study may be due to the absence of concomitant PI exposure. Our findings suggest that NNRTIs may have a less stressful effect on the *in utero* metabolic milieu than PIs.

Alcohol and smoking exposure were associated with abnormal ACP in this study, but when considered in the multivariate models did not change the ARV-specific effects. Alcohol is a known hepatotoxin[35] that may interact with PIs to cause hepatocyte injury in some patients.[36] While there is no evidence for a direct effect of smoking exposure on FAO, smoking can cause increased oxidative stress[37] that theoretically could adversely affect intramitochondrial pathways such as FAO.

Infants with abnormal ACP were more likely to have lower gestational age at birth. It is possible that metabolic immaturity may contribute to dysfunctional FAO in premature neonates since false-positive newborn screens are more common in premature and low-birth-weight infants,[38] but this association has been found mainly for disorders of amino acid and hormone metabolism rather than FAO disorders.[39,40] We do not yet know where dysfunctional FAO lies on the causal pathway for preterm birth.

There are well-documented maternal–fetal complications [fatty liver of pregnancy and the hemolysis, liver dysfunction, low platelet (HELLP) syndrome] in pregnancies in which the fetus has a classic disorder of FAO.[39] Preterm birth and intrauterine growth restriction are common in neonates with certain inherited FAO disorders and especially prevalent in those with long-chain FAO disorders.[41] Newborns in our study with abnormal ACP tended to have lower birth weights than those with normal ACP (gestational age adjusted *z*-score −0.68 vs. −0.54) and this effect seemed to be driven by those with a long-chain pattern of abnormal ACP (gestational age adjusted birth weight *z*-score −0.80, data not shown). Additionally, recent clinical studies have shown that *in utero* PI exposure is associated with preterm birth, although the mechanism is not yet clear.[42] Future studies should examine whether dysfunctional FAO in the context of *in utero* PI exposure plays a role in gestation length and birth weight in HEU neonates, both of which are important for future and adult health.

Of the clinical laboratory outcomes that we analyzed, only ALT was statistically higher in those with an abnormal ACP. FAO dysfunction can lead to both hepatocytic and cardiomyocytic injury in children with inherited FAO disorders.[43] Hypoglycemia and elevated CPK levels, seen in severe FAO disorders, were not more common in our HEU newborns with abnormal ACP, again suggesting that in the first week of life FAO dysfunction is subclinical and mild. Furthermore, lactate, which is a clinical biomarker of OXPHOS dysfunction, was not significantly increased in those newborns with abnormal ACP.

FAO is an active metabolic pathway in the neurons and is highly expressed in the developing central nervous system and retina.[44] Speech and motor delays occur in 54% of those with inherited FAO disorders identified on NBS.[45] Although we hypothesized that FAO dysfunction in HEU neonates might manifest as neurodevelopmental delay, reassuringly, neurodevelopmental indices at 1 year of age were similar between those with normal and abnormal ACP.

In conclusion, 17% of HEU newborns in our study had abnormal ACP indicative of perinatal FAO dysfunction, which was more than expected. Abnormal ACP appears to be more likely in newborns with *in utero* exposure to PI-containing compared to NNRTI-containing regimens as well as in those exposed to alcohol and smoking. While it is reassuring that we did not find any serious clinical manifestations associated with abnormal ACP during early postnatal life, there is accumulating evidence that *in utero* nutritional and metabolic stress have far-reaching implications for the development of adult-onset disorders such as diabetes, obesity, and cardiovascular disease.[46,47] Moreover, it is not yet known whether having abnormal acylcarnitine levels at birth has implications for the development of chronic diseases, as is the case in adults with elevated acylcarnitines.[48] For these reasons it will be important to follow the health of HEU newborns with abnormal ACP beyond early childhood. This is especially crucial in a world in which access to ARV for pregnant HIV-positive women is increasing[49] and the reduction of chronic disease-related morbidity and mortality is a global health priority.[50]

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