MITOCHONDRIAL PROTON LEAK IN INFANTS WITH IN UTERO HIV/ART EXPOSURE IN BOTSWANA #00696

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BACKGROUND

- Mitochondria play a crucial role as the cellular powerhouse, governing cellular respiration and harnessing energy from glucose and other nutrients.
- Data are limited on mitochondrial (mt) function in infants with *in utero* HIV/ARV exposure who are uninfected (HEU).
- We evaluated mt function in infants HEU using a comparison group of infants HIV-unexposed uninfected (HUU) in Botswana.

METHODS

Study population

- The Tshilo Dikotla study enrolled pregnant women with HIV and HIV-seronegative pregnant women as well as their offspring from 2016-2019 in Botswana to assess metabolic effects of *in utero* HIV and ARV exposure.
- This analysis included infants HEU and HUU at 1 month of age with available mt function results.

Primary outcome

Mt oxygen consumption was measured in viable peripheral blood mononuclear cells from infants at 1 month of age using a Seahorse XFe96 to assess the following:

- Basal and maximal respiration
- ATP production
- Proton leak
- Spare respiratory capacity
- Non-mitochondrial respiration

Exposure of interest

In utero HIV/ARV exposure

Statistical analysis

- Linear regression models assessed the association between *in utero* HIV/ARV exposure and each log-transformed mt function parameter, adjusting for confounders.
- Infant HEU subgroup analyses assessed the association of *in utero* ARVs [tenofovir (TDF)/emtricitabine or lamivudine (XTC)/dolutegravir (DTG) vs. TDF/XTC/efavirenz (EFV)] with each mt outcome.

In this cohort from Botswana, infants with *in utero* HIV/ARV exposure had higher mitochondrial proton leak compared to infants without HIV/ARV exposure at 1 month of age, indicating immune cell mitochondrial uncoupling.

RESULTS

- N=202 infants (n=133 HEU, n=69 HUU)
- Maternal age (median 29.3 vs. 25.6 yr, *p<0.01*) was higher among women of infants HEU vs HUU. (Table 1)
- Median levels of mt proton leak were higher in infants HEU vs HUU [12.45 vs 10.78 pmol/min, *p=0.02*] in univariate analysis. (Table 2)
- **This relationship persisted even after adjusting for maternal age, GDM, infant sex, preterm birth, WAZ, exclusive breastfeeding, and HOMA (mean difference in proton leak was 0.15 log units higher in HEU vs HUU, *p<0.01*).**
- No differences in any other mt parameters were noted between groups.
- Among infants HEU, there was no association of *in utero* ARV with any mt function parameter.

Table 2. Mitochondrial Oxygen Consumption Rate (OCR) Measures by HIV/ART Exposure Status

	HEU infants (n=133) (pmol/min)	HUU infants (n=69) (pmol/min)	*p* value
Basal respiration	45.66 (32.82 - 63.94)	47.16 (30.96 - 60.52)	*0.40*
Maximal respiration	121.60 (86.68 -176.30)	118.20 (71.49 -155.90)	*0.18*
ATP production	33.01 (23.72 - 47.77)	36.35 (21.57 - 50.46)	*0.45*
Proton leak	12.45 (8.35 - 16.95)	10.78 (6.43 - 14.59)	*0.02*
Spare respiratory capacity	73.71 (45.48 - 113.6)	62.77 (35.49 - 103.7)	*0.10*
Non-mitochondrial respiration	22.07 (13.41 - 27.83)	19.56 (13.20 - 27.45)	*0.20*

Table 1. Characteristics of women and infants at enrollment

	Infants HEU (n=133)	Infants HUU (n=69)	*p* value
MATERNAL CHARACTERISTICS			
Sociodemographic			
Age at delivery (years)	29.3 (26.0, 34.7)	25.9 (22.0, 29.7)	*<0.01*
Annual income (USD)			
>$1200	60 (45.1)	36 (52.2)	*0.42*
$240 - $1199	20 (15.0)	10 (14.5)	
<$240	52 (39.1)	21 (30.4)	
Unsure/Unknown	1 (0.8)	2 (2.9)	
Married	8 (6.0)	9 (13.0)	*0.09*
Employed	65 (48.9)	31 (44.9)	*0.59*
Past Obstetric History			
Gravidity	3.0 (2.0, 4.0)	1.0 (1.0, 3.0)	*<0.01*
BMI at 1 month postpartum (kg/m²)	24.2 (21.4, 28.5)	24.5 (21.9, 29.1)	*0.18*
Hypertensive*	6 (4.5)	2 (2.9)	*0.57*
Gestational diabetes	10 (7.5)	5 (7.2)	*0.94*
CD4 cell count (cells/mm³)			
<=200	6 (4.5)	--	--
CD4 201-500	62 (46.6)	--	--
CD4 >500	63 (47.4)	--	--
Missing	2 (1.5)	--	--
HIV RNA level <40 (copies/mL)	123 (92.5)	--	--
On ART at conception	68 (51.1)	--	--
ART regimen			
TDF/XTC/DTG	91 (68.4)	--	--
TDF/XTC/EFV	42 (31.6)	--	--
INFANT CHARACTERISTICS			
Gestational age at delivery (weeks)	39.3 (37.7, 40.3)	39.7 (38.6, 40.7)	*0.06*
1 Month weight z score	0.3 (-0.2, 0.9)	0.4 (-0.1, 1.1)	*0.22*
1 Month length z score	0.2 (-0.6, 0.9)	0.1 (-0.8, 0.7)	*0.27*
Exclusive breastfeeding at 1 month	90 (67.7)	50 (72.5)	*0.48*
HOMA-IR at 1 month	0.7 (0.5, 1.1)	0.7 (0.4, 1.0)	*0.23*

All continuous variables expressed as median (interquartile range) and categorical variables as n (%). P values from Wilcoxon and Chi-Square tests as appropriate.
**Defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg; ART=antiretroviral therapy, BMI=body mass index, DTG=dolutegravir, EFV=efavirenz, XTC=emtricitabine or lamivudine, HOMA-IR=homeostatic model assessment for insulin resistance*

CONCLUSIONS

- In this Botswana cohort, mt function in infants HEU compared to infants HUU at 1 month of age was largely similar. However, infants HEU had higher mt proton leak, indicating immune cell mt uncoupling.
- While mt uncoupling may be a compensatory mechanism, it is also associated with increased oxidative stress which could potentially lead to metabolic diseases later in life.
- Future studies in larger cohorts are needed to confirm these findings and understand the long-term significance of mt dysfunction in infants HEU on metabolic health.

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