Abstract #376
Poster Session #P-U5

Insulin Resistance in HIV-Infected Youth is Associated with Decreased Mitochondrial Respiration

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ABSTRACT

Background: Antiretroviral therapy (ART) has dramatically reduced vertical HIV transmission and prolonged survival of HIV-infected children. However, these advances may lead to progressive long-term metabolic complications including insulin resistance (IR). We hypothesize that HIV and/or ART cause mitochondrial dysfunction, decreasing cellular respiration and thereby increasing oxidative stress and lactate/pyruvate levels, resulting in IR.

Methods: A subset of children perinatally infected with HIV (pHIV) enrolled in the Pediatric HIV/AIDS Cohort Study were co-enrolled in a mitochondrial substudy. Fasting venous lactate, glucose, and insulin levels were measured. IR was defined by Homeostasis Model Assessment (HOMA-IR) >4.0. Mitochondrial respiration was measured from peripheral blood mononuclear cells utilizing the Seahorse XF24 analyzer (http://www.seahorsebio.com) in a random sample of 25 with IR (IR+) and 50 without (IR-). Unadjusted differences in mitochondrial respiration between IR+ and IR- were evaluated using the Wilcoxon rank sum test. Correlations between mitochondrial respiration (basal, ATP production, proton leak, maximal, spare, and non-mitochondrial respiration) and metabolic measurements were evaluated by Spearman correlation.

Results: IR+ were similar to IR- in age (median 16.5 v 15.6 yr), males (60% v 50%), Non-Hispanic black (68% v 72%), and current CD4 counts (574 v 686 cells/mm3). IR+ trended toward a lower nadir CD4% (12% v 17%, p=0.07) and a higher peak viral load (607K v 185K, p=0.07). There were no significant differences in past or current type of ART. Median venous lactate (1.6 v 1.1 mM , p<0.001), pyruvate (0.12 v 0.09 mM, p=0.003), and glucose (88 v 85 mg/dL, p=0.007) were higher in IR+. Furthermore, proton leak (585 v 790 pmoles, p=0.03), maximal respiration (1815 v 2499 pmoles, p=0.025), and spare respiratory capacity (1162 v 2017 pmoles, p=0.03) were significantly lower in the IR+ group. Basal respiration, ATP production, proton leak, maximal respiration, and spare respiratory capacity were negatively associated with HOMA-IR (p<0.009), while non-mitochondrial respiration was positively associated with glucose (p=0.02).

Conclusions: Insulin resistance is associated with lower mitochondrial respiratory activity in pHIV youth. These findings provide a possible mechanism for metabolic dysfunction in pHIV youth.

BACKGROUND

- Insulin resistance (IR) is common among pHIV children.[1]
- Mitochondrial dysfunction, induced by ARV or chronic viral infection, is a possible mechanism for metabolic dysfunction.[2,3]

OBJECTIVES

- To compare mitochondrial function of HIV+ with and without IR
- To identify relationships between mitochondrial and clinical parameters

REFERENCES

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METHODS AND RESULTS

•Protocol: The Adolescent Master Protocol (AMP) of the Pediatric HIV/AIDS Cohort Study (PHACS), is a prospective cohort study conducted at 12 US sites designed to define the impact of HIV infection and ARV on pre-adolescents and adolescents with perinatal HIV infection. The Mitochondrial Determinants Component (MDC) is a separately funded study (R01 NR012885) that has co-enrolled AMP participants since May 2011. The objective of MDC is to determine the influence of mitochondrial abnormalities on metabolic outcomes in HIV+ children enrolled in PHACS from 7 years of age until their 16th birthday. HIV exposed but uninfected are also enrolled, to serve as controls, but not included in the analysis. Fasting venous lactate, glucose, insulin, and lipid levels were measured. IR was defined by homeostasis model assessment (HOMA-IR) >4.0, as all children were at Tanner stage 2-5. Medical history was abstracted from medical charts and anthropometric measurements were performed by standard techniques.

•Population: 75 HIV+ children were studied; 25 insulin resistant (IR+) and 50 non-insulin resistant (IR-) with 2 or more banked vials.

•Main outcomes: IR measured by HOMA-IR [normal: Tanner stage 2+ (≤ 4)], total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides.

•Mitochondrial parameters included: Mitochondrial respiratory parameters were assessed from a MitoStress Test in PBMCs (Seahorse XF24, Seahorse Biosciences, MA).

•Statistical analyses: IR+ and IR- were compared by different patient characteristics, mitochondrial parameters, and lipid levels. Wilcoxon test for continuous variables and Fisher's exact test or Kruskal Wallis test for categorical variables. Spearman correlation was used to compare two continuous variables.

Table 1. Patient Characteristics

Characteristic	IR+ (25)	IR- (50)	P value
Age, median (yr)	16.54	15.59	0.104*
Male (%)	15 (60)	25 (50)	0.468**
Race, NHB (%)	17 (68)	36 (72)	1.00**
Tanner Stage			
Tanner Stage 2 (%)	2 (8)	8 (16)	0.521**
Tanner Stage 3 (%)	1 (4)	5 (10)	
Tanner Stage 4 (%)	3 (12)	8 (16)	
Tanner Stage 5 (%)	19 (76)	29 (58)	
CDC Class; n (%)			
A—Mildly Symptomatic	7 (28)	10 (20)	0.339**
B—Moderately Symptomatic	12 (48)	17 (34)	
C—Severely Symptomatic	4 (16)	13 (26)	
N—Not Symptomatic	2 (8)	10 (20)	
Current HAART Regimen			
HAART with PI	17 (68)	31 (62)	0.971**
HAART without PI	4 (16)	9 (18)	
Non-HAART ARV	1 (4)	4 (8)	
Not on ARV	2 (8)	5 (10)	
CD4 Count,§ %, [IQR]	29.1 [18, 34.5]	34.5 [24.85, 38.4]	0.138*
Viral Load, copies/mL¥ Median, [IQR]	180 [40, 5071]	349 [48, 5878]	0.442*
BMI Z-score, Median, [IQR]	1.11 [0.57, 1.87]	0.18 [-0.44, 0.75]	**<0.001***
Weight Z-score, Median, [IQR]	0.78 [0.28, 1.53]	0.17 [-0.52, 0.70]	**0.009***
Height Z-score, Median,[IQR]	-0.41 [-1.51, 0.41]	-0.05 [-1.17, 0.59]	0.416*

Table 1. Patient Demographics. Unless otherwise indicated n for IR+ is 25 and n for IR- is 50. BMI: body mass index. §N of 24/44; ¥N of 23/46. Significant differences were observed in BMI and weight Z-score. *Wilcoxon and **Fisher's Exact tests.

Table 2. Mitochondrial Respiratory Parameters. The differences in mitochondrial respiratory parameters, expressed as area under the curve.

Figure 1. Mitochondrial Respiration in Peripheral Blood Mononuclear Cells

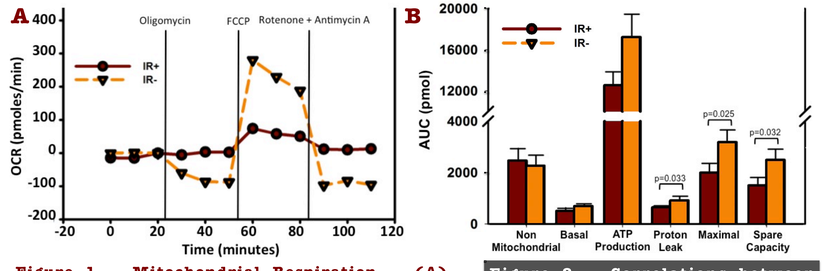


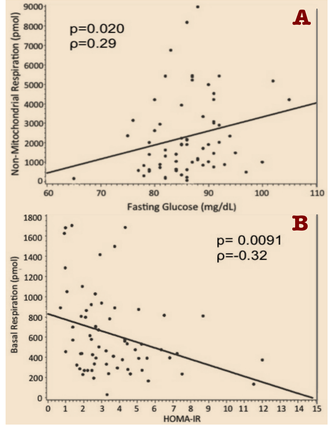
Figure 1. Mitochondrial Respiration. **(A)** Representative Respiration Curve of an Insulin-Resistant (IR+) and a Non-Insulin-Resistant Subject. Injections of inhibitors (oligomycin [ATP synthase], FCCP [uncoupler], and rotenone and antimycin A [CI and CIII]. OCR, oxygen consumption. **(B)** Differences in Mitochondrial Respiratory Parameters in 22 Insulin-Resistant and 44 Non-Insulin-Resistant Adolescents. AUC, area under the curve.

Figure 2. Correlations. **(A)** Relationship Between Non-Mitochondrial Respiration and Fasting Glucose. **(B)** Relationship Between Basal Respiration and HOMA-IR.

Table 2. Mitochondrial Respiratory Parameters

AUC (pmol) median, [IQR]	IR+ (22)	IR- (44)	P-value*
Non-Mitochondrial Respiration	1562.92 [895.18, 4215.83]	1912.09 [785.83, 3055.51]	0.881
Basal Respiration	417.50 [281.67, 560.83]	597.50 [335.42, 905.84]	0.074
ATP Production	11513.34 [7760.83, 15220.00]	15203.09 [9164, 224450.00]	0.078
Proton Leak	584.59 [487.50, 1057.92]	790 [590.00, 1057.92]	**0.033**
Maximal Respiration	1815.84 [889.17, 2652.50]	2499.17 [1446.67, 4492.92]	**0.025**
Spare Respiratory Capacity	1162.5 [585.83, 2225.00]	2017.08 [1088.75, 3718.75]	**0.032**

Figure 2. Correlations between Mitochondrial Parameters and (A) Glucose and (B) HOMA-IR



RESULTS

Table 3. Clinical Metabolic and Anthropometric Characteristics

Metabolic Outcome	N (IR+/IR-)	IR+	IR-	P-value
Glucose (mg/dL)	25/50	88 [84, 93]	85 [80, 89]	**0.007***
Insulin (mU/mL)	25/50	24.7 [22.4, 32.2]	10.9 [6.9, 13.5]	**<0.001***
HOMA-IR	25/50	5.9 [4.6, 7.4]	2.3 [1.4, 2.8]	**<0.001***
Total cholesterol (mg/dL)	25/49	182 [151, 198]	153 [129, 172]	**0.018***
Triglycerides (mg/dL)	25/49	110 [88, 179]	79 [54, 98]	**<0.001***
LDL-cholesterol (mg/dL)	25/49	101 [68, 126]	86 [73, 100]	0.078*
HDL-cholesterol (mg/dL)	25/49	49 [43, 56]	50 [41, 58]	0.923*
Venous Lactate (mM)	24/46	1.6 [1.2, 1.9]	1.10 [0.8, 1.2]	**<0.001***
Venous Pyruvate (mM)	24/45	0.12 [0.09, 0.17]	0.09 [0.06, 0.11]	**0.003***
TSF (mm)	18/43	13.9 [7.0, 21.3]	11.7 [7.0, 15.1]	0.752**
BS (mm)	19/46	9.0 [4.0, 7.7]	7.3 [4.0, 9.0]	0.569**
SISF (mm)	19/44	15.6 [7.0, 21.7]	11.3 [4.7, 14.1]	0.056**
SSSF (mm)	17/43	15.4 [9.0, 18.2]	11.1 [7.7, 13.3]	0.065**
MUAC (cm)	21/49	28.9 [26.5, 30.3]	26.3 [23.7, 28.5]	**0.016****
MWC (cm)	21/49	84.2 [76.0, 89.5]	75.4 [68.6, 82.0]	**0.004****
MHC (cm)	21/49	91.4 [83.0, 98.1]	87.3 [79.7, 94.5]	0.204**
WHR	21/49	0.92 [0.87, 1.0]	0.86 [0.82, 0.91]	**0.015****

Table 3. Metabolic and Anthropometric Characteristics. Differences in metabolics and anthropometrics were observed. HOMA-IR: homeostasis model of assessment of insulin resistance; SISF: suprailiac skin fold; SSSF: subscapular skin fold; MUAC: mid-upper arm circumference; MWC: mid-waist circumference; WHR: waist-to-hip ratio. *Wilcoxon Test. **Fisher's Exact test.

CONCLUSIONS

- IR+ children were heavier and had greater BMI than IR- children.
- Mitochondrial respiratory parameters (basal respiration, ATP production, proton leak, maximal respiration, and spare respiratory capacity) were lower in the IR+ than IR- subjects, and were negatively correlated to HOMA-IR, indicating lowered respiration because of insulin resistance, thus suggesting less production of energy.
- Non-mitochondrial respiration was higher in IR+, but also correlated with fasting glucose, suggesting an alternative method (glycolysis) for producing energy.
- Clinical and anthropometric differences were observed between IR+ and IR-, suggesting that altered utilization of substrates are used for energy production.
- In summary, IR+ subjects are heavier, had higher lipid levels, and were not producing energy efficiently through the electron transport chain.

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