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Maternal post-absorptive leucine kinetics during late pregnancy in US women with HIV taking antiretroviral therapy: a cross-sectional pilot study

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Abstract

Background—Despite the success of combination antiretroviral therapy (cART) for the prevention of mother to child transmission of HIV, infants exposed to cART *in utero* frequently are born smaller and have mild cardiac abnormalities. The mechanisms responsible for lower birth weight and cardiac abnormalities in children exposed to cART are unclear but could be related to dysregulation of maternal amino acid metabolism during pregnancy. Previous data in HIV(–) women have shown a relationship between abnormal maternal protein metabolism during pregnancy and low infant birth weight and animal data demonstrate a relationship between altered maternal protein metabolism and increased risk for offspring cardiovascular abnormalities.

Objective—The objectives of this study were to:

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1. characterize post-absorptive maternal leucine kinetics during late pregnancy and
2. examine the relationships between maternal leucine kinetics and offspring birth weight and cardiac function.

Design—Post-absorptive maternal leucine kinetics (evaluated by using stable isotope tracer methodology) in 16 HIV(+) women receiving cART and 14 HIV(−) US women during the 3rd trimester of pregnancy were compared. Relationships between post-absorptive maternal leucine kinetics, cardiac function (echocardiography) and birth weight were statistically examined.

Results—Maternal plasma leucine concentration (HIV(−): 82.8 ± 10.7 vs. HIV(+): 72.3 ± 13.5 μ M, $p=0.06$) and leucine oxidation rate (HIV(−): 6.1 ± 1.6 vs. HIV(+): 4.9 ± 1.8 μ mol/kgBW/min, $p=0.03$) were lower in HIV+ women compared to controls. Total leucine turnover rate, non-oxidative leucine disposal rate and post-absorptive maternal glucose and palmitate kinetics did not differ between groups. Left ventricular fractional shortening tended to be lower in children born to HIV(+) compared to controls (HIV(−): 42 ± 1 vs. HIV+: 36 ± 5 %, $p=0.08$) and associated with lower maternal plasma leucine concentration ($r=0.43$, $p=0.08$).

Conclusions—Preliminary results indicate that post-absorptive maternal leucine metabolism during late pregnancy is mildly altered in HIV+ US women taking cART. The clinical significance of maternal leucine metabolism on adverse infant outcomes is unclear and should be further explored in more expansive studies.

Keywords

HIV; metabolism; pregnancy; amino acid; nutrition

Introduction

Annually over 1.4 million children are born to women with HIV-infection world-wide(1). Combination antiretroviral treatment (cART) is effective in preventing the vertical transmission of Human Immunodeficiency Virus (HIV) from mother to child during pregnancy and parturition (2–4). However children exposed to cART *in utero*, regardless of their HIV status appear to be at a greater risk for lower birth weight (5, 6) and stunted growth and wasting(7, 8), particularly in less developed countries. In addition, children exposed to cART *in utero* are born with minor cardiac abnormalities (9) that persist into pre-adolescence (10).

Mechanisms for lower birth weight and impaired cardiac abnormalities in offspring exposed to cART are unclear. In HIV(−) women, abnormalities in protein metabolism (i.e. intake) during pregnancy is associated in lower offspring birth weight (11, 12). In non-HIV animal models, maternal protein restriction during pregnancy results in lower offspring birth weight(13) and abnormalities in molecular regulators of cardiac growth(14). In addition, in a rodent model of intrauterine growth restriction (a condition where offspring are born smaller and frequently have cardiovascular abnormalities), maternal leucine turnover, concentration and fetal leucine delivery is blunted(15, 16).

Disruptions in amino acid metabolism are well-known in HIV(+) adults both taking and not taking cART(17–19), however, little is known regarding maternal amino acid metabolism

during HIV(+) pregnancy and its potential effects on infant birth weight and cardiac function. Based on previous findings of insulin resistance(20, 21) and impaired fatty acid oxidation(22) in non-gravid HIV(+) adults, we hypothesized that maternal leucine utilization (i.e. oxidation rate) during HIV(+) pregnancy would be higher in order to meet maternal energy needs (i.e. to compensate for insulin resistance and lower fatty acid oxidation) leaving less leucine (i.e. lower plasma concentration and leucine non-oxidative disposal rate) available for fetal growth and metabolism. Subsequently, reduced maternal amino acid delivery to the fetus could contribute to lower birth weight and cardiac function in the offspring by limiting the amount of leucine or by disrupting growth signaling needed for fetal organ growth and maturation. Therefore, our primary aim was to compare post-absorptive maternal leucine kinetics in late pregnancy between women with and without HIV taking cART. Our secondary aim was to examine the relationships between maternal post-absorptive leucine kinetics and infant birth weight and cardiac function. We also measured maternal glucose and fatty acid kinetics in order to examine leucine kinetics in the context of overall maternal substrate metabolism. Identification of mechanisms for abnormalities in infant body composition and cardiac structure and function in children exposed to HIV and cART may lead to nutritional optimization strategies for HIV+ pregnancy; especially important in resource limited countries and in those who are socio-economically disadvantaged, where nutrition is often sub-optimal.

Participants

Pregnant women were recruited from January 2009–December 2010. HIV-infected women (n=16) were recruited from the AIDS Clinical Trials Unit and the Infectious Diseases Clinics at Washington University School of Medicine (WUSM), and HIV-negative women (n=14) were recruited from the WUSM/Barnes Jewish Hospital Women's Health Clinic. Twenty HIV(+) pregnant women were screened and 16 agreed to participate and were enrolled; and 19 HIV(−) pregnant women were screened and n=14 agreed to participate and were enrolled in the study (total n=30). All HIV(+) women were infected less than 10 years and did not have Acquired Immune Deficiency Syndrome (AIDS). HIV(+) women were taking combination antiretroviral therapy (cART) during their pregnancy and all but three HIV(+) women had undetectable viral loads at the time of the metabolic study (Table 1). Twelve (n=12) of 16 HIV(+) women were taking combivir (lamivudine/zidovudine) and all were taking a protease inhibitor. Eleven (n=11) of 16 HIV(+) women received intravenous zidovudine during parturition. All enrolled HIV(+) and HIV(−) women were excluded if they had current or a history of gestational diabetes, were not sedentary (exercise 2x/week), had multiple pregnancies, had a fetal abnormality (as determined by routine standard of care ultrasonography), and if they were currently using illegal drugs or had greater than moderate alcohol consumption (by self-report). The Human Studies Committee at Washington University approved the study and all women provided informed consent before participating.

Methods

Fasting Blood Chemistry and Physical Examination

Using a cross sectional design, all women were studied in the 3rd trimester of pregnancy, between gestational weeks 30–36. Upon enrollment, all women provided comprehensive medical history and underwent a physical examination. Fasting blood chemistry were obtained and included complete blood cell count, endocrine profiles (insulin, triglycerides (TG), total-, LDL-, and HDL-cholesterol), serum lipid, lipoprotein, and plasma HIV RNA quantitation (Roche Amplicor HIV-1 Monitor®; Roche Diagnostics Corporation, Indianapolis, IN). Plasma analyses were performed as previously described (20).

Substrate Kinetics Study

The evening prior (1800h) to the metabolic study, participating women consumed a standardized meal, based on body weight (BW)(12 kcal/kg BW) with 55% of calories from carbohydrate, 30% from fat and 15% from protein. In order to ensure adequate muscle and hepatic glycogen stores the women ingested a high carbohydrate liquid beverage (80 g carbohydrates, 12.2 g fat, 17.6 g protein) (Ensure™; Ross Laboratories, Columbus, OH) following the meal (1900h). Participating women then remained fasted (excluding water) until completion of the study the following day. The following morning at 0700h, participants reported to the Clinical Research Unit at the Washington University Institute of Clinical and Translational Sciences. An intravenous catheter for isotope infusion was inserted into a forearm vein and a contralateral intravenous catheter was inserted into hand vein (heated to 55°C) for arterialized venous sampling. At 0800h (time 0 min), a constant infusion of [2,2-²H]palmitate (0.04 µmol/kgBW/min) bound to 25% serum albumin (Centeon, LLC, Kankakee, IL), a primed, constant infusion of 1-¹³C leucine (0.06 µmol/kgBW/min) with a 1-¹³C bicarbonate prime (1.2 µmol/kgBW, in 0.9% saline), and a primed, constant infusion of [6,6-²H] glucose (0.25 µmol/kgBW/min) dissolved in 0.9% saline were started and maintained for 180 min as previously described (22, 23). Leucine, an essential amino acid, was selected for use as a tracer in the current study because leucine is oxidized in skeletal muscle(24) and has a regulatory effect on other branched chain amino acids(25) and muscle protein synthesis(26). Thus, we hypothesized that leucine might have a regulatory role in fetal muscle and heart development. All tracers were obtained from Cambridge Isotope Laboratories (Andover, MA).

Baseline blood samples were collected before starting the study and additional blood samples were collected every 10 min during the last half hour of the study (t=160, 170, 180 min) to determine leucine, glucose and palmitate tracer-to-tracee ratios (TTRs) and plasma substrate and hormone concentrations(20, 22, 23). Vial preservative EDTA was used for substrate kinetics and trysolol for hormone concentrations. Following collection, blood samples were immediately placed in ice and within 30 min, plasma was separated by centrifugation (3000 rpm, 10 min, 4°C, 1006 g) and stored at -80°C until analysis. ¹³CO₂ breath enrichment was obtained by collecting expired breath in evacuated tubes at the same time intervals as the blood samples(22). Indirect calorimetry was used to measure 15 min whole body oxygen consumption (VO₂) and CO₂ production rates (VCO₂) at t=135 min

(ParvoMedics, Sandy, UT) for the calculation of resting energy expenditure and leucine oxidation rate.

^2H -glucose, ^2H -palmitate, and ^{13}C -leucine plasma tracer-to-tracee ratios were quantified using capillary gas chromatography–mass spectrometry (GC-MS; Agilent 6890 N gas chromatograph and Agilent 5973 N mass selective detector; Agilent, Palo Alto, CA, USA) as previously described(19, 27). For enrichment quantification of ^2H -glucose, cold acetone was used to precipitate plasma proteins, lipids were extracted into hexane, and the aqueous phase was dried (Labconco, Kansas City, MO, USA). Glucose was derivatized to form the heptafluorobutyric derivative of glucose, and GC electron ionization (EI)-MS and selective ion monitoring was used to determine ^2H -glucose enrichment. Plasma leucine was isolated using cation exchange chromatography. Plasma leucine was derivatized to the heptafluorobutyric propyl ester derivative. GC-MS in negative-chemical ionization mode was used to determine ^{13}C - leucine enrichment. Plasma leucine concentration was determined using an internal standard ([U- $^{13}\text{C}_6$]-leucine). For ^2H -palmitate enrichment, cold acetone was used to precipitate plasma proteins, lipids were extracted into hexane, and iodomethane and dichloromethane were used to form fatty acid methyl esters. GC-EI-MS with selective ion monitoring was used to determine plasma ^2H -palmitate enrichment. The GC-MS instrument response was calibrated using gravimetric standards of known isotope enrichment. Breath $^{13}\text{CO}_2$ enrichment was measured using gas isotope ratio mass spectrometry (IRMS; Finnigan Delta + XL, Bremen, Germany)(28).

Calculations

Plasma leucine, palmitate, and glucose rates of appearance were calculated by dividing each tracer infusion rate by the average tracer-to-tracee (TTR) ratio obtained during the last 30 min of the study (time 160, 170, 180 min). Leucine oxidation rate was determined by multiplying the average breath $^{13}\text{CO}_2$ TTR obtained during the last 30 min of the study (time 160, 170, 180 min) by the CO_2 production rate from indirect calorimetry ($^{13}\text{CO}_2$ TTR x VCO_2) divided by the plasma leucine TTR(22). Kinetic rates were expressed per kilogram body weight where appropriate.

Infant Cardiac Function and Birth Weight

Infant anthropometrics including birth weight were obtained from the woman's medical record. Cardiac function was measured at 1 month post-partum by 2D, Doppler and tissue Doppler echocardiography at the Heart Station at St. Louis Children's Hospital. Specifically, transthoracic echocardiographic images were acquired using a GE Vivid 7™ imaging system (General Electric Medical Systems, Milwaukee, WI). All images were obtained at frame rates ranging from 60 to 130 fps and consisted of three consecutive heart cycles. The images were obtained by an experienced sonographer, digitally archived for subsequent analyses, and analyzed by one investigator.

Indexes quantifying systolic and diastolic left ventricular (LV) function were determined from analyses of M-mode images to determine LV fractional shortening ($\text{FS} = [\text{LV end diastolic diameter} - \text{LV end systolic diameter}] / \text{LV end diastolic diameter}$) and 2D images were utilized to measure LV ejection fraction using the modified Simpson method(29). The

Tei Index(30) and isovolumetric contraction and relaxation times describing cardiac function were determined from analyses of mitral Doppler flow and tissue Doppler (mitral annulus) patterns, respectively(31).

Statistical Analyses

Maternal and infant group differences in demographics, substrate kinetics and cardiac function were determined by Independent Samples Mann-Whitney U test (due to $n < 30$ and non-normal distribution on some variables) and chi-square analyses where appropriate. Relationships between maternal leucine kinetics and infant birth weight and cardiac function were determined by Pearson Product correlation analyses. Results are expressed as means $\pm$ SD. Statistical significance was determined at $p < 0.05$ level.

Results

Maternal demographics and plasma metabolites

Age, height, weight, and gestational weight gain (GWG) were similar between the 2 groups of women (Table 1). Fasting maternal plasma HbA1C, glucose, insulin, triglycerides, growth hormone, cortisol, beta-hydroxybutyrate and cholesterol levels were not different between groups. Maternal plasma hematocrit was significantly lower and free fatty acid ($p = 0.06$) concentration was significantly higher in HIV(+) than HIV(−) (Table 1). Maternal plasma concentration of insulin-like growth factor-1 tended to be lower in HIV(+) than HIV(−) (Table 1).

Infant demographics and cardiac function

All infants were born HIV(−). Gestational birth week, birth length, head circumference, chest circumference, and delivery mode were not different between groups (Table 2). There was no difference in birth weight between groups (Table 2). A greater percentage of males ($p = 0.07$) and those admitted to special care nursery were born to HIV(+).

Echocardiography parameters were only obtained in $n = 23$ infants because only 14 HIV(+) and 9 HIV(−) mother/infant pairs returned for the 1-month post-partum echocardiogram. Reasons for lack of follow up included change of contact information and not returning phone calls after repeated attempts. Heart rate was significantly higher in infants born to HIV(+) than HIV(−) (Table 2). Fractional shortening tended ($p = 0.08$) to be lower and late filling during late diastole measured by tissue Doppler was higher in infants born to HIV(+) compared to HIV(−). Other cardiac parameters were not different between groups of infants (Table 2).

Maternal substrate kinetics

All participants were studied during the mid 3rd trimester of pregnancy (mean: 32.4 ± 2.4 weeks gestation). Total energy expenditure expressed per kg body weight tended ($p = 0.09$) to be lower in HIV(+) compared to HIV(−) (Table 3). Maternal post-absorptive glucose and palmitate turnover rates expressed in absolute or per kg body weight were not different between groups. Maternal fasting plasma leucine concentration was significantly lower ($p = 0.06$) in HIV(+) compared to HIV(−). Leucine oxidation rate expressed per kg body

weight was significantly lower in HIV(+) vs. HIV(-) (Table 3). Non-oxidative leucine disposal rate was not different between groups (Table 3). Maternal substrate kinetics are reported in Table 3.

Correlation analyses

Lower cardiac fractional shortening tended to correlate with lower fasting plasma leucine concentration ($r = 0.43$, $p = 0.08$) and smaller birth weight ($r = 0.43$, $p < 0.07$). There were no significant associations between maternal leucine kinetic rates, infant birth weight or cardiac function.

Discussion

Overall

To our knowledge, this is the first study to quantify maternal substrate kinetics in HIV(+) pregnant women taking cART, and relate these to infant birth metrics, and cardiac functional parameters. The main finding was that maternal post-absorptive leucine kinetics were mildly altered in HIV+ pregnant women and there were trends towards relationships between maternal leucine concentration, infant birth weight and cardiac function. Currently, optimal nutritional requirements for HIV+ women during pregnancy are not clear(32) but these data suggest that they are similar in virologically well-controlled HIV(+) and HIV(-) pregnancy. These data do appear to highlight the importance of well-controlled HIV infection and pre-natal care during HIV(+) pregnancy as all of the HIV(+) women except three had undetectable plasma viremia and attended a similar amount of pre-natal visits as their uninfected peers (~9–10 pre-natal visits for all women). However, the current study did not examine maternal substrate metabolism under the absorptive or insulin-stimulated (i.e. hyperinsulinemic clamp) condition and therefore cannot make any conclusion under these conditions; thus additional research should be performed. Accordingly, the NIH/WHO Working Group executive summary recently recommended further research be performed on the effect of cART on nutritional status during HIV+ pregnancy and its impact on maternal and infant health outcomes(33).

Leucine Metabolism

We found small differences in post-absorptive leucine metabolism in virologically suppressed HIV+ women during the 3rd trimester of pregnancy. Women with HIV had no demonstrable increase in leucine turnover rate but had lower leucine oxidation rate which was contrary to our hypothesis. The effect of HIV and cART on leucine oxidation is unclear as it is elevated in virologically suppressed HIV+ men but is no different between those with AIDS wasting and non-infected controls(19). Higher plasma free fatty acids levels might have inhibited leucine oxidation in HIV(+) women(34). It is possible that lower leucine oxidation rate in HIV+ women during late pregnancy was driven, at least partially, by the lower plasma leucine concentration in the HIV(+) group. The mechanism behind lower plasma leucine concentration in HIV(+) pregnant women is unclear. Fat and carbohydrate intestinal absorption is impaired in individuals with HIV taking cART(35, 36) however the effect of HIV/cART on protein absorption is not known. Lower maternal plasma leucine concentration might lead to lower leucine delivery to the fetus, however, we did not measure

cord blood leucine concentration and therefore, this is speculative. Overall, differences in leucine metabolism between well-controlled HIV(+) and HIV(-) pregnancy are small and the clinical meaning is unclear however; these findings might be more clinically important in populations where food insecurity is high and maternal nutrition (i.e. protein) is suboptimal.

Glucose and Fatty Acid Metabolism

In the current study, post-absorptive glucose and fatty acid metabolism kinetics were not different between HIV(+) and HIV(-) women during late pregnancy. This finding was surprising in light of the evidence supportive of dysregulated glucose(37, 38) and fatty acid(20, 22, 39) metabolism in non-gravid HIV(+) adults taking cART, including when women were examined independently(40). HIV(+) women had higher plasma free fatty acid level; however this was not associated with any infant outcome.

Birth Weight and Cardiac Function

Birth weight was not different in infants born to women with and without HIV in the current study. Studies examining the effect of HIV/cART on infant birth weight have been conflicting(5, 6, 41, 42). In studies demonstrating lower birth weight in infants born to HIV(+) women, low maternal CD4 count and high viral load were variables associated with this adverse outcome(43–45); associations not seen in our cohort. Some studies(7, 10) have shown a relationship between cART exposure and lower birth weight(46, 47) but this is not a universal finding(48, 49).

The effect on cardiac function from *in utero* exposure to HIV and cART is unclear. Infants exposed to HIV and cART had significantly higher heart rate and lower systolic contractile function (fractional shortening) 1-month after birth. Higher heart rate in infants exposed to HIV and cART has been previously reported(50, 51) however these infants were perinatally infected with HIV where infants in the current study were not. Lipshultz et al.(51) showed increased fractional shortening in infants exposed HIV/cART at birth; contrary to the current study's findings. The current study suggests a hemodynamically significant effect of HIV and cART exposure on infant cardiac function. This intrinsic effect might be a continuum of cardiac dysfunction later in life, evidenced by pre-adolescents exposed to HIV/cART *in utero* having higher resting heart rate and lower diastolic function when compared to their unexposed peers(10). Well-characterized follow up cardiac function studies in adolescents and adults exposed to HIV/cART are warranted to fully determine the effect of HIV and cART *in utero* exposure on long-term cardiovascular health and heart function.

Mechanisms for adverse infant outcomes in children exposed to HIV and cART have not been fully elucidated. The relationship between altered maternal amino acid metabolism and infant cardiac abnormalities has been previously demonstrated in non-HIV animal models(13, 14). However, we were not able to confirm this in HIV(+) human pregnancy as maternal leucine kinetics were not associated with infant birth weight or cardiac function. There tended to be a relationship between lower maternal leucine concentration and lower fractional shortening in the infants but in the absence of abnormal maternal kinetic rates or cord blood leucine concentrations, we cannot comment on the significance of this finding.

Future studies that examine both maternal and fetal amino acid levels and kinetics and their relationship to body composition and cardiac function in HIV and cART exposed infants are warranted.

Limitations

Maternal substrate kinetics were measured during the post-absorptive state thus the findings cannot be extrapolated to other conditions (fed, insulin-stimulated, exercise). Although pre-study meal composition was identical, habitual maternal diet was not measured. There was no difference between smoking prevalence between groups in the current study however smoking has been associated with altered protein metabolism(52) and low birth weight(53). We did not statistically control for the effect of smoking and thus small differences between the groups in smoking prevalence might have influenced outcomes. Maternal diet was not recorded and maternal physical activity was not objectively measured (e.g. accelerometry) rather by self-report. Umbilical cord blood was not collected thus cord blood levels of leucine, IGF-1, insulin and other metabolites were not measured which may have assisted in the interpretation of the effect of maternal substrate metabolism on infant outcomes. Participants were not stratified by cART composition or HIV duration so the independent effects of HIV and cART on protein metabolism could not be identified. Apart from birth weight, other obstetric outcomes were not analyzed but would be important to examine in larger, future studies.

Conclusions

Maternal leucine metabolism during late pregnancy is mildly altered in HIV(+) women however the clinical importance of this is not clear. Well-controlled viremia and comprehensive pre-natal care during HIV(+) pregnancy might be important to minimize abnormalities in maternal metabolism and adverse infant outcomes. Larger, well-characterized studies examining maternal substrate metabolism during different conditions and the effect on infant outcomes are needed in order to quantify maternal nutritional needs during pregnancy in both well-nourished and food insecure environments.

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References

1. World Health Organization. HIV/AIDS Fact Sheet. 2014. Available from: <http://www.who.int/mediacentre/factsheets/fs360/en/>
2. Whitmore SK, Zhang X, Taylor AW, Blair JM. Estimated number of infants born to HIV-infected women in the United States and five dependent areas, 2006. *J Acquir Immune Defic Syndr*. 2011; 57(3):218–22. [PubMed: 21372725]
3. de Vincenzi I. Kesho Bora Study G. Triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child

transmission of HIV-1 (Kesho Bora study): a randomised controlled trial. *The Lancet infectious diseases*. 2011; 11(3):171–80. [PubMed: 21237718]

4. Prevention CfDCA. Diagnoses of HIV infection and AIDS in the United States and dependent areas 2010. HIV Surveillance Report. 2012; 22
5. Young S, Murray K, Mwesigwa J, Natureeba P, Osterbauer B, Achan J, et al. Maternal nutritional status predicts adverse birth outcomes among HIV-infected rural Ugandan women receiving combination antiretroviral therapy. *PLoS One*. 2012; 7(8):e41934. [PubMed: 22879899]
6. Hernandez S, Moren C, Lopez M, Coll O, Cardellach F, Gratacos E, et al. Perinatal outcomes, mitochondrial toxicity and apoptosis in HIV-treated pregnant women and in-utero-exposed newborn. *AIDS*. 2012; 26(4):419–28. [PubMed: 22156962]
7. McDonald CM, Kupka R, Manji KP, Okuma J, Bosch RJ, Aboud S, et al. Predictors of stunting, wasting and underweight among Tanzanian children born to HIV-infected women. *Eur J Clin Nutr*. 2012; 66(11):1265–76. [PubMed: 23031850]
8. Ram M, Gupte N, Nayak U, Kinikar AA, Khandave M, Shankar AV, et al. Growth patterns among HIV-exposed infants receiving nevirapine prophylaxis in Pune, India. *BMC Infect Dis*. 2012; 12:282. [PubMed: 23114104]
9. Lipshultz SE, Shearer WT, Thompson B, Rich KC, Cheng I, Orav EJ, et al. Cardiac effects of antiretroviral therapy in HIV-negative infants born to HIV-positive mothers: NHLBI CHAART-1 (National Heart, Lung, and Blood Institute Cardiovascular Status of HAART Therapy in HIV-Exposed Infants and Children cohort study). *J Am Coll Cardiol*. 2011; 57(1):76–85. [PubMed: 21185505]
10. Cade WT, Waggoner AD, Hubert S, Krauss MJ, Singh GK, Overton ET. Reduced diastolic function and left ventricular mass in HIV-negative preadolescent children exposed to antiretroviral therapy in utero. *AIDS*. 2012; 26(16):2053–8. [PubMed: 22874520]
11. Godfrey K, Robinson S, Barker DJ, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ*. 1996; 312(7028):410–4. [PubMed: 8601112]
12. Godfrey KM, Barker DJ, Robinson S, Osmond C. Maternal birthweight and diet in pregnancy in relation to the infant's thinness at birth. *British journal of obstetrics and gynaecology*. 1997; 104(6):663–7. [PubMed: 9197868]
13. Dudele A, Lund S, Jessen N, Wegener G, Winther G, Elnif J, et al. Maternal protein restriction before pregnancy reduces offspring early body mass and affects glucose metabolism in C57BL/6J Bom mice. *Journal of developmental origins of health and disease*. 2012; 3(5):364–74. [PubMed: 25102266]
14. Lie S, Sim SM, McMillen IC, Williams-Wyss O, MacLaughlin SM, Kleemann DO, et al. Maternal undernutrition around the time of conception and embryo number each impact on the abundance of key regulators of cardiac growth and metabolism in the fetal sheep heart. *Journal of developmental origins of health and disease*. 2013; 4(5):377–90. [PubMed: 24970731]
15. Ross JC, Fennessey PV, Wilkening RB, Battaglia FC, Meschia G. Placental transport and fetal utilization of leucine in a model of fetal growth retardation. *Am J Physiol*. 1996; 270(3 Pt 1):E491–503. [PubMed: 8638698]
16. Marconi AM, Paolini CL, Stramare L, Cetin I, Fennessey PV, Pardi G, et al. Steady state maternal-fetal leucine enrichments in normal and intrauterine growth-restricted pregnancies. *Pediatr Res*. 1999; 46(1):114–9. [PubMed: 10400144]
17. Reeds DN, Cade WT, Patterson BW, Powderly WG, Klein S, Yarasheski KE. Whole-body proteolysis rate is elevated in HIV-associated insulin resistance. *Diabetes*. 2006; 55(10):2849–55. [PubMed: 17003352]
18. Yarasheski KE, Smith SR, Powderly WG. Reducing plasma HIV RNA improves muscle amino acid metabolism. *Am J Physiol Endocrinol Metab*. 2005; 288(1):E278–84. [PubMed: 15367396]
19. Yarasheski KE, Zachwieja JJ, Gischler J, Crowley J, Horgan MM, Powderly WG. Increased plasma gln and Leu Ra and inappropriately low muscle protein synthesis rate in AIDS wasting. *Am J Physiol*. 1998; 275(4 Pt 1):E577–83. [PubMed: 9755075]

20. Reeds DN, Yarasheski KE, Fontana L, Cade WT, Laciny E, DeMoss A, et al. Alterations in liver, muscle, and adipose tissue insulin sensitivity in men with HIV infection and dyslipidemia. *Am J Physiol Endocrinol Metab.* 2006; 290(1):E47–E53. [PubMed: 16118251]
21. Arama V, Tiliscan C, Streinu-Cercel A, Ion D, Mihailescu R, Munteanu D, et al. Insulin resistance and adipokines serum levels in a caucasian cohort of hiv-positive patients undergoing antiretroviral therapy: a cross sectional study. *BMC endocrine disorders.* 2013; 13:4. [PubMed: 23351215]
22. Cade WT, Reeds DN, Mittendorfer B, Patterson BW, Powderly WG, Klein S, et al. Blunted lipolysis and fatty acid oxidation during moderate exercise in HIV-infected subjects taking HAART. *Am J Physiol Endocrinol Metab.* 2007; 292(3):E812–9. [PubMed: 17106066]
23. Matthews DE, Motil KJ, Rohrbach DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C]leucine. *Am J Physiol.* 1980; 238(5):E473–9. [PubMed: 6769340]
24. Odessey R, Goldberg AL. Oxidation of leucine by rat skeletal muscle. *Am J Physiol.* 1972; 223(6): 1376–83. [PubMed: 4641630]
25. Hambraeus L, Bilmazes C, Dippel C, Scrimshaw N, Young VR. Regulatory role of dietary leucine on plasma branched-chain amino acid levels in young men. *J Nutr.* 1976; 106(2):230–40. [PubMed: 1249648]
26. Stipanuk MH. Leucine and protein synthesis: mTOR and beyond. *Nutrition reviews.* 2007; 65(3): 122–9. [PubMed: 17425063]
27. Cade WT, Spencer CT, Reeds DN, Waggoner AD, O'Connor R, Maisenbacher M, et al. Substrate metabolism during basal and hyperinsulinemic conditions in adolescents and young-adults with Barth syndrome. *J Inher Metab Dis.* 2013; 36(1):91–101. [PubMed: 22580961]
28. Mittendorfer B, Fields DA, Klein S. Excess body fat in men decreases plasma fatty acid availability and oxidation during endurance exercise. *Am J Physiol Endocrinol Metab.* 2004; 286(3):E354–62. [PubMed: 14625204]
29. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, et al. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. *J Am Soc Echocardiogr.* 2010; 23(5):465–95. quiz 576–7. [PubMed: 20451803]
30. Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function--a study in normals and dilated cardiomyopathy. *J Cardiol.* 1995; 26(6):357–66. [PubMed: 8558414]
31. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur J Echocardiogr.* 2009; 10(2):165–93. [PubMed: 19270053]
32. Papatheakis, P.; Rollins, N., editors. Consultation on nutrition and HIV/AIDS in Africa: Evidence, lessons and recommendations for action. Durban, South Africa: World Health Organization; 2005. HIV and nutrition: pregnant and lactating women.
33. Raiten DJ, Mulligan K, Papatheakis P, Wanke C. Executive summary--nutritional care of HIV-infected adolescents and adults, including pregnant and lactating women: what do we know, what can we do, and where do we go from here? *Am J Clin Nutr.* 2011; 94(6):1667S–76S. [PubMed: 22089438]
34. Lang CH. Elevated plasma free fatty acids decrease basal protein synthesis, but not the anabolic effect of leucine, in skeletal muscle. *Am J Physiol Endocrinol Metab.* 2006; 291(3):E666–74. [PubMed: 16684854]
35. Poles MA, Fuerst M, McGowan I, Elliott J, Rezaei A, Mark D, et al. HIV-related diarrhea is multifactorial and fat malabsorption is commonly present, independent of HAART. *The American journal of gastroenterology.* 2001; 96(6):1831–7. [PubMed: 11421246]
36. Jimenez-Exposito MJ, Garcia-Lorda P, Alonso-Villaverde C, de Virgala CM, Sola R, Masana L, et al. Effect of malabsorption on nutritional status and resting energy expenditure in HIV-infected patients. *Aids.* 1998; 12(15):1965–72. [PubMed: 9814864]

37. Mondy K, Overton ET, Grubb J, Tong S, Seyfried W, Powderly W, et al. Metabolic syndrome in HIV-infected patients from an urban, midwestern US outpatient population. *Clin Infect Dis*. 2007; 44(5):726–34. [PubMed: 17278068]
38. Capeau J, Bouteloup V, Katlama C, Bastard JP, Guiyedi V, Salmon-Ceron D, et al. Ten-year diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral treatment. *AIDS*. 2012; 26(3):303–14. [PubMed: 22089377]
39. Reeds DN, Mittendorfer B, Patterson BW, Powderly WG, Yarasheski KE, Klein S. Alterations in lipid kinetics in men with HIV-dyslipidemia. *Am J Physiol Endocrinol Metab*. 2003; 285(3):E490–7. [PubMed: 12746213]
40. Sobieszczyk ME, Hoover DR, Anastos K, Mulligan K, Tan T, Shi Q, et al. Prevalence and predictors of metabolic syndrome among HIV-infected and HIV-uninfected women in the Women's Interagency HIV Study. *J Acquir Immune Defic Syndr*. 2008; 48(3):272–80. [PubMed: 18545157]
41. Ezechi OC, Gab-Okafor CV, Oladele DA, Kalejaiye OO, Oke BO, Ohwodo HO, et al. Pregnancy, obstetric and neonatal outcomes in HIV positive Nigerian women. *Afr J Reprod Health*. 2013; 17(3):160–8. [PubMed: 24069778]
42. Patil S, Bhosale R, Sambarey P, Gupte N, Suryavanshi N, Sastry J, et al. Impact of maternal human immunodeficiency virus infection on pregnancy and birth outcomes in Pune, India. *AIDS Care*. 2011; 23(12):1562–9. [PubMed: 21711178]
43. Joseph O, Biodun O, Michael E. Pregnancy outcome among HIV positive women receiving antenatal HAART versus untreated maternal HIV infection. *J Coll Physicians Surg Pak*. 2011; 21(6):356–9. [PubMed: 21711992]
44. Yu L, Li WY, Chen RY, Tang ZR, Pang J, Gui XZ, et al. Pregnancy outcomes and risk factors for low birth weight and preterm delivery among HIV-infected pregnant women in Guangxi, China. *Chin Med J (Engl)*. 2012; 125(3):403–9. [PubMed: 22490393]
45. Rollins NC, Coovadia HM, Bland RM, Coutoudis A, Bennish ML, Patel D, et al. Pregnancy outcomes in HIV-infected and uninfected women in rural and urban South Africa. *J Acquir Immune Defic Syndr*. 2007; 44(3):321–8. [PubMed: 17195768]
46. Darak S, Darak T, Kulkarni S, Kulkarni V, Parchure R, Hutter I, et al. Effect of highly active antiretroviral treatment (HAART) during pregnancy on pregnancy outcomes: experiences from a PMTCT program in western India. *AIDS Patient Care STDS*. 2013; 27(3):163–70. [PubMed: 23477457]
47. Ekouevi DK, Coffie PA, Becquet R, Tonwe-Gold B, Horo A, Thiebaut R, et al. Antiretroviral therapy in pregnant women with advanced HIV disease and pregnancy outcomes in Abidjan, Cote d'Ivoire. *AIDS*. 2008; 22(14):1815–20. [PubMed: 18753864]
48. Schulte J, Dominguez K, Sukalac T, Bohannon B, Fowler MG. Declines in low birth weight and preterm birth among infants who were born to HIV-infected women during an era of increased use of maternal antiretroviral drugs: Pediatric Spectrum of HIV Disease, 1989–2004. *Pediatrics*. 2007; 119(4):e900–6. [PubMed: 17353299]
49. Tuomala RE, Shapiro DE, Mofenson LM, Bryson Y, Culnane M, Hughes MD, et al. Antiretroviral therapy during pregnancy and the risk of an adverse outcome. *N Engl J Med*. 2002; 346(24):1863–70. [PubMed: 12063370]
50. Lipshultz SE, Easley KA, Orav EJ, Kaplan S, Starc TJ, Bricker JT, et al. Cardiovascular status of infants and children of women infected with HIV-1 (P(2)C(2) HIV): a cohort study. *Lancet*. 2002; 360(9330):368–73. [PubMed: 12241776]
51. Lipshultz SE, Williams PL, Wilkinson JD, Leister EC, Van Dyke RB, Shearer WT, et al. Cardiac status of children infected with human immunodeficiency virus who are receiving long-term combination antiretroviral therapy: results from the Adolescent Master Protocol of the Multicenter Pediatric HIV/AIDS Cohort Study. *JAMA Pediatr*. 2013; 167(6):520–7. [PubMed: 23608879]
52. Petersen AM, Magkos F, Atherton P, Selby A, Smith K, Rennie MJ, et al. Smoking impairs muscle protein synthesis and increases the expression of myostatin and MAFbx in muscle. *Am J Physiol Endocrinol Metab*. 2007; 293(3):E843–8. [PubMed: 17609255]

53. Haeri S, Shauer M, Dale M, Leslie J, Baker AM, Saddlemire S, et al. Obstetric and newborn infant outcomes in human immunodeficiency virus-infected women who receive highly active antiretroviral therapy. *Am J Obstet Gynecol.* 2009; 201(3):315, e1–5. [PubMed: 19733286]

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Table 1**Maternal Demographic and Metabolic Variables**

Variable	HIV(-)(n=14)	HIV(+)(n=16)	p-value
Age (yrs)	26 ± 5(23,29)	26 ± 4(24,28)	0.89
Race (n, White/Black)	4/10	2/14	0.13
Height (cm)	164.7 ± 4.7(162.2,167.2)	165.5 ± 8.6(161.3,169.7)	0.68
Weight at study (kg)	86.0 ± 24.3(73.3,98.7)	85.3 ± 22.1(74.5,96.1)	0.70
GWG (kg)	17.2 ± 6.9(13.6,20.8)	17.7 ± 21.2(7.3,28.1)	0.20
Smoking during pregnancy (n)	4	7	0.39
HIV duration (yrs)	N/A	5.3 ± 3.4(3.6,8.7)	
3rd trimester CD4 (cells/dL)	N/A	509 ± 205(409,609)	
3rd trimester viral load (copies/mL)			
<400 (%)	N/A	88	
400–1000 (%)	N/A	12	
Hematocrit	31.0 ± 1.9(30,32)	28.7 ± 3.3(27.1,30.3)	0.05
HbA1C (%)	5.1 ± 0.8(4.7,5.5)	5.4 ± 1.4(4.7,6.1)	0.65
Fasting Glucose (mg/dL)	77.4 ± 5.8(74.4,80.4)	76.9 ± 7.1(73.4,80.4)	0.76
Fasting Insulin (μU/mL)	12.9 ± 12.3(6.5,19.3)	9.1 ± 5.4(6.5,11.8)	0.79
TG (mg/dL)	150.2 ± 36.2(131.2,169.2)	160.3 ± 61.1(130.4,190.2)	1.00
HDL (mg/dL)	65.5 ± 15.6(57.3,73.7)	60.0 ± 12.1(54.1,72.1)	0.36
LDL (mg/dL)	109.1 ± 22.9(97.1,121.1)	86.8 ± 36.6(68.9,104.7)	0.24
Chol (mg/dL)	204.7 ± 38.9(184.3,225.1)	178.9 ± 38.2(160.2,197.6)	0.10
FFA (μmol/L)	467.9 ± 103.7(413.6,522.2)	555.0 ± 122.5(495.0,615.0)	0.06
βHB (μmol/L)	318.1 ± 149.7(239.7,239.7)	317.2 ± 166.4(235.7,398.7)	0.92
Growth Hormone (ng/ml)	0.48 ± 0.69(0.12,0.84)	0.39 ± 0.34(0.22,0.56)	0.53
Cortisol (ug/dL)	23.4 ± 6.9(19.8,27.0)	21.7 ± 4.7(19.4,24.0)	0.53
IGF-1 (ng/mL)	375.8 ± 211.2(164.6,486.4)	249.8 ± 101.6(200.0,299.6)	0.11

Values are mean ± SD (95% CI). GWG: gestational weight gain, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, Chol: total cholesterol, FFA: free fatty acid, βHB: beta hydroxy butyrate, IGF-1: insulin-like growth factor 1.

Table 2

Infant Demographics and Echocardiographic Variables

Variable	Born to HIV(-) (n=14)	Born to HIV(+) (n=16)	p-value
<i>Demographics</i>			
Gestational age (wks)	38 ± 2	38 ± 2	0.55
Sex (Male/Female, %)	36/64	67/33	0.07
Delivery mode (Spontaneous/C-section, %)	86/14	69/31	0.27
Birth weight (g)	2977 ± 561	3187 ± 626	0.22
Birth length (cm)	50.3 ± 3.1	49.6 ± 2.8	0.58
Head circumference (cm)	33.6 ± 1.9	34.2 ± 1.7	0.25
Chest circumference (cm)	31.6 ± 2.4	31.8 ± 2.5	0.82
APGAR 1-min	7.8 ± 0.7	6.3 ± 2.8	0.27
APGAR 5-min	8.8 ± 0.4	7.9 ± 1.8	0.53
Disposition (Nursery/Special Care, %)	83/17	62/38	0.04
<i>Echocardiography</i>			
HR (bpm)	153 ± 18	170 ± 15	0.02
MM LVPWD (cm)	0.37 ± 0.06	0.40 ± 0.04	0.20
MM IVSD (cm)	0.42 ± 0.09	0.45 ± 0.05	0.40
MM LVEDD (cm)	1.95 ± 0.26	1.98 ± 0.16	0.75
MM LVESD (cm)	1.14 ± 0.18	1.28 ± 0.15	0.15
FS (%)	42 ± 1	36 ± 5	0.08
2D LVEDV (ml)	7.6 ± 1.7	7.4 ± 2.1	1.00
2D LVESV (ml)	2.7 ± 0.8	2.4 ± 0.4	0.62
EF (%)	62 ± 6	65 ± 6	0.28
Doppler E'	0.9 ± 0.2	1.0 ± 0.2	0.10
Doppler A'	1.0 ± 0.2	1.0 ± 0.2	0.24
Doppler AoV (m/s)	1.0 ± 0.2	1.0 ± 0.2	0.71
Doppler AoV ET (ms)	179.0 ± 15.4	186.0 ± 9.0	0.24
Tai index	0.49 ± 0.04	0.46 ± 0.08	0.92
LV Vs (cm/s)	5.8 ± 1.5	6.3 ± 0.9	0.18
LV Ve (cm/s)	8.6 ± 3.0	10.4 ± 3.1	0.16
LV Va (cm/s)	10.3 ± 2.9	11.5 ± 2.8	0.04

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Variable	Born to HIV(−) (n=14)	Born to HIV(+) (n=16)	p-value
IVCT (ms)	38.0 ± 6.4	39.8 ± 5.5	0.48
IVRT (ms)	41.6 ± 8.2	40.4 ± 6.3	0.70

Values are mean ± SD. HR: heart rate, MM LVPWD: M-mode left ventricular posterior wall diameter, MM IVSD: M-mode intraventricular septum diameter, MM LVEDD: M-mode left ventricular end diastolic diameter, MM LVESD: M-mode left ventricular end systolic diameter, FS: fractional shortening, 2D LVEDV: two dimensional left ventricular end diastolic volume, 2D LVESV: two dimensional left ventricular end systolic volume, EF: ejection fraction, Doppler E: early filling velocity measured by Doppler echocardiography during diastole, A': filling velocity measured by Doppler echocardiography during diastole, Ao V: aortic velocity, Ao V ET: aortic velocity ejection time, LV Vs: left ventricular wall velocity measured by tissue Doppler imaging during systole, LV Ve: left ventricular wall velocity measured by tissue Doppler imaging during early diastole, LV Va: left ventricular wall velocity measured by tissue Doppler imaging during late diastole, IVCT: intraventricular contraction time, IVRT: intraventricular relaxation time.

Table 3**Post-Absorptive Substrate Metabolism**

Variable	HIV(-) (n=14)	HIV(+)(n=16)	p-value
Basal			
Energy expenditure (kcal/day)	1959 ± 594	1727 ± 374	0.61
Energy expenditure (kcal/kgBW/day)	22.9 ± 2.9	20.7 ± 3.9	0.14
Glucose turnover rate (μmol/min)	5408 ± 1313	5198 ± 1310	0.67
Glucose turnover rate (μmol/kgBW/min)	64.2 ± 8.5	62.0 ± 10.7	0.53
Palmitate turnover rate (μmol/min)	183.9 ± 86.1	177.9 ± 87.4	0.98
Palmitate turnover rate (μmol/kgBW/min)	2.1 ± 0.7	2.1 ± 0.9	0.68
Fasting plasma leucine (μM)	82.8 ± 10.7	72.3 ± 13.5	0.06
Leucine turnover rate (μmol/hr)	6760 ± 1233	6541 ± 1415	0.58
Leucine turnover rate (μmol/kgBW/hr)	81.5 ± 16.3	78.8 ± 15.1	0.76
Leucine oxidation rate (μmol/hr)	510.3 ± 152.4	419.2 ± 179.3	0.07
Leucine oxidation rate (μmol/kgBW/hr)	6.1 ± 1.6	4.9 ± 1.8	0.03
Non-oxidative leucine disposal rate (μmol/hr)	6250 ± 1140	6122 ± 1252	0.86
Non-oxidative leucine disposal rate: (μmol/kgBW/hr)	75.4 ± 15.4	73.9 ± 14.0	0.92

Kcal: kilocalorie, BW: body weight, g: gram, μM: micromolar, hr: hour, min: minute