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INSULIN RESISTANCE AND INTESTINAL INTEGRITY IN CHILDREN WITH AND WITHOUT HIV IN UGANDA

Sahera DIRAJLAL-FARGO^{1,2,3}, Lingpeng SHAN³, Abdus SATTAR³, Emily BOWMAN⁴, Janelle Gabriel⁴, Manjusha KULKARNI⁴, Nicholas FUNDERBURG⁴, Rashidah NAZZINDA⁵, Victor MUSIIME⁵, Grace A McCOMSEY^{1,2,3}

¹University Hospitals Cleveland Medical Center; Cleveland, OH ²Rainbow Babies and Children's Hospital, Cleveland, OH ³Case Western Reserve University, Cleveland, OH ⁴Ohio State University School of Health and Rehabilitation Sciences, Columbus, OH ⁵Joint Clinical Research Centre, Kampala, Uganda

Abstract

Background: The risk of cardiometabolic complications in children with perinatally acquired HIV (PHIVs) and exposed uninfected children (HEU) and their relationship to systemic inflammation and markers of gut integrity is not well established.

Methods: This is a cross-sectional study in PHIV, HEU and HIV unexposed uninfected (HIV–) children aged 2–10 years old enrolled in Uganda. PHIVs were on stable ART with HIV-1 RNA < 400 copies/mL. Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR). We measured markers of systemic inflammation, monocyte activation and gut integrity. Kruskal-Wallis tests were used to compare markers by HIV status, Pearson correlation and multiple linear regressions were used to assess associations of HOMA-IR.

Results: Overall, 172 participants were enrolled (57 HIV+, 59 HEU and 56 HIV–). Median (IQR) age was 7.8 (6.39, 8.84) years, 55% were females and median BMI was 15 (14.3, 15.8) kg/m². Among PHIV, median CD4% was 34%, 93% had viral load < 20 copies/mL. PHIV had higher waist hip ratio, HDL cholesterol, triglycerides, and HOMA-IR (p = 0.02). Factors correlated with HOMA-IR included higher BMI, HDL, and lower sTNFRI (p = 0.02). There was no correlation between any of the other inflammatory or gut biomarkers with HOMA-IR (p = 0.05). After adjusting for age, and sTNFRI, BMI remained independently associated with HOMA-IR ($\beta=0.16$, p<0.01).

Corresponding author: Grace McComsey, MD, FIDSA, Professor of Pediatrics and Medicine, University Hospitals Cleveland Medical Center and Case School of Medicine, 2061 Cornell Rd- Mail Stop: 5083, Cleveland, OH 44106, grace.mccomsey@uhhospitals.org. **Alternate corresponding author:** Sahera Dirajlal-Fargo, MS, DO, Assistant Professor of Pediatrics, University Hospitals Cleveland Medical Center and Case School of Medicine, 11100 Euclid Avenue-Mailstop: 6008A, Cleveland, OH 44106, Sahera.dirajlal-fargo@uhhospitals.org.

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Conclusions: Despite viral suppression, Ugandan PHIVs have disturbances in glucose metabolism. Higher BMI, and not immune activation or alteration in gut integrity, was associated with insulin resistance in this population.

Keywords

insulin resistance; inflammation; immune activation; gut integrity; HIV exposed uninfected

Introduction

HIV infection has become a chronic disease with the potential for long-term survival. Non-communicable comorbidities such as cardiovascular and metabolic disorders have become increasingly more prevalent in adults living with HIV (ALHIV) [1]. Children living with perinatally acquired HIV (PHIV) are also at higher risk of cardiometabolic diseases. The presence of insulin resistance has deleterious health consequences in the general population and contributes to the progression of metabolic and cardiovascular derangements, including diabetes [1, 2]. In addition, it is associated with increased mortality in non-diabetic adults [3]. In HIV uninfected children, abnormal insulin resistance as measured by the homeostasis model assessment (HOMA-IR) index has been associated with early carotid atherosclerosis and endothelial dysfunction [4, 5]. High prevalence of insulin resistance has been reported in PHIV children [6–9], and its etiology is likely multifactorial. Studies in PHIVs have shown an association between body mass index (BMI) and higher waist circumference with measures of insulin resistance [6]. Our group has demonstrated an association between HOMA-IR and monocyte activation markers in young ART-naïve Ugandan PHIVs, 48 weeks after antiretroviral therapy (ART) initiation [10]. Heightened immune activation and inflammation, despite viral suppression on ART, is known to be associated with increased mortality and several comorbidities including metabolic and cardiovascular diseases in ALHIV [11–18]. The mechanisms causing HIV-associated immune activation remain unclear but the role of alteration of intestinal integrity and the resultant translocation of microbial products from the intestinal lumen to the systemic circulation appears to be a central factor that determines the severity of HIV-associated chronic immune activation [19, 20]. To our knowledge, no study has reported on the relationship between gut integrity markers and insulin resistance in PHIVs, specifically in sub-Saharan Africa which has the highest burden of pediatric HIV. Differences in ethnicity, genetics, nutrition, environmental factors, and co-infections could impact the role of immune activation, alteration of the gut integrity and resultant microbial translocation on metabolic outcomes.

In this current study, we assessed insulin resistance as estimated by HOMA-IR in PHIVs who are virally suppressed on stable ART compared to perinatally HIV-exposed but uninfected children (HEUs) and HIV unexposed and uninfected children (HIV–) in Uganda. We explored potential associations among intestinal damage and the resultant bacterial and fungal translocation on disturbances of glucose metabolism in these Ugandan children.

Methods

Study Design

This is a cross sectional study of PHIV, HEU and HIV unexposed uninfected children prospectively enrolled at the Joint Clinical Research Center in Kampala, Uganda. All participants were 2–10 years of age. PHIV participants were on stable ART for at least 6 months with HIV-1 RNA < 400 copies/mL in the last 6 months. HIV– participants were either HIV– siblings of the PHIV or recruited from the community using community liaison volunteers from the JCRC. All participants lived in Kampala or peri-urban surroundings. History of acute infections (malaria, tuberculosis, helminthiasis, pneumonia, meningitis) in the last 3 months, moderate or severe malnutrition, and diarrhea in the last 3 months were exclusionary. Participants with known diabetes and cardiovascular diseases were also excluded.

Study Evaluations

Blood was drawn after an 8-hour fast. Blood was processed and plasma and serum were cryopreserved for shipment to University Hospitals Cleveland Medical Center, Cleveland, Ohio. The samples were used for measurement of glucose, insulin, lipids, and soluble markers of monocyte activation, systemic inflammation and gut integrity. Insulin was measured by ELISA sandwich immunoassay (ALPCO, Salem, New Hampshire, USA) and the derived homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as described^[21], and insulin resistance was defined categorically as levels of HOMA-IR greater than 2.5 for prepubertal children.

Inflammation, monocyte activation and gut integrity markers

Plasma markers of monocyte activation (sCD163 and sCD14), systemic inflammation [soluble TNF α receptor I and II (sTNFRI and II), high sensitivity C-reactive protein (hsCRP), interleukin 6 (IL-6)], coagulation (d-dimer), and oxidized lipids (oxidized LDL) were measured by ELISA (R & D Systems, Minneapolis, Minnesota, USA and ALPCO, Salem, New Hampshire, USA and Mercodia, Uppsala, Sweden). The marker of fungal translocation Beta D Glucan (BDG, Mybiosource Inc. CA), lipopolysaccharide-binding protein (LBP, Hycult Biotech Inc. PA), an indirect marker of microbial translocation; zonulin (Promocell Germany), a marker of intestinal permeability and intestinal fatty acid binding protein (I-FABP, R & D Systems, Minneapolis, Minnesota, USA), a marker of intestinal integrity were measured by ELISA. The intra-assay variability ranged between 4–8% and inter-assay variability was less than 10% for all markers. All assays were performed on batched samples, never previously thawed, at Dr. Funderburg's laboratory at Ohio State University, Columbus, OH. Laboratory personnel were blinded to group assignments and clinical characteristics.

Statistical Analyses

The primary objective of this analysis was to compare HOMA-IR as a continuous variable between PHIV, PHEU and HIV– children. The secondary objectives were to determine the association between HOMA-IR and biomarkers, as well as clinically relevant factors.

We performed descriptive analyses on all of the covariates (including age, sex, race, nadir and current CD4, ART duration and type) and outcomes of interests: HOMA-IR and measured biomarkers. Kruskal-Wallis tests were used to compare continuous variables and Fisher's exact test for categorical variables, by HIV status. Pearson correlation was used to assess correlations with HOMA-IR and each biomarker as well as HIV and anthropometric risk factors.

Multivariable regression was used to model HOMA-IR. In the multiple linear regression analysis, we included covariates that were significantly ($p<0.1$) associated with HOMA-IR in the correlation analysis. We also adjusted the analysis for known risk factors of insulin resistance such as age, sex, BMI and family history of diabetes.

All the statistical analyses were performed using Stata 15 and R 3.4.1

Results

Baseline Characteristics

Overall, 172 participants were enrolled in this study and included in this analysis: 57 PHIV, 59 HEU and 56 HIV– children. Median [IQR] age was 7.8 years [6.39, 8.84], 55% of participants were female, median BMI was 15.2 [14.38, 15.81]. As shown in Table 1, HDL cholesterol, triglycerides and waist hip ratio was higher in PHIVs (<0.01). Known family history of diabetes was higher in HIV– children ($p=0.01$).

Among PHIVs, 93% had a viral load <20 copies/mL and median CD4 % was 37 [27, 41]. The median time on ART was 72 months [63, 76 months], 51% were on a nevirapine based regimen and 23% on lopinavir/ritonavir.

BDG, zonulin and sCD14 were higher in the PHIV group compared to both HEU and HIV– groups ($p<0.01$). Some of the inflammatory and monocyte activation markers including sCD163, sTNFRI and II and D-dimer were lower in PHIV ($p<0.05$). There were no differences in the gastrointestinal markers and systemic inflammatory markers between the HEU and HIV– children ($p=0.1$).

HOMA-IR

As shown in Table 1 and Figure 1, HOMA-IR was higher in PHIV ($p<0.02$). In the PHIV group, 4 participants had insulin resistance defined as HOMA-IR >2.5 , including one with HOMA-IR >3.16 . None of the participants in the HEU or HIV– group had HOMA-IR >2.5 . There was no difference in HOMA-IR between HEU and HIV– children ($p=0.89$).

HOMA-IR was not different between sexes ($p=0.76$).

There was no correlation with CD4 or viral load ($p>0.4$). HOMA-IR was not associated with being on protease inhibitor ($p=0.08$), any of the NNRTI ($p=0.32$) or being on abacavir ($p=0.92$).

HOMA-IR and biomarkers

There was no correlation between HOMA-IR and markers of systemic inflammation, monocyte activation, gut integrity, microbial or fungal translocation or oxidized lipids among all combined participants (Figure 2). Higher HOMA-IR only correlated with lower sTNFRI ($r=-0.19$, $p=0.01$). The results were similar when assessing the correlation between HOMA-IR and biomarkers within groups (Table 2).

HOMA-IR and metabolic risk factors

HOMA-IR correlated with BMI and HDL ($p=0.01$) but not with waist hip ratio ($p=0.4$). In single predictor regression analyses, only BMI remained associated with HOMA-IR ($\beta=0.15$, $p<0.01$). After adjusting for age and sTNFRI, higher BMI remained independently associated with HOMA-IR ($p<0.01$). No other covariates were associated with HOMA-IR in this model (Table 3).

Discussion

For the first time in PHIV, we investigated the role of markers of gut integrity and translocation on insulin resistance. We found that Ugandan PHIVs have disturbances in glucose metabolism when compared to HEU and HIV– children. Despite evidence of alteration in gut integrity and translocation with significantly higher sCD14, zonulin and BDG in PHIV, higher BMI was the only predictive factor of HOMA-IR in this population.

Insulin resistance can be broadly defined as a subnormal biological response to normal insulin concentrations. Insulin resistance has been previously reported in PHIV^[6, 22,23], however data in African children are limited. Furthermore, data may be difficult to interpret due to the lack of a control arm which is important since norms of HOMA-IR for African children are not available. We have previously shown that in young ART-naïve Ugandan children (median age 2.8 years), HOMA-IR significantly increased after 48 weeks of ART, specifically in children on abacavir^[10]. Here, we found that, at the same site in Uganda, older children remain at risk of insulin resistance after 6 years on ART, regardless of the regimen, with higher HOMA-IR compared to age and sex matched HEU and HIV– children. To our knowledge, only one other study has compared PHIV to controls in an African setting. The authors reported that insulin resistance in older South African children (9–14 years) was not significantly different in PHIV compared to age-matched uninfected controls, however, the overall prevalence of insulin resistance was high in both group (18%)^[24]. Puberty is associated with a decrease in insulin sensitivity and we hypothesize that findings in youth with HIV during this time of considerable metabolic and hormonal change may be difficult to extrapolate to our prepubertal study participants, additionally genetic predisposition to insulin resistance may be difference between black South African children and Ugandan children.

HIV infection, inflammation, ART, gut microbiota and immune activation likely all play a role in insulin resistance^[25]. The innate immune system and insulin signaling are integrated. We have previously shown that sCD163, a marker of monocyte activation, was associated with greater HOMA-IR in a cohort of young Ugandan PHIV 48 weeks after ART

initiation^[10]. Increases in proinflammatory cytokines and lipopolysaccharide can also result in a down regulation in insulin signaling^[26]. Bacterial translocation in HIV has been associated with impaired glucose homeostasis^[27]. In the cohort described here, we have found evidence of gut dysfunction with higher levels of sCD14, zonulin (a marker of intestinal permeability) and fungal translocation (as measured by Beta-d glucan, BDG) in PHIV compared to HEU and HIV– children. In addition, differences in biomarkers between PHIV, HEU and HIV– children in our study have been further explored and may be secondary to breastfeeding history, all PHIV being on co-trimoxazole or socioeconomic and dietary differences between the groups^[28]. In ART-naïve adults, markers of gut dysfunction has been shown to predict increase in adipose tissue^[29]. For this study, we were interested in further exploring the relationship of inflammation and immune activation, as well as intestinal damage and the resultant bacterial and fungal translocation, on disturbances of glucose metabolism in PHIV. Despite alteration in intestinal integrity, these markers do not appear to play a role in HOMA-IR in PHIV. We hypothesize that the interaction between intestinal integrity, the intestinal microbiota, the innate immune system and metabolic dysfunction may be complex and may also change as children age. We hypothesize that the relationship between gut integrity and metabolic complications, specifically insulin resistance, may be different in non-obese children. Another possible explanation is that since only four children reached the cut off of insulin resistance, the disturbances in glucose homeostasis may not be large enough in young children to explore these associations accurately.

Several different factors may be driving insulin resistance in this setting. Insulin resistance commonly occurs in association with obesity. In the Pediatric HIV/AIDS Cohort Study (PHACS, a prospective cohort study in vertically infected children in the US), insulin resistance in PHIV and HEU was higher than what is reported in uninfected youth, however similar to what is reported in uninfected obese youth^[6]. In South African youth, insulin resistance was associated with higher waist circumference after adjusting for age, sex, BMI and tanner stage^[24]. We did not find a correlation with waist circumference, however, we found that insulin resistance in our young PHIV cohort was independently associated with higher BMI. Different predictors of insulin resistance have been associated with insulin resistance in children vs adolescents with obesity^[30]. In a large retrospective study in children and adolescents with obesity in the Netherlands, BMI was associated with insulin in children and adolescents, however waist circumference was associated with insulin resistance in adolescents only^[30]. The authors hypothesized that this may be secondary to different regional fat distribution in children vs adolescents. This may also be true in young African PHIV with lower levels of subcutaneous abdominal fat. Our findings are clinically significant, despite the lack of frank obesity and differences between groups, young PHIV with viral suppression, have disturbances in insulin homeostasis which may be secondary to body composition. PHIV in this study already possess several cardiometabolic risk factors associated with Metabolic Syndrome in adults including high waist hip ratio, insulin resistance and elevated triglycerides compared to uninfected children highlighting the need for continued vigilant attention to CVD risk screening in this population.

Despite having averted HIV infection, HEU children have heightened systemic inflammation and immune activation in infancy^[31], and are known to have increased

morbidity and mortality compared to unexposed children [32,33]. We did not find evidence of disturbances in glucose metabolism or persistent inflammation in young HEU children compared to HIV– children.

We did not find any correlation between abacavir and HOMA-IR or any other ART.

Our study is limited by the small sample size and the cross sectional nature precluding us from drawing conclusions about the temporal relationship of HOMA-IR and biomarkers. Specifically, in regards to systemic inflammation and gut biomarkers, our study may not have adequate power to detect a small correlation with HOMA; however, the nearly absent observed correlations between HOMA and the biomarkers are unlikely to significantly change with a larger sample size. Some of our findings may be secondary to type I errors and may not reflect true associations. Additionally, we did not explore diet and gut microbiome dysbiosis which could affect glucose metabolism. Our study is strengthened by our detailed evaluations of inflammation, immune activation, gut integrity and bacterial and fungal translocation. Additionally, the inclusion of an age and sex matched comparison groups from the same community allows for adequate comparisons.

In conclusion, we show that young PHIV in Uganda have evidence of disturbances in glucose homeostasis and fungal translocation. However BMI, and not inflammation or gut integrity markers, was associated with insulin resistance. Our results highlight the importance of body composition in insulin resistance even in the setting of a chronic inflammatory state like HIV. Sub-Saharan Africa is facing an increase burden in cardiovascular diseases due to rapid demographic, sociocultural and economic transitions. With obesity becoming more frequent in the HIV population, our findings support the need for future preventive interventions aimed at reducing premature mortality from cardiometabolic diseases in the 12 million PHIV under the age of 17 who reside in sub-Saharan Africa.

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References

1. Paula AA, Schechter M, Tuboi SH, Faulhaber JC, Luz PM, Veloso VG, et al. Continuous Increase of Cardiovascular Diseases, Diabetes, and Non-HIV Related Cancers as Causes of Death in HIV-Infected Individuals in Brazil: An Analysis of Nationwide Data. *PloS one* 2014; 9(4):e94636. [PubMed: 24728320]

2. Blaha MJ, DeFilippis AP, Rivera JJ, Budoff MJ, Blankstein R, Agatston A, et al. The relationship between insulin resistance and incidence and progression of coronary artery calcification: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes care* 2011; 34(3):749–751. [PubMed: 21292863]
3. Tenenbaum A, Adler Y, Boyko V, Tenenbaum H, Fisman EZ, Tanne D, et al. Insulin resistance is associated with increased risk of major cardiovascular events in patients with preexisting coronary artery disease. *American heart journal* 2007; 153(4):559–565. [PubMed: 17383294]
4. Ausk KJ, Boyko EJ, Ioannou GN. Insulin resistance predicts mortality in nondiabetic individuals in the U.S. *Diabetes care* 2010; 33(6):1179–1185. [PubMed: 20200308]
5. Atabek ME, Pirgon O, Kivrak AS. Evidence for association between insulin resistance and premature carotid atherosclerosis in childhood obesity. *Pediatric research* 2007; 61(3):345–349. [PubMed: 17314695]
6. Valle Jimenez M, Estepa RM, Camacho RM, Estrada RC, Luna FG, Guitarte FB. Endothelial dysfunction is related to insulin resistance and inflammatory biomarker levels in obese prepubertal children. *European journal of endocrinology* 2007; 156(4):497–502. [PubMed: 17389466]
7. Geffner ME, Patel K, Jacobson DL, Wu J, Miller TL, Hazra R, et al. Changes in insulin sensitivity over time and associated factors in HIV-infected adolescents. *Aids* 2018; 32(5):613–622. [PubMed: 29280758]
8. Geffner ME, Patel K, Miller TL, Hazra R, Silio M, Van Dyke RB, et al. Factors associated with insulin resistance among children and adolescents perinatally infected with HIV-1 in the pediatric HIV/AIDS cohort study. *Hormone research in paediatrics* 2011; 76(6):386–391. [PubMed: 22042056]
9. Innes S, Abdullah KL, Haubrich R, Cotton MF, Browne SH. High Prevalence of Dyslipidemia and Insulin Resistance in HIV-infected Prepubertal African Children on Antiretroviral Therapy. *The Pediatric infectious disease journal* 2016; 35(1):e1–7. [PubMed: 26421804]
10. Dirajlal-Fargo S, Musiime V, Cook A, Mirembe G, Kenny J, Jiang Y, et al. Insulin Resistance and Markers of Inflammation in HIV-infected Ugandan Children in the CHAPAS-3 Trial. *The Pediatric infectious disease journal* 2017; 36(8):761–767. [PubMed: 28067719]
11. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS medicine* 2008; 5(10):e203. [PubMed: 18942885]
12. Tenorio AR, Zheng Y, Bosch RJ, Krishnan S, Rodriguez B, Hunt PW, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. *The Journal of infectious diseases* 2014; 210(8):1248–1259. [PubMed: 24795473]
13. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. *The Journal of infectious diseases* 2011; 203(6):780–790. [PubMed: 21252259]
14. McComsey GA, Kitch D, Sax PE, Tierney C, Jahed NC, Melbourne K, et al. Associations of inflammatory markers with AIDS and non-AIDS clinical events after initiation of antiretroviral therapy: AIDS clinical trials group A5224s, a substudy of ACTG A5202. *Journal of acquired immune deficiency syndromes* 2014; 65(2):167–174. [PubMed: 24121755]
15. Longenecker CT, Jiang Y, Orringer CE, Gilkeson RC, Debanne S, Funderburg NT, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. *AIDS (London, England)* 2014; 28(7):969–977.
16. Hileman CO, Longenecker CT, Carman TL, McComsey GA. C-reactive protein predicts 96-week carotid intima media thickness progression in HIV-infected adults naive to antiretroviral therapy. *Journal of acquired immune deficiency syndromes* 2014; 65(3):340–344. [PubMed: 24220288]
17. Brown TT, Tassiopoulos K, Bosch RJ, Shikuma C, McComsey GA. Association between systemic inflammation and incident diabetes in HIV-infected patients after initiation of antiretroviral therapy. *Diabetes care* 2010; 33(10):2244–2249. [PubMed: 20664016]
18. Gupta SK, Kitch D, Tierney C, Melbourne K, Ha B, McComsey GA, et al. Markers of renal disease and function are associated with systemic inflammation in HIV infection. *HIV medicine* 2015; 16(10):591–598. [PubMed: 25990642]

19. Brechley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nature medicine* 2006; 12(12): 1365–1371.
20. Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. *Clinical microbiology reviews* 2013; 26(1):2–18. [PubMed: 23297256]
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7):412–419. [PubMed: 3899825]
22. Blazquez D, Ramos-Amador JT, Sainz T, Mellado MJ, Garcia-Ascaso M, De Jose MI, et al. Lipid and glucose alterations in perinatally-acquired HIV-infected adolescents and young adults. *BMC infectious diseases* 2015; 15:119. [PubMed: 25880777]
23. Paganella MP, Cohen RA, Harris DR, de Souza Kuchenbecker R, Sperhake RD, Kato SK, et al. Association of Dyslipidemia and Glucose Abnormalities With Antiretroviral Treatment in a Cohort of HIV-Infected Latin American Children. *Journal of acquired immune deficiency syndromes (1999)* 2017; 74(1):e1–e8. [PubMed: 27570910]
24. Frigati LJ, Jao J, Mahtab S, Asafu Agyei NA, Cotton MF, Myer L, et al. Insulin Resistance in South African Youth Living with Perinatally Acquired HIV Receiving Antiretroviral Therapy. *AIDS research and human retroviruses* 2019; 35(1):56–62. [PubMed: 30156434]
25. Pedro MN, Rocha GZ, Guadagnini D, Santos A, Magro DO, Assalin HB, et al. Insulin Resistance in HIV-Patients: Causes and Consequences. *Frontiers in endocrinology* 2018; 9:514. [PubMed: 30233499]
26. Liang H, Hussey SE, Sanchez-Avila A, Tantiwong P, Musi N. Effect of lipopolysaccharide on inflammation and insulin action in human muscle. *PloS one* 2013; 8(5):e63983. [PubMed: 23704966]
27. Moreno-Perez O, Giner L, Reus S, Boix V, Alfayate R, Frances R, et al. Impact of circulating bacterial DNA in long-term glucose homeostasis in non-diabetic patients with HIV infection: cohort study. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2018; 37(2):313–318.
28. Dirajlal-Fargo S, El-Kamari V, Weiner L, Shan L, Sattar A, Kulkarni M, et al. Altered intestinal permeability and fungal translocation in Ugandan children with HIV. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2019.
29. El Kamari V, Moser C, Hileman CO, Currier JS, Brown TT, Johnston L, et al. Lower pretreatment gut integrity is independently associated with fat gain on antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2018.
30. Lentferink YE, Elst MAJ, Knibbe CAJ, van der Vorst MMJ. Predictors of Insulin Resistance in Children versus Adolescents with Obesity. *Journal of obesity* 2017; 2017:3793868. [PubMed: 29375912]
31. Dirajlal-Fargo S, Mussi-Pinhata MM, Weinberg A, Yu Q, Cohen R, Harris DR, et al. HIV-exposed uninfected infants have increased inflammation and monocyte activation. *AIDS (London, England)* 2019.
32. Brahmabhatt H, Kigozi G, Wabwire-Mangen F, Serwadda D, Lutalo T, Nalugoda F, et al. Mortality in HIV-infected and uninfected children of HIV-infected and uninfected mothers in rural Uganda. *Journal of acquired immune deficiency syndromes* 2006; 41(4):504–508. [PubMed: 16652060]
33. Koyanagi A, Humphrey JH, Ntozini R, Nathoo K, Moulton LH, Iliff P, et al. Morbidity among human immunodeficiency virus-exposed but uninfected, human immunodeficiency virus-infected, and human immunodeficiency virus-unexposed infants in Zimbabwe before availability of highly active antiretroviral therapy. *The Pediatric infectious disease journal* 2011; 30(1):45–51. [PubMed: 21173675]

Figure 1: Box plots of HOMA-IR in the 3 group.

Median shown by the line that divides the box, quartiles 2 and 3 represented by the upper and lower border of the box; whiskers represent quartiles 1 and 4.

Figure 2: Scatter plot between HOMA-IR and biomarkers among all groups

r= Pearson correlation

BDG: beta D glucan, HOMA: Homeostatic assessment insulin resistance, hsCRP: high-sensitivity C reactive protein, IFAB: intestinal fatty acid binding protein, IL6: interleukin 6, LBP: lipopolysaccharide binding protein, OxLDL: oxidized low density lipoprotein, TNFRI and II: soluble tumor necrosis factor α I and II, sCD14: soluble CD 14, sCD163: soluble CD 163

Table 1:

Comparison of baseline characteristics between study groups

Variables	Overall	PHIV	HEU	HIV–	p-value
	n=172	n=57	n=59	n=56	
Demographics					
Age (y)	7.9 [6.4, 8.8]	7.7 [6.6, 8.8]	7.9 [6.2, 8.7]	7.9 [6.4, 9]	0.93
Female (%)	94 (54.7)	31 (54.4)	33 (55.9)	30 (53.6)	0.97
Family history of diabetes mellitus (%)	43 (25.4)	9 (16.7)	15 (25.4)	19 (33.9)	0.01
Height (m)	1.22 [1.14, 1.30]	1.20 [1.14, 1.26]	1.23 [1.12, 1.32]	1.23 [1.16, 1.29]	0.52
Weight (kg)	22.5 [19.7, 25.5]	21.7 [20, 24.6]	22.5 [18.7, 25.7]	23 [20, 26]	0.52
Cardiovascular Risk Factors					
BMI (kg/m²)	15.02 [14.38, 15.81]	14.93 [14.48, 15.91]	14.92 [14.06, 15.60]	15.17 [14.57, 16.25]	0.08
Waist (cm)	56.50 [53.50, 59.00]	57.00 [55.00, 59.25]	56.00 [53.00, 59.25]	56.75 [53.22, 58.47]	0.37
Hip (cm)	62.00 [58.00, 67.00]	60.00 [57.75, 65.50]	62.00 [58.00, 66.85]	63.75 [61.00, 67.12]	0.05
Waist:Hip Ratio	0.91 [0.87, 0.94]	0.92 [0.89, 0.98]	0.91 [0.87, 0.93]	0.89 [0.85, 0.92]	<0.01
Systolic Blood Pressure (mmHg)	102.00 [96.00, 108.00]	98.50 [95.00, 105.00]	101.00 [93.50, 106.50]	105.00 [98.75, 109.50]	0.04
Diastolic Blood Pressure (mmHg)	63.00 [58.00, 67.00]	63.00 [58.00, 67.75]	63.00 [58.00, 67.00]	64.00 [59.75, 67.25]	0.53
Cholesterol (md/dL)	145.00 [129.00, 164.50]	152.00 [130.00, 168.00]	143.00 [129.00, 165.00]	143.00 [128.50, 160.00]	0.42
HDL (mg/dL)	46.00 [37.25, 52.50]	49.90 [38.90, 57.10]	43.40 [35.80, 51.40]	42.40 [37.15, 48.50]	0.01
Non-HDL (mg/dL)	98.40 [84.30, 117.65]	95.70 [83.20, 122.10]	97.90 [81.15, 117.65]	98.80 [88.55, 113.15]	0.84
Cholesterol:HDL ratio	3.30 [2.80, 3.80]	3.00 [2.70, 3.70]	3.30 [2.85, 3.85]	3.50 [3.10, 3.80]	0.10
LDL (mg/dL)	82.00 [69.00, 102.00]	80.00 [69.00, 102.00]	79.00 [68.00, 106.00]	86.00 [73.00, 101.50]	0.54
VLDL (mg/dL)	15.00 [11.00, 21.00]	17.00 [13.00, 23.00]	14.00 [11.00, 22.00]	13.00 [10.00, 17.00]	0.02
Triglycerides (mg/dL)	73.00 [56.00, 104.50]	83.00 [64.00, 113.00]	72.00 [56.50, 109.50]	65.00 [51.50, 86.50]	0.02
Insulin (μIU/mL)	3.00 [2.00, 5.00]	5.00 [3.00, 7.00]	3.00 [2.00, 4.00]	3.00 [2.00, 4.00]	0.00
HOMA-IR	0.73 [0.43, 1.16]	0.94 [0.62, 1.51]	0.57 [0.42, 1.06]	0.68 [0.41, 1.08]	0.02
HIV Variables					
Viral Load (copies/mL)	20.00 [0.00, 20.00]	20.00 [0.00, 20.00]			
Absolute CD4 (cells/μL)	1266.00 [851.00, 1737.50]	1266.00 [851.00, 1737.50]			
CD4%	37.00 [27.00, 41.50]	37.00 [27.00, 41.50]			
Absolute CD4 Nadir (cells/μL)	1194.00 [678.00, 1641.00]	1194.00 [678.00, 1641.00]			
ART duration (months)	71.92 [63.37, 76.42]	71.92 [63.37, 76.42]			
Abacavir (%)	29 (50.9)	29 (50.9)			
Lopinavir/ritonavir (%)	13 (22.8)	13 (22.8)			
Efavirenz (%)	16 (28.1)	16 (28.1)			

Variables	Overall	PHIV	HEU	HIV–	p-value
	n=172	n=57	n=59	n=56	
Nevirapine (%)	29 (50.9)	29 (50.9)			
Markers of Gastrointestinal Translocation and intestinal integrity					
sCD14 (ng/mL)	1819.84 [1545.27, 2153.71]	2071.46 [1685.31, 2560.62]	1824.41 [1568.31, 2007.96]	1665.53 [1414.74, 1854.60]	<0.001
BDG (pg/mL)	169.39 [122.43, 215.57]	199.54 [178.49, 242.68]	128.84 [115.55, 169.71]	163.46 [131.53, 207.81]	<0.001
I-FABP (pg/mL)	2677.89 [1788.93, 3712.61]	2747.97 [1979.67, 4043.56]	2838.78 [1738.09, 3726.87]	2333.37 [1589.81, 3364.01]	0.15
Zonulin (ng/mL)	6.74 [4.44, 11.22]	10.95 [9.77, 12.11]	5.42 [4.64, 6.58]	5.54 [2.33, 11.31]	<0.001
LBP (ng/mL)	13224.30 [5628.14, 17436.99]	10829.41 [5524.94, 16793.43]	13736.70 [5406.26, 17501.17]	14172.99 [9744.67, 19179.22]	0.19
Systemic Markers of Inflammation					
hsCRP (ng/mL)	689.95 [191.03, 2849.43]	640.47 [289.67, 2597.90]	730.37 [175.65, 3363.64]	663.53 [147.24, 2233.24]	0.48
IL6 (pg/mL)	1.69 [0.94, 3.15]	1.64 [0.95, 3.63]	1.80 [1.02, 2.95]	1.59 [0.70, 2.46]	0.52
sCD163 (ng/mL)	432.37 [282.41, 640.64]	328.85 [240.25, 490.64]	491.57 [323.20, 737.27]	459.48 [314.52, 592.42]	0.01
sTNFRI (pg/mL)	915.63 [747.80, 1032.66]	786.27 [653.17, 960.39]	942.71 [814.95, 1062.35]	927.35 [805.52, 1082.79]	0.009
sTNFRII (pg/mL)	2550.05 [2099.09, 3223.80]	2263.95 [1907.57, 3179.02]	2725.86 [2259.09, 3192.89]	2575.30 [2147.08, 3451.92]	0.04
D-dimer (ng/mL)	411.21 [300.78, 662.73]	343.73 [250.42, 405.67]	515.13 [362.44, 820.82]	430.52 [331.82, 685.66]	<0.001
Oxidized LDL (U/L)	32038.43 [25609.59, 39392.03]	32369.86 [25591.08, 39146.01]	30241.77 [25300.76, 36626.90]	32727.03 [27028.51, 42753.30]	0.32

Median [Interquartile range]

Bold values represent $p < 0.05$

ART: antiretroviral therapy, BMI: body mass index, HDL: high density lipoprotein, HOMA-IR: Homeostatic assessment insulin resistance, LDL: low-density lipoprotein, NRTI: nucleotide reverse transcriptase inhibitor, PI: protease inhibitor, NNRTI: non-nucleotide reverse transcriptase inhibitor, VLDL: very low density lipoprotein,

Table 2:

Correlation between HOMA and biomarkers by groups

	Overall	PHIV	HEU	HIV–
Markers of Gastrointestinal Translocation and intestinal integrity				
sCD14 (ng/mL)	0.03	–0.15	0.11	–0.05
BDG (pg/mL)	0.01	–0.07	–0.05	<–0.01
I-FABP (pg/mL)	–0.11	–0.19	–0.20	<0.01
Zonulin (ng/mL)	<–0.01	–0.07	–0.13	–0.10
LBP (ng/mL)	–0.05	–0.01	–0.01	–0.04
Systemic inflammation				
hsCRP (ng/mL)	–0.04	–0.05	–0.14	0.08
IL6 (pg/mL)	–0.10	–0.16	–0.16	0.02
sCD163 (ng/mL)	–0.02	0.17	–0.14	0.13
sTNFR1 (pg/mL)	–0.18	–0.15	–0.09	–0.15
sTNFR2 (pg/mL)	–0.10	–0.06	–0.12	–0.02
D-dimer (ng/mL)	–0.08	–0.04	0.01	–0.13
Oxidized LDL (U/L)	0.08	0.21	0.20	–0.10

Pearson correlation between HOMA-IR and biomarkers by group.

Bold values represent $p < 0.05$

BDG: beta D glucan, HOMA: Homeostatic assessment insulin resistance, hsCRP: high-sensitivity C reactive protein, IFAB: intestinal fatty acid binding protein, IL6: interleukin 6, LBP: lipopolysaccharide binding protein, OxLDL: oxidized low density lipoprotein, TNFR1 and 2: soluble tumor necrosis factor α 1 and 2, sCD14: soluble CD 14, sCD163: soluble CD 163,

Table 3:

Adjusted Model of HOMA-IR

	β Coefficient	Standard Error	95% Confidence interval	P value
Age (years)	0.04	0.03	−0.02, 0.10	0.20
Body Mass Index ((kg/m ²))	0.16	0.04	0.08, 0.25	<0.01
sTNFRI (pg/mL)	<−0.01	<0.01	<−0.01, <0.01	0.16

Bold values represent p< 0.05

sTNFRI: soluble tumor necrosis factor α I