BMJ Open Associations of HIV infection with insulin and glucose levels in antiretroviral-naïve Rwandan women: a cross-sectional analysis

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ABSTRACT
Objectives: The purpose of these analyses was to determine the associations of HIV infection and related immune dysfunction with a glucose homeostasis in the population of antiretroviral-naïve HIV-infected and uninfected Rwandan women. We hypothesise that insulin resistance and its consequences in the developing countries may be further elevated with HIV infection itself regardless of antiretroviral therapy.

Study design: Cross-sectional analysis of a longitudinal cohort.

Setting: Community-based women’s associations.

Participants: In 2005, 710 HIV-infected (HIV positive) antiretroviral naïve and 226 HIV-uninfected (HIV negative) women were enrolled in the Rwanda Women’s Interassociation Study and Assessment (RWISA). Clinical and demographic parameters, CD4 count, fasting insulin and glucose levels, anthropometric measurements and Bioelectrical Impedance Analysis (BIA) were obtained. Linear models were fit to log-transformed Homeostasis Model Assessment (HOMA) with results exponentiated back to a multiplicative effect on the original scale.

Primary outcome measures: The outcome, insulin resistance, was measured by the HOMA, calculated as fasting insulin (µU/mL)xfasting glucose (mmol/L)/22.5.

Results: In adjusted models, HIV-positive women were less insulin resistant than HIV-negative; an HIV-positive woman tended to have 0.728 times as much (95% CI 0.671 to 0.800) HOMA than a comparable HIV-negative woman. Among the HIV-positive women, those with CD4 <200 cells/µL tended to have 0.741 times as much (95% CI 0.601 to 0.912) HOMA than a comparable HIV-negative woman tended to have 0.728 times as much (95% CI 0.681 to 0.861) HOMA than a comparable HIV-negative woman. The older age was independently associated with a lower HOMA insulin resistance. After adjusting for body mass index, fat and fat-free mass were not independently associated with HOMA.

Conclusions: This study found that HIV infection and more advanced HIV infection (CD4 counts <200 cells/µL) were associated with greater insulin sensitivity in antiretroviral naïve African women. These findings provide baseline information for the interpretation of future studies on the effect of antiretroviral therapy on metabolic insulin sensitivity derangements in African population.

INTRODUCTION
Potent antiretroviral therapy (ART) in patients with HIV disease has been associated with changes in lipid metabolism and glucose homeostasis in the developed countries.1 2 The Women’s Interagency HIV-study (WIHS), in five US cities, found that longer cumulative nucleoside reverse transcriptase inhibitor exposure was associated with a greater insulin resistance (IR) in HIV-infected women.2 However, HIV-positive patients in the developed countries are typically treated with ART prior to advanced CD4 depletion. This complicates separating the effects of advanced HIV-related immune dysfunction from the effects of antiretroviral agents on IR.

The greatest burden of HIV infection worldwide is in sub-Saharan Africa with 70% of HIV infection. More than 60% of HIV-infected Africans are women.3 However, metabolic changes, especially glucose and insulin homeostasis, among HIV-positive antiretroviral naïve patients in Africa have not been well described.
The prevalence of IR and metabolic conditions in the industrialised societies is elevated, affecting as many as 30–40% of adults. While this is largely due to factors other than HIV infection and highly active antiretroviral therapy, such as obesity and advanced age, there are still concerns that IR and its consequences in the developed countries may be further elevated with HIV infection and/or its treatment. While IR and type 2 diabetes mellitus (T2DM) are currently rare in the developing countries, the WHO predicts large increases in the incidence and prevalence of T2DM in the African participants due to better access to food and transition to modern lifestyles. Thus, in sub-Saharan Africa, with high rates of HIV infection and ~16% predicted increase in non-HIV-related T2DM from 2000 to 2030, there may be a greater potential for the impact of HIV infection and antiretroviral therapy on IR.

The purpose of these analyses was to determine the associations of HIV infection and related immune dysfunction with glucose homeostasis in a population of antiretroviral-naïve HIV-infected and uninfected Rwandan women. Most of the women in this study were thin with a mean body mass index (BMI) of ~21 kg/m², suggesting that this analysis could serve as a normative baseline for future analyses if the standard of living improves and Rwandans become heavier. The fact that the HIV-infected women participants were ART naïve at study enrolment makes this a unique data source on the association of HIV infection with IR in the absence of ART.

METHODS
Setting and participants
The Rwanda Women’s Interassociation Study and Assessment (RWISA) was an observational prospective study of 710 HIV-infected and 226 HIV-uninfected Rwandan women enrolled in 2005. Participants were recruited through grassroots women’s organisations and clinical care sites that served HIV-infected patients. Eligible participants were 25 years or older, willing to give informed consent and to be tested for HIV infection, and able to return for bi-annual visits. Women were excluded if they had previously received any antiretroviral treatment, with the allowable exception of prior single-dose nevirapine to prevent mother-to-child HIV transmission. Each participant provided written informed consent after watching a video demonstrating the study procedures.

Laboratory data
Full blood counts, serum liver function values and lipoprotein levels were determined by standard methods at the King Faisal Hospital clinical laboratory in Kigali Rwanda. Insulin was measured with the Perkin Elmer alphalISA human insulin kit on a Perkin Elmer Empire Plate reader and glucose was measured using the Olympus America glucose hexokinase kit on an Olympus AU400 chemistry autoanalyser, at the Albert Einstein College of Medicine, Bronx, New York. CD4 counts were measured at the National Reference Laboratory of Rwanda by a FACS counter (Becton and Dickinson, Immunocytemetry Systems, San Jose, California, USA). The diagnosis of HIV infection was performed by a testing algorithm, which required two positive results from commercial HIV-1 antibodies ELISA kits (HIV Vironostika, the Netherlands and Murex HIV-1,2, Oxford, UK).

Statistical analysis
We performed two different sets of analyses: one compared HIV-positive women with HIV-negative women and the other compared HIV-positive women by disease severity defined by CD4 categories as follows: CD4>350, CD4 200–350, and CD4 <200 cells/µL. Categorical variables, such as menopausal status, were presented by number and percentage while continuous variables such as age and BMI were summarised by mean and SD for normally distributed variables or median and IQR for variables violating normal distribution assumption (absolute values of skewness greater than 3). Comparisons of continuous and categorical characteristics among groups were made by Pearson χ² tests and analysis of variance or Kruskal Wallis tests. To assess for the independent associations of our variables of interest with log₁₀ HOMA, we performed stepwise multivariable linear regression analysis, with p=0.10 for entry and retention. Because of its skewed distribution, HOMA (skewness=4.34) was log₁₀ transformed in all linear regression analyses (skewness log HOMA=0.19). Coefficients from univariate and multivariate linear regression models on log₁₀ HOMA were converted back to the HOMA scale by exponentiation (10^x). This exponentiated value reflects the multiplicative association of an increase in one unit of that variable on the central tendency of HOMA. Analyses were conducted using STATA V.11.1 (StataCorp LP, College Station, Texas, 2010).

Exposure and outcome variables
The primary exposure variables were HIV serostatus (HIV positive vs negative) and CD4 count among HIV-positive women categorised into three groups: >350, 200–350 cells/µL and <200 cells/µL. The secondary exposure variables included age, waist-to-hip ratio (WHR), self-reported menopausal status, having had a stage IV WHO AIDS defining illness, BMI calculated as weight in kilograms divided by (height in meters)², fat mass index (FMI) and fat-free mass index (FFMI) calculated from resistance and reactance using Kotler’s formulae and standardised by dividing by height². The outcome, IR, was measured by the Homeostasis Model Assessment (HOMA), calculated as fasting insulin (µU/mL)×fasting glucose (mmol/L)/22.5. HOMA correlates well with IR measured by the gold standard euglycemic-hyperinsulinemic clamp.

The purpose of these analyses was to determine the associations of HIV infection and related immune dysfunction with glucose homeostasis in a population of antiretroviral-naïve HIV-infected and uninfected Rwandan women. Most of the women in this study were thin with a mean body mass index (BMI) of ~21 kg/m², suggesting that this analysis could serve as a normative baseline for future analyses if the standard of living improves and Rwandans become heavier. The fact that the HIV-infected women participants were ART naïve at study enrolment makes this a unique data source on the association of HIV infection with IR in the absence of ART.
RESULTS

A total of 212 HIV-negative women and 551 HIV-positive were included in this analysis. The HIV-negative women were older (mean age 42 vs 34 years in HIV positive) and more likely to be postmenopausal than were the HIV-positive women (p<0.001 for each comparison; table 1). There were no significant differences in the metabolic and anthropometric parameters (FFMI, FMI, BMI and WHR) between the HIV-positive women and the HIV-negative women, p>0.05 for all (table 1). These parameters also did not differ in comparisons of the means among the three CD4 groups among HIV-positive women. Overall, these women had a high prevalence of malnutrition defined by WHO as BMI <18.5 kg/m²; 156 (20.5%) of all women (data not shown), 18% of the HIV-negative women and 24% of the HIV-negative women had a mean BMI less than 18.5 kg/m² (table 1).

Fasting glucose concentration were highest in the HIV-negative women with a mean (mg/dL) and ±SD of 81.8±18.9 compared with 77.5±19.8 in the HIV-positive participants (p<0.001). The mean glucose was 81.1±34.5, 77.1±9.1 and 75.5±10.8 in the HIV-positive women with CD4>350, CD4 200–350 and CD4 <200 cells/µL respectively (p<0.001). The median insulin was significantly higher: 4.29 μU/mL (2.2–7.7) in the HIV-negative women compared with 3.45 μU/mL (1.6–6.2) in the HIV-positive women (p=0.005). The HIV-positive women with CD4>350 cells/µL had a higher median insulin of 4.1 (2.2–7.2) compared with the HIV-positive women with CD4 200–350 cells/µL (3.6 (1.8–6.5)) and <200 cells/µL (2.8 (1.4–10.2)) p=0.01. The HIV-positive women were more insulin sensitive than the HIV-negative women, as defined by lower HOMA scores: median HOMA and IQR 0.66 (0.3–1.2) for HIV-positive women versus 0.83 (0.4–1.5) for HIV-negative women, p<0.001, with very low values in the HIV-positive women with CD4 count <200 cells/µL: median (IQR) 0.52 (0.21–1.00) compared with 0.65 (0.34–1.32) and 0.80 (0.43–1.52) in the HIV positive women with CD4 200–350 and >350 cells/µL, respectively, p<0.001.

Table 2 presents the results of univariate linear regression models on log-HOMA. Columns 2 and 3 are from models fit to all participants and include HIV status (HIV positive versus HIV negative) as a covariate. Columns 4 and 5 are restricted to HIV-positive participants and include CD4 count category, CD4 200–350 and CD4>350 (each vs CD4 <200 cells/µL as reference) as covariates. Columns 2 and 4 contain log HOMA scale coefficients while columns 4 and 5 contain HOMA scale coefficients. Log HOMA scale coefficients are the actual results of the linear regression on log₁₀ HOMA while HOMA scale coefficients are 10 exponentiated to the power of the log HOMA scale coefficients and reflect the multiplicative association of an increase in one unit of that variable on the central tendency of HOMA. For example, using all subjects, on the log HOMA scale an HIV-positive woman, on an average, has (as in the second column of table 2) 0.101 lower log units 95% CI –0.164 to –0.038 units than an HIV-negative women. However, log units, while being the actual results of the linear models, are hard to interpret, so going to column 3 of table 2, on the HOMA scale an HIV-positive woman tends to have 0.793 (=10⁻⁰.¹⁰¹) times as much HOMA as an HIV-negative women. If the central tendency of an HIV-positive woman’s HOMA score was 1.00, then the central tendency of an HIV-negative woman’s HOMA score would be 0.793×1.00=0.793. The 95% CI for this central tendency in the HIV-positive women is 0.686 to 0.916 units times as much HOMA as in the HIV-negative

Table 1 Baseline characteristics of study participants (n=763)

| Characteristic | All participants (n=763) | HIV-positive participants (n=551) |
|---------------|------------------------|---------------------------------|
|               | HIV negative (n=212)   | HIV positive (n=551)            | CD4 >350 (n=143) | CD4 200–350 (n=195) | CD4 <200 (n=213) |
| Age (years)*  | 42.4±10.5              | 34.8±6.9                        | <0.001            | 34.1±6.9              | 35.3±6.5            | 34.8±7.1            | <0.001          |
| Menopause (yes vs no) | 42 (21.8)                | 29 (5.6)                         | <0.001            | 9 (6.6)               | 9 (5.1)             | 11 (5.6)             | <0.001          |
| WHO class 4 (yes vs no) | 13 (6.1)                 | 170 (30.8)                      | <0.001            | 33 (23.1)              | 60 (30.7)           | 77 (36.1)            | <0.001          |
| Waist–hip ratio* | 0.88±0.07               | 0.88±0.07                       | 0.99              | 0.88±0.07              | 0.89±0.08           | 0.87±0.08            | 0.21            |
| Body mass index* (kg/m²) | 21.3±3.7                | 21.6±3.8                        | 0.06              | 4.1±3.2                | 4.6±3.3             | 4.1±3.1              | 0.60            |
| Fat mass index* (kg/m²) | 3.8±3.10               | 4.3±3.2                         | 0.69              | 17.4±1.4               | 17.4±1.6            | 17±1.5               | 0.21            |
| Fat-free mass Index* (kg/m²) | 17.3±6.0               | 17.3±0.07                       | 0.79              | 510±150                | 269±40              | 123±50               | <0.001          |
| CD4* (cells/µL) | NA                     | 275±175                         | 0.005             | 4.29 (2.2–7.7)         | 3.45 (1.6–6.2)      | 2.84 (1.4–10.2)      | 0.21            |
| Glucose* (mg/dL) | 81.8±18.9              | 77.5±19.8                       | <0.001            | 81.1±54.5              | 77.1±9.1            | 75.5±10.8            | <0.001          |
| Insulin† (µU/mL) | 4.29 (2.2–7.7)         | 3.45 (1.6–6.2)                  | 0.005             | 4.1 (2.2–7.2)          | 3.6 (1.8–6.5)       | 2.84 (1.4–10.2)      | 10.2            |
| HOMA score†     | 0.83 (0.4–1.5)          | 0.66 (0.3–1.2)                  | <0.001            | 0.80 (0.4–1.5)         | 0.65 (0.3–1.3)      | 0.52 (0.2–1)         | <0.001          |
| Log HOMA score* | −0.09±0.4              | −0.19±0.4                       | <0.001            | −0.11±0.4              | −0.17±0.4           | −0.25±0.4            | <0.001          |

Categorical variables are presented as frequency (percentage).

*Normally distributed variables are presented as mean±SD.

†Not normally distributed variables are presented as median (IQR).

HOMA, Homeostasis Model Assessment.

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women. When reporting data in tables 2 and 3 we focus more on the HOMA scale, which is easier to interpret.

We report here the rest of the univariate results from models on all subjects (columns 2 and 3) which are essentially similar to those from models restricted to the HIV-positive subjects only. The higher values of body composition and anthropometric parameters were each significantly associated with an increased IR in unadjusted analysis: for FFMI, estimate in HOMA units (95% CI) was 1.083/k/m² (1.035, 1.133); for FMI 1.072/k/m² (1.035, 1.113); for WHR 1.110 (1.015, 1.215) and for BMI 1.061/k/m² (1.044, 1.081).

We assessed HIV infection’s independent association with HOMA by forward stepwise selection in multivariable linear regression models that adjusted for age, BMI and WHR (table 3). Among all participants, the HIV-positive women were less insulin resistant as measured by HOMA, tending to have only 0.728 times as much HOMA, 95% CI (0.681 to 0.861 units) compared with a similar (with respect to the other variables in the model) HIV-negative woman. In analyses restricted to the HIV-positive women, the HOMA score of an HIV-positive woman with CD4 lymphocyte count <200 cells/µL tended to be only 0.741 (0.601, 0.912) times that of comparable HIV-positive women with CD4>350 cells/µL. However, there was not a significant association when women with CD4 200–350 were compared with those with CD4 >350 cells/µL: 0.826 95% CI (0.667, 1.021). As

### Table 2

| Participant characteristics | All participants | HIV-positive participants |
|----------------------------|------------------|--------------------------|
|                            | Log HOMA scale* | HOMA scale†             |
| HIV positive vs HIV negative| −0.101 (−0.164 to −0.038) | 0.793 (0.668 to 0.916) |
| CD4 200–350 vs CD4 >350   | NA               | NA                       |
| CD4 <200 vs CD4 >350      | NA               | NA                       |
| Age (per 5 years)          | −0.010 (−0.027 to 0.006) | 0.976 (0.941 to 1.013) |
| WHR×10                    | 0.045 (0.006 to 0.085)  | 1.110 (1.015 to 1.215)  |
| BMI per kg/m²              | 0.026 (0.019 to 0.033)  | 1.061 (1.044 to 1.078)  |
| FFMI per kg/m²             | 0.035 (0.015 to 0.054)  | 1.083 (1.035 to 1.133)  |
| FMI per kg/m²              | 0.030 (0.021 to 0.039)  | 1.072 (1.049 to 1.095)  |
| Menopause (yes vs no)      | −0.055 (−0.153 to 0.044) | 0.882 (0.703 to 1.107) |

*Numbers for log HOMA are coefficients from univariate linear regression models on log HOMA.
†Numbers for the HOMA are exponentiation of the numbers on the log HOMA and reflect the multiplicative effect on the central tendency for HOMA.
NA, this variable was not considered for the model.
BMI, body mass index; FFMI, fat-free mass index; FMI, fat mass index; HOMA, Homeostasis Model Assessment.

### Table 3

| Participant characteristics | All participants | HIV-positive subjects |
|----------------------------|------------------|----------------------|
|                            | Log HOMA scale‡ | HOMA scale§           |
| HIV positive vs HIV negative| −0.138 (−0.210 to −0.065) | 0.728 (0.681 to 0.861) |
| CD4 200–350 vs CD4 >350   | NA               | NA                   |
| CD4 <200 vs CD4 >350      | NA               | NA                   |
| Age (per 5 years)          | −0.024 (−0.043 to 0.004) | 0.946 (0.906 to 1.009) |
| WHR×10                    | 0.042 (−0.001 to 0.085) | 1.110 (0.998 to 1.216) |
| BMI per kg/m²              | 0.026 (0.018 to 0.034)  | 1.062 (1.042 to 1.081) |

†Models from forward stepwise selection using all variables in table 2.
‡Numbers for log HOMA are coefficients from multivariate linear regression models on log HOMA.
§Numbers for the HOMA are exponentiation of the numbers on the log HOMA and reflect the multiplicative effect on the central tendency for HOMA.
NA, this variable was not considered for the model.
BMI, body mass index; FFMI, fat-free mass index; FMI, fat mass index; HOMA, Homeostasis Model Assessment; WHR, waist-to-hip ratio.
In this study of the association of HIV infection with insulin homeostasis in antiretroviral-naive HIV-infected and uninfected Rwandan women, HIV-infection and more significantly HIV infection with advanced CD4 cell depletion were associated with a greater insulin sensitivity, independent of BMI or other body composition measures in univariate and multivariate models. The HIV-uninfected women and the HIV-infected participants with less advanced HIV disease, that is, CD4 lymphocyte counts above 350 cells/µL, had a significantly higher IR as indicated by higher HOMA scores. Of note, even in adjusted analysis, women with the lowest CD4 cell counts <200 cells/µL had a significantly lower HOMA scores than did the HIV-positive women with CD4≥350 cells/µL. Hommes et al.3 previously demonstrated that HIV-infected men with AIDS, even in the absence of acute illness, had increased resting energy expenditure (REE) rates. This higher REE might be responsible for the low levels of glucose and HOMA score that we observed in women with CD4 <200 cells/µL. (table 1), suggesting that more advanced HIV disease may have been directly associated with lower glucose levels, resulting in the decrease in serum insulin and HOMA.

Our findings are similar to the results from the Women’s Interagency HIV Study,14 which observed a higher BMI to be independently associated with a higher HOMA IR in HIV-positive women not on antiretroviral treatment, by glucose tolerance testing. However, in contrast to another study,15 in which WHR was found to be associated with IR, we found that WHR was not independently associated with HOMA IR after controlling for BMI. Since WHR is considered as an index of body composition, it should independently (ie, after adjustment for BMI) predict fasting hyperinsulinemia and therefore IR. To that end we did find a borderline (ie, p<0.10) statistical association between WHR and HOMA score in adjusted analyses on all participants. Another prior study,16 found that increased adipose tissue mass and obesity were associated with IR and abnormalities in glucose metabolism. In our study, FMI was statistically associated with IR in univariate models but did not remain so after controlling for BMI and other potential covariates.

Our study differs from the previous published reports of correlates of IR in several ways. The participants in our study were women from a developing country, were thinner and had a relatively high prevalence of malnutrition (>20% had mean BMI <18.5 kg/m²).17 Most of the women in this study were HIV infected and in general had advanced HIV disease (74% of the HIV-positive women had CD4 count <350 cells/µL) and all were antiretroviral naïve. The differences in demographic, clinical and metabolic profiles between our study and other cohorts may explain the discrepancies between our findings and the previously published results, and may explain the low values of insulin and glucose in our study compared with studies in the USA (table 1).

A surprising finding here was the inverse association of age with IR. We found that younger women were more insulin resistant than older women. These findings differ from the vast majority of epidemiological literature that finds older age being associated with a greater IR,18 frequently because of higher BMI. Our finding of an association of younger age with IR, rather than insulin sensitivity, may represent a ‘cohort effect’ of malnutrition-related diabetes mellitus: IR that is more prevalent in adults who in childhood experienced malnutrition.19 In the earlier decades, the population of Rwanda was much smaller than its current ~11 million people, and thus malnutrition may have become more common over the course of several decades. However, whether malnutrition-related diabetes exists as a distinct entity is under debate.18 Further study on the associations of age with IR in the Africans is warranted.

This study has some limitations, including a cross-sectional design which does not allow for observing temporal patterns in association. In addition, we used a surrogate marker of IR, and not the gold standard euglycemic insulin clamp. Further, while the HOMA index has been validated in the North Americans,4 this has not been carried out for the Africans who have a lower prevalence of obesity. In addition, the fact that REE increases in case of HIV/AIDS and therefore decreases insulin and glucose levels makes it difficult to distinguish the cause of lower HOMA score (either REE or increased insulin sensitivity).

In summary, this study of antiretroviral naïve HIV-infected and uninfected women found that HIV infection and, in particular, advanced HIV infection (CD4 counts <200 cells/µL among HIV-positive women) were associated with a significantly greater insulin sensitivity. The findings of this study may provide the key information to investigators for the interpretation of future studies of the effect of antiretroviral therapy on metabolic parameters in HIV-infected women from African population.

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