Insulin resistance in acute and early HIV-1 subtype C infection

Sara Auld

A. Background and Study Rationale

A thorough characterization of the acute and early phases of HIV-1 infection is necessary to better understand the natural history of this disease. The period following seroconversion of subtype B of the virus has been studied extensively in North America and Europe. However, there is a need for better data on subtype C infection which is responsible for greater than 95% of heterosexual infections in South Africa.[i] Previous studies have show that disease progression may vary according to subtype; additionally, subtype C has been shown to have differences in viral load trajectory that may indicate it is a more aggressive subtype.[ii]

Nutritional and metabolic disorders are increasingly being recognized as a complication of HIV but have been studied most often in the context of chronic illness and among patients receiving highly active antiretroviral therapy (HAART), particularly protease inhibitors (PI). However, changes in lipid and glucose metabolism were noted in HIV patients prior to the availability of HAART so it is reasonable to postulate that the infection itself impacts these metabolic parameters.[iii,iv] It is important to better understand the nutritional and metabolic characteristics of HIV infection during the early stages following seroconversion as early derangements of nutritional and metabolic homeostasis could potentially indicate a predisposition towards development of more significant abnormalities or could impact disease progression.

Lipodystrophy syndrome, characterized by peripheral fat wasting, central adiposity, hyperlipidemia and insulin resistance, is one of the metabolic disorders that develops in the context of HIV infection. As with other nutritional and metabolic disorders seen in HIV infection, the etiology of this syndrome is likely multifactorial. Effects of antiretroviral therapies and direct effects of the virus contribute to the disturbances that cause lipodystrophy syndrome. An excess of inflammatory cytokines is postulated to be one of the causes directly related to the HIV infection.[v] HIV patients have been found to have increased levels of TNF-α, IL-6 and IFN-α. Studies in non-HIV patients with obesity and diabetes mellitus have show that inflammation is a trigger for insulin insensitivity and that circulating levels of IL-6 strongly correlate with insulin resistance.[vi] Furthermore, viral factors have also been implicated in insulin resistance. Two accessory proteins of the virus, Vpr and Tat, have been found to increase tissue sensitivity to glucocorticoids as well as having direct modulatory effects on adipogenesis and tissue sensitivity to insulin.

In 1999, Hadigan and colleagues published cross sectional data suggesting an association between HIV infection and insulin resistance.[vii] HIV-infected women in this study, including those less than 90% of ideal body weight, were found to have significant fasting hyperinsulinemia and an increased insulin-to-glucose ratio that was unrelated to PI use. The fasting glucose concentrations in these lipodystrophy patients often remain normal although, as this study demonstrated, they will manifest fasting hyperinsulinemia. Most likely, these patients have reached a state of compensated normoglycemia that masks an underlying insulin resistance.[viii]

Anthropomorphic measures have been extensively studied as an inexpensive, reliable and non-invasive means of characterizing patients with lipodystrophy syndrome. Notably, HIV-infected patients with lipodystrophy have been shown to have an increased waist-to-hip ratio, increased waist circumference, reduced hip circumference and reduced mid-thigh circumference when compared with age- and BMI-matched control subjects.[ix] Among these HIV patients with fat redistribution, 35% had evidence of glucose intolerance in contrast to 5% of their matched control subjects. A study by Meininger, et al. found that fat redistribution contributes to hyperinsulinemia in HIV-positive men and

that this association was independent of BMI and PI use.[x] They proposed that waist-to-hip ratio (WHR) is an integrated index of body-composition changes and strongly predicts fasting hyperinsulinemia in HIV-positive men.

This prospective study of South African women will better define the relationship between insulin resistance and HIV-1 infection with subtype C. First it will define the baseline prevalence of insulin resistance among a high-risk population of South African women. Previous studies of South African factory workers and of urban South Africans have found the prevalence of impaired glucose tolerance and diabetes mellitus to be from 5.1-5.5% and from 4.5-5.2% respectively.[xi,xii] Next, the study will determine if acute and early HIV infection is associated with an increase in the incidence of insulin resistance. Finally, the study will determine if there is a correlation between the presence of insulin resistance and disease progression as measured by CD4+ count.

The secondary objective of the study will be to assess other markers of metabolic derangements, namely waist-to-hip ratio (WHR) and body-mass index (BMI), and assess if there is a correlation between these parameters, insulin resistance and disease progression.

A better characterization of the prevalence of insulin resistance along with the changes that occur during the acute and early stages of the infection will allow clinicians to better manage these metabolic disturbances. Recent cross-sectional data suggests an increased risk of MI and cardiovascular events in HIV-infected patients.[xiii] Moreover, as antiretroviral medications become increasingly available in South Africa, a thorough understanding of metabolic abnormalities, such as insulin resistance, that will predispose to long-term cardiovascular morbidity is even more important. This study will provide a framework for future studies that can evaluate the effects of various interventions, i.e. metformin, thiazolidinediones, on insulin resistance, lipodystrophy syndrome, and progression of HIV disease.

B. Study Design and Statistical Analysis

This is a prospective observational cohort study of South African women with acute HIV infection. The study participants will be identified from a cohort of female sex workers in KwaZulu-Natal and from research cohorts in Vulindlela.

The primary outcome will be a diagnosis of insulin resistance. The participants will be screened at baseline and then followed for the development of insulin resistance following seroconversion at 6, 12 and 18 months. There are a number of indices that have been identified as reliable assays of insulin sensitivity. In this study, insulin resistance will be determined by the Quantitative Insulin Sensitivity Check Index (QUICKI).[1] QUICKI is an index of insulin sensitivity that can be determined from a fasting blood sample and correlates well to the "gold standard" of the hyperinsulinemic euglycemic glucose clamp technique for both non-obese and obese patients. The index has been touted as an inexpensive, accessible measure of insulin sensitivity for use in clinical investigation.[xiv,xv] Insulin resistance will be defined as a QUICKI of less than 0.333. However, the study data will examined prior to analysis to ensure that QUICKI provides a normal distribution. If the data transformations of QUICKI do not result in a normal distribution of data points, a different index may be considered. Secondary outcomes in the study will be WHR and BMI. Study staff will be trained in how to measure these parameters in a standardized fashion.

CD4+ T cell counts will be measured from collected blood specimens at all study visits. The CD4+ T cell counts will be measured using the FACSCalibur flow cytometer. CD4+ counts of the study participants with and without insulin resistance will be compared using an unpaired t-test.

A total of 100 participants will be enrolled from the two cohorts mentioned above. A 10% attrition rate is expected thus leaving 90 active participants. The baseline prevalence of insulin resistance is expected to be 10% among this population. If an additional 10% of participants develop insulin resistance during the first 18 months following seroconversion, 18 patients in this study will meet criteria for insulin resistance. Control subjects will be selected from study participants that have seroconverted

[1] QUICKI = $1/[\log(I_0) + \log(G_0)]$

but have not developed insulin resistance. In an attempt to better delineate any association between insulin resistance and CD4+ counts, the control subjects will be selected for having the least tendency towards insulin resistance (i.e. the highest QUICKI). The variability of CD4+ count from the Multicenter AIDS Cohort Study at 6 months following seroconversion as estimated from the interquartile range was found to be 284.[xvi,xvii] Hence, for a set considering an alpha of 0.05 and a power of 80%, the detectable effect size given a sample size of 18 will be a CD4+ count of 273.

C. Study Procedure

As study participants are identified from the cohorts described above, they will be screened at baseline, prior to seroconversion. This initial screening will document an existing diagnosis of diabetes mellitus, a physical exam including WHR and BMI and a blood draw for fasting glucose and fasting insulin levels as well as CD4+ count. For the WHR, waist circumference will be measured at the thinnest area below the rib cage and above the umbilicus; the hip will be measured at the greatest circumference around the hip or buttocks with the subject standing relaxed, with feet together and arms by the side.[2] BMI will be calculated as follows: BMI = (weight in kgs)/(height in meters)^2.

Subsequent visits will be conducted at 6, 12 and 18 months following seroconversion. At follow-up visits, participants will undergo a physical exam including WHR and BMI and a blood draw for fasting glucose and insulin levels and CD4+ count.

D. Study Drugs

This study will not involve the use of any drugs.

E. Medical Devices

This study will not involve the use of any investigational devices.

F. Study Questionnaires

This study will not use any questionnaires.

G. Study Subjects

Study participants will include female sex workers age 18 and above that will be screened from among 1500 sex workers located around 15 truckstops in the Midlands area of KwaZulu-Natal province. HIV negative sex workers will be identified and followed monthly for evidence of seroconversion. Time of infection will be defined as the midpoint between the last HIV negative test and the first HIV positive test. Acute infections will be diagnosed based on the detection of HIV-1 replication in the absence of HIV-1 antibodies or based upon detection of HIV-1 antibodies with a negative HIV-1 antibody test within the previous three months. Acute infection is defined as the three-month period following HIV diagnosis and early infection is defined as the period from 3 months to 12 months following diagnosis. All sex workers, including those not participating in the study, will receive access to clinical care, access to condoms and treatment for sexually transmitted infections.

A second group of study participants will include a community research cohort in Vulindlela that has been recruited from uninfected adolescents (> 14 years) and adults attending antenatal and family planning services at public health care clinics. This cohort will be followed quarterly for evidence of seroconversion as described above.

[2] WHR norms for men are <0.9 and <0.85 for women

Inclusion criteria are as follows: women must be able to provide adequate locator information for study retention purposes, willing to participate in the study follow-up, willing to receive an HIV test result, and willing and capable to provide informed consent. Likewise, women are ineligible for participation if they plan to travel away from the study center for > 3 months during the study period, if they are unable to provide informed consent, if they have taken or are taking HAART.

All subjects will undergo an informed consent process for their participation. The consent forms will be available in Zulu and will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. For issues pertaining to subjects that are minors, refer to the section "Minors in Research Study" below.

H. Recruitment of Subjects

For the cohort of female sex workers, a network of community liaison persons (CLPs) will schedule screening visits and assist with transport and logistical arrangements in consultation with a study coordinator.

For the Vulindlela community research cohort, a study nurse will monitor HIV pretest counseling and will disseminate information about the study. On seroconversion, participants will be asked if they would like to enroll in this study.

Once enrolled in the study, the study site will make every effort to retain the subject to minimize bias associated with loss-to-follow-up.

I. Confidentiality of Study Data

To maintain subject confidentiality all laboratory specimens, evaluation forms, reports and other records that leave the study site will be identified by a unique coded number. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject except as necessary for monitoring.

All study procedures will be conducted in private, and every effort will be made to protect participant privacy and confidentiality to the extent possible.

J. Potential Conflict of Interest

The investigator declares no conflict of interest.

K. Location of the Study

The study will take place at the Doris Duke Medical Research Institute, the Nelson R Mandela School of Medicine of the University of Natal, and the Centre for the AIDS Programme of Research in South Africa (CAPRISA) Vulindlela research facility.

L. Potential Risks

This study carries limited risks for the patients. There is a small risk associated with blood draws of discomfort, bruising and/or anxiety. Additionally, patients may experience anxiety or psychological distress prior to or after a HIV test or after receiving a diagnosis of HIV. Trained counselors will be available to help participants deal with these feelings.

M. Potential Benefit

There may be no direct benefits to participants of this study. However, participants may benefit in the future from information gathered by this study. Study participants will receive HIV and STI counseling and testing and regular physical examinations.

N. Alternative Therapies

This study does not involve any treatment or interventions. The study subjects have the alternative of not participating in the study.

O. Compensation to Subjects

Participants will be reimbursed for costs associated with travel to study visits, time away from work, and child care. Reimbursement amounts will be specified in the study informed consent forms.

P. Costs to Subjects

There will be no cost to study participants.

Q. Minors

Adolescent minors above the age of 14 will be included in the Vulindlela research cohort. Section 39(4) of South Africa's Child Care Act stipulates that minors who are between the ages of 14 and 18 are legally capable of independently consenting to medical treatment. However, the Child Care Act does not discuss the age of consent for participation in research. In an attempt to resolve this discrepancy, the South African Medical Research Council (MRC) has equated medical treatment to *therapeutic* research. The MRC deems the pursuit of additional parental consent as "desirable" but not mandatory in such situations. With regards to non-therapeutic research, the MRC states that such research is not permissible without parental consent. While this study is technically a non-therapeutic observational study, participation in the study would result in benefits to participants such as identification of HIV positive status and referrals for treatment.

Furthermore, adolescent women comprise a significant and growing proportion of the HIV positive population in South Africa. Recent studies have demonstrated incidence rates as high as 25.8% among adolescent women.[xviii] It is critical that these women are included in studies such as this one since they do comprise such a notable cohort of HIV-infected individuals. The Vulindlela study site already has community advisory groups established that are able to provide input specific for adolescent issues. This protocol has been endorsed by the advisory group as an important contribution to our understanding of HIV.

R. Radiation or Radioactive Substances

This study will not expose the participants to any additional radiation beyond their standard clinical management.

S. References

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