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Risk Factors for HIV-Associated Lipodystrophy in Men and Women

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Risk Factors for HIV-Associated Lipodystrophy Syndrome in Men and Women

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2006

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An abstract of
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Abstract

Risk Factors for HIV-Associated Lipodystrophy in Men and Women

By Bradley J Klos

Purpose: The aim of this analysis was to evaluate the prevalence and risk factors for HIV-Associated Lipodystrophy Syndrome (HALS) in the Study to Understand the Natural History of HIV/AIDS in the Era of Effective Treatment (SUN Study).
Methods: The SUN study is Data collected at baseline was used for this analysis. HALS was defined using both Fat Mass Ratio in males and females and Lower Limb to Trunk Fat Ratio (LLTFR) in males only. Univariate analysis was performed using MH test statistics. Multivariate analysis was performed using Poisson regression to estimate Prevalence Ratios (PR).
Results: Population prevalence was 13% using FMR and 38% using LLTRF to define HALS. In females, only aerobics and having taken stavudine were significant in the final model. In males, using FMR to define HALs, risk factors included white non-Hispanic (2.32) and Hispanic (PR 2.49) compared to black non-Hispanic, decreased adiponectin (2.11), use of testosterone (1.87), current smokers (0.51), having taken ziduvodine (1.53), having taken stavudine (2.12), and increased age (PR 1.69 per 10 year increase) were all significant. In males, using LLTRF to define HALS, model was similar but dropped testosterone and smoking status.
Conclusions: FMR did not provide a useful HALS definition for model building in females, but did appear to be associated with expected risk factors in males. LLTRF was similar in males, but generally provided measures of association closer to the null. White non-Hispanic and Hispanic races appear to be at an increased risk for HALS. Lack of temporal data limits causal interpretation of models, however several traditional risk factors, such as age and HAART therapy, were confirmed while several new risk associations, such as race and a seemingly protective effect of current smoking status, were identified.

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Background

HIV – Associated Lipodystrophy Syndrome

Lipodystrophy refers to a broad array of metabolic conditions that lead to irregular and unwanted patterns of body-fat redistribution. These adipose tissue redistribution patterns can either be inherited due to genetic factors or acquired due to specific drug or behavioral pattern combinations. Lipodystrophy is a rare disorder in the general population, however is much more common is specific subpopulations, such as people with specific gene mutations and patients with Human Immunodeficiency Virus (HIV) on anti-retroviral (ARV) therapy (Chong 2010).

The most prevalent form of lipodystrophy in the United States today is acquired HIV-Associated Lipodystrophy Syndrome (HALS). HALS was first formally described in 1998, soon after the introduction of effective combination ARV therapy dramatically prolonged the expected time of HIV-infection prior to development of AIDS (Asha 2011, Barbaro 2007). HALS is most commonly associated with the new gold standard in HIV treatment, Highly Active Anti-Retroviral Therapy (HAART), and prevalence estimates ranging between 40-70% among HAART patients. Diagnosis is typically made on the basis on subjective findings from a physical examination, but efforts have been made to find an objective definition using more quantified methods of advanced clinical imaging, such as dual-energy x-ray absorptiometry, or DEXA (Fiorenza 2011). HALS is distinct from HIV-wasting in that it only affects adipose tissue as opposed to general body mass, and the pattern of tissue loss is markedly different. HALS typically manifests as lipoatrophy, or loss of fatty tissue, occurring in the face, arms, legs, and subcutaneous layer. Lipohypertrophy, or accumulation of fat mass, also occurs in the abdomen, including both visceral fat mass and hump-like formations on the chest and back (Fiorenza 2011, Kotler 2003, Moyle 2010). It was initially assumed that both lipoatrophy and lipohypertrophy

were physical manifestations of the same underlying mechanism. Current evidence suggests that these patterns are each caused by a variety of factors, and that fat loss and accumulation might occur independently of each other (Tsiodras 2010).

As HALS is defined by a variety of symptoms and attributed to several causes, an objective definition has been under debate since the identification of the syndrome itself (Sweeney 2007). Clinical diagnosis has been considered the gold standard in many studies, but is typically only made with identifiable regional fat loss of at least 30%. This tends to exclude both cases with only mild fat loss or where visceral fat increase is the main symptom. Obstacles in establishing an objective definition include the natural variability of fat distribution by age, sex, presence of metabolic syndromes which cause similar symptoms, and whether HAART treatment is considered during diagnosis (Carr 2003).

Several methods have been developed which use body imaging technology to quantify regional fat and create a consistent clinical description. DEXA, one such method, uses body mass density measurements to classify body tissue as one of three categories: bone, fat mass, and lean mass. This can be used to determine several measures which are associated with HALS. The Fat Mass Ratio (FMR), or the ratio of lower limb fat percentage to trunk fat percentage, is one of the more researched measures. Patients with lipodystrophy exhibit both an increased proportion of body fat in the trunk and reduced thigh fat (Dinges 2005), both of which increase FMR. Defining HALS as having an FMR of greater than or equal to 1.961 in males and 1.329 in females has a sensitivity of 58.3% and a specificity of 83.7% (Bonnett 2005, Freitas 2010). Other measurements are being researched as well, but have not been widely validated in existing literature. One study in Indian men determined that defining HALS has having a ratio of limb fat over total body fat of over 0.35 is 86.6% sensitive and 60.0% specific, a ratio of trunk fat to total limb fat (LLTFR) of over 1.68 is 73.3% sensitive and 70.0% specific, and the ratio of trunk

fat to lower limb fat of over 2.28 is 86.7% sensitive and 70% specific (Asha 2011). An HIV-associated lipodystrophy case definition (LDCD) score was developed by Andrew Carr in 2003, which incorporates clinical variables of HIV progression, metabolic parameters, DEXA and CT scan data (10 total variables). This model was shown to have 79% sensitivity and 80% specificity, but includes data which is not commonly available in all research settings. An alternative LDCD which dropped the CT scan data maintained 76% sensitivity and 80% specificity, but did not alleviate all feasibility concerns (Law 2005). Few of these methods agree well with subjective clinical diagnosis, making it difficult for researchers to establish a true prevalence of the syndrome. Research examples in this paper use either clinical diagnosis or measurements of regional fat gain/loss over time.

Health Impacts of HALS

Some researchers prefer to define lipodystrophy based on clinical measurements which are more strongly associated with outcomes of concern, such as metabolic syndrome and increased cardiovascular risk. HIV-associated metabolic syndrome is one of the main health outcomes that often follows the development of HALS. Specific metabolic features associated with lipodystrophy include hypertriglyceridemia, hypercholesterolemia, lowered high-density lipoprotein cholesterol levels, increased insulin resistance, type 2 diabetes mellitus, and elevated liver enzymes (Carr 2003). Visceral adipose tissue is strongly associated with metabolic syndrome and cardiovascular risk in the general public (Fox 2007), and this trend is thought to be the same in HIV patients. The incidence of metabolic syndrome increased from 2000 to 2007 (Moyle), although it is possible that increased surveillance played a role in this increase as well.

The effects of metabolic syndrome negatively impact heart health, and the risk of myocardial infarction (MI) increases with duration of both HIV infection and HAART treatment (Kim 2011, Turcinov 2010). It is currently under debate whether this effect is caused directly by

lipodystrophy, indirectly through metabolic syndrome, or by other complications with chronic HIV infection or treatment(Grunt 2006, Freitas 2011). Associations have been found between anti-retroviral (ARV) treatment and cardiovascular endothelial and inflammatory markers which cause atherosclerosis (Calmy 2009). HIV viremia, or increased HIV-mRNA viral load, and low CD4 counts have also been associated with endothelial dysfunction, hypercoagulability, vascular injury, and elevated levels of C-reactive protein (CRP)(Troll 2011). High levels of visceral adipose tissue (VAT) and low levels of subcutaneous adipose tissue (SAT), both caused by HALS, are also tied to increased heart risk. An increase in cardiovascular risk among HAART patients has been documented, although it appears that this risk peaked in 2001-2002, and traditional risk factors (i.e. smoking status, BMI, etc.) have the more predictive value than HIV/AIDS related factors. Still, MI risk does appear to increase more rapidly with age in HIV-positive patients than in the general population. In 2008 one study examined endothelial function in a cohort of HIV-patients beginning ARV treatment, with results indicating that ARV naïve patients actually had decreased endothelial function when compared to those on HAART (Kotler 2008). However, lipodystrophy has been associated with endothelial dysfunction when controlling for ARV regimen and traditional risk factors (Masia 2010). Additionally, Framingham Risk Scores, an objective evaluation of cardiac risk which includes age, smoking status, cholesterol levels, and other factors associated with cardiac risk, has been consistently higher in HIV-infected individuals after controlling for traditional risk factors (Lake 2010). HIV patients with lipodystrophy showed an odds ratio of 4.36 when compared to HIV patients without lipodystrophy for developing an increased coronary artery score, which is highly predictive of CV events in the general population (Moyle 2010). The effect of lipodystrophy on cardiac health is currently one of the main health concerns associated with HALS.

Several other side effects have been documented, but have received less attention in recent clinical research. Lipodystrophy in HIV patients has been tied to decreased bone density, which occurs independently of HAART therapy when controlling for other factors. This trend was strongly associated with increased levels of visceral fat, but not with decreased subcutaneous fat (Huang 2001). The changes in physical appearance also correlated with decreased self-esteem, and this negative body image can lead to symptoms of anxiety and depression (Moyle 2010). There is a strong sentiment that these impacts on body image and attitude will have a negative impact on HAART and ARV adherence (Lo 2010, McComsey 2004). As adipose tissue decreases, triglyceride storage increases in the fatty tissue of the liver. If these fat masses continue to increase, hepatic steatosis can develop (Javor 2005). These factors add substantially to the morbidity burden of HALS, and potentially the efficacy of HAART treatment.

Causes and Risk Factors

HIV Disease Status

As HALS is restricted by definition to events within the HIV-infected population, associations with markers of HIV infection were the first to be analyzed. Known associations include diagnosis of progression to AIDS, stage of disease (A/B/C), HIV-RNA viral count and CD-4 + cell count (both at baseline and in response to HAART) (Carr 2003, Han 2011). It has generally been hypothesized that the disease progression and the state of the immune system affect the onset of HALS and related symptoms (Tsiodras 2010). Even low levels of HIV-replication increase chronic inflammatory markers, which can affect fat mass storage and related metabolic conditions (Falutz 2011). Furthermore, the most significant predictor of a normalized lipid profile is a reduction in HIV-RNA count (Masia 2010). These risk factors are not thought to be the predominant cause of HALS, but may play a significant role in risk, particularly in aging HIV-patients.

Important Adipokines

While body adipose tissue has long been viewed as little more than a site for energy storage, it is now known to serve as an endocrine organ which interacts with other body functions on a systemic scale. One mechanism for this is the active secretion of proteins called adipokines from adipocytes, or fat cells (Bluher 2009). These adipokines have a wide array of functions which affect satiety, metabolism, and immune response. Adipokines which have known or suspected associations with HALS include leptin, adiponectin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-Reactive Protein (CRP).

Leptin is a 167-amino acid protein involved in satiety and the body response to starvation. It is mainly secreted by adipocytes, although small amounts have been found in the intestines and in smooth muscle as well. After secretion, it is transported through the blood-brain barrier and binds to areas which involve control of feeding and energy balance. Elevated leptin levels suppress some anorexigenic neuropeptides, and increase production of other anorexigenic peptides, such as α–MSH, which are associated with satiety. Conversely, low leptin levels occurring during body response to starvation can trigger hyperphagia, reduced thermogenesis, and suppression of thyroid hormone production. It is thought this might be a defense mechanism which increases body efficiency in times of need (Unger 2004). The body response to starvation has been noted as much more reactive than the response to overnutrition (Savage 2010). Leptin levels also impact blood glucose, insulin, triglycerides, and hepatic steatosis. Increased leptin has been linked to reduced feeding behaviors, thought to be attributable to its impact on satiety (Ahima 2004). The main role of leptin in the body is to indicate adequate fat energy storage to the parts of the brain which impact feeding behaviors, metabolism, and energy use (Bluher 2009, Fain 2004).

Leptin was one of the first biomarkers with widely identified abnormalities in HALS patients. However, no significant correlation between leptin and levels of HIV-RNA (Calmy 2009). Initially leptin levels were thought to be decreased in HALS patients, although this was most apparent in patients with an overall fat loss (Giralt 2011). Some studies suggest the association with leptin is due to a loss in overall fat mass rather than HALS status itself (Luo 2009, Mynarcik 2002, Tao 2009). Leptin levels are consistently higher in patients with clinical lipohypertrophy and lower in patients with clinical lipoatrophy, which is consistent with the hypothesis that it is related to total body fat rather than the presence of lipodystrophy itself (Sweeney 2006). Blood serum levels of circulating leptin are controlled by a balance of fat loss and gene expression (Tsiodras 2010). Leptin is also significantly associated with insulin resistance (Nagy 2003). While leptin has been used to successfully treat some symptoms of lipoatrophy, studies show contradictory results concerning the potential etiologic impact of pre-existing low level levels on HAART therapy and HALS (Wunder 2005).

Adiponectin, a 244-amino acid protein involved in the systemic regulation of glucose and lipid metabolism and inflammatory markers (Trinca 2011), is the most highly secreted gene product from adipocytes. It is mainly produced in subcutaneous fat bodies, and typically shows an inverse relationship to abdominal visceral fat (Luo 2009). Low adiponectin levels are strongly associated with insulin resistance and type-2 diabetes (Deloumeaux 2010), and are often found in acquired lipodystrophy syndromes such as HALS. In animal models, this happens due to the increased oxidation of fatty acids in muscle and liver tissue due to inhibited gluceoneogenesis and glucose release (Sweeney 2007). Elevated levels are typically found in obese subjects following weight loss. Adiponectin has anti-inflammatory, anti-atherosclerotic, antithrombotic, antioxidant, and hypolipidemic properties (Tsiodras 2010). Adiponectin levels are inversely

correlated to leptin levels in the general population, including most non-HALS lipodystrophy syndromes (Giralt 2011).

Adiponectin is strongly associated with HALS, althought the exact mechanism behind this associations in not well understood. Adiponectin is increased in some lipodystrophy and metabolic related syndromes, however is typically decreased in HALS patients and other individuals who show fat redistribution (as opposed to lipoatrophy). Adiponectin levels appear to drop shortly after HIV infection regardless of treatment, however decrease further due specific antiretroviral medications and/or HALS symptoms. This effect is most pronounced in patients who show HALS fat distribution patterns (Leszczyszyn-Pynka 2005, Luo, Mynarcik 2002). This association appears to be independent of age, disease status, or leptin levels (Tsiodras 2010). Recently, adiponectin has received increasing clinical research attention as the predominant HALS-associated adipokine.

TNF-α, IL-6, and CRP are pro-inflammatory markers involved in immune response and cell regulation. TNF-α has cytotoxic properties, and high levels are known to cause fever and body mass wasting (Locksley 2001). TNF-α is negatively associated with adiponectin serum levels, and it has been postulated that TNF-α inhibits adiponectin gene expression and protein secretion. IL-6 is involved in acute response to infection and trauma, and deregulation is associated with inflammatory diseases such as rheumatoid arthritis and irritable bowel syndrome (Heinrich 2003). Increased IL-6 levels are independently associated with incidence of type-2 diabetes. One study founds links between both TNF-α and IL-6 to HAART treatment and body fat redistribution (Vigouroux 2003). CRP is also typically elevated, and is used as a marker for existing tissue damage. Elevated levels are indicative of future risk of both diabetes and cardiovascular health (Pepys 2003). Elevated CRP levels have been found in HIV-infected individuals with both metabolic syndrome and HALS (Samaras 2007, Tadayyon 2008). The pro-

inflammatory response to HAART treatment coupled with the deregulation of these cytokines is one theory behind the mechanism causing HALS (Lihn 2003), however the true nature of their interaction is poorly understood.

HAART Regimen

The introduction of HAART therapy dramatically improved the lifespan for patients after HIV diagnosis. It was not until shortly after its introduction that HALS-like patterns of fat redistribution began to appear as anecdotal observations in the literature. As the syndrome had initially been noticed in HAART patients, the new drug therapy was immediately suspected as a potential cause, and numerous studies were launched to determine which classes of drugs showed the strongest and most consistent associations. It was soon determined that HALS occurs almost exclusively in patients on one or more ARV medication, despite the presence of other risk factors (Carr 2003). This differential appearance of symptoms has led many to speculate that HALS might be impacted by a genetic predisposition to side effects of these drugs. For example, a single mutation in the resistin gene has been associated with hyperlipidemia. However, the pharmacogenetics are not fully understood. Conflicting evidence has been gathered to support this theory (Vidal 2011). Protease inhibitors (PI) and nucleoside reverse transcriptase inhibitors (NRTI) are two such classes of HAART drug which appeared to show the most consistent harmful effects (Fiorenza 2011).

Protease Inhibitors (PIs) are a class of drug which bind to the catalytic site of HIV protease to inhibit retroviral reproduction. Examples include indinavir and saquinavir. They show evidence of interaction and/or competitive binding with homologous proteins which play a role in lipid metabolism (Barbaro 2007, Carr 2000, Carr 2003, Fiorenza 2011). They also impede adipocyte differentiation through several mechanisms (Raboud 2010, Troll 2010). They have several known toxicities, including renal calculi, nausea, and diarrhea, but these are

typically mild and do not persist throughout treatment. It has also been associated with hyperglycemia, hyperlipidemia, increased levels of reactive oxidative species, and decreased adipokine secretions. Not all PI drugs and combinations show the same level of effect. For example patients taking ritonavir boosted atazanavir have shown significantly lower levels of fat loss than patients taking atazanivir alone (McComsey 2009). Similarly, HAART patients switching to a combination of atazanavir and ritonavir experienced an increase in limb and visceral fat when compared to patients switching to a saquinavir and ritonavir combination (Moyle 2010).

Nucleoside Reverse Transcriptase Inhibitors (NRTIs) are a class of drugs which binds the catalytic site of HIV reverse transcriptase molecules, inhibiting the creation of HIV-DNA. Examples include stavudine and zidovudine. They have been known to cause mitochondrial toxicity by inhibiting mitochondrial polymerase, which is essential in DNA repair and results in a decrease in mitochondrial DNA in fat cells (Barbaro 2007, Fiorenza 2011, Garrabou 2011, Troll 2010). Patients taking only NRTIs have reported lipoatrophy and the incidence of "buffalo hump", a fat mass on the upper back which is characteristic of HALS patients. These abnormalities have also been noticed in HIV-infected who are not on ARV medications and who are experiencing mitochondrial toxicity due to infection (Carr 2003). Not all NRTIs have the same degree of effect, and studies have constructed the hierarchy (from highest mitochondrial polymerase inhibition to lowest) of zalcitabine > didanosine > stavudine > lamivudine > zidovudine > abacavir (McComsey 2004-A, McComsey 2005).

Other HAART regimen drugs have shown less conclusive associations to HALS. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have shown no significant association to either lipodystrophy or metabolic abnormalities when controlling for other HAART medications, although some evidence of harmful interactions have been identified (Fiorenza 2011). Some have proposed that a switch from NRTI to NNRTI may have a beneficial impact in HALS patients,

however current studies have shown mixed results (Moyle 2010). As more information regarding long term side effects of ARV medications come to light, research must continue to explore these associations in order to maximize the effectiveness of HAART treatment.

Behavioral and Environmental Factors

While HIV-related risk factors have been the focus of surveillance and preventative research, a growing emphasis has been placed on the impact which personal behaviors, such as exercise level and exposure to tobacco smoke and illicit drugs, which also impact metabolism.

Exercise levels and fat distribution/metabolism are intrinsically linked to the human body's energy balance. Despite this, attempts to quantify the preventative impact of different exercise levels suffer from study design hindrances which limit external validity, or comparability between a study and the general population. For example, many studies define exercise differently, or use different measurements of fat redistribution. In patients with increased levels of visceral fat, there is some evidence of exercise causing visceral adipose tissue loss, combatting the typical HALS lipoatrophy pattern. Unadjusted data on male patients who engaged in aerobic and resistance training exercise showed a 2% reduction in total body fat, with most of the loss occurring in the trunk. HIV-positive women who exercise have a lower average waist size, but no change in overall fat levels. Another study found that a 4 month aerobic program led to a 12% reduction in VAT levels and improved triglyceride and HDL levels. Physical therapy programs have been highly recommended as one method of combatting HALS symptoms, and one recent study determined that physically active HIV-patients (as opposed to sedentary) had a 79% reduction in HALS prevalence (Segatto 2011). Diet interventions have also been tested, but with mixed success (Moyle 2010), and a comparison of diet to BMI and fat content/distribution in HAART patients found only saturated fats to differ significantly (Samaras

2009). Few studies have specifically addressed the association between exercise levels and the development of HALS, and more research in needed in this area.

Little research has been performed on how smoking, environmental tobacco smoke (ETS), alcohol consumption, and illicit drug use contribute to cardiovascular risk and fat metabolism in HIV patients, and no studies analyze this effect in the context of HALS specifically. While some research has confirmed an association between cardiovascular risk and smoking status in HIV and AIDS patients, it has not been established whether or not this risk is significantly different from that of the general population (Salyer 2006). Smoking and ETS are well established risk factors for obesity, and have shown evidence for interaction with several components of body metabolism (Verngaud 2011). Alcohol consumption also impacts metabolism and weight gain, although appears to have a different impact in different populations (Pajari 2010, Schroder 2007, Wakabayashi 2011). Marijuana and other illicit drugs are thought to have some impact on weight gain and metabolism, however their impact in U.S. HIV-positive populations is not as well understood (Ades 2010, Freedhoff 2011). As these factors may have metabolic impacts independent of associated dietary contributions, it is necessary to establish whether these behaviors have an influence on HALS incidence independently or on combination with other factors.

Other Suspected Factors

While research has focused on the potential risk factors listed above, several other non-preventable or poorly understood variables are known to have an impact. Gender is a major determinant of body fat composition, and women have generally shown a higher susceptibility to HALS like symptoms (Asha 2011, Carr 2003, Deloumeaux 2010, Freitas 2011). Low levels of antioxidants have been found in some HALS patients with mitochondrial toxicity, however this trend appears to be tied to NRTI use rather than an independent risk factor, and antioxidant

supplement therapies had a minimal positive effect and actually increased insulin resistance (McComsey 2003). Age also has a strong association with increased risk. This is partially attributed to a temporal relationship to HAART toxicity and other cardiovascular risk factors which typically increase with age, however interaction between age or time of HAART exposure and other factors is also possible (Moyle 2010, Onen 2010, Salyer 2006). In conclusion, many factors, some treatable or preventable and others inherent qualities, seem to contribute to the occurrence of lipodystrophy symptoms.

Current HALS Treatment Methods

While several of the major risk factor for HALS, such as gender and age, are difficult or unfeasible to address, new prevention methods are currently be developed to help mitigate the symptoms and adverse health outcomes. Exercise regimens focusing on aerobics have been success in limited trials, and will continue to be a focus of future research. One trial showed that administering an analogue of growth hormone releasing factor was effect at reversing both lipoatrophy and lipohypertrophy without affecting insulin resistance in patients with type 2 diabetes (Abribat 2009). Metformin, an insulin sensitizing agent, was recently shown to reduce insulin resistance and visceral adipose tissue levels as well (Hadagin 2011). Alternate HAART regimens, which replace specific medications with similar analogues that present a lower risk of fat redistribution or metabolic complications, are often the first resort to HALS symptoms (Carr 2003, Moyle 2010, Troll 2010). Leptin supplements have shown signs of success in treating patients with high insulin resistance and diminished leptin levels (Ahima 2004, Bluher 2009, Chong 2009, Oral 2002), although their use is currently limited (Savage 2010). Leptin supplements are also associated with reduced incidence of hepatic steatosis (Javor 2005). Research is ongoing about supplements which help boost adiponectin levels as well. Several

thiazolidinediones have also been used, although with mixed results (Raboud 2010). A variety of treatment options are being implemented to address specific symptoms related to HALS.

An increased understanding of the population at risk for HALS is necessary to both develop new prevention and intervention methods, as well as target these solutions at appropriate high risk sub-group of HIV-infected individuals. The goal of this analysis is to further elucidate existing HAART therapy associations, to determine which covariates most strongly affect the association of lipodystrophy with circulating adipokines, and to explore the impacts of exercise, smoking, drinking, and other illicit drugs on HALS, which are currently poorly understood.

Methods

Data Collection

All data analyzed in this study was collected as part of the Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy (SUN). SUN is a prospective cohort study funded by the Centers for Disease Control and Prevention (CDC). It follows HIV-positive individuals recruited from 7 sites in 4 U.S. cities: Minneapolis, Minnesota; Providence, Rhode Island; St. Louis, Missouri, and Denver, Colorado. Enrollment occurred between March 1, 2004, and June 30, 2006.

Recruitment centers were told to actively recruit women, African-Americans, and Hispanics to maximize external validity to the general population. Eligibility was limited to individuals who were ARV naïve or who had experienced HAART only (defined as either ≥ 3 NRTI or ≥ 3 ARV of at least 2 different classes). Additional inclusion criteria included being at least 18 years of age, having at least 2 scheduled appointments at the clinic which they were enrolled at, having received less than 30 days of cumulative mono or dual ARV treatment, being expected to live and be treated at the clinic for at least the next two years, and who had not experienced an

AIDS defining illness in the previous 60 days. Patients were excluded if they were incarcerated, anticipating incarceration, or unable to provide informed consent. CD4+ T-lymphocyte cell counts were also evaluated at enrollment to increase the likelihood of at least 5 years of participation. Individuals with at least 30 days of exposure to HAART therapy were required to have a CD4+ count of ≥ 100 (minimizes low survival rates of enrolled patients), while individuals with less than 30 days of exposure to HAART had to have a count of ≥ 100 and ≤ 500 (maximal potential for effective HAART treatment). Following enrollment, data was collected at baseline and at 6 month intervals which coincided with scheduled appointments.

Much of the information was already being collected as a part of routine care, as was abstracted from clinic medical records into a central electronic database. Data collected in this way includes height, weight, blood pressure, CD4+ T-Lymphocyte counts, HIV viral load, liver function tests, and fasting serum lipid and glucose concentrations. Other data which was abstracted from clinic records includes socio-demographic information, current and previous medications, symptoms and medical diagnoses, laboratory tests, and historical information on conditions relevant to the study's objectives.

Data on behavioral patterns and family history was collected using a computer assisted interview. Use of alcohol, tobacco, and other recreational drugs were assessed using the format of the Behavioral and Risk Factor Surveillance System (NCDDPHP 2008). Employment status and psychosocial function were assessed using the Medical Outcomes Survey short Form-12 (SF-36 2008). Depression was assessed using PRIME-MD (Spitzer 1999). Data was also collected on HAART adherence, use of supplemental hormone treatments, and sexual activity.

Screening was conducted for sexually transmitted infections (STI) and blood-borne pathogens, including Hepatitis C. Syphilis and Hepatitis C were screened using blood serum. All testing was performed at central laboratories owned or contracted by the CDC except for *N.*

gonorrhoeae, *C. trachomatis*, and syphilis, which were performed at the participating clinics (Vellozi 2009).

Body fat and lean mass composition were assessed using non-invasive Dual-Energy X-ray Absorbiometry (DEXA) (Vellozi 2009). This non-invasive scanning process makes rectilinear cross sections of the individual. It is commonly used due to its wide availability, relatively small exposure to radiation, and has high precision. Accuracy has been difficult to judge, as direct methods to calculate body mass composition are not currently available. The DXA process assumes that all body tissue can be classified into 3 sections based on X-ray photon attenuation properties: fat mass, lean (fat-free) mass, and bone mineral. Questions have been raised about consistency between manufacturers, who might use different algorithms to calculate total body mass from individual data (Plank 2005). DXA scans have been increasingly used to measure changes in regional fat mass, and are currently being assessed as an objective method of quantifying the impacts of HALS and other lipodystrophy syndromes (Asha 2011, Bonnett 2005).

Data Analysis

In order to simplify the case definition and maximize the number of individuals included in the model, lipodystrophy cases were defined in two ways. The first was having an FMR of at least 1.961 in men and 1.329 in women. This cutoff point, thought to be the best single objective measure, has been previously found to give a sensitivity of 58.3% and a specificity of 83.7 % in men and a sensitivity of 51.4% and a specificity of 94.6% in women. The second method was having an LLTFR of at least 1.38. This method was found to be is 73.3% sensitive and 70.0% specific, however has only been validated in males. Separate analyses were performed for each gender and definition of HALS.

Continuous variables including leptin, adiponectin, TNA-α, IL-6, and CRP were converted into binary variables by spitting at the median of the sample population (see Table 1), as cutoff

points with clinical significance in HALS diagnosis are not known at this time. CD-4 count was converted to a binary variable by using the cutoff for an AIDS diagnosis (200 x 10^6/L). Viral count was turned into a binary variables by evaluating whether HIV-RNA copies/mL were above 400, a common limit of detection.

For univariate analysis, information regarding all dichotomous variables being considered was compared to the dichotomous case definition using a 2 x 2 table to calculate prevalence ratios. Significance was evaluated using Mantel-Haenszel chi square test statistic. For age, the only continuous variable evaluated, a Poisson model (explained below) was developed including only age.

Given the dichotomous outcome variable and the preference for prevalence ratios over odds ratios, it was decided to use Poisson regression modeling rather than logistic modeling to determine effect estimates. Prevalence ratios are preferred for point-prevalence studies, as odds ratios are biased away from the null in common diseases (Barros 2003, Coutinho 2008, Petersen 2008, Zhou 2004). As both measures of outcome were above 10% prevalence, prevalence ratios are a more appropriate measure.

Poisson modeling assumes a Poisson distribution, meaning the conditional mean is equal to the conditional variance, but is otherwise conceptually similar to linear or logistic modeling. Assumptions include independence of observations, and a linear relationship between the outcome and the log of variables included in the model. Several studies have shown that Poisson regression with robust variance provide adequate estimates of prevalence ratios from cross sectional data regardless of baseline prevalence of the outcome (Barros 2003, Petersen 2008). The adjustment to a robust Poisson model using a sandwich error term is described by Zhou in a paper regarding Poisson analysis of binary outcome data (Zhou 2004). In short, Poisson modeling allows for a certain amount of variation, or error, in the covariates of

interest. When working with binary data, this can lead to conservative estimates and larger confidence intervals. The sandwich error method corrects for this using the traditional variance measure for the delta method:

$$\hat{\text{var}}(R\hat{R}) = \frac{1}{a} - \frac{1}{n_1} + \frac{1}{c} - \frac{1}{n_0}.$$

where a= count of exposed cases, n_1=total count of exposed, c=count of unexposed cases, and n_0=count unexposed.

Variables for age, BMI category, diabetic status, and ARV medications AZT and D4T were included for both male models regardless of significance, as these are known confounders for HALS associations. Only age and diabetic status were included in the female model, as all models including BMI categories failed to converge. Other risk factors were included if associations identified in the literature were confirmed as significant during univariate analysis. Statistical interactions were not addressed in this model due to the increased complication in interpreting results.

For all analyses, separate models were developed for men and women. All models were developed using the GENMOD procedure in SAS v9.3 (SAS Institute, Cary NC), which uses Maximum Likelihood Estimates to calculate all coefficients. P-values of less than 0.05 were considered statistically significant, although this was not the only factor considered when evaluating inclusion in the final model.

Results

Population Characteristics

Of 700 patients enrolled in the study, 620 had complete data for analysis (Table 1). Of these, 488 (78%) were male, 548 (88%) were currently on a HAART regimen, 373 (60%) were white non-Hispanic, 173 (28%) were black non-Hispanic, 54 (9%) were Hispanic, 20 (3%) were

Table1: Study Population Characteristics By Gender

Covariate	Male (n=488)		Female (n=132)		Total (n=620)	
	N/Median	*%/ (IQR)*	*N/Median*	*%/ (IQR)*	*N/Median*	*%/ (IQR)*
Age (Years)	42	(36-48)	39	(31-45)	41	(35-47)
White (non-Hispanic)	331	67.8	42	31.8	373	60.2
Black (non-Hispanic)	101	20.8	72	54.6	173	27.9
Hispanic	48	9.8	6	4.5	54	8.7
Other Race/Ethnicity	8	1.6	12	9.1	20	3.2
BMI	25.1	(22.6-27.8)	27.4	(23.3-31.9)	25.5	(22.8-28.5)
Years HIV+	5	(2.2-8.0)	5	(2.5-8.2)	5	(2.3-8.1)
HAART (ever)	431	88.3	117	88.6	548	88.4
Ziduvodine (ever)	274	56.2	82	62.1	356	57.4
Stavudine (ever)	137	28.1	29	22	166	26.8
CD4 + count*	471	(328-675)	460	(335-699)	468	(332-679)
Viral Load < 400	372	76.2	94	71.2	466	75.2
Diabetic	27	5.5	12	9	39	6.3
Current Smoker	209	42.8	69	52.3	278	44.8
C-Reactive Protein (mg/L)**	1.61	(0.76-3.71)	3.15	(0.78-6.91)	1.77	(0.76-4.42)
Leptin (ng/mL)	3.11	(1.81-5.44)	16.1	(9.03-27.57)	4.18	(2.09-8.61)
Adiponectin (µg/mL)	7955	(4960-11895)	8710	(6618-14658)	8132	(5195-12470)

* data only available for 485 males

** data only available for 484 males

classified as “other race/ethnicity”, 39 (6%) were diabetic, 466 (75%) had an undetectable HIV-RNA viral load (defined as <400 copies/mL), 356 (57%) had taken ziduvodine, 166 (27%) had taken stavudine, and 278 (43%) were current smokers. Median age of the study population was 41 (inter-quartile range: 35-47), median length of HIV-infection in years was 5 (2-8), median BMI was 25.5 (22.8-28.5), median B-CD4 count was 468 (332-679), median blood serum adiponectin level was 8132 (5195-12470), and median blood-serum C-Reactive Protein (CRP) level was 1.77 (0.76-4.42). Female subjects had a median blood serum leptin level of 16.10 (9.03-27.57), while male subjects had a median level of 3.11 (1.81-5.44).

Univariate Analysis

Due to biological differences between the fat distributions in males and females, analysis for risk factors for HALS. As a cutoff for LLTFR cutoff has only been validated in male populations, this outcome was only used for males. FMR was used for both females and males, with separate cutoff points.

Factors significantly associated with HALS in females, defined as having an FMR greater than 1.329, include reduced adiponectin levels (defined as below the median value of 2.36) (prevalence ratio: 25.13; 95% confidence interval: 1.50-420.19), having taken stavudine for at least 30 days (PR 4.21, 3.19-63.27), white non-Hispanic race compared to black non-Hispanic race (PR 5.00, 1.36-18.38), currently participating in aerobic exercise (PR 10.27, 2.29-46.13), and having an undetectable viral load (true PR undefined, logit estimate of PR: 8.62, 0.52-143.55). Despite being established as risk factors in previous studies, having taken ziduvodine, diabetic status, and BMI classifications of underweight, overweight, and obese were not statiscially significant (Table 2).

Table 2: Univariate (unadjusted) Analysis for Associations Between HALS (Defined using FMR and LLTFR) in Males and Females

Covariate	Female (HALS defined as FMR > 1.33)			Male (HALS defined as FMR > 1.96)			Male (HALS defined as LLTFR > 1.38)		
	Prev. Ratio	95% Conf. Int.	p-value	Prev. Ratio	95% Conf. Int.	p-value	Prev. Ratio	95% Conf. Int.	p-value
White Non-Hispanic	**5.00**	**(1.36-28.38)**	**0.007**	**2.08**	**(1.17-3.68)**	**0.009**	**1.77**	**(1.32-2.37)**	**<0.001**
Black Non-Hispanic	**0.92**	**(0.83-0.95)**	**0.106**	**0.89**	**(0.83-0.95)**	**0.007**	**0.69**	**(0.61-0.79)**	**<0.001**
Hispanic	0.42	(0.02-7.63)	0.300	0.88	(0.39-1.88)	0.701	0.85	(0.56-1.29)	0.419
Other Race/Ethnicity	0.85	(0.09-16.22)	0.475	0.87	(0.14-5.51)	0.881	1.63	(0.94-2.82)	0.165
Adiponectin (< 8132 µg/mL)	**25.13**	**(1.50-420.19)**	**<0.001**	**5.19**	**(2.80-9.64)**	**<0.001**	**2.36**	**(1.82-3.05)**	**<0.001**
Leptin (> 4.18 ng/mL)	0.66	(0.09-4.64)	0.679	**0.59**	**(0.35-0.97)**	**0.033**	0.89	(0.70-1.13)	0.327
TNF-α (> 13.8 µg/mL)	0.87	(0.25-3.10)	0.833	**0.55**	**(0.34-0.88)**	**0.012**	**0.79**	**(0.62-0.99)**	**0.042**
CRP (> 1.77 mg/L)	0.94	(0.28-3.19)	0.930	**0.61**	**(0.38-0.97)**	**0.032**	1.00	(0.80-1.25)	0.999
Viral Load < 400 copies/mL	8.62	(0.52-143.55)	0.037	**5.15**	**(1.92-13.81)**	**<0.001**	**2.25**	**(1.53-3.30)**	**<0.001**
Cd-4 + Count < 200	0.53	(0.03-8.33)	0.348	0.20	(0.03-1.36)	0.051	0.60	(0.32-1.10)	0.066
Ziduvodine (ever)	2.44	(0.54-11.03)	0.227	**2.44**	**(1.45-4.08)**	**<0.001**	**2.29**	**(1.74-3.02)**	**<0.001**
Stavudine (ever)	**4.21**	**(3.19-63.27)**	**<0.001**	**2.71**	**(1.77-4.15)**	**<0.001**	**1.76**	**(1.42-2.18)**	**<0.001**
Diabetic Status	1.11	(0.15-8.04)	0.918	0.76	(0.26-2.27)	0.622	1.16	(0.75-1.79)	0.531
Current Smoker	0.39	(0.11-1.45)	0.144	**0.33**	**(0.19-0.58)**	**<0.001**	**0.73**	**(0.58-0.93)**	**0.009**
Testosterone (ever)	NA			**2.84**	**(1.73-4.67)**	**<0.001**	**1.73**	**(1.32-2.28)**	**0.001**
Drink more than 3 nights/week	1.59	(0.44-5.76)	0.481	**0.58**	**(0.33-1.00)**	**0.046**	**0.75**	**(0.57-0.98)**	**0.027**
Current Aerobic Exercise	**10.27**	**(2.29-46.13)**	**<0.001**	**1.59**	**(1.00-2.50)**	**0.045**	1.13	(0.90-1.41)	0.292
Exercise Monthly	2.95	(0.65-13.35)	0.137	1.46	(0.85-2.42)	0.133	1.07	(0.84-1.35)	0.603
Age (10 year increase)	0.97	(0.55-1.71)	0.917	**1.70**	**(1.37-2.11)**	**<0.001**	**1.38**	**(1.23-1.55)**	**<0.001**

Bold labels indicate statisticallt significant (p-value < 0.050) associations in univariate analysis.

Factors significantly associated with HALS in males, defined as having an FMR greater than 1.961, include reduced adiponectin levels (PR 5.19, 2.80-9.64), increased leptin levels (defined as higher than the median level of 8132) (PR 0.59, 0.35-0.97), increased TNF-α (PR 0.55, 0.34-0.88), increased CRP (0.61, 0.38-0.97), having taken ziduvodine (PR 2.44, 1.45-4.08), having taken stavudine (2.71, 1.77-4.15), white non-Hispanic race compared to black non-hispanic race (PR 2.08, 1.17-3.68), being a current smoker (PR 0.33, 0.19-0.58), currently participating in aerobic exercise (PR 1.59, 1.00-2.50), currently taking testosterone supplements (PR 2.84, 1.73-4.67), drinking alcohol at least 3 times a week (PR 0.58, 0.33-1.00), and having an undetectable viral load (PR 5.15, 1.92-13.81). Diabetic status and BMI groupings of underweight, overweight and obese were not statistically significant.

Factors significantly associated with HALS in males, defined as having an LLTFR greater than 2.28, included reduced adiponectin levels (PR 2.36, 1.82-3.05), increased TNF-α (PR 0.79, 0.62-0.99), having taken ziduvodine (PR 2.29, 1.74-3.02), having taken stavudine (1.76, 1.42-2.18), white non- smoker (PR 0.33, 0.19-0.58), currently participating in aerobic exercise (PR 1.59, 1.00-2.50), currently taking testosterone supplements (PR 2.84, 1.73-4.67), drinking alcohol at least 3 times a week (PR 0.58, 0.33-1.00), and having an undetectable viral load (PR 5.15, 1.92-13.81). Diabetic status and BMI groupings of underweight, overweight and obese were not statistically significant.

Factors significantly associated with HALS in males, defined as having an LLTFR greater than 2.28, included reduced adiponectin levels (PR 2.36, 1.82-3.05), increased TNF-α (PR 0.79, 0.62-0.99), having taken ziduvodine (PR 2.29, 1.74-3.02), having taken stavudine (1.76, 1.42-2.18), white non-Hispanic race compared to black non-Hispanic race (PR 1.77, 1.32-2.37), being a current smoker (PR 0.73, 0.58-0.93), currently taking testosterone supplements (PR 1.73, 1.32-2.28), drinking alcohol at least 3 times a week (PR 0.75, 0.57-0.98), and having an undetectable

viral load (PR 2.25, 1.53-3.30). Diabetic status and BMI groupings of underweight, overweight and obese were not statistically significant.

Multivariate Analysis

Factors which remained significant in multivariate analysis for HALS in females, using FMR to define HALS, include having taken stavudine (PR 19.03, 4.95-73.16) and participating in aerobic exercise (PR 10.00, 2.54-39.46). Other factors included in the model were having taken zidovudine (PR 2.56, 0.96-6.83), diabetic status (PR 0.93, 0.46-1.91), and age (PR for a 10 year increase: 0.73, 0.51-1.04) (Table 3).

Factors which remained significant in multivariate analysis for HALS in males, using FMR to define HALS, include decreased adiponectin (PR 4.87, 2.75-8.62), increase CRP (PR 0.50, 0.33-0.75), white non-Hispanic race compared to black non-Hispanic race (PR 2.32, 1.10-4.88), having taken stavudine (PR 2.12, 1.45-3.09), currently taking testosterone supplements (PR: 1.87, 1.16-3.01), being a current smoker (PR 0.51, 0.30-0.86), having an undetectable viral load (PR 4.44, 1.89-10.46), and increasing age (PR for a 10 year increase: 1.69, 1.34-2.13). Other factors included in the model were having taken ziduvodine (PR 1.53, 0.97-2.41), Hispanic compared to black non-Hispanic race (PR 2.49, 0.98-6.36), other compared to black non-Hispanic race (PR 2.12, 0.56-8.04), diabetic status (PR 0.69, 0.25-1.90), and categorical variables for BMI grouping of underweight (PR 6.94, 2.39-20.16), overweight (PR 1.00, 0.67-1.49), and obese (PR 0.58, 0.25-1.36) compared to the normal range.

Factors which remained significant in multivariate analysis for HALS in males, using LLTFR to define HALS, include decreased adiponectin (PR 2.24, 1.75-2.86), white non-Hispanic race compared to black non-Hispanic race (PR 2.36, 1.62-3.45), Hispanic race compared to black non-hispanic race (PR 2.10, 1.26-3.53), other race/ethnicity compared to black non-Hispanic race (PR 3.52, 1.94-6.38), having taken ziduvodine (PR 1.66, 1.29-2.14), having taken stavudine

Table 3: Multivariate (adjusted) Analysis for Associations Between HALS (Defined using FMR and LLTFR) in Males and Females

	Female (HALS defined as FMR > 1.33)			Male (HALS defined as FMR > 1.96)			Male (HALS defined as LLTFR > 1.38)		
Covariate	**Prev. Ratio**	**95% Conf. Int.**	**p-value**	**Prev. Ratio**	**95% Conf. Int.**	**p-value**	**Prev. Ratio**	**95% Conf. Int.**	**p-value**
White Non-Hispanic*		NA		2.32	(1.10-4.88)	0.026	2.36	(1.62-3.45)	<0.001
Hispanic*		NA		2.49	(0.98-6.36)	0.056	2.10	(1.26-3.53)	<0.001
Other Race/Ethnicity*		NA		2.11	(0.56-8.04)	0.271	3.52	(1.94-6.38)	0.005
Adiponectin (< 8132 µg/mL)		NA		4.87	(2.75-8.61)	<0.001	2.24	(1.75-2.86)	<0.001
CRP (> 1.77 mg/L)		NA		0.50	(0.33-0.75)	0.001		NA	
Viral Load < 400 copies/mL		NA		4.44	(1.89-10.46)	0.001	1.63	(1.17-2.27)	0.004
Ziduvodine (ever)	2.56	(0.96-6.83)	0.061	1.53	(0.97-2.41)	0.066	1.66	(1.29-2.14)	<0.001
Stavudine (ever)	19.03	(4.95-73.16)	<0.001	2.12	(1.45-3.09)	<0.001	1.55	(1.27-1.88)	<0.001
Current Smoker		NA		0.51	(0.30-0.86)	0.012		NA	
Testosterone (ever)		NA		1.87	(1.16-3.01)	0.010		NA	
Aerobic Exercise	10.00	(2.54-39.46)	0.001					NA	
Age (Decade)	0.73	(0.51-1.04)	0.077	1.69	(1.34-2.13)	<0.001	1.33	(1.18-1.50)	<0.001
Diabetic Status	0.93	(0.46-1.91)	0.850	0.69	(0.25-1.90)	0.467	0.90	(0.62-1.30)	0.563
Underweight (BMI < 18)**		NA		6.94	(2.39-20.16)	<0.001	1.08	(0.20-5.99)	0.926
Overweight (26 ≤ BMI <30)**		NA		1.00	(0.67-1.49)	0.993	0.96	(0.77-1.19)	0.714
Obese (BMI > 30)**		NA		0.58	(0.25-1.36)	0.210	1.02	(0.74-1.39)	0.918

* compared to Black non-Hispanic race

** compared to reference group (18 ≤ BMI < 26)

(PR 1.66, 1.29-2.14), having taken stavudine (PR 1.55, 1.27-1.8), having an undetectable viral load (PR 1.63, 1.17-2.27), and increasing age (PR for a 10 year increase: 1.33, 1.18-1.50). Other factors included in the model were diabetic status (PR 0.69, 0.25-1.90), and categorical variables for BMI grouping of underweight (PR 6.94, 2.39-20.16), overweight (PR 1.00, 0.67-1.49), and obese (PR 0.58, 0.25-1.36) compared to the normal range.

Discussion

The final model for women only showed significant associations for stavudine and aerobic exercise, although zidovudine did show a positive if not significant association. In general, it would be ideal to have more test subjects for this model. Age appeared to be a protective factor in this model. This contrasts the existing body of knowledge on the subject, however was not significant. The high prevalence ratio associating aerobics with HALS in women is likely indicative of a response to HALS symptoms developing, rather than a true causal relationship. Poisson models including the dichotomous adiponectin variables and categorical variables for race and BMI category would not converge. This is surprising, especially given the known association with adiponectin, and the strong association in univariate analysis (PR 25.13). This might be indicative of a skewed distribution for these categories. Nevertheless, the drug associations found matched those the author expected to find.

The analysis of risk factors for HALS in males, when defined by FMR, was the largest and most complex of the models included here. The HAART drug stavudine was highly significant (PR 2.12, p value < 0.001), however AZT was not statistically significant when controlling for other factors (PR 1.53, p value=0.066). Race appeared to be associated with HALS, with white non-Hispanic and Hispanic subjects both being twice as likely to have HALS when compared to black non-Hispanic patients. This association might be caused by genetic factors, socioeconomic factors, environmental factors, or some interaction which was not measured here. Smoking

appeared to be a protective factor., which contradicts the hypothesis that other sources of tobacco smoke, including ETS, are likely risk factors for HALS. It is possible that patients who develop HALS symptoms are more likely to quit smoking, however data of time of exposure compared to time of disease was not available for this analysis. Adiponectin was highly significant when controlling for other factors (4.87, < 0.001). While leptin appeared significant during univariate analysis, it was not significant in any model which also controlled for adiponectin. HALS did not appear to be associated with several classic markers of HIV disease status, including length of infection and CD4 count, but it was strongly associated with a decreased viral load. This might be explained if increased viral load led to fat wasting, which would counteract the central adiposity which typically allows for HALS diagnosis. The presence of undetectable viral load as a positive risk factor is highly surprising, and contrasts almost all existing literature on the subject. It is also possible that this association arose from the inclusion of HAART naïve patients in the model, who would have low risk of HALS and high risk of a detectable viral load due to minimal exposure to HAART and other ARV medications. Increased CRP levels appeared to be a protective factor, which was not expected. Men currently taking testosterone appeared to have an increased risk of HALS (PR 1.87, 0.010). Prevalence increased with age, with a 10 year age increase associated with a prevalence ratio fo 1.69. Among BMI groups, only the underweight group appeared to be significant when compared to normal. It should be noted that this group had a very small samples size.

Using FMR as a cutoff point created a much more robust model in males than in females. One potential reason is that FMR has shown to be a better measure for HALS in men than in women using epidemiological validation measures of sensitivity and specificity. The sample size in males was also much higher, 580 compared to 132. Despite this, the ARV drug associations appeared to be similar in both groups, although stavudine had a stronger

association in females. Aerobics was significant for females only. It is possible that females were more likely to report engaging in aerobic activities. Diabetes was slightly protective in both models, although not significantly so in either. It was interesting that age was a protective factor in females, while an increased risk factor as expected for males. It is possible that this association has been confounded in the female model, although it should be noted that in females age was note quite significant (p=0.077). It appears that using FMR to define HALS shows more associations with risk factors in males than in females.

The model evaluating risk factors for HALS in males, using LLTFR rather than FMR, was remarkably similar. All variables except for CRP, testosterone, and current smoking status remained in the model. It should be noted that in the former model, CRP and smoking status showed relationships which contrasted previous literature. In most cases, the variable coefficients in the LLTFR model were closer to the null of 1.00. It is relevant to point out that LLTFR is a more sensitive measure, but not as specific. This means that while it correctly identifies more "true" cases of HALS, it will also include more false positives. This is most obvious when comparing the prevalence of HALS when using the two measures: 13% when using FMR and 38% using LLTFR. All male subjects above the FMR cutoff were also above the LLTFR cutoff. This is not surprising, as the two measures are closely related. It is likely that the measures of association presented here are stronger in the more extreme cases identified by FMR. Due to this, effect estimates appear to be stronger, and more variables showed significant associations. In a broader sense, this might indicate that using FMR is a better measure for identifying extreme cases, however LTFR likely captures more of the true population at risk for HALS. The agreement between the models help to validate each other, however this is to be expected as they were derived from the same study population.

In attempting to identify behavioral and environmental factors which showed significant associations with either measure of HALS, most fell out of significance in the multivariate models. Exposure to alcohol and tobacco smoke both appeared to be protective during univariate analysis, however only smoking remained significant when controlling for other variables. Exercise and aerobics were positively associated with HALS (FMR) in both sexes, however after controlling for other factors exercise was not significant for either and aerobics was only significant in women. These exercise related variables possibly indicate an increase in planned aerobic activities to combat HALS symptoms, however the temporal element of these exposures was not addressed in this analysis. Illicit drug use was not found to be significant, although only occurred in a relatively small sample of the study population. Information on dietary intake and other environmental factors was not available.

This analysis contributes to the validation of existing risk factors for HALS, the identification of new risk factors and associations, and the body of literature regarding the strengths and weaknesses of defining HALS as an outcome, for which no "gold standard" method of diagnosis currently exists. Known associations with ARV medications such as ziduvodine and stavudine were confirmed in males and females, and stavudine was confirmed to show a stronger association with HALS regardless of gender or outcome definition. This analysis provides further evidence that cytokines such as leptin and adiponectin and inflammatory markers such as CRP and TNFA do show associations with some definitions of HALS, however that only the association with adiponectin remains meaningful and significant when controlling for other factors. Viral load was also confirmed as the marker of HIV progress with is most strongly associated with HALS. The consistent presence of smoking as a protective factor might indicate that tobacco smoke, while increasing risk of cardiovascular disease, cancer, and overall mortality, does not increase risk for HALS. Most interesting was the appearance of

increased prevalence in white non-Hispanic and Hispanic populations when compared to Black non-Hispanic subjects, a trend which had not been identified previously. As HALS is usually analyzed in specific populations, it is possible that an analysis including race has not been feasible until this time.

While this study contributes to the body of knowledge concerning HALS and the growing HIV-positive population in the US today, it also helps to identify some gaps which still need to be addressed. The most pressing dearth of information concerns women living with HIV. As the burden has historically focused on male populations, MSM specifically, much less is known about the occurrence of HALS and other HIV-related complications. The poor outcome definition and smaller sample size in females here reflect a larger problem of this increasing burden. More research needs to be done to help bridge this gender gap in knowledge. Further research should also be done to help clarify or explain the differential risk identified among different race groups. Hopefully continued research, both in the SUN study and other HIV/AIDS cohorts around the US, can help to address these concerns in the future.

Strengths of study include a high sample size, a diverse study population, and highly systematic data collection methods. The SUN study allowed for a cohort of over 600 subjects, including individuals of varied demographic backgrounds for comparison. This allowed for the concurrent robust analysis of multiple risk factors.

Weaknesses of this study include a lack of temporal data and little information concerning behavioral and environmental variables which are known to impact body fat distribution. The analysis of data as a point-prevalence study limits interpretations of causality, as it us unknown whether exposure preceded disease.

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