BODY HABITUS CHANGES, METABOLIC ABNORMALITIES, AND SUBCLINICAL CORONARY ATHEROSCLEROSIS ASSOCIATED WITH LONG-TERM ANTIRETROVIRAL THERAPY

by

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Submitted to the Graduate Faculty of

the Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Public Health

University of Pittsburgh

2008

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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The public health significance of this work reflects the HIV/AIDS epidemic and the growing concerns of long-term consequences of antiretroviral therapy. The advent of highly active antiretroviral therapy (HAART) has greatly improved survival among those with HIV-infection. As a corollary, clinicians and researchers face a range of long-term complications previously of little importance to HIV-infected patients. HIV-associated lipodystrophy syndrome (HIV-LS) was first described in 1998 and involves a constellation of metabolic and morphologic abnormalities. Whereas AIDS wasting syndrome has been associated with immunosuppression and high viral burden, HIV-LS has been documented with immunocompetence and suppressed viral concentration. Participants of the local site of the Multicenter AIDS Cohort Study (MACS) consented to photography of their lipodystrophic body habitus changes during routine clinic appointments. The compilation of these photographs was used to develop a manual for clinicians at all four of the MACS sites in order to accurately document the syndrome, and permitted initial classification of HIV-LS into two phenotypes. A third phenotype was identified following a preliminary observation of lipoaccumulation extending bilaterally and symmetrically from the breasts laterally into

the axilla. Additional cases were subsequently identified within the MACS; all subjects had pre-existing lipoaccumulation of at least one other anatomical site. It was speculated as to whether this represented a previously unrecognized evolution of HIV-LS. Our next project involved studying the health related quality of life (HRQL) of men with HIV-LS. We found HIV-LS does not negatively affect HRQL or exacerbate depressive symptoms above and beyond the diagnosis of HIV-infection. The metabolic abnormalities of HIV-LS include insulin resistance and dyslipidemia, both of which are considered pro-atherogenic risks. The final segment of this project involved detecting coronary artery calcification via electron beam computed tomography among HIV-infected men treated with HAART. In this male population with well controlled HIV-infection, chronic use of HAART did not impact the progression of subclinical coronary atherosclerosis. In contrast, traditional atherosclerosis risk factors of smoking and advancing age were predictive of coronary atherosclerosis. HIV-infection requires life-long combination treatment. Clinicians, researchers and patients recognize dyslipidemia, peripheral lipoatrophy and central lipohypertrophy as significant consequences of this combination therapy, and hope that concerns regarding increasing cardiac risk are not warranted.

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1.0 BACKGROUND AND LITERATURE REVIEW

Prior to the advent of highly active antiretroviral therapy (HAART), increased fasting triglycerides (1) and decreased serum cholesterol levels (2) were among the first metabolic abnormalities associated with immunodeficiency virus (HIV) infection. (1-5) A range of metabolic abnormalities are found among patients with acquired immunodeficiency syndrome (AIDS), including lower high-density lipoproteins (HDL) levels and a greater proportion of low density lipoprotein (LDL)-B phenotype, which is strongly associated with insulin resistance. (6) In aggregate, hyperlipidemia, insulin resistance, fasting hyperglycemia, central obesity, and peripheral lipoatrophy comprises HIV lipodystrophy syndrome (HIV-LS). This syndrome was first described in 1998 in response to self-reports by HIV-infected individuals. (3) The reported prevalence of HIV-LS varies widely among cross-sectional studies, reflecting the absence of a standardized case definition. (3,7) Techniques used to establish the diagnosis range from self-report only to those which include some form of imaging, such as magnetic resonance imaging (MRI), computed tomography (CT) or dual energy X-ray absorptiometry (DEXA), though the use of these imaging approaches have not been supported or justified by existing data. Most often, the diagnosis relies on a combination of self-report and clinician assessment, and is quantified by the degree of change in the distribution of body fat. Whereas AIDS wasting syndrome has been characterized by muscle mass loss, associated with immunosuppression and high viral burden, HIV-LS has been documented with immunocompetence, suppressed viral load (3) and a disproportionate loss of adipose tissue. (3,7)

Initially, protease inhibitor (PI) use was implicated as the cause of HIV-LS; however, more extensive research and follow-up efforts have documented such findings in patients with HIV unexposed to PI. (7) These metabolic abnormalities, however, can be exacerbated by additional metabolic derangements associated with commonly used highly active antiretroviral therapies (HAART) regimens. HAART regimens, particularly their PI components, have revolutionized the treatment of HIV infection through potent viral suppression and have led to a significant reduction in new AIDS cases and deaths. These therapies, however, have been associated with elevations in serum triglycerides, insulin resistance, fasting hyperglycemia, and lipodystrophy with central adiposity. (3,7) HAART-associated hyperglycemia and lipid abnormalities may emerge rapidly after drug therapy begins and may be quite extreme, with hypertriglyceridemia exceeding 1000 mg/dL at times. (4) Hewitt and colleagues reported increases in serum triglycerides from a median of 298 mg/dL to 1803 mg/dL after a mean treatment period of 223 days among patients on a PI-containing regimen. (8) Carr, et al reported lipid abnormalities among 74% of patients taking PI for a median of 21 months. (3) Additionally, these effects are not limited to adults; dyslipidemia has been reported among children taking HAART even among those without a history of familial hypercholesterolemia. (9) While HIV-LS is intriguing to clinicians and researchers, it is disturbing to those who experience it. Individuals are often concerned that abnormal body habitus changes will make their HIV-infection obvious to others, and they may alter activities of daily living in order to conceal these changes. Such activities may negatively impact their quality of life and adherence to their antiretroviral medications.

The advent of HAART has greatly improved survival among those with HIV, meaning clinicians and patients now face a range of long-term complications previously of little importance to HIV patients. During the era of untreated HIV-infection, morbidities and mortalities resulted primarily from infection and malignancy. Now, the long-term consequences of HIV-infection and the side effects of potent therapies, including metabolic and body habitus changes pose different concerns. (3,7,10,11) Specifically, as those with HIV-infection age, they are subject to the same complications of increased serum triglycerides and insulin resistance as their non-HIV infected peers, including coronary artery disease, myocardial infarction, peripheral vascular disease, cerebrovascular accidents, pancreatitis and type II diabetes mellitus. (10,11) Several cases of premature atherosclerosis and cardiac events have been described among patients treated for HIV-infections among patients with and without traditional risk factors. (12) Thus, the relationship between metabolic toxicities, HAART therapy and increased cardiovascular risk has become an area of intense research, especially as increasing numbers of patients face decades of antiretroviral therapy.

1.1 TREATMENT OF HIV INFECTION

Since the development of zidovudine (ZDV) in 1987 and the subsequent approval of other nucleoside reverse transcriptase inhibitors (NRTIs), the treatment for HIV infection has evolved dramatically. NRTIs effectively suppress viral replication by incorporating into the viral DNA as it enters the human cell nucleus. ZVD monotherapy was used for several years until viral resistance rendered it ineffective. Subsequently, combination regimens including NRTI components dominated treatment until the mid-1990s when PIs were introduced.

PIs inhibit viral replication by preventing reassembly of viral proteins and release from infected human CD4 cells. With the introduction of PIs, the standard of HIV care incorporated both NRTIs and PIs. The addition of non-nucleoside reverse transcriptase inhibitors (NNRTIs) to the anti-HIV arsenal in 1998 further broadened clinicians' options. NNRTIs bind directly to reverse transcriptase thereby preventing the conversion of viral RNA to viral DNA.

Enfuvirtide (Fuzeon®), a fusion inhibitor is one of the newest HIV treatments, approved for experimental use in the United States March 13, 2003. Enfuvirtide has a unique mechanism of action which blocks viral particles from adhering to, and thus prevents entry into, human CD4+ cells. The latest research and development has focused on inhibiting CCR5 entry and integrase. CCR5 is a receptor on T-lymphocytes that circulating HIV uses to attach to the cell. CCR5 entry inhibitors alter the shape of the CCR5 which prevents HIV from successfully adhering to the cell. Integrase inhibitors interfere with the enzyme necessary to permit HIV from integrating its genetic material into human DNA for replication.

The current standard of care is to prescribe potent regimens consisting of at least two NRTIs, *and* at least one PI or at least one NNRTI; or triple nucleoside therapy in the case of prior treatment failure. Since these classes of medications are effective in different stages of viral replication, using them in combination most efficiently reduces viral burden. HAART is not curative, however, and HIV-infected patients can expect to take some combination of antiretroviral medications for the rest of their lives. When taken as prescribed, these potent regimens reduce viral load to undetectable levels in the blood in most patients. While HAART has dramatically decreased the rates of opportunistic infections, delayed the diagnosis of AIDS, and improved life expectancy, there are serious side effects which may alter patients' adherence rates. These side effects include gastrointestinal upset, hyperglycemia, nephrolithiasis,

pancreatitis, accumulation and redistribution of body fat and severe lipid abnormalities (1,3-5,8,11) and their degree and impact can vary significantly among individuals. For some, treatment effects can negatively impact quality of life. Reflecting the growing awareness of medication-related toxicities, adverse reactions and complications, many researchers and clinicians are advocating postponing treatment. In the past, guidelines recommended pharmacologic therapy for patients with CD4 counts below 500 cells/mm^3 and a viral load above 20,000 copies/ml. (13) The most recent Adult and Adolescent HIV treatment guidelines were released January 29, 2008 and recommend delaying therapy in asymptomatic patients until the CD4 count is below 350 cells/mm^3. (14)

1.2 HIV-ASSOCIATED LIPODYSTROPHY SYNDROME

HIV-LS involves a constellation of metabolic abnormalities and body habitus changes including hyperlipidemia, insulin resistance, hyperglycemia, central adiposity and peripheral lipoatrophy. (3,7,15) Neither strict diagnostic criteria nor case definition currently exist. In 2003, Carr and colleagues proposed a detailed HIV-LS case definition however, as it required medical imaging, many clinicians and researchers considered it cost prohibitive. (7) Currently, documentation of the syndrome relies on subjective measures such as patient self-report and/or clinician report.

1.2.1 Glucose homeostasis

Normal glucose homeostasis is an intricate process that requires both adequate pancreatic beta cell production of the hormone, insulin, and the appropriate binding of this hormone to cell surface insulin receptors. The pairing of the hormone with its receptor facilitates the entry of glucose into cells. Abnormalities of any part of the process can lead to glucose intolerance or

frank diabetes. Diabetes mellitus type I results from absolute pancreatic beta cell failure and a complete absence of insulin. In contrast, those with diabetes mellitus type II have a relative insulin deficiency resulting in hyperglycemia or insulin receptor abnormalities which lead to resistance to the actions of insulin even in the presence of normal or high levels of the hormone.

The American Diabetes Association defines diabetes mellitus (DM) as fasting blood glucose greater than 126 mg/dL or a post-prandial glucose exceeding 200 mg/dL. In the absence of true DM, two other related pathologies can exist: impaired glucose tolerance, defined as postprandial hyperglycemia associated with normal fasting glucose levels, and insulin resistance, characterized by impaired insulin utilization by peripheral tissues. In the presence of insulin resistance, high concentrations of insulin are required to drive glucose into muscle and fat cells.

Insulin resistance was first described in the early 1940s. At the time, endogenous insulin levels could not be measured; however, Himsworth noted that in some diabetic patients the administration of exogenous insulin did not cure their diabetes and he postulated other mechanisms were responsible for their hyperglycemia. (16) Decades later, Yallow and colleagues reported elevated insulin levels among patients with type II DM. (17) Insulin resistance has been identified as a risk factor for coronary artery disease, (18) a relationship likely mediated through associated endothelial dysfunction and the prothrombotic, proinflammatory effects of vascular injury. (19,20)

Disorders of glucose homesostasis, including impaired glucose tolerance, insulin resistance and diabetes mellitus, are more prevalent among patients taking HAART than among the general public. Conservative estimates of insulin resistance in HIV-infected individuals range from 1%-6%, (21) and impaired glucose tolerance likely affects an even greater percentage. The findings appear attributable, in some measure to HIV itself and some to its treatment. Noor and

colleagues described new-onset insulin resistance among a cohort of HIV-negative subjects who were given protease inhibitors for as little as 4 weeks. (22,23) Data by Dube et al also support a treatment-related effect; in their work, withdrawing protease inhibitors reversed some hyperglycemia associated with medication use. (24)

In 2004, a large prospective study was conducted within the Multicenter AIDS Cohort Study (MACS) to ascertain the prevalence and incidence of diabetes mellitus among HIV-infected men. (25) DM was defined as a fasting glucose measurement of at least 126mg/dL, a self-reported history of DM or use of diabetes medication. After adjusting for age and body mass index, the authors found a DM prevalence of 14% prevalence among HIV-infected men treated with HAART compared to a 5% among seronegative men (prevalence ratio 4.6; 95% CI, 1.85-9.16). (25) After a mean follow-up time of 2.4 years, 5% (38/680) of those men initially without DM met the diagnostic criteria. Twenty-four of these new cases (63%) were among HIV-infected men treated with HAART while the remaining four cases were among HIV-infected patients treated with non-HAART regimens. These results supported previous findings of an increased prevalence of DM among HIV-infected individuals undergoing treatment and underscored the particular association of DM with HAART therapy as these patients had DM rates four times higher than seronegative male counterparts. (25)

1.2.2 Dyslipidemia and HAART

Elevated serum cholesterol, LDL and triglycerides, and low levels of HDL are independent risk factors for coronary artery disease. (25 -28) The Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) identified high and optimal serum lipids

relative to coronary artery disease risk for patients with and without additional risk factors. (28) (Table 1)

Table 1. Serum lipid concentrations relative to coronary artery disease risk

Serum Values	Stratification of high serum concentration	Optimum concentration
Total Cholesterol	$\geq$ 240 mg/dL	< 200 mg/dL
LDL Cholesterol **goals are based on risk factors, especially existing coronary artery disease*	$\geq$ 160 mg/dL * without coronary artery disease and < 2 risk factors	< 100 mg / dL *with history of coronary artery disease
Triglycerides	> 400 mg/dL	< 200 mg/dL

Antiretroviral therapy negatively impacts both lipid transport and metabolism. (21) The exact mechanism of antiretroviral induced hyperlipidemia is beyond the scope of this work, but in summary, involves increased production of very low density lipoproteins (VLDL), excessive free fatty acid mobilization and impaired lipoprotein clearance. (29) These abnormalities most likely result from concomitant insulin resistance that prevents efficient fat cell storage of triglycerides. These triglycerides circulate, manifesting as elevated free fatty acid concentrations that facilitate hepatic secretion of VLDL.

Antiretroviral associated dyslipidemia has been reported to occur within weeks of initiation. (1) Carr and colleagues found a 74% prevalence of lipid abnormalities in patients taking protease inhibitors for a median of 21 months. (3) Protease inhibitors have even caused elevations in lipids in HIV-infected children without familial hypercholesterolemia. (9) Both HIV and the antiretroviral therapies have been suspected in causing the hyperlipidemia, although

no exact mechanism has been proven. (2,5) There are conflicting results with regard to the incidence and severity of antiretroviral therapy induced dyslipidemia.

Protease inhibitors were the first components of HAART to be implicated in treatment-induced hyperlipidemia. Initial prevalence reports varied, though, from 8% to 66%. (30) Hewitt documented a 6-fold increase in serum triglycerides (mean 298 – 1803 mg/dL) among patients who had received a protease inhibitor regimen for a median of 223 days. (8) In that study, triglycerides were lowered to a median level of 300 mg/dL after three months of treatment with gemfibrozil. (8)

One retrospective study has been performed comparing pre-seroconversion lipid profiles to those after approximately three years of treatment with HAART (N=50 men). (1) Authors described a mean decline of -30 mg/dL of cholesterol, and a mean LDL-C decline of -22 mg/dL after HIV-infection. Following treatment with HAART, the triglycerides and total cholesterol levels increased. It was suggested that the post-treatment increases in triglycerides and cholesterol represented a re-approximation to baseline levels. These findings support the concept of increasing age causing the increase in serum lipids rather than treatment with HAART. (1) The magnitude of antiretroviral mediated dyslipidemia appears to vary by drug. For example, the protease inhibitor ritonavir, and the NRTI stavudine (d4T, zerit) have been associated with extreme dyslipidemia. (2,5) Although all protease inhibitors have been associated with some degree of dyslipidemia, ritonavir is the one most often implicated in severe abnormalities. (5) With longer periods of observation, nucleoside reverse transcriptase inhibitors are also now known to be involved.

Some researchers have investigated the putative role of protease inhibitors in HIV-related hyperlipidemia by substituting different medications and observing changes in serum

lipids. Results have been inconsistent. (Table 2) Periard reported improvement in serum cholesterol and triglycerides in seven patients 29-63 days after substituting nelfinavir or indinvavir for ritonavir. (31) In a larger study among 40 patients, Tebas described a 31% decrease in triglycerides (p=0.005) and an 11% decrease in cholesterol (NS) during a 24 week follow up period when subjects' protease inhibitors were replaced with nevirapine. (32) Among twenty patients with undetectable viral load and HIV-LS syndrome, the investigators from the Scandinavian Simvastatin Survival Study (4S) reported a 31% triglycerides reduction (p=0.03) within 6 months following substitution of efavirenz for the patients' protease inhibitors. (33) Total cholesterol did not change. In the Tabas trial, one patient whose viral load was initially undetectable developed detectable virus after protease inhibitor discontinuation. (32) Upon protease inhibitor re-initiation, the viral load again became undetectable. (32) In the 4S trial, all study participants had undetectable viral loads at baseline and one developed detectable virus within the 6 month follow-up period. (33)

Some researchers have found no lipid improvement when PI were excluded from HIV regimens. Gharakhanian reported that among thirty-one subjects who substituted efavirenz for their PI, no change in serum lipids emerged after 10 months of observation. (34) In a study by Vicinana and colleagues, thirty-nine patients received efavirenz in place of their protease inhibitor. (35) During follow-up time, total cholesterol, LDL and triglycerides actually increased slightly. (35)

Barriero completed the largest study; 138 subjects were randomized to either continue PI or replace a PI with nevirapine. (36) Both groups had similar lipid profiles at baseline. In the PI group, mean triglycerides were 284 (±346) mg /dL and mean cholesterol was 249 (±77) mg/dL. The nevarapine-treated group had mean triglycerides of 292 (±352) mg/dL and mean cholesterol

of 242 (± 102) mg/dL. After 6 months, no changes in cholesterol, triglycerides or viral suppression developed. (36)

Table 2. Changes in antiretroviral regimens and effect on serum lipids

Researcher / Study	N	Medication change	Outcome
Periard 1999	7	Rit → Nelfinavir or Indinavir	↓ cholesterol and triglycerides after 29-63 days
Tebas 2000	40	PI → Nevirapine	↓ cholesterol by 11% (NS) and ↓ triglycerides by 31% (p=0.005) after 24 weeks
Scandinavian (4S) 1994	20	PI→ efavirenz	↓ triglycerides 31% (p=0.03) and no change in cholesterol
Viciana 2000	39	PI→ efavirenz	Slight ↑ in cholesterol, LDL, triglycerides and HDL
Gharakhanian 2000	31	PI→ efavirenz	No change in lipids after 10 months
Barreiro 2000	138	Randomized to continue PI or → Nevirapine	No change in lipids after 6 months

1.2.3 Pharmacologic treatment for dyslipidemia

In response to the prevalence of antiretroviral associated dyslipidemia, most clinicians now routinely monitor fasting triglycerides and cholesterol in 3-4 month intervals in patients who are infected with HIV and treated with antiretroviral therapy. Those HIV-positive patients with triglycerides in the range of 500-1000 mg/dL, LDL exceeding 160 mg/ dL, and those with LDL exceeding 130 mg/dL in the presence of coronary artery disease, often require more frequent assessments.

Among patients taking HAART, hypertriglyceridemia may be complicated by additional metabolic abnormalities, further complicating the management of HIV-infected individuals.

Goals of lipid lowering treatment are based on the presence of additional cardiac risk factors. Lipid-lowering agents are effective for secondary prevention as well, and decrease the risk for subsequent cardiac events among those with existing CAD by improving endothelial function, decreasing platelet activity and decreasing inflammation.

Although there are some very specific guidelines for treating hyperlipidemia in the general population, (4) the management in HIV-infected individuals is much more complex. Guidelines have been proposed for treatment of antiretroviral- induced hyperlipidemia. (4) An additional challenge in the treatment of those with antiretroviral-associated hyperlipidemia is that most patients exhibit combined lipid abnormalities encompassing both cholesterol and triglyceride abnormalities. (1,3,4) Furthermore, common lifestyle recommendations for cholesterol management such as aerobic exercise and weight reduction may not suit some HIV-infected patients struggling to maintain or regain weight lost due to HIV wasting syndrome. Specific low-fat diets may also conflict with antiretroviral regimens. For example, saquinavir (invirase) should be taken within two hours of a high fat meal. These examples highlight the particular limitations of non-pharmacologic techniques among those infected with HIV. Thus, lipid lowering agents often become the first-line treatment options. These agents must be prescribed with caution; however, as patients frequently take several medications which increases the risk for drug interactions. Additionally, many individuals with HIV-infection have concomitant hepatitis B or hepatitis C virus infection which may be associated with hepatic insufficiency and abnormal clearance of hepatically metabolized medications, such as statins.

Table 3: Effects of lipid-lowering medication and potential adverse effects

Medication Class	Serologic effects	Potential Side Effects
HMGCoA reductase inhibitors (Statins)	↓ LDL ↓TG ↑HDL	Hepatic dysfunction Myopathy
Bile acid sequestrants	↓ LDL ↑HDL	Gastrointestinal upset Drug/drug interactions
Nicotinic acid	↓ LDL ↓TG ↑ HDL	Gastrointestinal upset Hyperglycemia Hyperuricemia Hepatic dysfunction
Fibric Acid	↓ LDL ↓ TG ↑ HDL	Myopathy Dyspepsia Gallstones

The primary goal of lipid lowering therapy is to reduce levels of atherogenic low-density lipoproteins. There are several classes of lipid-lowering medications currently available and include statins, bile acid resins, fibric acid derivatives, nicotinic acid and fibric acid. (Table 3)

The most common class of lipid lowering medications is HMG-CoA reductase inhibitors, or statins. They lower plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and preventing cholesterol synthesis in the liver. They also increase the number of hepatic LDL receptors on the cell-surface which facilitates uptake and catabolism of LDL and reduce LDL production. HMG-CoA reductase inhibitors also decrease the lipid content of atherosclerotic plaques, thereby making them more stable and less prone to rupture. Among the statins, atorvastatin (Lipitor) is most commonly prescribed to HIV-infected individuals because of its potent antihypertriglyceridemic effects. Pravastatin (Pravachol) is also commonly used in the HIV-infected population. Two other statins, simvastatin (Zocor) and lovastatin (Mevacor) are not recommended for use with protease inhibitors as both undergo cytochrome 3A4 metabolism and this shared metabolic pathway raises the likelihood of adverse effects.

Bile acid resins or bile acid sequestrants bind cholesterol-containing bile in the intestines; it is subsequently eliminated in the stool. Resins lower both serum cholesterol and LDL and are commonly used in combination with other lipid-lowering drugs. However, such combinations may raise triglycerides and therefore may not be optimal for use in those with mixed-abnormalities as is commonly found in HIV-infection. (4,5) Resins also require dosing three times daily at least two to three hours before or after other medications, a potentially unrealistic schedule for those already taking a complex HIV treatment regimen. When taken too soon after or before other medications, such as antiretrovirals, bile-acid resins may adversely affect their absorption.

Among the other agents to treat high cholesterol, clinicians generally avoid the use of niacin because of the possibility in exacerbating insulin resistance. (5) Another class of agents, the fibric acid derivatives, effectively lowers serum triglycerides between 30% and 55%; however, they are not used as first-line therapy due to their limited effects on total cholesterol and LDL levels. (5) HIV-infected patients with extreme hypertriglyceridemia frequently benefit from a combination of gemfibrozil and a statin, although such combinations increase the risk of rhabdomyolysis. (5) When lipid lowering therapy is initiated, lipid profiles generally improve within 6 months.

1.2.4 Body habitus changes

HIV lipodystrophy syndrome was first described in 1998 following self-reports among HIV-infected patients. The syndrome involves four important components: hyperlipidemia, fasting hyperglycemia, insulin resistance and body habitus changes. The lipodystrophic body habitus changes fall into two patterns. Phenotype I involves lipoatrophy only while phenotype II involves a combination of lipoatrophy and lipohypertrophy, often referred to as mixed

lipodystrophy. Lipoatrophy presents as loss of subcutaneous fat in the nasolabial folds and temporal regions of the face, extremities and buttocks. Lipohypertrophy presents to varying degrees in both genders as increased fats stores in the breasts, abdominal visceral compartment and the dorsocervical fat pad. (37) The latter is not the result of hypercortisolism. (37) Other areas may undergo lipohypertrophy as reports of occipital fat and circumferential neck accumulation, so called "Magedelen's collar" or "horse collar" have emerged.

Changes in visceral fat are generally assessed subjectively by clinician or patient report complemented by quantification using anthropometric measures. Attempts to measure visceral fat more precisely have included MRI, DEXA, and CT imaging. (7) These approaches provide more via continuous, rather than the dichotomous data which notes only the absence or presences of lipodystrophy. The costs for imaging modalities, however, are prohibitive for routine clinical use.

The reported prevalence HIV-LS has varied widely, and initially, protease inhibitors were hypothesized to be the primary cause. Particularly when severe, HIV-LS can be distressing enough patients may request a change in antiretroviral regimens even if such regimens have been successful in viral suppression. In response, new antiretroviral regimens have been developed which do not include this class of medications. Additional potential advantages of these protease inhibitor-sparing combinations may include fewer drug interactions, fewer pills which may enhance compliance, and fewer side effects, including lipodystrophy. It is not known, however, whether such protease-sparing regimens will suppress viral replication as well. Furthermore, as data have accrued, similar findings have been documented in HIV-infected patients not receiving protease inhibitors and among non-HIV infected patients.

2.0 SPECIFIC AIMS

The primary purpose of this work was to document long-term effects of HAART on body habitus and to determine the risk of subclinical coronary atherosclerosis among a cohort of HIV-infected men treated with HAART.

Specific Aim #1 – Photograph and characterize patterns of body habitus changes consistent with HIV-associated lipodystrophy syndrome (HIV-LS) in HIV-infected men treated with HAART, and develop a manual for clinicians to use in the Multicenter AIDS Cohort Study (MACS) to accurately and consistently document HIV-LS.

Specific Aim #2 – Describe a third phenotype of HIV-LS among subjects within the MACS.

Specific Aim #3 - Describe quality of life patterns among HIV-infected men with body habitus changes and HIV-LS.

Specific Aim #4 – Document prevalence of coronary artery calcification via electron beam computed tomography among a cohort of 80 HIV-infected men taking HAART, and document risk factors for progression of coronary artery calcification via electron beam computed tomography.

3.0 PREFACE TO FIRST PUBLICATION

Body habitus changes can be difficult to appreciate due to the natural variation of body shape, contour and size. Some people many appear to have "mild lipodystrophy" as a consequence of weight gain or aging. While experienced clinicians are able to distinguish true, even subtle HIV-associated lipodystrophic changes from age or obesity-related phenomena, such distinctions may be challenging, particularly in settings staffed by significant numbers of less experienced rotating residents and fellows, such as found at several MACS sites. Within the MACS, HIV-LS-associated body habitus changes have been assessed using self-report, clinician report, and anthropometric measurements obtained at the iliac crest, across the buttocks, at the midpoint of the arm and the midpoint of the thigh (estimated to the closest centimeter for the waist and hips and the closest 0.1 cm for the extremities). Skin-fold measurements were initially used but were discontinued after such data were deemed unreliable.

MACS investigators determined that a comprehensive manual providing technique to facilitate a thorough examination and providing illustrations of the potential range of HIV-LS severity would improve the quality and reliability of HIV-LS assessments. Thus, the manual, "Documenting HIV-associated Lipodystrophy Syndrome (HIV-LS) within the Multicenter AIDS Cohort Study," was created to establish consistent assessment of HIV-LS among all four of the MACS sites.

Clinicians within the MACS assessed each subject during every clinic visit for the presence and severity of HIV-LS using physical examination and study participant report.

Clinicians initiated participant self-report by asking about changes in the distribution or amount of body fat (either loss or gain) in the previous 6 months. If such changes were acknowledged, participants were then asked to identify affected areas (face, arms, legs, buttocks or dorsocervical region), and to describe the severity of changes in each. Subjects were also asked to describe increases, if any, in neck or waist circumference, using increments of less than 1 inch, between 1and 2 inches or exceeding 2 inches.

Using prospectively collected data, HIV-LS cases within MACS were characterized as "stable," defined as those whose phenotype has remained consistent over time, "developmental," defined as those unaffected or only mildly affected at baseline who are subsequently found to have developed lipodystrophic changes, or "transitional," defined as those with evidence of one lipodystrophic phenotype at baseline and a different phenotype at subsequent follow-up.

These descriptions were accompanied with photographs which were found to be vital to both identifying and monitoring HIV-LS onset and progression. Of note, many study participants were grateful that photographic evidence validated the changes they described.

The severity of HIV-LS was quantified as "mild," "moderate" or "severe" defined as follows. "Mild" defined changes evident to the clinician with close inspection; "moderate" defined changes evident without specific inspection and "severe" reflected changes evident to non-medical person by casual observation. In addition, objective data including standardized anthropometric measurements of height, weight, and arm, thigh, waist and hip circumferences and laboratory testing, including lipid profiles, glucose, insulin and glycosolated hemoglobin, were obtained.

Subjects in the MACS who were identified as having lipodystrophy were aware of our attempt to identify risk factors and granted permission for photography of HIV-LS affected body

areas. Photographs were taken exclusively by a Pitt Men's Study clinician for use exclusively in education and research.

3.1 SPECIFIC AIM #1

Specific aim #1, photograph and characterize patterns of body habitus changes consistent with HIV-LS, is addressed in an internal publication within the MACS, and represents the most comprehensive photographic compilation of HIV-associated lipodystrophy in the country. This 45 page manual , "Documenting HIV-associated Lipodystrophy Syndrome (HIV-LS) within the Multicenter AIDS Cohort Study" provides information on performing the clinical assessment for HIV-LS and includes photographic examples of mild, moderate and severe physical findings. This manual was circulated to all four of the MACS sites during the summer of 2007. (Appendix 1) The manual was reviewed favorably by clinicians and was reformatted and condensed into a pocket version in the summer of 2008. (Appendix 2)

4.0 PREFACE TO SECOND PUBLICATION

As previously described, the MACS assessment for HIV-LS involved inspection, anthropometric measurements, subcutaneous fat caliper measurements and photography. Initially, MACS researchers proposed 2 phenotypes of HIV-LS: phenotype I with isolated lipoatrophy and phenotype II which involved concurrent lipoatrophy and lipohypertrophy. (15) During a clinical encounter with a seropositive MACS patient, however, a distinct and unique pattern of lipoaccumulation was noted. This pattern was characterized by supradiaphramatic fat which extended laterally from the breasts in the absence obvious breast masses or fluid accumulation. This band-like accumulation of fat was non-tender and without overlying skin changes. No aspects of the patient's medical history accounted for these changes. In consultation with clinicians from the other MACS sites, these photographs were distributed to ascertain prevalence of this abnormal fat distribution. Once the photographs were viewed, additional cases were identified in Chicago, Baltimore and Los Angeles. It appeared that a new pattern of lipodystrophy notable for simultaneous fat accumulation and lipoatrophy had been recognized.

4.1 SPECIFIC AIM #3

Specific aim #2, to describe a third phenotype of HIV-LS, is addressed in a manuscript originally published in T*he Journal of the American Medical Association*. Permission for reprint in this doctoral dissertation was granted March 13, 2008.

4.2 A NOVEL PATTERN OF LIPOACCUMULATION IN HIV-INFECTED MEN

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JAMA 2006 296(7):766-68.

4.2.1 Introduction

Body habitus changes among persons infected with human immunodeficiency virus (HIV) who receive highly active antiretroviral therapy (HAART) are major concerns, in part because of their frequent association with insulin resistance and hyperlipidemia. The desire to avoid such changes

has influenced selection of antiretrovirals and timing of HAART initiation. Lipoatrophy is of particular cosmetic concern to affected individuals. It is most commonly manifested as limb and gluteal fat loss with consequent apparent thinning of the extremities and facial fat loss with a "sunken cheek" appearance. (7,38) Lipoaccumulation most often appears as visceral abdominal adiposity with increased abdominal girth. Other manifestations include dorsocervical fat pads ("buffalo humps") and neck lipoaccumulation ("bull neck").

We report what we believe to be a novel manifestation of lipoaccumulation in 8 men with HIV infection.

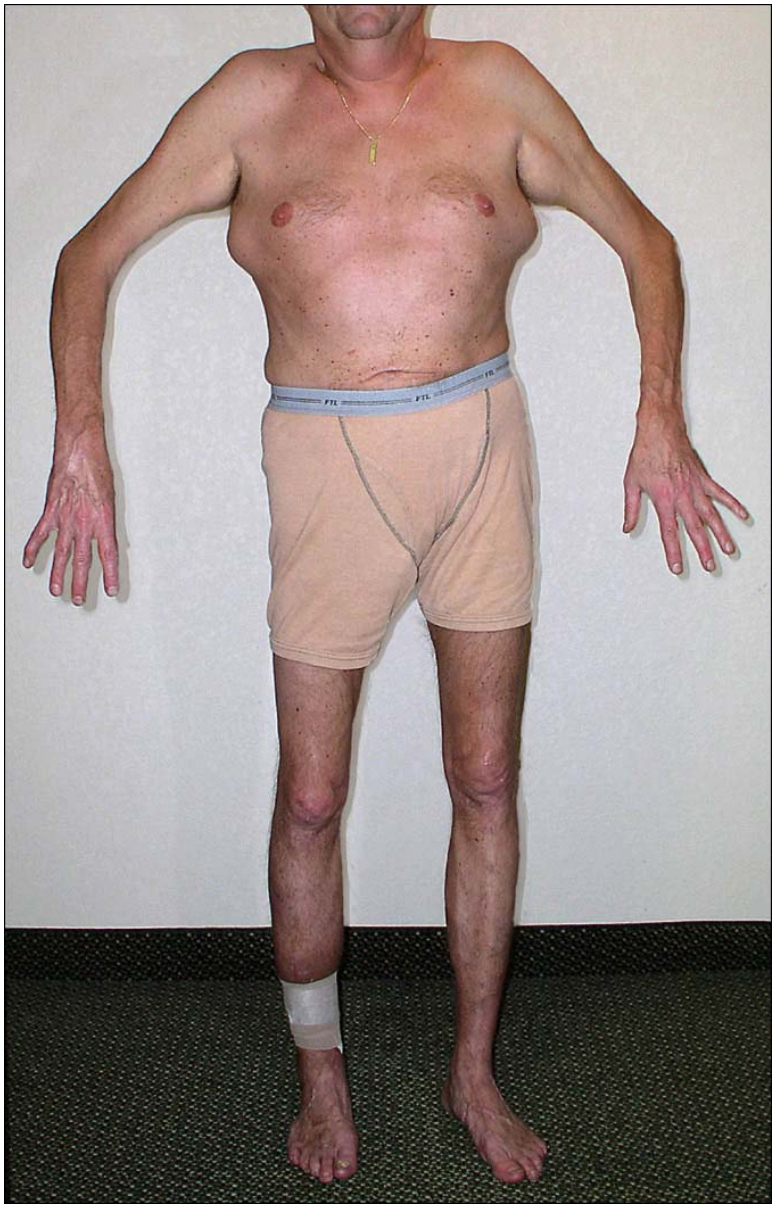

Figure 1. Example of a novel type of lipoaccumulation in men infected with Human Immunodeficiency Virus

4.2.2 Methods

All individuals are HIV-infected participants in the Multicenter AIDS Cohort Study (MACS), a longitudinal study of men who have sex with men. The original cohort included 5622 men. Since 1984, MACS participants have undergone clinical evaluation twice yearly. Since 1999, anthropometrics (height; weight; and circumference of chest, waist, hip, thigh, and mid proximal arm) and examiner- and self-reported body habitus changes have been recorded, as have diagnoses of hyperlipidemia, hyperglycemia, diabetes mellitus, and hypertension. Blood measurements include serum lipid levels, glucose levels, and CD4 cell count determinations. Patients self-designate race. Institutional review board approval was obtained for MACS and all participants gave written informed consent.

4.2.3 Results

An initial observation of a novel type of lipoaccumulation was made by a MACS clinician in Pittsburgh, PA. Seven other cases were subsequently identified study-wide. Mean age was 44.6 year (range, 39-52 years). Six patients were white and 2 were Asian/Pacific Islander.

All patients had bandlike accumulations of lipomatous-appearing tissue extending bilaterally and symmetrically from the breasts laterally into the axillae. (FIGURE 1) In some patients, this fatty tissue extended further posteriorly, with a near circumferential distribution. None of these persons had notable soft tissue nodularity, tenderness, evidence of mastitis or other acute inflammation or infection, or the presence of new or enlarged axillary lymphadenopathy. None had hypogonadism. All had preexisting lipoaccumulation (evidenced in at least 1 other bodily area [eg abdomen, dorsocervical area]) before the unique pattern of lipoaccumulation was noted but not before HAART was initiated; 6 had limb and facial lipoatrophy. (TABLE 4) Body

mass index measurements were not indicative of obesity. Four of the 8 participants reported a history of hyperlipidemia; none reported a history of hyperglycemia or diabetes mellitus.

One participant had a history of an AIDS-defining illness; 3 had a nadir $CD4^+$ cell count of less than 200/μL. (TABLE 5) Mean number of years of antiretroviral therapy received at the time of identifying the lipoaccumulation was 7.8 (range, 4.5-13 years). (TABLE 6) Six patients were receiving HAART at the time of identification, 4 with regimens including protease inhibitors. All participants had elevation in at least 1 measurement of serum low-density lipoprotein cholesterol, triglycerides, or total cholesterol during at least one visit.

Table 4. Patient clinical characteristics

Patient	Years ^	Baseline BMI*	Most recent BMI*	Lipoatrophy	Abd girth	Dorsocervical fat pad	Breast enlargement	Chest circumference (cm)	Arm Circumference (cm)
1	11.3	23.6	23.4	++	+	+	+	NA	NA
2	0.0	27.1	27.1	++	_	+	+	103.0	28.0
3	16.7	21.7	23.2	++	+	+	+	101.0	28.5
4	13.7	27.0	31.2	++	_	+	_	123.0	38.5
5	17.1	20.4	25.2	++	+	+	_	115.0	35.5
6	16.6	21.9	27.1	_	+	_	_	104.0	35.0
7	16.3	21.9	24.3	++	+	+	+	108.0	32.5
8	16.2	27.7	35.1	_	+	+	_	120.0	43.0
Mean	13.5	23.9	27.1					110.6	34.4

^ since baseline

* BMI , body mass index, calculated as weight in kilograms divided by the height in meters^2

Table 5. Patient laboratory measurements

Patient	Cholesterol (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	Triglycerides (mg/dL)	Nadir CD4+ (cells/mm3)	Recent CD4+ (cells/mm3)
1	239	122	53	NA	400	590
2	226	132	57	189	461	461
3	196	97	72	546	130	436
4	158	74	36	240	452	856
5	166	117	23	146	501	815
6	229	132	44	118	19	782
7	210	111	49	252	108	436
8	182	105	41	413	298	1364
Mean	201	111	47	272	296	718

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 6. Patient antiretroviral therapy duration

	Total duration of antiretroviral therapy in years							
	Duration of protease inhibitor therapy in years							
Patient	Any ART	Indinavir	Ritonavir	Saquinavir	Nelfinavir	Total	Stavudine	Zidovudine
1	11.0	0	0	^	0	^	9.0	1.0
2	7.5	0	0	0	**	**	5.5	**
3	10.5	0	0	4.5	0	4.5	0	10
4	5.0	0.5	0	0	0	0.5	5.0	0.5
5	4.5	0	0	0	3.0	3.0	3.0	1.5
6	5.0	5.0	0	0	0	5.0	0	0
7	5.5	2.5	3.0	2.5	0	5.0	3.0	2.5
8	13.0	0	0	1.5	2.5	5.5	1.5	9.0
Mean	7.8							

Abbreviations: ART, antiretroviral therapy;

^ There were temporal gaps in this patient's study visits between 1995 and 2001, so the exact duration of his protease inhibitor-based therapy is unknown.

**Most medication exposure was prior to study enrollment, so duration of receipt of some antiretroviral medication is not specified in the research records.

4.2.4 Comment

We believe that these patients demonstrate a previously unreported manifestation of lipoaccumulation. We were not able to identify the specific cause. Lipoatrophy has been associated with stavudine or zidovudine use, lipoaccumulation and insulin resistance with

protease inhibitor use, and hyperlipidemia with any of these therapies. (39-41) Other possible risk factors are HIV disease stage, age, race, and sex. (42) Since all 8 men had preexisting lipoaccumulation of some kind, this type of lipoaccumulation may represent a progression for some of the individuals. None were obese. Treatment-related etiologic factors were not readily apparent, but all had received protease inhibitor-containing HAART.

Our database has limitations on potentially relevant clinical information. Because we have not yet been able to undertake systematic review of all MACS participants for the presence of lipoaccumulation, a prevalence estimate cannot yet be reliably ascertained. There is not a single objective measure that can be systematically applied to all MACS participants specifically to discern this phenomenon. In addition, retrospective analyses do not allow for precise measurement of time of onset. Comparative evaluation and prospective observation are needed to further characterize this clinical entity.

4.2.5 Acknowledgement of funding / support

This work was supported by the National Institute of Allergy and Infectious Diseases, with additional supplemental funding from the National Cancer Institute (grants U01-A1-35042, 5-M01-RR-00052 [GCRC], U01-A1-35043, U01-A1-37984, U01-A1-35039, U01-35040, U01-A1-37613, and U01-A1-35041.

5.0 PREFACE TO THIRD PUBLICATION

The development of HIV-LS can be disturbing to patients who fear that their HIV-infection may become obvious to others. Clinicians within the MACS are aware of anecdotal examples of HIV-LS negatively impacting quality of life. Several subjects have reported changes in social behaviors as the result of their body habitus changes, including avoidance of swimming and sunbathing in public, wearing shorts and sleeveless shirts, and being uncomfortable in many everyday situations.

5.1 SPECIFIC AIM #3

Specific aim #3, describe quality of life patterns among HIV-infected men with HIV-LS, is addressed in a manuscript originally published in *AIDS Patient Care & STDs*. Reprint permission was granted for inclusion in this doctoral dissertation March 3, 2008.

5.2 EFFECTS OF LIPODYSTROPHY ON QUALITY OF LIFE AND DEPRESSION IN HIV-INFECTED MEN ON HAART

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AIDS PATIENT CARE and STDs 2006;20(8):35-45.

5.2.1 Abstract

The aim of the study was a prospective assessment of the possible consequences of a diagnosis of lipodystrophy on health-related quality of life (HRQL) and depressive symptomatology in HIV-seropositive men who have sex with men. A standardized physical assessment for lipodystrophy was introduced within a prospective study in April 1999. Over a 2-year follow-up, 37 HIV-seropositive men who met the criteria for lipodystrophy were longitudinally compared to 92 HIV-seropositive men without lipodystrophy and 88 HIV-seronegative men on measures of HRQL and depression. A series of questionnaires, which included the Medical Outcomes Study Short-Form 36 (SF-36) and the Center for Epidemiological Studies-Depression (CES-D), were administered to assess HRQL and depression, respectively. SF-36 scores were summarized using the mental and physical components; CES-D results were reported as both dichotomous (with or with clinical depression) and continuous scores. Neither the mental nor physical components of the SF-36 showed any significant differences between patients with lipodystrophy versus HIV-seropositive patients without lipodystrophy. Similarly, lipodystrophy status was not significantly associated with either continuous depression scores or presence of clinical depression. However,

consistent with previous results, HIV-seropositive men without lipodystrophy (compared to HIV-seronegative men) reported higher scores on both components of the SF-36 scales and both categorizations of the CES-D. The results of this study suggest that lipodystrophy does not negatively affect HRQL or depression, above and beyond, the diagnosis of HIV infection, although the impact of the severity of lipodystrophy on these conditions will require further study.

5.2.2 Introduction

Since the advent of highly active antiretroviral therapy (HAART), new challenges have been encountered by persons infected with HIV and clinicians treating those with HIV infection. Lipodystrophy is one of the most profound side effects of antiretroviral therapy and/or long-term infection with HIV. Lipodystrophy is characterized as a heterogeneous array of metabolic and morphologic changes including central adiposity (truncal fact accumulation), peripheral lipoatrophy (loss of subcutaneous fat on the arms and legs), facial lipoatrophy (loss of fat from the face), and accumulation of fat on the back of the neck. Metabolic alterations such as hyperinsulinemia and hyperlipidemia are often observed in combination with these morphologic changes. (43). Although still not known, the metabolic changes are suspected to increase the risk for diabetes and coronary heart disease in persons with lipodystrophy. (44, 45) Lenert and colleagues, suggested that the diagnosis of lipodystrophy was associated with 1% greater risk of death (46).

The prevalence of lipodystrophy varies according to the individual rating the bodily changes (patient versus physician), demographic variables such as age and gender, and methods of measurement (47). The methods of measuring morphologic changes associated with lipodystrophy are not universal and the syndrome appears to be heterogeneous and there

continues to be no widely accepted definition. Kingsley has described two separate phenotypes associated with lipodystrophy; the first is characterized by moderate to severe subcutaneous fat loss in two or more areas (i.e., arms, legs, buttocks or face) and termed peripheral lipoatrophy. (15) The second phenotype, mixed lipodystrophy, includes moderate to severe peripheral lipoatrophy (as described above) in addition to moderate to severe fat accumulation in the abdomen or breasts, with or without additional fat at the back of the neck. The mixed lipodystrophy group is therefore a subset of those men with peripheral lipoatrophy. For simplicity, men in either group (i.e., having peripheral lipoatrophy with or without mixed lipodystrophy) will be subsequently referred to as having lipodystrophy syndrome or HIV-LS.

Tien and colleagues reported that HIV-infected women were twice as likely to develop peripheral and central lipoatrophy compared to HIV-uninfected women. (48). Although inconsistent results have been reported, there appears to be a strong correlation between the use of protease inhibitors (PIs) and lipodystrophy, however not all persons taking PIs develop lipodystrophy and persons who have not taken a PI have reported lipodystrophy. Some studies have found that the development of lipodystrophy is associated with lack of response to HAART. (15,43) Others have noted an associated with the use of HAART and low CD4 count. Mauss and colleagues reported that the use of stavudine and a CD4 count less than 200 cells per microliter was associated with increased risk for lipodystrophy while non-nucleoside reverse transcriptase inhibitor (NNRTI) use is related to a reduced risk (43,49).

Relatively few treatment options have become available for patients suffering from lipodystrophy. Treatment with recombinant human growth hormone (rhGH) or testosterone has demonstrated some effect at reducing truncal adiposity as well as the fat accumulation on the back of the neck. (50) A recent prospective, randomized trial was reported by Kotler and

colleagues and found that the 1 mg daily of rhGH warrants further research for the treatment of excess trunk fat. (51) Researchers have also noted that a change from PIs to nevirapine may lead to a reversal of symptoms associated with lipodystrophy but this has not been effective in all patients. (52,53) Substitution of abacavir for AZT or d4T leads only to small, clinically insignificant decreases in lipoatrophy. (54)

The underlying mechanisms and methods of treatment of lipodystrophy are not well understood or established, however, the morphologic and metabolic changes are believed to have a significant impairment on the patient's HRQL, particularly the patients' psychological well-being. As with other diseases, the benefits and consequences of treatment must be weighed. HRQL, as well as survival, must be recognized as an important outcome in the treatment of HIV.

Few studies have been published concerning the effects of lipodystrophy on HRQL or reported depressive symptoms in HIV-seropositive patients diagnosed with lipodystrophy (55-57). Falutz found that of 82 patients with lipodystrophy, 67% described the morphologic and metabolic changes as tolerable while 70% of patients reported depressed mood as a result of body habitus changes. (55) The facial lipoatrophy appeared to have the greatest impact on patients followed by central adiposity (55). Eighty-six percent of patients described the changes in their body in a negative way (e.g., "I no longer appear healthy"). Falutz and colleagues' investigation, which only included single item measures of HRQL, indicated that the development of lipodystrophy may have profound effects on HRQL and mood. (55)

A second study by Goetzenich and colleagues found that a diagnosis of lipodystrophy was correlated with decreased social contact (63%), changes in daily performance (68%), changes in sexual functioning (68%), and decreased self-esteem (83%). (56). A detailed analysis found that sexuality was most affected by changes in the abdomen and neck while ratings in self-

esteem were primarily associated with changes in the abdomen. Decreased social contact was correlated with changes in the neck, legs, breast while daily performance was related to changes in the legs and arms. The strengths of this study were that it identified the various effects of lipodystrophy on specific aspects of physical and psychological functioning. Although this study provided greater detail in regard to the correlation between body habitus changes and its impact on specific areas of HRQL, the study lacked a standardized measure of HRQL and did not include a control group.

In one of the first studies to use standardized measures to assess the effects of lipodystrophy on HRQL, women who were HIV-seropositive reported that bodily changes associated with lipodystrophy influenced dressing behavior (65%), produced shame (49%), and disrupted their sex life (27%). Furthermore, women with breast lipoaccumulation had greater psychosocial impairment (57). More recently, Nicholas and colleagues reported that those individuals with lower CD4 counts and lipodystrophy reported lower HRQL on the Medical Outcomes Study (MOS; SF-36.) (57,58) Nineteen percent of participants reported depressive symptoms within the clinical range of the Center for Epidemiological Studies-Depression Scale (CES-D), however, no association was found between depressive symptoms and lipodystrophy. (58)

In contrast with these studies, researchers have also found that in HIV-seropositive patients treated with HAART, a diagnosis of lipodystrophy was not found to be associated with the individuals' attitude about their health condition or their overall well-being. However, patients reported that they were about twice as likely to feel recognizable as an HIV-positive person by their physical appearance. (59) It should be noted that this study used single items to

assess their attitudes regarding their health condition, well-being, and feelings of being recognized as an HIV-seropositive person.

Depression has also been studied separately in men who have sex with men. A paucity of research has been conducted concerning the psychological sequelae of lipodystrophy on patients. A study by Tsiodras and colleagues found that patients who were taking psychotropic medications (e.g., antidepressants and benzodiazepines) were more likely to have lipodystrophy. (60) Given the cross-sectional nature of this study, however, it is not clear if development of lipodystrophy led to depression or if the depression, or use of psychotropic medications, preceded the diagnosis of lipodystrophy.

The aims of the present study were to longitudinally investigate associations between HRQL and lipodystrophy, and between depression and lipodystrophy. To advance the previous research, the present study used standardized instruments and control groups of HIV-seropositive patients without lipodystrophy and HIV-seronegative patients. In addition, diagnosis of lipodystrophy is based on measurements by a trained physician assistant rather than patient self-report.

5.2.3 Materials and methods

Participants. The participants of this study were drawn from Pittsburgh, Pennsylvania, one of the four clinical centers forming the Multicenter AIDS Cohort Study (MACS). The Pittsburgh MACS has enrolled 490 HIV-seropositive and 756 HIV-negative men (n = 1252 total) who have sex with men (n = 1252). The MACS is an ongoing multicenter prospective cohort study of HIV infection in homosexual and bisexual men, of whom most (88%) were enrolled between 1984 and 1991. Briefly the MACS follows a 6-month visit schedule including detailed interviews, medical history, physical examination, and collection of biologic specimens. Further detail can

be found in Kaslow et al. (61) A specific lipodystrophy questionnaire and physical assessment were instituted at the 31st MACS visit (April 1, 1999 to September 30, 1999) to study the development of HIV-LS in this population. For the purposes of this analysis, the cohort was limited to 217 consecutive male patients seen at the Pittsburgh center. Of the 217 men, 129 were HIV-seropositive and 88 HIV-seronegative. None of the 88 HIV-seronegative patients had lipodystrophy and they are not used in any analyses evaluating the effect of lipodystrophy. However, we included these patients to provide a baseline comparison.

Thirty-seven of the HIV seropositive participants had at least one physical examination consistent with lipodystrophy. Lipodystrophy measurements were first collected in April 1999, approximately 3 years after HAART became widely available. To maintain the prospective nature of the study, the analysis is primarily restricted to all outcomes (SF-36 and CES-D scores) from April 1999 to September 2001. Baseline measurements for SF-36 and CES-D scores were calculated as the mean score of visits 0 to 26 (i.e., mean score before first initiation of HAART).

Measurement of lipodystrophy. For the purposes of this study, lipodystrophy was assessed by a physician assistant experienced in care of HIV/AIDS patients. The examiner rated the patient on two criteria of body change, fat wasting, and fat accumulation. These ratings included fat wasting of the face, arms, legs, and buttocks, and fat accumulation of the face, abdomen, back of neck, and breasts. Each area was rated according to severity as either (1) none (patient does not exhibit any signs of fat maldistribution), (2) mild (mild signs of fat maldistribution are noticed by clinician without looking for it), (3) moderate (signs of fat maldistribution are noticed by clinician without specifically looking for it or patient may complain that current clothing has become tighter), or (4) severe (signs of fat maldistribution

easily noted by causal observer). These measurements were then utilized to define lipodystrophy status, as previously described.

HRQ Life. HRQL was measured using the MOS SF-36. The SF-36 yields a profile of eight domains of HRQL including physical, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health. (62,63) Each component score was calculated by summing item responses after reversing selected items. Raw scores are then linearly transformed to a scale from 0 to 100, with a high score indicating a better HRQL. The SF-36 has been widely used and validated. (62) Two summary scores of the SF-36 scores, physical and mental functioning, were calculated using the weighted sum of appropriate questions, using the principal components derived by Ware. (63)

Depressive symptomatology. Depressive symptomatology was assessed using the CES-D. The scale includes 20 items assessing depressive symptomatology in the previous week. (64) A Likert-type scale is used with ratings from 0 (rarely or none of the time-less than 1 day in the past week) to 3 (most of or all of the time-5-7 days in the past week). Total scores range from 0 to 60, with a score of 16 or more indicating possible clinical depression. The instrument has been demonstrated to be valid and have a high internal consistency and test-retest reliability (8-week interval $r = 0.59$). (64)

Procedures. The participants in the present study were all enrolled prior to the initiation of HAART. All participants provided written informed consent prior to their participation in the study. Every 6 months after enrollment into the study, participants completed a battery of questionnaires, underwent a physical examination, and had extensive laboratory testing performed. The physical examination included measurements of fat wasting and fat accumulation (as previously described).

Data analysis. Statistical analyses were limited to the cohort of 217 men who received at least one physical examination for the assessment of lipodystrophy. Both HRQL and depression scores were analyzed using a repeated measures analysis with autoregressive correlation (other correlation structures were also tested but did not significantly affect results). HIV status alone was assessed through comparing seronegative men to the group of seropositive men without lipodystrophy, after adjusting for patient age. Within HIV-seropositive men, the significance of lipodystrophy status was evaluated after controlling for patient therapy, age and baseline response (i.e. mean score for depression or HRQL before first HAART initiation at visit 26). After recording CES-D as a dichotomous response (< 16 versus >= 16), analyses were then repeated using generalized linear mixed models. The mixed model can be described by the following equation, where *Y* is the observed outcome vector, X[beta] represents the covariate values and corresponding model coefficients, and *Zu* denotes the random effects component corresponding the given repeated measures design and correlation structure.

5.2.4 Equation

$$Y = X\beta + Zu + e$$

The generalized linear model, which is used for modeling repeated binary outcomes follows basically the same structure, except that a logistic link function relates the outcome variable to the linear combination of fixed and random predictors (on the right hand side of the equation). All statistical runs were conducted with SAS 8.01 (SAS Institute, Cary, NC) using either proc mixed (for continuous outcomes) or the glmm macro (for the outcome of presence/absence of clinical depression). Missing data for individual SF-36 items were imputed using the average score across all other questions in that component. The same procedure was used for missing data on the CES-D questionnaire.

5.2.5 Results

The demographic characteristics of the participants are described in Table 7. Mean age of participants (at visit 31) was 37 with a range of 23 to 69 years. Twenty percent of the men (*n* = 32) were below age 30 at visit 31; 73% (*n* = 116) were age 30 to 50; only 6% (*n* = 10) were above age 50. The majority of men were Caucasian (94%), followed by African American (4%), and Native American (1%). Nearly 100% of participants reported at least a high school education with 51% completing a 4-year degree. The median viral load (at visit 31, which was the baseline for lipodystrophy measurements) was 100 with a range of 80 (or less; values not reported below that threshold) to 166,400. The median CD4 count (at visit 31) was 634 with a range of 80 to 1642. HIV status was known for all 217 men; 129 were HIV-seropositive and 88 were HIV-seronegative.

Table 7. Demographic and disease-specific characteristics of HIV-seropositive men

Age	
Mean	37
Range	23 – 69
Ethnicity (%)	
Caucasian	94
African American	4
Native American	1
Other	1
Education (%)	
High school diploma	99
College graduate	51
Postgraduate/professional	
Lipodystrophy phenotype (%)	
Total	54
Peripheral	40
Mixed	14
CD4 Count (Visit 31)	
Median	634
Range	50 – 1642

Table 7 Cont. Demographic and disease-specific characteristics of HIV-seropositive men

Viral load (Visit 31)	
Median	100
Range	<80 – 166, 400

Of the HIV-seropositive, 37 (28.7%) were diagnosed with peripheral lipoatrophy during at least one visit, while 12 (9.3%) were also diagnosed with the mixed form of lipodystrophy during at least one visit. The overall proportion of subjects with peripheral lipoatrophy ranged between 9% (at visit 31) to approximately 40% (at visits 34 and 35). The prevalence of mixed lipodystrophy was much lower, with a maximum of only 13% of subjects having mixed lipodystrophy at visit 35 (April to September 2001; (Table 8).

Table 8. Lipodystrophy status within HIV-positive subjects by visit

Visit	Status	Frequency	Percent
31	Mixed	1	1.0
	Peripheral only	8	7.8
	None	94	91.3
32	Mixed	4	4.1
	Peripheral only	15	15.5
	None	78	80.4

Table 8 Cont. Lipodystrophy status within HIV-positive subjects by visit

Visit	Status	Frequency	Percent
33	Mixed	11	11.0
	Peripheral only	24	24.0
	None	65	65.0
34	Mixed	11	11.7
	Peripheral only	26	27.7
	None	57	60.6
35	Mixed	12	12.9
	Peripheral only	25	26.9
	None	56	60.2

HAART was initiated at visit 26, when approximately 20% of patients in this cohort received HAART. That percentage steadily increased to over 70% at visit 30, and has remained constant at approximately 80% over the last 3 visits (18 months). In contrast, the percentage of people receiving mono/combination treatment, or no treatment, has decreased steadily over the last 4 to = years (i.e., approximately since visit 25).

The number of people with at least one severe condition of fat wasting as reported by the physician assistant was 4 of 9 of those starting with at least moderate lipodystrophy (44.4%) on visit 31. Nine of 19 (47.4%) on visit 32; 21 of 35 (60.0%) on visit 33; 21 of 37 (56.8%) on visit 34; and 29 of 37 (78.4%) on visit 35 were diagnosed with lipodystrophy. In terms of the relationship between lipodystrophy and quality of life outcomes, we again found a very inconsistent relationship (quite possibly due to the small sample sizes which are even more

limited when restricting the outcome definition to severe conditions). For instance, the mean CES-D scores were higher for those with at least one severe condition at visits 31 (14.0 versus 8.4), 34 (11.7 versus 6.5) and 35 (11.1 versus 8.5), but lower at visits 32 (10.5 versus 12.2) and 33 (10.5 versus 14.4). This inconsistent relationship was similar in terms of an overall pattern, or lack thereof, as compared to the overall analyses of (at least moderate) lipodystrophy presented in the results section.

The prevalence of lipodystrophy was slightly higher in patients on HAART as compared to mono-combination therapy. The prevalence for those with peripheral lipoatrophy ranged from 10% (at visit 31) to 47% (at visits 35) for patients taking HAART; 7%-36% (at visits 31 and 35, respectively) for mono/combination therapy; and up to 11% (at visit 35) for patients not taking any HIV-related medications.

Patient therapy (adjusted for age and baseline scores) was not significantly associated with either the physical or mental component scores on the SF-36 ($p = 0.99$ and $p = 0.40$, respectively). In addition, therapy was not significantly associated with either continuous CES-D scores ($p = 0.94$) or presence of clinical depression ($p = 0.43$). Based on these results, therapy was not considered in the following models of lipodystrophy status.

Separate analyses (not shown here) were initially conducted for the peripheral lipoatrophy group without mixed lipodystrophy and the mixed lipodystrophy group. Primarily because of small sample sizes within the mixed lipodystrophy group (maximum of $n = 12$ over visits 31-35), and since no significant inconsistencies were detected in the relationship between these two categorizations and the (HIV-positive) group without lipodystrophy, further analyses focused on the peripheral lipoatrophy group (with or without mixed lipodystrophy; maximum of $n = 37$) versus the HIV-positive group without lipodystrophy. These subsequent comparisons

will be referred to as the lipodystrophy group versus the (HIV-positive) group without lipodystrophy. In all analyses, lipodystrophy (as well as all other variables considered) is measured as a time-dependent variable in the repeated measures model.

For the mental component of the SF-36, scores were not consistently higher or lower in the lipodystrophy group (Table 9). Rather, the lipodystrophy group had similar scores at visits 31 and 32, slightly lower (by less than a standard error) scores at visit 33, and slightly higher scores at visits 34-35 (by 1-1.5 standard errors). The HIV-negative group had consistently higher (SF-36 mental component) scores in almost all cases.

Table 9. Mean SF-36 mental and physical component scores by lipodystrophy status

Visit	Status	Mental Mean (SE)	Median	95% CI
1.5	Lipodystrophy +	45.5 (3.0)	46.8	(38.4, 52.5)
	Lipodystrophy -	45.4 (1.2)	49.2	(42.9, 47.8)
	HIV -	48.6 (1.2)	52.3	(46.3, 50.9)
32	Lipodystrophy +	45.2 (2.9)	50.3	(39.1, 51.3)
	Lipodystrophy -	45.7 (1.4)	50.7	(42.9, 48.6)
	HIV -	48.8 (1.2)	52.2	(46.4, 51.2)
33	Lipodystrophy +	44.2 (2.2)	48.6	(39.6, 48.7)
	Lipodystrophy -	46.2 (1.6)	51.1	(43.0, 49.3)
	HIV -	48.4 (1.2)	52.0	(45.9, 50.8)
34	Lipodystrophy +	48.3 (1.8)	51.0	(44.6, 52.0)
	Lipodystrophy -	47.0 (1.5)	52.4	(44.0, 50.0)
	HIV -	48.4 (1.3)	52.3	(45.9, 50.9)
35	Lipodystrophy +	49.1 (1.8)	52.9	(45.4, 52.9)
	Lipodystrophy -	46.4 (1.8)	52.8	(42.8, 50.0)
	HIV -	48.4 (1.2)	51.5	(45.9, 50.8)
Visit	**Status**	**Physical Mean (SE)**	**Median**	**95% CI**
31	Lipodystrophy +	47.2 (2.7)	49.5	(40.9, 53.4)
	Lipodystrophy -	51.4 (1.0)	55.4	(49.3, 53.4)
	HIV -	55.2 (0.7)	57.0	(53.7, 56.6)
32	Lipodystrophy +	48.4 (2.8)	53.2	(42.5, 54.3)
	Lipodystrophy -	52.5 (0.95)	55.7	(50.6, 54.4)
	HIV -	55.2 (0.8)	57.0	(53.6, 56.7)
33	Lipodystrophy +	46.9 (1.9)	49.1	(43.0, 50.9)
	Lipodystrophy -	53.0 (1.0)	55.9	(51.0, 55.0)
	HIV -	55.2 (0.7)	56.8	(53.8, 56.6)
34	Lipodystrophy +	48.8 (1.9)	51.6	(45.0, 52.6)
	Lipodystrophy -	54.2 (1.1)	56.9	(52.0, 56.5)
	HIV -	54.5 (0.9)	56.7	(52.7, 56.3)
35	Lipodystrophy +	48.9 (1.9)	51.8	(45.0, 52.8)
	Lipodystrophy -	52.7 (1.1)	55.4	(50.5, 55.0)
	HIV -	54.3 (0.9)	56.0	(52.5, 56.0)

Lipodystrophy + includes peripheral lipoatrophy with or without mixed lipodystrophy; Lipodystrophy – includes only HIV patients without lipodystrophy.
SE, standard error; CI, confidence interval.

Regarding the physical component scores, the lipodystrophy group showed more consistently decreased levels (Table 9). The mean score for the lipodystrophy group ranged between 47 and 49, whereas the group without lipodystrophy had mean scores ranging from 51 to 54. The HIV-negative group again showed higher mean scores across all time points.

To evaluate the statistical significance of associations between lipodystrophy and SF-36 summary scores (mental and physical component summaries), age, and baseline SF-36 summary scores (see Materials and Methods section) were entered as covariates in the mixed model. Although some consistent patterns were evident for the mean physical component scores, lipodystrophy was not significantly associated with either the physical component summary score ($p = 0.70$) or the mental component summary score ($p = 0.81$). The comparisons between the HIV-positive and HIV-negative groups were all highly significant ($p < 0.01$ for the physical component and $p = 0.03$ for the mental component).

CES-D scores did not show any consistent association between lipodystrophy and depression (Table 10). Both of the HIV-positive groups, with and without lipodystrophy, did however show increased depression scores versus the HIV-negative group across most all time points. Results of the repeated measures model also indicated that lipodystrophy was not significantly associated (after adjusting for age and baseline CES-D) with CES-D scores ($p = 0.55$). Further, after categorizing CES-D scores as 16 or greater versus less than 16, lipodystrophy was not significantly associated with presence of clinical depression ($p = 0.28$) (Table 11). HIV status alone, however, again led to highly significant results for both the continuous ($p < 0.01$) and dichotomous ($p = 0.01$) CES-D scores.

Table 10. Mean CES-D scores by lipodystrophy status

Visit	Status	Mean (SE)	Median	95% CI
31	Lipodystrophy +	10.9 (3.1)	8.0	(3.6, 18.1)
	Lipodystrophy -	12.6 (1.2)	10.0	(10.3, 15.0)
	HIV -	8.7 (1.0)	6.0	(6.8, 10.6)
32	Lipodystrophy +	11.4 (2.4)	9.0	(6.5, 16.4)
	Lipodystrophy -	11.1 (1.3)	7.5	(8.6, 13.6)
	HIV -	8.4 (1.0)	6.0	(6.4, 10.4)
33	Lipodystrophy +	12.3 (2.0)	10.0	(8.2, 16.3)
	Lipodystrophy -	10.9 (1.5)	7.0	(8.0, 13.8)
	HIV -	9.5 (1.1)	6.0	(7.2, 11.7)
34	Lipodystrophy +	9.2 (1.6)	6.5	(6.0, 12.3)
	Lipodystrophy -	9.9 (1.4)	6.5	(7.0, 12.8)
	HIV -	9.3 (1.2)	5.0	(6.9, 11.6)
35	Lipodystrophy +	10.2 (1.8)	7.4	(6.5, 13.9)
	Lipodystrophy -	11.6 (1.7)	8.0	(8.1, 15.0)
	HIV -	8.8 (1.1)	5.1	(6.7, 11.0)

Lipodystrophy + includes peripheral lipoatrophy with or without mixed lipodystrophy; Lipodystrophy – includes only HIV+ patients without lipodystrophy.

CES-D, Center for Epidemiological Studies – Depression; SE, standard error; CI, confidence interval.

Table 11. Clinical depression by peripheral lipoatrophy status

Visit	Status	CES-D ≥ 16 (%)	CES-D < 16 (%)
31	Lipodystrophy +	2 (22.2)	7 (77.8)
	Lipodystrophy –	30 (32.3)	63 (67.7)
	Hiv –	17 (21.5)	62 (78.5)
32	Lipodystrophy +	3 (16.7)	15 (83.3)
	Lipodystrophy –	26 (33.3)	52 (66.7)
	Hiv –	16 (20.0)	64 (80.0)
33	Lipodystrophy +	10 (28.6)	25 (71.4)
	Lipodystrophy –	16 (24.6)	49 (75.4)
	Hiv –	18 (22.8)	61 (77.2)
34	Lipodystrophy +	7 (19.4)	29 (80.6)
	Lipodystrophy –	14 (25.0)	42 (75.0)
	Hiv –	18 (23.1)	60 (76.9)
35	Lipodystrophy +	8 (22.9)	27 (77.1)
	Lipodystrophy –	16 (29.1)	39 (70.9)
	Hiv –	15 (19.2)	63 (80.8)

Lipodystrophy + includes peripheral lipoatrophy with or without mixed lipodystrophy; Lipodystrophy – includes only HIV+ patients without lipodystrophy.

CES-D, Center for Epidemiological Studies – Depression.

5.2.6 Discussion

Previous research has found, when using nonstandardized questionnaires, that lipodystrophy is reported to affect HRQL, particularly in the area of interpersonal and sexual functioning. (66) Goetzenich and colleagues reported that a diagnosis of lipodystrophy was associated with decreased social contact, changes in daily performance, problems with sexual relations, and decreased self-esteem. (56) In contrast, we found that, among HIV-seropositive participants, a diagnosis of lipodystrophy, in general, was not found to be associated with decreased HRQL. (48,56) Although it was expected that patients with lipodystrophy would have a lower HRQL, particularly on scales measuring social and emotional well-being, 7,15 no significant differences were identified in either the mental or physical summary scores measured by the SF-36.

Falutz and colleagues 14 found that approximately 70% of patients with lipodystrophy reported some symptoms of depression; however a standardized instrument to measure depression or clinical interview were not used. (55) Therefore, it is difficult to ascertain how severely the participants' mood was affected by the diagnosis of lipodystrophy. The results of the present study found that lipodystrophy, over and above a diagnosis of HIV, was not associated with elevated scores on the CES-D. Although the number of participants with lipodystrophy in this sample was relatively small, the use of a standardized instrument to assess depressive symptoms provided greater validity and reliability in the measure of depressive symptoms in these patients.

Prior research has demonstrated that patients who reported facial atrophy were three times more likely to seek treatment than patients with other types of lipodystrophy. (57). *Post hoc* analyses revealed that the presence of lipodystrophy in various areas of the body (e.g., face, trunk) was not found to be related to decrements in HRQL or mood.

The prevalence of lipodystrophy in this study was consistent with previous studies assessing body habitus changes in HIV-seropositive patients. At the last patient visit recorded (April to September 2001), approximately 47% of patients taking HAART met the criteria for lipodystrophy. Dukers and colleagues found a similar prevalence in which approximately 50% of patients were self-diagnosed. (66) A strength of the present study is that the diagnosis of lipodystrophy was made by a trained physician assistant.

Although no previous study has been conducted specifically addressing the effects of lipodystrophy on mood, a study by Tsiodras and colleagues found that patients who were taking psychotropic medications (e.g., antidepressants and benzodiazapines) were more likely to have lipodystrophy than those who have not been diagnosed with lipodystrophy. (60) If the same is true for the present study, the lack of differences in reported depressive symptomatology may be a result of the depression that was treated in patients with lipodystrophy.

Although MACS does offer a unique environment for studying the effects of lipodystrophy, the study was somewhat limited by a relatively small sample size. The mixed lipodystrophy group was particularly small ($n = 12$). Some of the analyses showed a trend toward some effect, particularly in the case of mental component scores (worse in the lipodystrophy group) and clinical depression (better in the lipodystrophy group than the other HIV-seropositives). Our follow-up of persons receiving HAART after lipodystrophy was also limited. It would be premature to conclude that the effects of lipodystrophy on HRQL would remain minimal beyond our observation period, as both lipodystrophy severity and HAART-associated metabolic toxicities increase. Despite this caution, it is encouraging that the minimal changes in HRQL reported herein were observed in this HAART-experienced population. The results of the study suggest that, although the adverse effects of changes in body habitus as a result of HAART

are profound, as a whole, the symptoms do not significantly effect HRQL or mood (as measured with the SF-36 and CES-D) above and beyond that of a diagnosis of HIV.

It may be that the differences in HRQL and mood between people diagnosed with lipodystrophy and those without cannot be detected statistically. Further research concerning clinically meaningful differences between scores on the SF-36 as well as the CES-D should be performed. Although not the goal of this study, previous research has demonstrated that although lipodystrophy may not affect HRQL or mood, symptoms of lipodystrophy are associated with decreased medication adherence.(67) As a result of decreased adherence to medication regimens, patients who develop lipodystrophy may experience increased viral loads, opportunistic infections, and possibly increased mortality compared to patients without lipodystrophy. The discontinuation of HAART secondary to lipodystrophy warrants further research, particularly if it is associated with increased morbidity and mortality. Furthermore, although the HRQL instruments may not detect statistically significant differences in HRQL and mood in people with lipodystrophy.

5.2.7 Acknowledgement of funding / support

This study was supported by the National Institute of Allergy and Infectious Disease with additional supplemental funding from the National Cancer Institute UO1-AI-35041.

6.0 PREFACE TO FOURTH PUBLICATION

HIV-infection can damage the myocardium, endocardium and pleura. Autran and colleagues first described a case of pericardial Kaposi's sarcoma in a patient with AIDS in 1983. (68) Since then, clinicians have gained a comprehensive understanding of the many cardiac manifestations of HIV-infection and AIDS. These include pericardial effusions, endocarditis, dilated cardiomyopathy, and pulmonary hypertension. While HAART, has decrease the prevalence of cardiac diseases caused by opportunistic infections and malnutrition, the increasing prevalence of antiretroviral-associated metabolic abnormalities have raised concerns about potential atherosclerosis mediated cardiac morbidity and mortality. Several cases of premature atherosclerosis and cardiac events have been described among patients treated for HIV-infections among patients with and without traditional risk factors. (10,12,69) Thus, the relationship between metabolic toxicities, HAART therapy and increased cardiovascular risk has become an area of intense research, especially as increasing numbers of patients face years of antiretroviral therapy.

6.1 PATHOGENESIS OF CORONARY ARTERY DISEASE

6.1.1 Introduction

Atherosclerotic disease occurs in the high-pressure coronary vasculature because of defects in the endothelium. The initiating abnormality is most often sub-intimal accumulation of plaques. These plaques are likely the result of high levels of circulating low-density lipoproteins (LDL). Associated with the recruitment of monocytes to the area, these accumulated lipid particles undergo oxidative changes and are then referred to as foam cells, which reflects the foamy appearance of the cytoplasm. The sub-intimal defects continue to enlarge mainly due to a prolonged inflammatory response and develop into fatty streaks. Over time, these fatty streaks become calcified and have the potential to cause stenosis of the vessel if a large enough area is involved. The degree of calcification usually correlates with advancing lesions. (70) If the stenosis develops gradually, arteriogenesis occurs and collateral vessels are used to perfuse the area of the myocardium affected by the stenosis. However, if a thrombus forms in an existing stenotic vessel, the collateral arteries are often inadequate to maintain normal cardiac perfusion. In addition to forming a stenotic area, the plaques can rupture. Rupture of vulnerable plaques leading to coronary thrombosis accounts for most acute coronary syndromes. The rupture exposes the endothelial cells to the oxidized LDL and inflammatory cells. Increased levels of platelet derived growth factor (from infiltrating macrophages) and plasminogen activator inhibitor (from oxidized LDL) cause clot formation in the vasculature and propagate thrombus formation. In normal vasculature, the appropriate response is vasodilation of the vessel in order to continue to perfuse the more distal area. However, oxidized LDL has been shown to induce the expression of endothelin which inhibits the vasodilation. The result is a thrombus in a

constricted vessel which may lead to a partial or totally occlusion. When a coronary artery becomes partially or totally occluded, myocardial ischemia becomes evident.

6.1.2 Risk Factors for coronary artery disease

Atherosclerotic disease of the coronary arteries and peripheral vasculature remains the leading cause of death in the United States and worldwide. Given the increasing prevalence of coronary artery disease (CAD), efforts have been put forth toward primary, secondary and tertiary prevention. Through post-mortem examination of young adults, it is now widely understood that the process of atherosclerosis begins decades before the disease becomes clinically evident. The risk factors for coronary artery disease in the general population have been well documented and include, cigarette smoking, hypertension, male age $\geq$ 45 years, female age $\geq$ 55 years (or postmenopausal without estrogen replacement), and first degree family history of premature coronary artery disease. In response to the epidemiologic studies that have illustrated the relationship between hyperlipidemia and coronary artery disease, the National Cholesterol Education Program (NCEP) has identified additional risk factors for the development of CAD. (28) These risk factors include HDL < 40 mg/dL, total cholesterol (TC) > 200 mg/dL, and LDL >130 mg/dL. The most recent NCEP III guidelines omit diabetes and other forms of atherosclerotic disease (peripheral vascular disease, abdominal aortic aneurysm and symptomatic carotid artery disease) as risk factors, and now consider them as coronary heart disease equivalents. (28)

Primary prevention for coronary artery disease includes strategies that are initiated before the pathogenesis has begun. For example, programs which discourage children from ever starting to smoke, and encouraging a physically active lifestyle would be considered primary prevention for coronary artery disease. Secondary prevention includes attempts to minimize

complications or clinical outcomes from disease once it has been diagnosed. For example, once an individual is found to have coronary artery disease and hyperlipidemia, lipid lowering medications should be prescribed. Finally, tertiary prevention involves attempts to minimize debilitation or death from disease and may involve surgical bypass grafting for those with severe or multi-vessel coronary artery disease. In order to reduce the risk of major coronary events, efforts involving diet modification, aerobic exercise and weight reduction are often the first to be instituted once hyperlipidemia is diagnosed. If sub-optimal results are obtained, or if the patient already adheres to these practices and maintains normal body weight, pharmacologic interventions may be necessary.

6.1.3 Methods of detecting coronary artery disease

Coronary artery disease cannot be detected during a routine physical, although, the complications of risk factors for CAD are easily detected during a thorough examination. For example, hypertensive patients may develop an additional heart sound, S4, arterial bruits, and/or fundoscopic changes. Diabetic patients may develop peripheral neuropathy, renal insufficiency, superficial ulcerations, and fundoscopic changes. When such findings are present, the clinician has compelling evidence that the disease (HTN, DM, etc) has progressed to the point of end-organ damage which may include atherosclerosis of the coronary, cerebrovascular and peripheral vascular beds. There are no blood tests that are confirmatory for coronary artery disease. Serial cardiac enzymes and troponin I levels are helpful in establishing the diagnosis of acute myocardial infarction, however, they offer no value in the progression or long-term follow-up of coronary artery disease. Lipid levels and C-reactive protein appear to be the only laboratory tests of value in terms of disease surveillance. C-reactive protein is an inflammatory marker and elevated levels are associated with an increased risk of coronary events. The addition of C-

reactive protein to standard lipid testing panels has been shown to improve outcome prediction in the primary prevention of acute coronary events. Apolipoprotein B levels represent the protein component of the LDL molecules and serum levels can easily be measured. Apolipoprotein B levels are not traditionally used as a screening tool, although, they have been used in clinical research trials which attempt to identify easily measurable surrogates for CAD risk.

Similar to most other medical conditions, the first step in the evaluation process for coronary artery disease involves non-invasive studies. The most common and inexpensive one is electrocardiography (EKG). During acute myocardial infarction, characteristic EKG findings include inverted T-waves, and ST-segment changes. Typically, ST-segment depression indicates subendocardial ischemia while ST-segment elevation is consistent with the more severe transmural ischemia. Findings on EKG can also suggest conditions resulting from CAD such as conduction defects or an old infarction. Mortality from acute myocardial infarction usually occurs when infarction leads to ventricular tachycardia or ventricular fibrillation. Electrocardiography will not provide information regarding the presence/absence of subclinical atherosclerosis. Chest X-rays are of only limited value in the work-up of coronary artery disease and most likely show only subsequent congestive heart failure or cardiomegaly. Echocardiography is a non-invasive ultrasound of the heart. It is most helpful in the evaluation of valvular deformities or vegetations, ventricular size and function and the presence of atrial thrombi, or pericardial effusions. Since the coronary arteries are not adequately visualized, this testing offers no value for screening for or quantifying the degree of coronary artery disease. Magnetic resonance imaging (MRI) has many functions and has been traditionally used for imaging soft-tissue injuries to the knee or hip. MRI does not appear to be a reliable tool for assessing and quantifying CAD because the cardiac and respiratory motions distort the images

and makes interpretation difficult and unreliable. However, MR angiography (MRA) does have a role in detecting peripheral and cerebral atherosclerosis.

In order to most accurately assess the true severity of atherosclerotic lesions medical imaging of the vasculature is necessary. Typically, patients with angina experience pain only with physical activity, and remain symptom free at rest. If an individual has a history of intermittent chest pain and is found to have abnormalities on EKG testing, the next step in evaluation is cardiac stress testing. During this evaluation patients are exercised, typically on a treadmill, in order to stress the heart and increase cardiac demands. Any signs or symptoms consistent with cardiac disease during the procedure are documented. While the test is being performed, the individual is evaluated for respiratory effort, heart rate, blood pressure and is also monitored by cardiac leads. This test is considered positive when symptoms of chest pain, diaphoresis or hypotension occur, or when there are EKG changes consistent with cardiac ischemia. As a second component of this examination, cardiac scanning with thallium-201 for myocardial perfusion is performed. For those individuals in which physical activity is not possible, (such as those with concurrent pulmonary disease or other conditions which limit their capacity for activity), the heart rate can be elevated with persantine or adenosine. Uptake of these pharmacologic agents by the myocardium is directly proportional to the to coronary blood flow. By using adenosine, (or persantine) as an adjunct to thallium-210, areas of the myocardium which are perfused by vessels with stenotic lesions can be identified. If significant coronary artery disease is suspected from the findings on the stress (or adenosine) thallium study, the next step is cardiac catheterization. This extremely reliable test is very common and allows for immediate catheter assisted intervention in some instances. The procedure involves cannulating the femoral artery and advancing the catheter to the heart where a contrast material

is infused into the coronary arterial circulation. Images of the coronary vasculature can then be reviewed for evidence of arterial stenosis and perfusion defects. Due to the cost and invasiveness of the procedure, this study is not performed as screening in asymptomatic individuals. When CAD is documented, treatment would depend on the degree or severity of the disease. Conservative treatment may consist only of medication with stent placement being recommended for more significant disease. For those with multi-vessel disease, coronary artery bypass grafting with vein grafts may be necessary. These surgical procedures are not curative since patients may develop progression of disease in native vessels and/or develop stenotic lesions in the grafted vessels.

Flouroscopy can be used to detect calcification in coronary arteries. The utility of fluoroscopy is greatest when moderate to large calcifications are present and the reliability in detecting smaller areas of calcification is less. (70) Additionally, large body-habitus makes determining coronary calcium via fluoroscopy technically difficult. (70)

6.1.4 Electron beam computed tomography

Coronary artery scanning by electron beam tomography (EBCT) has been shown to noninvasively and accurately detect and quantify the degree of calcified atherosclerotic plaque. (71,72) It is ideal for screening asymptomatic patients who may be at risk for future cardiac events based on risk factors such as family history. .

To perform the test, the patient is positioned comfortably in the supine position. Three electrode patches are placed on the inferior-anterior chest and an EKG tracing is obtained. The participant is positioned within the gantry with arms over the head and resting comfortably on the armrest. The participant is asked to hold a breath while the scanner passes from the shoulders to the hips. The patient must hold his breath each time the scanner is operated. The

first image is used for calibrating the scanner to the diameter of the chest, subsequent images are obtained of the entire heart, taking a cross-sectional image every 3 mm. The images are obtained during the diastolic phase of the cardiac cycle, one image during each heart beat which is confirmed with the EKG tracing. The scan also images the aorta from the aortic arch to the iliac bifurcation and cross-sectional images are taken every 6 mm.

Based on these images, a calcium score can be calculated for each region of interest. The calcium score is generated using a Base Value Region of Interest computer program. This program extracts all pixels above 130 Hounsfield units within an operator-defined region measuring 3 mm of thickness. All pixels greater than 130 Hounsfield units and larger than 1 mm within the coronary arteries are considered to be calcium. A calcium score is then calculated by multiplying the area of all significant pixels by a grade number one, two, three, or four indicative of the peak CT number. The individual region-of-interest scores are then summed for a total coronary artery calcium score. The same procedure is used for the aorta. Because calcium is an early feature of atherosclerotic-plaque formation, measurement of coronary calcium correlates with the extent of atherosclerosis. High calcium scores have good sensitivity for coronary artery calcification, ranging from 80-90%, but low specificity, ranging from 40-60%. The total calcium volume score can predict future cardiac events. Calcification of arteries occurs only as a consequence of atherosclerosis. The degree of calcification correlates with hisotomorphometric measurements of calcium crystals. The radiation exposure to the patient during this study is minimal. Generally, external radiation exposure is described as either a dose for a particular body region, i.e., the skin the radiation passes through and exposure to the entire body. The dose of radiation to the patient during an EBCT is approximately 0.8 rads to the chest and 2.5 rads to the abdomen. This represents less than 2% of the radiation exposure limit set by the federal

regulations (50 rads) to any single organ or tissue allowed for radiation workers per year. There is no minimum level of radiation exposure that is recognized as being totally free of the risk of causing cellular abnormalities. The greatest advantages of this test are that it's non-invasive and that it takes only twenty minutes to properly position the patient, connect the EKG censors and complete the scanning. Screening patients with EBCT is convenient since there is no need for intravenous access, fluids or contrast material.

Coronary calcium scores have been archived for thousands of asymptomatic men and women allowing for the creation of a database of "normal values" based on age.(73) There are scores (age and gender stratified) which are believed to have clinical significance. (73) The development and progression of coronary atherosclerosis is gender dependent. (72). The cutoffs most often used are calcium scores of 0, <10, >10<100, >100 indicating no, low, intermediate and high risk for future cardiac events. Quintile scores have been determined for additional stratification of risk. (73) For example, a calcium score of 100 is at the 50th percentile for those at age 60, and at the 25th percentile for those who are 50 years old. (72)

The most significant limitation to EBCT is the correlation between coronary calcification and luminal narrowing. Essentially, calcium may be present which will lead to a high calcium score, but if the calcium is distributed circumferentially, the vessel lumen may not be significantly compromised. (72). Additionally, vulnerable plaques, frequently don't contain any calcium suggesting that a vulnerable plaque may not be detected via EBCT. (72) EBCT is not available to patients without the referral of a provider, is relatively expensive ($450) and not usually paid for by most third-party payers.

Women who are premenopausal have lower coronary calcium scores when compared to their male counterparts. Below the age of 50, it is uncommon to find coronary calcium scores

>10 in women, whereas 25% of men in the general public below the age of 50 years have scores > 10. (73) Women aged 50-59 continue to have scores significantly lower than men of the same age. Women aged 60-69 typically have scores similar to men who are 10 years younger. EBCT utilization is of greatest value among older, asymptomatic individuals. (72)

Both the American Heart Association and the American College of Cardiology publicly supported eight points of the 1996 Expert consensus on EBCT which include:

1. "A negative EBCT test makes the presence of atherosclerotic plaque, including unstable plaque very unlikely.
2. A negative test is highly unlikely in the presence of significant luminal obstructive disease.
3. Negative tests occur in the majority of patients who have angiographically normal coronary arteries.
4. A negative test may be consistent with a low risk of a cardiovascular event in the next 2-5 years.
5. A positive EBCT confirms the presence of a coronary atherosclerotic plaque.
6. The greater the amount of calcium, the greater the likelihood of occlusive CAD, but there is not a 1-to-1 relationship, and findings may not be site specific.
7. The total amount of calcium correlates best with the total amount of atherosclerotic plaque, although the true "plaque burden" is underestimated.
8. A high calcium score may be consistent with moderate to high risk of a cardiovascular event within the next 2 to 5 years." (72)

6.1.5 Cardiac disease and HIV-infection

Concerns about HAART-related atherosclerotic complications were amplified by initial results from the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) Study published in 2003, in which the authors concluded that the "the incidence of myocardial infarction increased with longer exposure to combination antiretroviral therapy" (69). DAD was an international collaboration that prospectively followed 23,437 HIV-infected subjects from Australia, Europe and the United States from December 1999 through April 2001. The study endpoint was the occurrence of a myocardial infarction (MI) during the observation window which lasted through February 2005. During this period, 93.6% of study participants were taking antiretroviral medications for a median of 6.9 years. The median exposure to protease inhibitors specifically was 4.0 years.(69)

MI was documented in 345 patients yielding an incidence of 3.65 per 1000 person-years and almost 1/3 of these (29.3%) were fatal myocardial. Among those suffering a fatal MI, median protease inhibitor exposure was 3.7 years. The incidence of MI or other ischemic vascular event increased directly with antiretroviral exposure, with a relative risk of 1.26 per additional year of therapy (95% confidence interval, CI, 1.12-1.41, $p<0.001$). (74) Risk increased in the presence of traditional cardiac risk factors such as older age, cigarette smoking, male sex, elevated serum cholesterol, elevated triglycerides and diabetes mellitus. Subsequent analysis determined that both protease inhibitors and non-nucleoside reverse transcriptase inhibitors increased risk with an incidence of 5.77 MI per 1000 person-years. (75)

One of the largest American studies, the HIV Outpatient Study (HOPS) reported an increased odds ratio for MI of 4.92 among those receiving protease inhibitors even after adjusting for other risks such as age, sex, cigarette smoking, hypertension and dyslipidemia.(76)

An alternative to documenting cardiac events is to document the development of subclinical atherosclerosis via EBCT in a cohort of HIV-infected men taking HAART. Three cross-sectional studies have investigated prevalence of coronary artery calcification (CAC) via EBCT among HIV-infected subjects. (77-79) Talwani, et al compared CAC scores among HIV-infected men older than age 40 years without a history of treated hyperlipidemia or coronary artery disease to HIV-negative age and race-matched controls to HIV-negative age, and race matched male controls. (77) Cases (n=60) included both HAART experienced and naïve subjects. Among HAART treated participants, the treatment duration was 25.9 months (range 6-61 months). CAC scores exceeding 0 for 4 coronary vessels were detected in 33% of cases and 39% of controls (p=0.26). Clinically significant CAC scores, defined as CAC total score for 4 vessels exceeding the 75th percentile, were documented in 18% of cases and 17% of controls. (77) Traditional risk factors predicted CAC in both cases and controls.(77)

Robinson and colleagues documented a higher proportion of subclinical atherosclerosis defined as increased coronary artery calcium, among treated HIV-infected men aged 30-60 years (n=27, mean age: 43.5) compared with HIV-negative age-matched controls (n=81). (78) Specifically, a higher proportion of HIV-infected men had CAC scores exceeding the 90th percentile compared to HIV-negative controls (p=.034). An important caveat, however, was that in this study, traditional atherosclerosis risk factors were not controlled for or matched. For example, nearly twice as many cases (81.5%) reported a smoking history compared to controls (41.7%). (78)

Lai et al attempted to document the effects specifically of protease inhibitors on lipid profiles, red blood cell indices, C-reactive protein levels, and coronary calcification among 98 HIV-infected African-American men and women. (79) Participants included both protease

inhibitor-treated patients (n-55, average age 39.3± 4.5 years) and those who were not (n=43, average age 37.8 ± 5.1years). Eighty-four percent of all subjects were current smokers. The only statistically significant finding with regard to coronary artery calcification was a small difference in the proportion of total calcium volume scores that exceeded 5 among non –PI users compared to users of nelfinavir ($p<0.05$) and ritonavir ($p<0.05$). (79)

To date, no longitudinal studies have evaluated coronary artery calcification among individuals treated for HIV-infection.

6.2 SPECIFIC AIM #4

Specific Aim #4, to document prevalence of CAC via EBCT among a cohort of HIV-infected men and document risk factors for progression of CAC, is addressed in a manuscript entitled, "Progression of subclinical coronary atherosclerosis (CAC) in HIV-infected men using highly active antiretroviral therapy (HAART)".

6.3 PROGRESSION OF SUBCLINICAL CORONARY ATHEROSCLEROSIS (CAC) IN HIV-INFECTED MEN USING HIGHLY ACTIVE ANTIERETROVIRAL THERAPY

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6.3.1 Introduction

Highly active antiretroviral therapy (HAART) has reduced AIDS incidence and mortality and has dramatically prolonged life expectancy of those infected with the human immunodeficiency virus (HIV). Despite the efficacy of HAART to suppress viral replication and promote immune reconstitution, HAART has been associated with a constellation of potentially pro-atherogenic metabolic derangements including hypertriglyceridemia (3,4,80-83), hyperglycemia (84), insulin resistance (85-89), and central obesity. (15,89) The long-term effects of these changes in HIV-infected individuals have not been fully explored, but in the general population those conditions are associated with microvascular and macrovascular changes resulting in atherosclerosis and impaired endothelial function. (10)

Several reports of myocardial infarction (MI) among protease inhibitor (PI)-treated patients without known coronary artery disease (CAD) risk factors were suggestive of increased atherosclerosis in HIV-infected patients. (90,91) . The HIV Outpatient Study (HOPS), one of the largest American studies, showed that the risk for MI was increased nearly five-fold among those with exposure to PIs, even after adjustment for age, sex, cigarette smoking, hypertension and

dyslipidemia. (76) The International Prospective Study of Cardiovascular Risk and Antiretroviral Therapy – Data Collection on Adverse Effects of Anti-HIV Drugs (D:A:D) Study reported an incidence of myocardial infarction among those not exposed to protease inhibitors as 1.53 per 1000 person-years and an incidence of MI among those treated with protease inhibitors of 6.01 per 1000 person-years (75). Investigators noted the incidence of MI increased proportionally with the duration of exposure to protease inhibitors, with an increased relative risk of 1.16 (95% CI, 1.10-1.23) per additional year of therapy. (75)

Coronary artery calcification (CAC) assessed by electron beam tomography (EBCT) can provide a sensitive and replicable measure of subclinical coronary atherosclerosis that strongly predicts subsequent cardiovascular disease. (71,72) EBT is non-invasive, and can detect CAC in the absence of associated vascular stenosis or occlusion. Therefore, it can detect subclinical disease and is ideal for screening asymptomatic patients. No studies have prospectively assessed CAC in HIV-infected patients treated with HAART. The purpose of this study was to examine risk factors for progression of CAC among HIV-infected men treated with HAART over an approximate three year period.

6.3.2 Subjects

Study participants were recruited from those participating in the Multicenter AIDS Cohort Study (MACS) at the University of Pittsburgh Graduate School of Public Health site. The MACS rationale and design has been described elsewhere. (61) Briefly, the study began in 1984 to evaluate the natural history of HIV-infection among gay and bisexual men. The study population includes both HIV-positive and HIV-negative men. Participants are seen every six months for completion of questionnaires (demographics, health and sexual behaviors, medical diagnoses and health care utilization) physical examination, and laboratory evaluation, including T-lymphocyte subset analysis, HIV RNA testing, metabolic and lipid testing.

Two hundred and twelve men completed a clinic visit between October and March 2001; 117 of whom were HIV-infected. Of the 117 potential participants for this substudy, 80 (68.3%) were enrolled. Eligibility criteria included infection with HIV, age > 35 years, negative personal history of CAD, and ability to provide written consent. Those not enrolled included men who had moved away (n=11), those that could not be contacted by phone (n=9), those with work conflicts that did not permit participation (n=8), those who declined participation (n=3), and those who did not meet eligibility criteria due to age (n=2), terminal illness (n=1), or preexistent CAD (n=3). The study was approved by the Institutional Review Board at the University of Pittsburgh and all participants gave written consent.

6.3.3 Data collection

Data regarding cardiovascular risks factors, including personal smoking history and family history of first-degree male relatives with CAD was elicited through self-administered questionnaire and chart review. Self-reported medication histories were used to determine participants' use of lipid-lowering agents and to determine cumulative exposure to HAART. For

the purpose of this study, HAART was defined as a regimen consisting of two nucleoside reverse transcriptase inhibitors and a protease inhibitor or two nucleoside reverse transcriptase inhibitors and a non-nucleoside reverse transcriptase inhibitor.

Diagnostic testing was performed upon enrollment and included fasting lipid testing or, for those unable or unwilling to fast, LDL-direct determination. Dyslipidemia was defined as total cholesterol > 240 mg/dL, high density lipoprotein (HDL) < 40 mg/dL, low density lipoprotein (LDL) > 160 mg/dL, or triglycerides > 200mg/dL.

High resolution, non-contrast electron beam tomography (EBCT) images of the heart, coronary arteries, and abdominal aorta were obtained both upon enrollment and at a median follow-up of 2.7 years utilizing an Imatron C-150 scanner. Coronary artery calcium scoring was performed according to standard protocol. Total CAC scores were generated using a Base Value Region of Interest computer program and incorporating the area and grade of calcification. All results were reviewed and interpreted by the same attending cardiologist who has expertise in this modality.

6.3.4 Statistical considerations

Summary statistics were determined for all variables of interest. Statistical differences in baseline characteristics, baseline lipid testing, and the use of lipid-lowering medications across (the three) categories of baseline CAC scores were assessed via the Kruskal-Wallis test (continuous variables) or chi-square test (categorical variables). The Kruskal-Wallis test was used in favor of the analysis of variance since some of the distributions were moderately to highly skewed. Statistical differences in characteristics of progressors versus non-progressors were assessed using the Wilcoxin rank-sum test (continuous variables) or chi-square test (categorical variables). Relationships between each factor and progression of CAC were

assessed via logistic regression, where progression of CAC was defined as a 1.5 increase of baseline CAC. For purposes of calculating the ratio of follow-up to baseline CAC, a value of 0.1 was imputed when the baseline was zero (since any change divided by zero would be undefined). The logistic model was used to estimate the odds ratio for each factor, initially without adjustment for other factors. All variables which showed at least marginal significance (i.e. $p<0.20$) with progression were included in the multivariate model.

6.3.5 Results

Characteristics of Study Population. All participants were HIV-infected men. At baseline, the median age of participants was 44.7 years (inter-quartile (IQ) range 42.2, 49.8 years). All but two participants were Caucasian (two African-American) and thirty-seven men (46.2%) were current or ex-smokers. Median CD4+ cell count was 619 cells/mm^3; median HIV RNA was < 50 cells / mm^3. Seventy-nine (98.8%) were taking HAART at baseline. The only subject not treated with HAART upon enrollment started nine months later. At baseline, the median duration of HAART was 5.01 years (IQ range 3.95, 5.68 years). Subjects were asked to identify the presence of CAD in first-degree relatives, with the majority denying such history.

Baseline Data. Of the 80 participants, 38 (47.5%) had a baseline total CAC score of zero. Nineteen (23.7%) had a CAC between 0 and 10, and 23 had CAC scores greater than 10. Only five individuals (6.2%) had CAC scores in excess of 100. Characteristics of study population are stratified by baseline CAC score in Table 12. The aortic calcium volume scores were highly skewed with a median of 15 and IQ range of 0.0, 156.5 (total range 0.0, 5612). The highest median aorta calcium score was seen in the group with CAC scores >10. Sixty percent of 23 subjects with CAC scores exceeding 10 were either current or ex-smokers; this was the highest proportion of smokers among the three groups stratified by CAC scores (0, <10, >10).

Serum lipid results at baseline are provided in Table X. Sixty-three participants had dyslipidemia as defined by elevated lipids or by the use of one or more lipid-lowering medications at baseline. The median total cholesterol was 202 mg/dL (IQ range 187.3, 239.3 mg/dL); 20 (25%) participants had levels in excess of 240 mg/dL. The median HDL was 39.6 mg/dL (IQ range 33.5, 45.6 mg/dL); 41 (51.2%) had levels lower than < 40 mg/dL. LDL-C was determined for 53 participants and the median level was 114.5 mg/dL. LDL-D was determined for 19 participants and the median level was 119mg/dL. Seventeen men had an LDL level >160 mg/dL. Among the sixty-five participants who had triglyceride levels measured, the median was 202 mg/dL (IQ range 118, 326 mg/dL). Serum triglyceride levels exceeded 400 mg/dL in 12 men including three men with extreme hypertriglyceridemia, defined as a triglyceride level greater than 1000 mg/dL. At baseline, twenty-two participants (27.5%) were taking lipid-lowering medications; six of these were taking 2 medications. Specific medications included: HMG-CoA reductase inhibitor (n=14; atorvostatin was used by 12/14), fibrate (n=6), niacin (n=1) and one was unspecified. The use of lipid-lowering medication was highest among those in the highest CAC category (43.4%). (Table 13)

Follow Up Data. After a median follow up time of 2.7 years, 73/80 (91.2%) of study participants underwent repeat lipid testing and EBT assessment. Follow up data were not available for seven participants due to death (N=4, all from non-cardiac causes), study withdrawal (n=1) and loss to follow up (n=2).

Follow-up laboratory testing revealed a median HIV RNA < 50 cells/mm^3 and a median CD4+ cell count of 590 cells/mm^3.

At follow-up 36/73 (49.3%) had CAC score exceeding 10, compared to 23/80 (28.7%) at baseline. Among 34 participants with baseline CAC score of 0, 25 (73.5%) has a score of 0 at

follow up. The remaining nine had a median CAC score of 19.6 and 6 of 9 had a follow-up CAC score > 10. Plots of the baseline CAC (horizontal axis) and follow-up CAC scores (vertical axis) are shown (Figure 2,3,4).

Progression of existing CAC (i.e. > 0) was defined as a 1.5 CAC increase. Based on our definition, thirty-eight subjects (52.0%) were "progressors" and thirty-five were identified as "non-progressors". "Progressors" and "non-progressors" were similar with regard to serum lipids, exposure to HAART, family history, and use of lipid-lowering medications. (Table 14)

Univariate analysis explored the relationship of a range of variables to CAC progression. (Table 15) Variables with a marginally significant relationship to progression (i.e., $p<0.20$) were considered in the multivariate model; these included baseline CAC, smoking, family history and aorta volume score. Analysis using only progression as the outcome of interest, revealed an odds ratio (OR) for age of 1.06 per year ($p=0.05$), equivalent to an approximate doubling in 10 years (OR 1.98). The OR for current smoking was 2.58 ($p=0.08$). (Table 15) Use of lipid-lowering medication was associated with lower odds of CAC score doubling, however was not significant.

6.3.6 Discussion

In this male population with well controlled HIV-infection, chronic use of HAART did not impact the progression of sub-clinical coronary atherosclerosis as measured by EBT. In contrast, traditional atherosclerosis risk factors of smoking and advancing age, were borderline predictive of subclinical coronary atherosclerosis. The median duration of treatment with HAART in this population was in excess of 5 years (at baseline), longer than that of participants in the three previous studies utilizing EBCT in HIV-positive populations. (77-79) Talwani and colleagues compared the CAC scores of HIV-infected men (n=60), aged 40 years and older (mean 47; range 40-66 years) to age and race-matched male HIV-negative controls (n=180). (77) Subjects in

either arm had no history of treated hyperlipidemia or coronary artery disease; those in the HIV-positive arm include both HAART-exposed (mean treatment duration 25.9 months) and HAART-naïve. CAC, defined as any score greater than 0 for 4 vessels, was detected in 33% of HIV-infected cases and 39% of controls (p=0.26). (77) Clinically significant CAC, as defined as a total score > 75th percentile, was documented in 18% of cases and 17% of controls, revealing no significant difference. (77) No significant differences in either total or age-specific CAC scores were found between pairs of cases receiving protease inhibitors and their controls. In both study arms, traditional coronary risk factors did predict the presence of CAC. (77) Conflicting results were reported by Robinson in a smaller study of CAC in treated HIV-infected patients. (78) In this work, 22% of HIV-infected men (n=27, mean age 43.5. range; 30-60 years) had CAC scores > 90th percentile compared to 7% of HIV-negative age-matched controls (n=81, p = .034).(78) This study, however, was limited by lack of adjustment for traditional CAD factors. Among HIV-infected patients, smoking rates exceeded 81% compared to a smoking rate of 41.7% in the control group. (78)

In a study conducted among HIV-infected African-American men and women (n=98), Lai and colleagues compared lipid profiles, red blood cell indices, C-reactive protein levels, and CAC in patients taking PI (n=55, mean age 39.3, +/- 4.5 years) and those who were not (n=43, mean age 37.8 +/- 5.1 years). (79) The only statistically significant finding was a small difference in the proportion of total calcium volume scores > 5 among non –PI users compared to users of nelfinavir ($p<0.05$), ritonavir ($p<0.05$) or saquinavir ($p<0.05$). (79) No significant differences were found in the proportion of total calcium volume scores > 5 among non-PI users compared to users of indinavir, lopinavir or saquinavir/ritonavir combination. (79) Our study is

the first to prospectively document CAC in men treated with highly active antiretroviral therapy for HIV-infection.

Dyslipidemia at baseline was relatively common among our cohort 63/80 (78.8%), which would be expected in this population given the duration of HAART. As concern about HAART-induced dyslipidemia on cardiac outcomes has increased, clinicians have been more aggressive to recommend lipid-lowering therapy. (4) Our data reflect this trend, as 27.5% of our subjects were on lipid- lowering medications at baseline and half (42.5%) of these had a baseline CAC exceeding 10. The true relationship, however, between dyslipidemia and CAC may be confounded by treatment with lipid lowering medications. The duration of treatment with the lipid lowering medications was not quantified and may further impact these results.

CAC scores between 0 and 10 generally indicate minimal risk for future cardiac events (24) therefore, we chose CAC scores of zero, <10 and >10 as our study stratifications. The prevalence of clinically significant CAC scores (CAC >10) was only 23/80 (28.7%) and was associated with age and family history of cardiovascular disease in our univariate analysis. The prevalence of clinically significant CAC scores in our study was similar that described by Talwani, et al, who documented "detectable" CAC (defined as CAC > 0) in 33% of HAART-treated HIV-infected subjects (n=60).(21) A notable difference, however was that the mean duration of HAART treatment in the Talwani study was only 25.9 months, significantly less than the mean duration of 4.69 years at baseline of this study (median 5.01 years). (77)

In our study, five individuals had CAC scores exceeding 100, a level that confers significantly increased cardiac risk (92). Of these five, follow-up scores were available for four, and calcium volume scores ranged from 267-597. These increases are consistent with reports of rapid atherosclerotic progression once sub-intimal inflammation and calcification begins.

The design of this study allowed for investigation of incidence of CAC over a period of 2.7 years. Fifty-two of the 57 participants with baseline CAC less than 10 had follow- up scans. Follow-up CAC scores exceeding 10 were found in 15 participants, yielding an incidence of clinically significant CAC of 28.8% over a 2.7 year period (Figure 1).

Research regarding calcification of the thoracic or abdominal aorta has not been as extensive as the coronary arteries using EBCT, and generally focuses on subpopulations with existing cardiovascular risk factors, such as dyslipidemia (93) or cardiovascular disease equivalent such as diabetes mellitus. (94). The process of atherosclerosis occurring simultaneously in multiple vascular beds is common, and patients with coronary artery disease often have peripheral and cerebral vascular disease. (95,96) In our study, the baseline abdominal aorta calcium score was at least marginally significant on a univariate scale (0.03 with rank-sum test and 0.10 with the logistic model), but was also correlated with the potentially significant predictors of age and baseline CAC, (with correlation coefficients of 0.45 and 0.66 respectively). It did not affect results of the presented multivariable models. These findings are similar to a previous study by Post and colleagues, who documented a strong association between increasing age and thoracic aortic calcification among a cohort of 614 Amish subjects. (97) Our results do not support the finding of abdominal aorta calcification as an independent predictor of progression of coronary calcification, which may be attributable to our small sample size and/or the variance of the aorta calcium scores.

This prospective study design allowed for identification and description of characteristics associated with CAC score progression. Progressors and non-progressors were similar in the majority of characteristics except smoking and age. HAART appeared to play no role in CAC

score progression, and in fact, the median duration of HAART treatment was *longer* among non-progressors (5.15 years) than progressors (4.8 years).

Duration of HAART was not associated with either prevalent or incident CAC. This finding contradicts previous work by Robinson et al that found a modestly elevated level of CAC in HIV-infected patients treated with HAART, compared to HIV-uninfected controls. (78) Robinson's study, however, did not control for smoking which varied significantly between the HIV-infected subjects (81.5%) and the controls (4.7%). (78).

Our study is limited in three important ways. It excluded women. Despite the increasingly recognized relevance of gender-related differences in CAD risk factors and outcomes, our study could not address this. Additionally, our overall sample size was small, as only 73 subjects had both baseline and follow up data. Larger prospective studies have now been initiated at all four MACS study sites. Finally, our study was limited by the relatively small numbers of participants with baseline CAC scores in excess of 100, as CAC progression is most clinically significant among those with higher baseline scores.

Shortly after protease inhibitors therapy became available, accounts of myocardial infarctions surfaced among HAART-treated patients without traditional CAD risk factors. These accounts prompted suspicion of accelerated atherosclerosis and increased cardiovascular morbidity and mortality in individuals treated for HIV-infection. As methods to measure sub-intimal calcium have become available and as such calcium accumulation has become correlated with the risk for future cardiac events, prospective studies have tried to evaluate the presence of CAC among those with HIV infection, with particular emphasis on those treated with HAART. Three such previously published cross-sectional studies of CAC scores in HIV-infected subjects have provided conflicting results. (77-79) No prior studies, however, have published data

regarding the progression of CAC in HIV-infected subjects. Our data do not support indicate that chronic exposure to HAART promotes the development or progression of subclinical atherosclerosis. Instead, our data indicate that the strongest risk factors for CAC score progression are increasing age and smoking, reinforcing the need stay vigilant in recommending and assisting with smoking cessation for all patients.

Table 12. Baseline characteristics stratified by baseline CAC scores

	Overall N=80	CAC= 0 N=38	CAC < 10 N=19	CAC > 10 N=23
Age (years) Median [IQR]	44.7 [42.2, 49.8]	43.8 [41.8, 46.9]	44.6 [40.8, 47.5]	49.8 [44.5, 55.5] p=0.0016
Duration of HAART (years) Median [IQR]	5.01 [3.95, 5.68]	5.11 [3.69, 5.62]	4.44 [3.78, 5.47]	5.57 [4.66, 5.75] p=0.2571
CD4+ count (cells / mm^3) Median [IQR]	619 [409, 885]	618.5 [385.2,883]	535 [403, 928]	679 [480, 957] p=0.7505
HIV Viral load (cells / mm^3) Median [IQR]	50 [50, 641]	50 [50, 50]	50 [50,50]	50 [50, 1400] p=0.9792
Positive family history of CAD (%)	29/79 ^^ 36.7%	11/38 28.9 %	4/18 22.2%	14/23 60.8% p=0.01507
Ever Smoked (versus Never) (%)	37/80 46.2 %	15/38 39.4 %	8/19 42.1 %	14/23 60.8 % p=0.2456
Abdominal aorta calcium volume score Median [IQR]	15 [0.0, 156.5]	2.41 [0.0, 19]	19 [0.0, 85.5]	191.0 [31.86, 739]

^^ Only 79 men could relate family history

Table 13. Baseline Lipid Testing and use of lipid lowering medication stratified by baseline CAC scores

	Overall N=80	CAC = 0 N=38	CAC <10 N=19	CAC >10 N=23
Total Cholesterol (mg/dL) Median [IQR]	202.0 [187.3, 239.3]	204.0 [179.8, 252.5]	204.0 [195,235.5]	197 [183.0, 223.0] p=0.4993
HDL (mg/dL) Median [IQR]	39.6 [33.5, 45.6]	40.4 [33.8, 46.0]	39.9 [37.4, 49.3]	38.9 [32.0, 43.2] p=0.5947
LDL (mg/dL) Median [IQR]	114.5 [96.8, 148.3]	128.0 [98.0, 167.0]	114.5 [107.0, 143.0]	107.0 [93.0, 137.0] p=0.2818
Triglycerides (mg/dL) Median [IQR]	202.0 [118.0, 326.0]	171.5 [111.3, 266.5]	240.5 [172.0, 480.8]	262.0 [116.0, 394.5] p=0.2284
N (%) taking lipid lowering medications	22/80 27.5%	5/38 13.1%	7/19 36.8%	10/23 43.4% p=0.0213

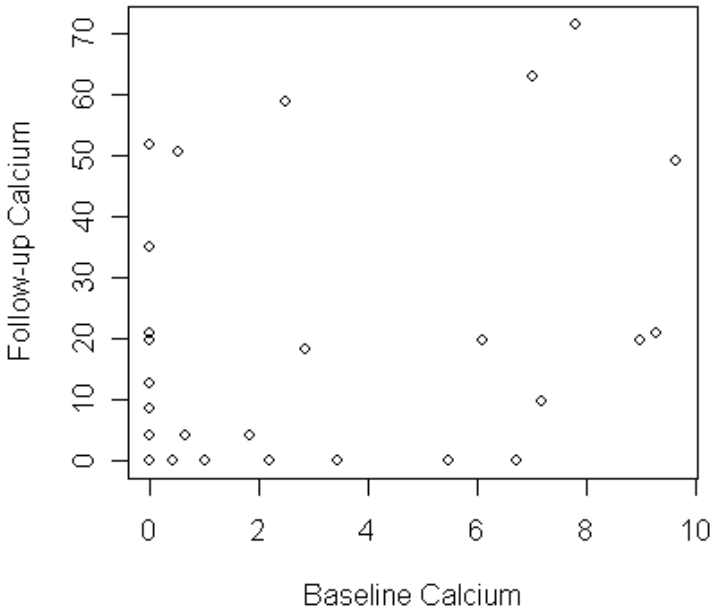

Figure 2. Plot of follow-up by baseline calcium levels – baseline levels < 10

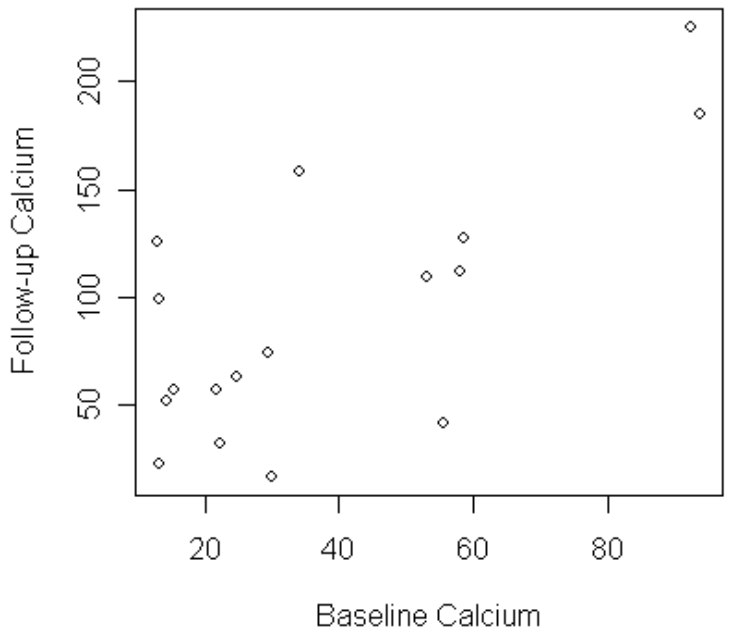

Figure 3. Plot of follow-up by baseline calcium levels – baseline levels ≥ 10 but < 100

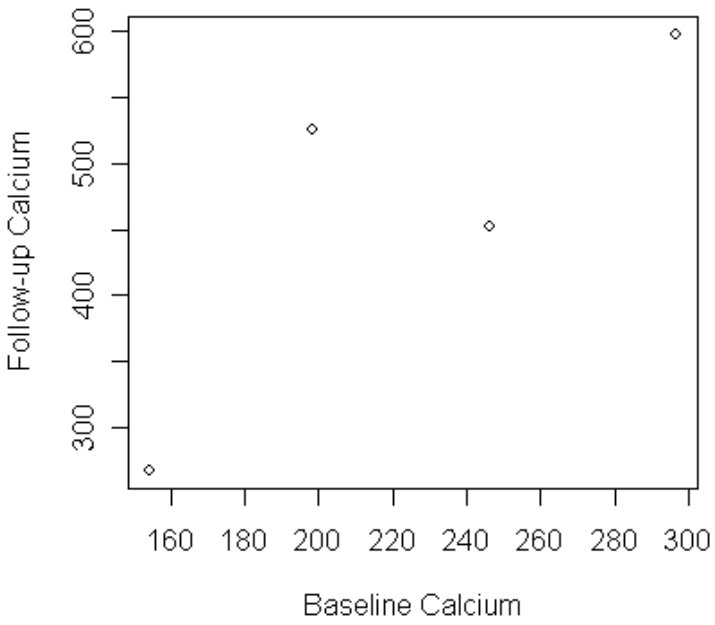

Figure 4. Plot of Follow-up by baseline calcium levels – baseline levels ≥ 100

Table 14. Characteristics of progressors and non-progressors

Variable	Progressors N=38	Non-progressors N=35
Age (years) Median [IQR]	46.03 [43.38, 52.34]	44.06 [40.87, 48.36] p=0.04447
Cholesterol (mg/dL) Median [IQR]	197.5 [188.0, 225.5]	202.0 [179.0, 241.0] p=0.7196
HDL (mg/dL) Median [IQR]	38.45 [32.38, 45.08]	42.10 [37.50, 47.85] p=0.1182
LDL (mg/dL) Median [IQR]	113.0 [96.0, 143.0]	119.50 [96.25, 149.80] p=0.6087
Triglycerides (mg/dL) Median [IQR]	222.5 [118.8, 417.8]	189.0 [112.5, 280.0] p=0.4423
Duration of HAART (years) Median [IQR]	4.865 [3.688, 5.678]	5.150 [4.105, 5.635] p=0.7114
Baseline CAC score Median [IQR]	9.4750 [0.5475, 33.100]	0.000 [0.00, 0.725] p=0.00001132
Current smokers N (%)	21/38 55.2 %	11/35 31.4 % p=0.07609
Positive family history of CAD N (%) ** unknown family history for one non-progressor	17/38 44.7 %	10/34 ** 28.5 % p = 0.2726
Users of lipid-lowering medications N (%)	11/38 28.9 %	9/35 25.7 % p = 0.9627
Abdominal aorta calcium volume score Median [IQR]	39 [3.66, 272.2]	4.0 [0.0, 58.5] p=0.031

Table 15. Unadjusted logistic models for predicting a 1.5 fold increase in CAC scores

Variable	*Unadjusted Odds Ratio*	*P-value*
Age	1.1065	0.0217
Cholesterol	0.998	0.628
HDL	0.985	0.428
LDL	0.994	0.300
Triglycerides	1.0009	0.375
Duration on HAART	0.981	0.911
Baseline Calcium Score	1.0558	0.0227
Ever Smoking	2.7999	0.0397
Family history of CVD	1.943	0.182
Lipid lowering meds	1.1769	0.757
Abdominal aorta score	1.0015	0.106

Table 16. Multivariable logistic model for predicting a 1.5-fold increase of CAC scores

Variable	*Adjusted Odds Ratio*	*P-value*
Age	1.068 (10 year OR 1.98)	0.053
Smoking	2.568	0.089

7.0 FINAL SYNTHESIS AND CONCLUSIONS

There is no dispute that highly active antiretroviral therapy (HAART) has dramatically decreased the rates of opportunistic infections, delayed the diagnosis of AIDS and improved life expectancy in those with HIV-infection. Initially, the immediate need to suppress the viral burden and improve immunologic status was paramount and considerations of the long-term consequences of these therapies were of minimal importance. However, with increasing follow-up times, clinicians now know that these therapies are associated with substantial atherogenic lipid changes and it is possible that they will cause significant morbidity and mortality related to accelerated atherosclerosis. Clinicians have become vigilant in screening for, and treating induced metabolic toxicities even in the absence of physical changes as they are likely to precede the physical lipodystrophic findings.

Treatment for HIV-infection is life-long. As such, the adverse effects of such therapy have become a growing public health concern. Although HIV–LS syndrome has just recently been acknowledged, it has already impacted trends in treatment, antiretroviral adherence and the physical appearance of those affected. Whereas AIDS wasting syndrome had some treatment options, (albeit not always successful) of high calorie diets, appetite stimulants, human growth hormone, enteral tube feedings, and total parenteral nutrition, HIV-LS has no recognized treatment. Savvy clinicians know that substitution of stavudine or zidovudine with abacavir can

improve lipoatrophy; (98) other than that, patients are left only with strategies of concealment rather than treatment for HIV-LS. Within the MACS, we've heard several anecdotal accounts of the aesthetic and cosmetic effects of the body habitus changes. Those most severely affected attempt to hide parts of their bodies because they fear recognition of their HIV-infection by others. Men have explained how they avoid wearing shorts in public and intentionally grow facial hair to minimize the appearance of their facial lipoatrophy. Photographing the body habitus changes has validated self-reports of both the development of, and worsening of, the syndrome. In the most extreme cases, patients have pursued surgical intervention for their HIV-LS. Unfortunately, such intervention is not curative and most often provides only temporary improvement. One Pitt Men's Study participant underwent liposuction of his growing dorsocervical fat pad, only to have the fat pad return several months later. Over the past several years, Pitt Men's Study participants with HIV-LS have graciously consented to photography knowing we were documenting the abnormal distribution of body fat as a consequence of treatment for HIV-infection. These photographs are now being used to improve the consistency and reliability of data collected MACS-wide. Clinicians, researchers and patients are eager to document the long-term effects of some of the newer antiretroviral medications. Hopefully, the negative effects on lipid profiles and related physical lipodystrophic changes will be fewer than with the earlier antiretroviral medications.

HAART induced dyslipidemia has been well described and clinicians now monitor serum lipids and treat with lipid lowering medications as indicated. Data from the MACS support this trend in treatment with a report of 34% of subjects prescribed lipid-lowering medications. (1) In our study utilizing EBCT, we documented dyslipidemia in 78% of subjects. Twelve subjects had serum triglycerides > 400 mg/dL, including three with levels in excess of 1,000mg/dL.

Pharmacologic treatment for hyperlipidemia was also common, with 22 participants treated at baseline. Effective management of antiretroviral induced hyperlipidemia will continue to be a challenge as HIV-infected patients enjoy delayed progression to AIDS with longer life expectancy.

Fasting hyperglycemia and insulin insensitivity are also important components of HIV-LS, and their prevalence have been widely documented. (3,13,22,24,25) Although the processes of hyperglycemia and insulin resistance are separate from hyperlipidemia, they are synergistic with regard to the development and progression of atherosclerosis. The extent to which atherogenesis is a complication of HIV-LS is still unknown. The current concern of accelerated atherosclerosis (and subsequent morbidities of cerebrovascular disease, coronary artery disease and peripheral vascular disease) is real. Among the general public, these vascular conditions generally commence at the same time, although the clinical manifestations may not appear simultaneously because of vessel size, development of collateral vessels and oxygen demands of the distal tissues. How these vascular conditions progress in the presence of a chronic immune activation due to HIV-infections remains unknown. The effect of HIV-infection and its treatment on cardiac risk is being explored in many studies. These studies vary widely in their outcome of interest and include myocardial infarction, coronary calcification and/or angiography findings. Despite the differences in outcomes, traditional risk factors of family history and smoking are consistently found to affect cardiac risk. Traditional advice to lower cardiac risk may not be feasible among those infected with HIV. For example; a diet low in saturated fat may be recommended for "heart health" yet the HIV-infected patient may be struggling to maintain body weight. Or, peripheral neuropathy may limit opportunities for aerobic exercise.

My EBCT study endorsed two important concepts of atherosclerosis. First, is how the process of atherosclerosis occurs simultaneously in multiple vascular beds. The subject with the highest abdominal aorta score at baseline (5612) was one of only five men who had a baseline CAC score > 100. Similarly, the subject with the highest baseline CAC score had a baseline aorta volume score of 1596. The other important concept illustrates that once the process of atherosclerosis begins, it continues to progress. The patient with greatest CAC score at baseline (297) also had the greatest score at follow-up (598). Both of these scores suggest likelihood of a future cardiac event.

The findings in my study utilizing EBCT are similar to findings found MACS-wide. Specifically, Kingsley and colleagues did <u>not</u> find that chronic use of HAART (>8 years) was associated with an increased prevalence of CAC>10 Agatston units. (99) Rather, this study found traditional cardiovascular risk factors of increasing age and smoking as contributors to the development of subclinical atherosclerosis. Kingsley's study had a larger cohort and was also able to identify increased LDL, hypertension and decreased HDL as predictors of risk. (99) Findings in our EBCT study, and the MACS study, are similar to results documented by Depairon who used ultrasound detected thickening of peripheral arteries as a surrogate for coronary artery disease.(100) In that study, a multivariate logistic regression analysis revealed age and cigarette smoking as significant risk factors and not exposure to protease inhibitors. (100) Some of the largest prospective studies in the world have documented increased risk for myocardial infarction. The MACS is now investigating additional measures of atherosclerosis. They are prospectively measuring the intima-media thickness of the carotid arteries and repeating EBCT imaging for the coronary vasculature. The three-year follow-up for that study will be completed in September 2008.

Early studies showed associations between exposure to protease inhibitors and myocardial infarctions. (10,31) One of the most recent studies evaluated cardiovascular risk among patients with HIV-infection and documented a relative risk (RR) of 1.75 (95% confidence interval 1.51-2.02; $p<0.0001$) of acute myocardial infarction after adjusting for age, gender, race, hypertension, diabetes mellitus and dyslipidemia. (101) The RR was even higher for women in the gender-stratified analysis (RR 2.98 confidence interval 2.33-3.75 $p<0.0001$). It is not known why hard outcomes continue to be so strongly associated with exposure to antiretroviral medications, while subclinical atherosclerosis is not. Perhaps the incubation time has not been long enough or maybe the myocardial infarctions related to HAART are the result of sudden complete arterial occlusion that is not preceded by sub-intimal calcification. This speculation warrants further investigation.

Historically, HIV related morbidity and mortality was from opportunistic infections and malignancies. It is reasonable to assume that HIV/AIDS will present ongoing challenges of viral resistance and new challenges long-term complications of antiretroviral therapy. In this work, we have addressed our specific aims and raised several new questions. Questions such as: are sub-intimal changes simultaneously occurring in other vascular beds, such as the peripheral vascular system or cerebrovascular system? How will peripheral and visceral adipocytes respond to 30 years + exposure to HAART? How will insulin sensitive tissues respond to 30 years + exposure to HAART? Does HIV-LS phenotype III occur as natural progression of lipohypertrophy? And finally, how many other phenotypes of HIV-LS exist?

APPENDIX A

Documenting HIV-associated Lipodystrophy Syndrome (HIV-LS) within the Multicenter AIDS Cohort Study

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Graduate School of Public Health
Multicenter AIDS Cohort Study (MACS)

Introduction

HIV-associated lipodystrophy syndrome (HIV-LS) was first described in 1998 and involves a constellation of findings including hyperglycemia, insulin resistance, hyperlipidemia and body habitus changes.

Unfortunately, no strict diagnostic criteria have been established, leaving documentation of the syndrome to the somewhat subjective patient self-report and/or clinician report.

HIV-Lipodystrophy Syndrome

Whereas AIDS wasting syndrome has been associated with immunosuppression and high viral burden, HIV-LS has been documented with immunocompetence and suppressed viral concentration.

Defining HIV-LS

HIV-LS consists of both lipoatrophy and lipohypertrophy.

Lipoatrophy involves the face (temporal area and cheeks) and extremities while the lipohypertrophy involves the breasts, dorsocervical fat pad and visceral fat of the abdomen.

Defining HIV-LS

Clinicians within the MACS evaluate each subject during every clinic visit for the presence and severity of HIV-LS via self-report and physical examination.

The severity of HIV-LS has been quantified by a scale using mild/moderate and severe descriptors for each of the affected body areas.

Mild is documented for changes that are evident to clinician with close inspection; **moderate** is used for changes that are evident to clinician without specifically looking for them; and **severe** reflects changes that are evident to a non-medical person by casual observation.

Defining HIV-LS

HIV-LS has been described within the MACS via three phenotypes.

Phenotype I involves lipoatrophy only, whereas **Phenotype II** involves mixed findings of peripheral fat loss and visceral fat accumulation. Recently, **Phenotype III** has been proposed which involves supra-diaphramatic accumulation of adipose tissue which extends laterally from the breasts.

HIV-LS

Using prospectively collected data, HIV-LS cases have been deemed as “stable” referring to individuals who are consistently observed with the same phenotype, “developmental” referring to individuals initially unaffected (or mildly affected) and on subsequent examination are found to be developing lipodystrophic changes, or “transitional” referring to progression from one phenotype to another.

HIV-LS

The quantification of lipodystrophic changes among MACS participants have been used in conjunction with standardized anthropometric measurements (NHANES III: including height, weight, arm, thigh, waist and hip circumferences) and laboratory testing for metabolic abnormalities (lipid profiles, glucose, insulin and glycosolated hemoglobin).

Defining HIV-LS

Body habitus changes can be difficult to appreciate due to the natural variation of body shape, contour and size. As such, the appearance of some individuals in the general population may initially be perceived as "mild lipodystrophy" as a consequence of weight gain or aging.

However, with close inspection and careful consideration, experienced clinicians are able to detect true lipodystrophic changes even when they are subtle.

Purpose

The purpose of this manual is to provide examples of mild, moderate and severe lipodystrophic findings in order to establish consistency of assessment of HIV-LS among all four of the MACS sites.

Performing the HIV-LS Assessment

General Instructions

In order to collect the most reliable data, assessment for HIV-LS should be performed on every MACS participant regardless of HIV serostatus.

The assessment should begin with the self-report questionnaire. Subjects are initially asked if they have noticed any change in the amount or distribution in body fat in the last 6 months or since their last MACS visit. If a change has been noticed, subjects are asked about specific body areas affected by lipodystrophy, and current severity of fat loss / gain in those areas.

Note that subjects are to describe the current severity of each area (as mild, moderate or severe) and not the degree of change since last visit.

Subjects are also asked about behaviors that may influence their body fat such as recent changes in dietary or exercise habits, use of growth hormones or steroids, change in HIV-treatment medications, or cosmetic surgery.

Inspection

Inspection must be completed with the body area fully exposed. The subject should be instructed to relax, and to avoid adapting or changing their posture or physique.

Inspection of the breasts, abdomen and buttocks should be done at eye-level.

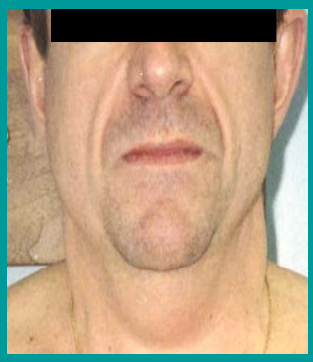
Inspection of the abdomen for the presence of visceral fat can be facilitated by also having the individual supine while inspecting for "bulging" of the flanks.

Facial Lipoatrophy

Lipoatrophy of the face is evident in the temporal areas and nasolabial folds. The sunken appearance of the face can be appreciated in the frontal and side-views.

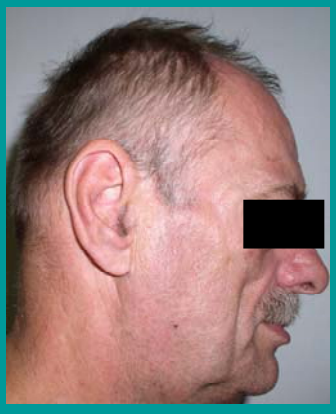
Mild Facial Lipoatrophy

- Note the pronounced nasolabial folds.



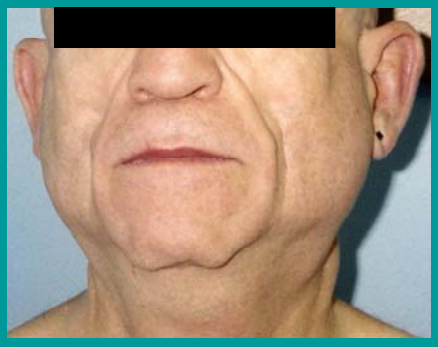
Moderate Facial Lipoatrophy

- As lipoatrophy of the face progresses, the prominence of the nasolabial folds fades and hollowing of the cheeks occurs just below and anterior to the zygomatic arch.



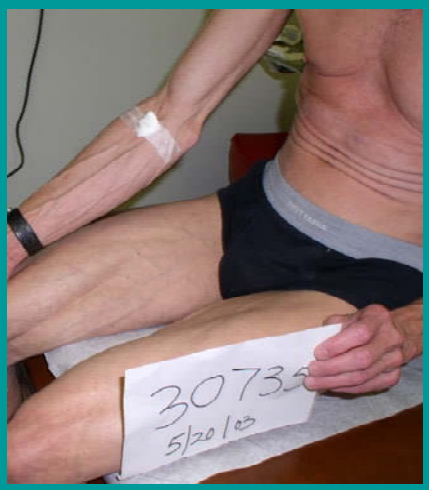
Severe Facial Lipoatrophy

- Severe facial lipoatrophy will be evident with both hollowing of the cheeks and a depression of the temporal region.



Lipoatrophy of Extremities

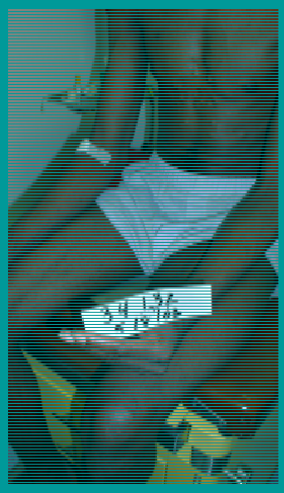
- Lipoatrophy of the extremities becomes evident with:
  - venous prominence
  - obvious bony landmarks and
  - increased muscle definition without an increase in muscle bulk.



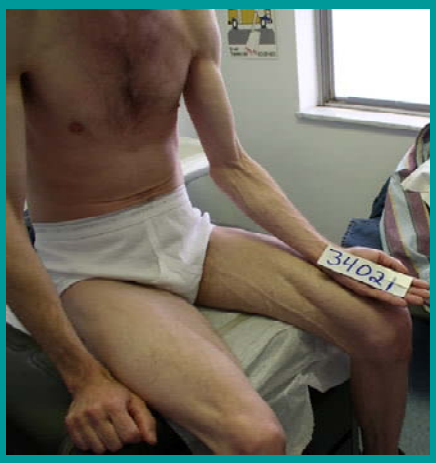
Mild Lipoatrophy of Extremities

- The venous prominence in this photograph suggests mild lipoatrophy of the extremities.

Moderate Lipoatrophy of the Extremities



Severe Lipoatrophy of the Extremities



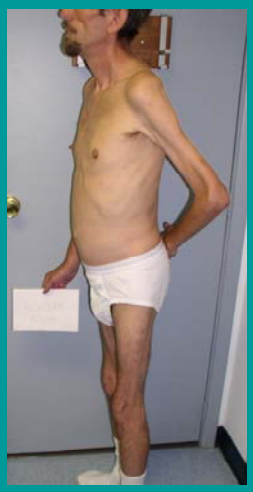
Severe lipoatrophy of the upper extremity

- This photograph clearly shows the loss of subcutaneous fat of the shoulder and arm.
- Facial atrophy is also evident in this participant.

Severe Lipoatrophy of Upper and Lower Extremities

- Fat loss of the extremities involves simultaneous loss of the arms and legs.

- This photograph clearly documents the severe lipoatrophy of the face, upper and lower extremities.

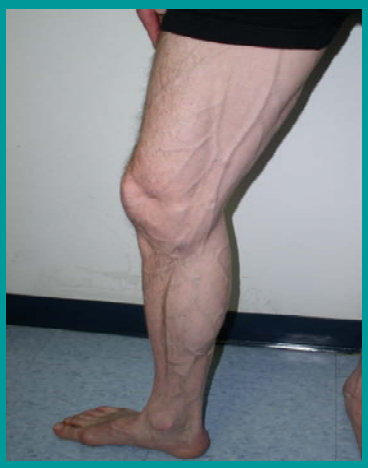


Deep Depression of the Medial Thigh

- Subcutaneous fat normally accumulates at the medial thigh. As lipoatrophy of the lower extremity progresses, a deep depression develops medial to the vastus medialis and adductor magnus as shown here.

Severe Lipoatrophy of the Legs

- Venous prominence is a characteristic finding in subcutaneous fat loss of the extremities as experienced by this subject.



Lipoatrophy of the Extremities

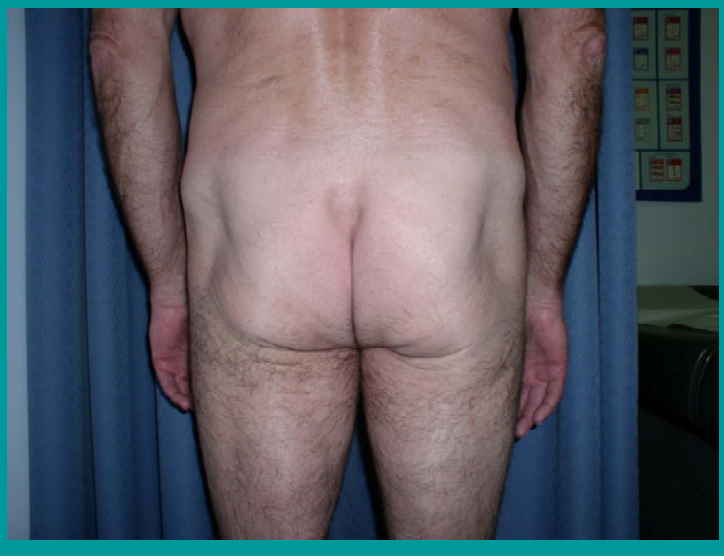
- Eventually, loss of all perigenicular fat becomes obvious, and the lower extremities look too thin to support the weight of the body as shown here.

Lipoatrophy of the Buttocks

Loss of fat in the gluteal region is common, and is seen with simultaneous loss of fat of the extremities.

With severe loss of the buttocks, striations of the gluteus maximus may become obvious.

Moderate Lipoatrophy of the Buttocks

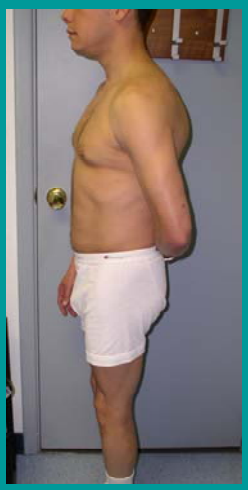


Severe Lipoatrophy of the Buttocks

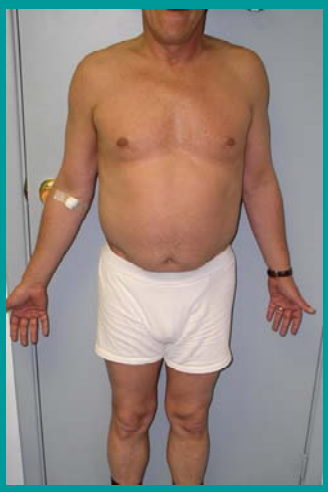
- Upon inspection from the side view, the loss of fat in the gluteal region is particularly evident. In this photograph, severe visceral fat accumulation can also be appreciated which is characteristic of phenotype II of HIV-LS.

Mild Hypertrophy of the Breasts

- Although more common among women with HIV-LS, breast enlargement has been documented in men.
- Hypertrophy of the breasts can be appreciated in the side and frontal views.

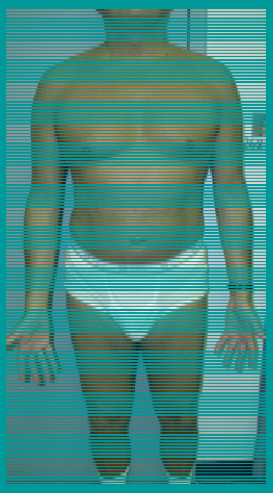


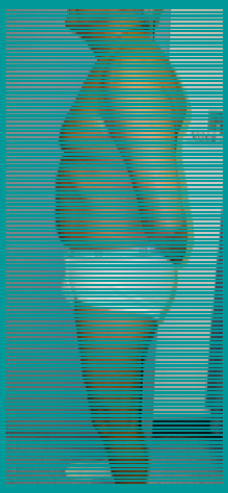
Mild Hypertrophy of the Breasts



** Both images are from the same study participant.

Moderate Lipohypertrophy of the Breasts



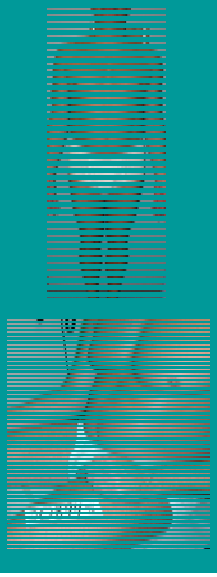


** Both images are of the same study participant

Moderate Lipohypertrophy of the Breasts

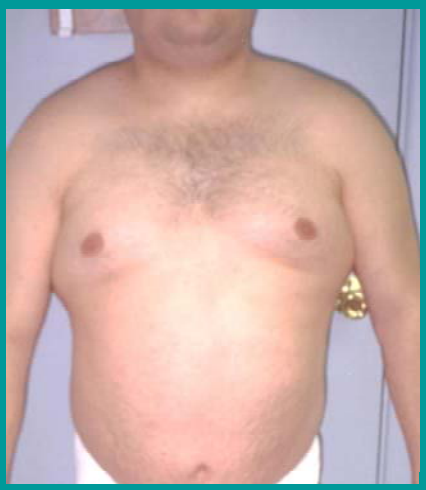
Severe Lipodhypertrophy of the Breasts

- As the breasts increase in size, the nipples become laterally displaced as evident here.



Severe Lipohypertrophy of the Breasts

Severe Lipohypertrophy of the Breasts

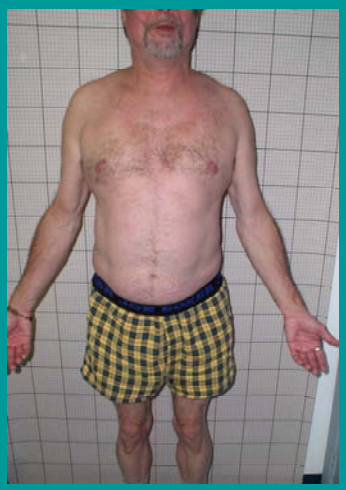


Visceral Fat Accumulation

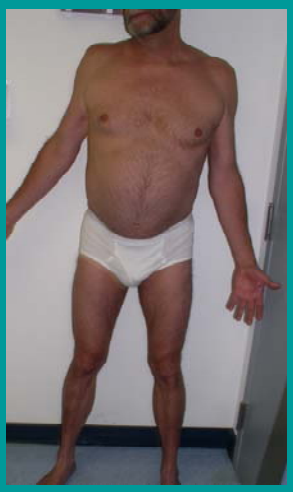
Visceral fat accumulation causes abdominal distention. Most often, the overlying skin of the abdomen is tight, which is in contrast to accumulation of subcutaneous fat which causes the skin to become loose and sagging. Visceral fat accumulation can be confused with ascites and is similar in appearance to a gravid abdomen.

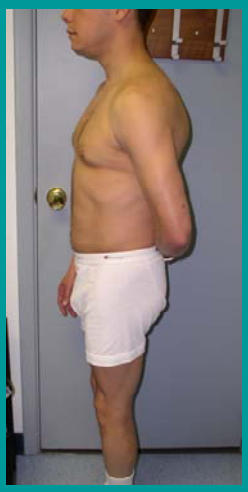
Visceral Fat Accumulation

- As visceral fat accumulates, the oblique and rectus abdominus muscles are defined, as if being pushed forward by the underlying viscera and fat. This is in contrast to accumulation of subcutaneous fat during which the definition of the oblique and rectus abdominus muscles is lost.

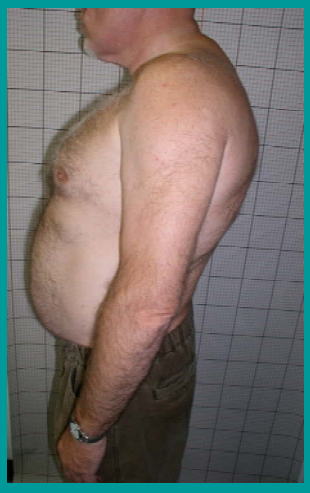


Mild Visceral Fat Accumulation

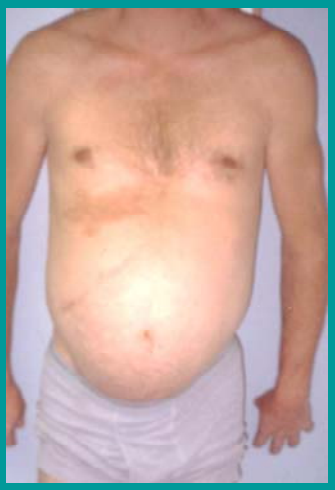


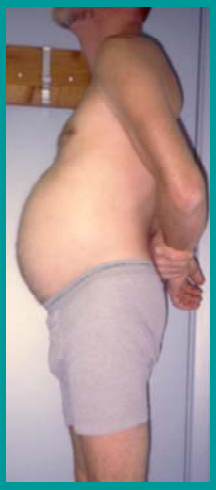


Moderate Visceral Fat Accumulation



Severe Visceral Fat Accumulation





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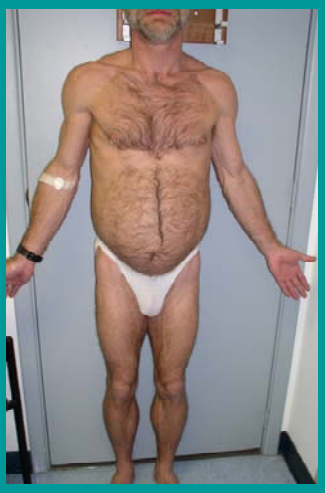
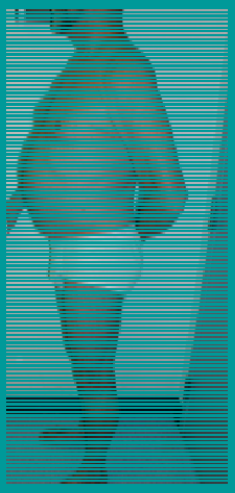
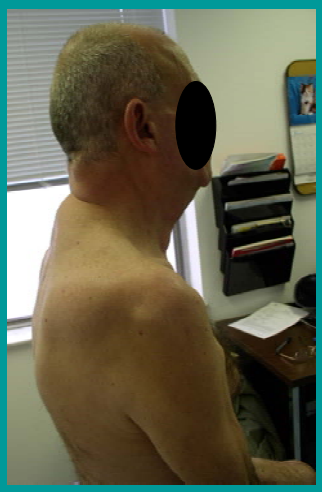
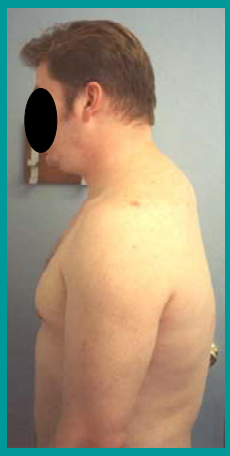
Severe Visceral Fat Accumulation





Mild Dorsocervical Fat Pad





Severe Dorsocervical Fat Pad

- This photograph illustrates a typical presentation of phenotype II. Note the lipoatrophy of the face with simultaneous fat accumulation of the abdomen and dorsocervical fat pad.

Acknowledgement and Appreciation

This work could not have been possible without the cooperation and dedication of the Pittsburgh participants of the MACS.

These men generously consented to photography knowing we were documenting the abnormal distribution of body fat as a consequence of treatment for HIV-infection.

APPENDIX B

Documenting HAART-associated body habitus changes within the Multicenter AIDS Cohort Study

Developed by Bridget C Calhoun, MPH, PA-C
University of Pittsburgh Graduate School of Public Health
for the MACS Metabolic Working Group

Body Habitus Changes

Clinicians within the MACS evaluate each subject during every clinic visit for the presence and severity of body habitus changes via self-report and physical examination.

The severity of the changes has been quantified by a scale using mild/moderate and severe descriptors for each of the affected body areas.

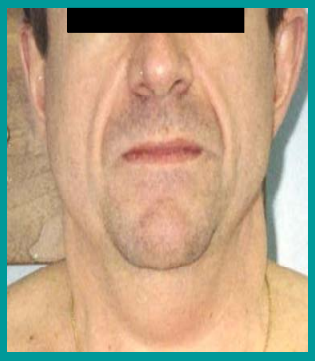
Mild is documented for changes that are evident to clinician with close inspection; **moderate** is used for changes that are evident to clinician without specifically looking for them; and **severe** reflects changes that are evident to a non-medical person by casual observation.

Purpose

The purpose of this manual is to provide examples of mild, moderate and severe findings in order to establish consistency of assessment among all four of the MACS sites.

Mild Facial Lipoatrophy

- Note the pronounced nasolabial folds.

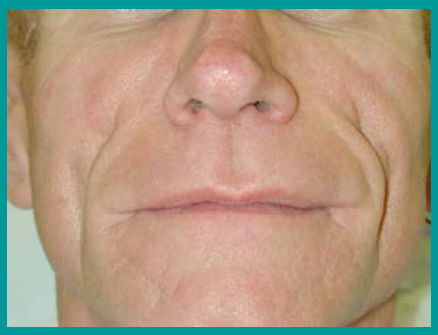


Moderate Facial Lipoatrophy

- As lipoatrophy of the face progresses, the prominence of the nasolabial folds fades and hollowing of the cheeks occurs just below and anterior to the zygomatic arch.

Severe Facial Lipoatrophy

- Severe facial lipoatrophy will be evident with both hollowing of the cheeks and a depression of the temporal region.

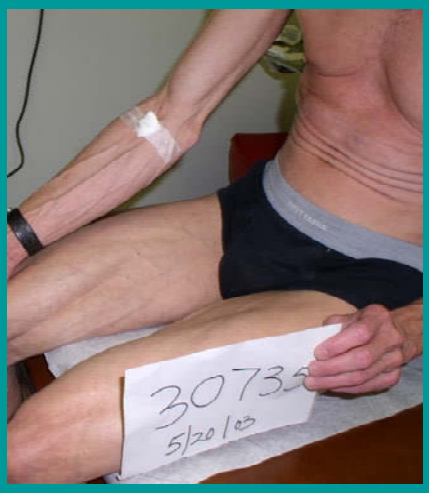


Mild Lipoatrophy of Extremities

- The venous prominence in this photograph suggests mild lipoatrophy of the extremities.

Moderate Lipoatrophy of Extremities

- Lipoatrophy of the extremities becomes evident with:
  - venous prominence
  - obvious bony landmarks and
  - increased muscle definition without an increase in muscle bulk.



Severe lipoatrophy of the upper extremity

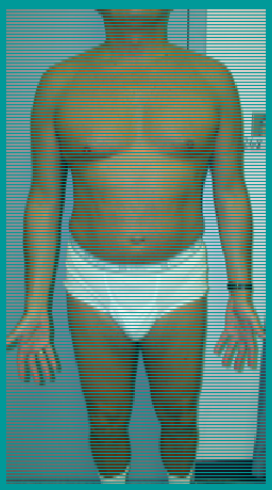
- This photograph clearly shows the loss of subcutaneous fat of the shoulder and arm.
- Facial atrophy is also evident in this participant.

Severe lipoatrophy of the lower extremity

- Subcutaneous fat normally accumulates at the medial thigh. As lipoatrophy of the lower extremity progresses, a deep depression develops medial to the vastus medialis and adductor magnus as shown here.

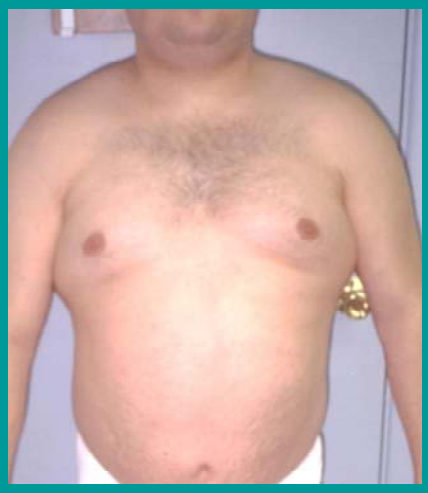
Mild Hypertrophy of the Breasts

Moderate Lipohypertrophy of the Breasts

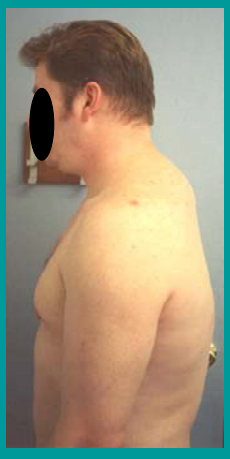


Severe Lipohypertrophy of the Breasts

- With severe lipohypertrophy of the breasts, the nipples become laterally displaced as shown in this participant.

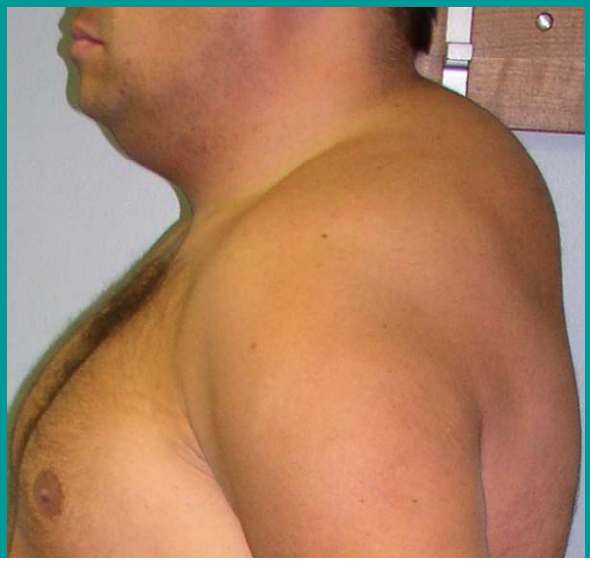


Mild Dorsocervical Fat Pad



Moderate Dorsocervical Fat Pad

Severe Dorsocervical Fat Pad



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Bridget C Calhoun, MPH
2008
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