PREVALENCE OF MORPHOLOGIC AND METABOLIC ABNORMALITIES IN VERTICALLY HIV-INFECTED AND UNINFECTED CHILDREN AND YOUTH

A Multicenter Trial of the Pediatric AIDS Clinical Trials Group

Sponsored by:

The National Institute of Allergy and Infectious Diseases

In Collaboration with:
The Adolescent Trials Network for HIV/AIDS Interventions (ATN)

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SCHEMA

PREVALENCE OF MORPHOLOGIC AND METABOLIC ABNORMALITIES IN VERTICALLY HIV-INFECTED AND UNINFECTED CHILDREN AND YOUTH

DESIGN: Cross-sectional study designed to evaluate the prevalence of morphologic and metabolic abnormalities in vertically HIV-infected children and youth and uninfected children and youth

SAMPLE SIZE: 450 subjects

POPULATION: Children and youth, 7 through < 25 years of age, who are:
- HIV-uninfected
- vertically HIV-infected and currently on a protease inhibitor (PI) containing regimen for ≥ 12 months, or
- vertically HIV-infected, and currently on a non-PI containing regimen for ≥ 12 months

STRATIFICATION:

<table>
<thead>
<tr>
<th>Group</th>
<th>A Tanner Stage 1</th>
<th>B Tanner Stage 2-3</th>
<th>C Tanner Stage ≥4</th>
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<tbody>
<tr>
<td>1</td>
<td>HIV-uninfected</td>
<td>n=50</td>
<td>n=50</td>
</tr>
<tr>
<td>2</td>
<td>HIV-infected; non PI-containing regimen</td>
<td>n=50</td>
<td>n=50</td>
</tr>
<tr>
<td>3</td>
<td>HIV-infected; PI-containing regimen</td>
<td>n=50</td>
<td>n=50</td>
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</table>
SCHEMA (Cont.)

REGIMEN: Treatment will not be provided through this study.

STUDY DURATION: Clinical and laboratory evaluations will be completed at up to three study visits within 30 days of study entry.

OBJECTIVES:

Primary

To compare the prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density in:

1. vertically HIV-infected children and youth on ART and HIV-uninfected children and youth.
2. vertically HIV-infected children and youth who are receiving PI-containing regimens and vertically HIV-infected children and youth who are not receiving PI-containing regimens.
3. vertically HIV-infected children and youth on ART and HIV-uninfected children and youth according to Tanner stage.

Secondary

1. To compare the prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density in vertically and horizontally HIV-infected females on ART through collaboration with ATN 021.
2. To explore the relationship of duration of exposure to highly active antiretroviral therapy (HAART) and the specific components of HAART therapy to prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density.
3. To explore the relationship between drug and non-drug factors and risk of specific aberrations in the morphologic and metabolic parameters that are being studied.
1.0 INTRODUCTION

1.1 Background

Despite unarguable advances in HIV care associated with highly active antiretroviral therapy (HAART), it is now apparent that many patients on these regimens are developing potentially deleterious metabolic effects, including insulin resistance, dyslipidemia, osteopenia and osteoporosis, and hyperlactatemia (1-11). Changes in body fat distribution often referred to as lipodystrophy have also been described. These changes involve both fat accumulation (abdominal visceral obesity, dorsocervical fat pad or buffalo hump, breast enlargement, lipomatosis) and fat loss (lipoatrophy in the face, limbs and gluteal regions). Although the long-term risks associated with this combination of complications in patients with HIV infection are unknown, there is mounting concern that these effects may impact the long-term prognosis in patients whose life expectancies have been significantly extended due to effective viral suppression by HAART. Moreover, adherence to otherwise life-saving antiretroviral therapy has been adversely influenced by the concern about the very obvious physical changes.

Early anecdotal reports led to the assumption that protease inhibitors (PIs) were directly responsible for both the metabolic and morphologic alterations (11-15). Indeed, there is considerable evidence from studies in both HIV-positive and HIV-negative subjects that some PIs can induce insulin resistance and increase triglyceride and cholesterol levels (8, 9, 11, 12, 14, 16-27). However, it is now clear that both metabolic changes and fat distribution abnormalities also occur in PI-naïve patients treated with nucleoside analogue reverse transcriptase inhibitors (NRTIs) (9, 28-41). In addition to class-specific effects, there is emerging evidence that there are differences with each class of drugs in the nature and magnitude of their metabolic effects (29, 42). In addition to exposure to both PI- and NRTI-containing regimens, a number of non-drug risk factors such as age, gender, race, and baseline body composition have been identified in cohort studies (43-46).

1.2 Metabolic and Morphologic Complications Associated with HAART

1.21 The HIV Lipodystrophy Syndrome (HLS)

Currently there is no consensus case definition of HIV-associated lipodystrophy. Carr et al. has attempted to define a single syndrome, based on a score that includes factors pertaining to both fat accumulation and fat loss, as well as metabolic parameters (47). Others assert that it is primarily a syndrome of lipoatrophy and isolated obesity should not be included in the definition (48, 49). Given the lack of consensus, the approach in the current
study will be to evaluate each metabolic and morphologic parameter independently and secondarily to assess the interrelationship among them.

Because metabolic and morphologic alterations were first recognized in the era of HAART, and especially in close temporal proximity to the introduction and widespread use of PIs, they were first believed to represent a complex adverse reaction to the PIs. This constellation of biochemical and physical aberrations is believed to carry a high, but as yet unquantifiable, risk of both cardiovascular disease and diabetes mellitus (DM) (50, 51). Other than PIs, NRTIs, chronic HIV infection itself, and immune reconstitution, as well as host factors such as age and body mass index have all been implicated as precipitants of HLS.

Some cohort studies have suggested that the severity of the underlying HIV disease and the duration of various combinations of antiretroviral therapies are the strongest predictors of HLS. For example, those variables assessed in the HIV Outpatients Study that were associated with a significantly increased risk of fat redistribution included: Age > 40 years; use of d4T for > 6 months; use of indinavir for > 2 years; results of the first recorded HIV serology dated > 7 years ago, and an AIDS diagnosis for > 2 years, among others (46, 52).

1.22 Prevalence of HIV Lipodystrophy Syndrome in Children

A number of studies have attempted to determine the prevalence of various lipodystrophic and metabolic abnormalities in children and to assess the risks associated with various antiretroviral therapy (ART) dosing regimens, age, viral load, and degree to which the immune system is compromised. In 1999, Babl, et al. described HLS in NIAID and NICHD pediatric clinical trials sites (53). Of nearly 2800 patients receiving some form of ART, about 1% displayed signs of fat maldistribution. Children receiving PIs were significantly more likely to show these morphologic changes. This study was limited by the retrospective and descriptive nature of the findings, the lack of any objective definition of abnormal fat distribution, and the lack of a standardized endocrine evaluation.

In a cross-sectional study of French children, 13 of 39 HIV-infected children displayed signs of lipodystrophy: 8 had truncal lipohypertrophy, 3 had peripheral lipoatrophy and 2 had both truncal lipohypertrophy and peripheral lipoatrophy (54). Combined lipodystrophies were observed only in adolescents and these were more severe than those seen in the younger patients. As a group, lipodystrophic children exhibited a greater degree of insulin resistance than those without lipodystrophy. Hypercholesterolemia and hypertriglyceridemia were observed in equal proportions in children with
and without changes in body habitus. In addition, approximately one-fourth of children without lipodystrophy had dyslipidemia. No significant associations could be ascertained between the lipid dystrophic and non-dystrophic groups with regard to ART regimen or length of treatment.

In a cross-sectional study of 40 HIV-infected children aged 2-16 years, Amaya, et al performed standardized physical assessments (55). Seven (18%) exhibited physical signs of fat redistribution, and 27 (68%), 11 (28%) and 3 (8%) displayed evidence of hypercholesterolemia, hypertriglyceridemia and insulin resistance, respectively. Twenty-eight percent of the cohort displayed no abnormalities. Statistical analyses did not reveal any significant associations between features of lipodystrophy and the patient’s age, viral load, exposure to specific ART regimens or duration of treatment with either PIs or NRTIs.

1.23 Morphologic Changes Associated with HLS

Body fat most often accumulates in a dorsocervical (“buffalo”) hump, the viscera (mesentery) and/or the breasts. Body fat is most often lost in the face and/or the subcutaneous tissues of the limbs. These aberrations may have psychological consequences and may result in poor adherence to the medical regimen.

Because the disorder has no standardized definition for surveillance purposes, the prevalence of the complete disorder, or of parts of the disorder, has been difficult to assess. A cohort study by Martinez, et al. has determined that the prevalence of each of these morphologic changes varies from 1% to 56% (24). These wide ranges indicate that aspects of the syndrome are not being recognized and that standardized anthropometric measures are not being applied. Some studies have examined the effects of switching antiretroviral regimens, primarily from those including PIs, to either non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing or exclusive NRTI-containing regimens; none has consistently resulted in reversal of the abnormal morphologic changes (56-59).

1.24 Dyslipidemia Associated with HLS

Elevations in serum triglyceride levels were observed in HIV-infected patients prior to the HAART era, but appear to have increased in frequency in the past five years (24). Dyslipidemia is not necessarily accompanied by fat redistribution in all instances (21). Many investigators have focused on the associations between various ART regimens and dyslipidemia (60-62). Although PIs, particularly ritonavir, seem to be generally associated with
dyslipidemia, it is now widely recognized that even patients briefly or never treated with PIs can develop these abnormalities (63-65). In data obtained by the Multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project patients grouped according to various treatment categories suggested strongly that both PIs and NNRTIs created a significantly greater risk for developing dyslipidemia than patients who received NRTIs alone (13). A study by Tsiodras, et al. also pointed to a strong association between PI use and risk of developing elevated triglyceride and cholesterol levels (60). Some studies suggest that switching from a PI to an NNRTI or to abacavir may improve the lipid profile (7-10, 17, 56-59, 66).

1.25 Relationships between Obesity, Inflammation and C-Reactive Protein (CRP)

There is an increasing body of evidence to suggest that weight gain, high levels of blood triglycerides and insulin resistance interact with one another and an unknown number of other clinical variables to induce a state of chronic inflammation (67). Mediators produced by adipose tissue and chronic infections (e.g., gingivitis, bronchitis, etc.) precipitate a cascade of events that results in the production of inflammatory and messenger cytokines that ultimately have a deleterious effect on the vascular endothelium. One widely recognized marker of this intersection of inflammatory responses is C-Reactive Protein (CRP). CRP levels are strongly linked to body mass index, to smoking and to excessive alcohol intake. CRP levels are a better predictor of heart disease than IL-6, certain cellular adhesion molecules or tumor necrosing factor (TNF). Higher levels of CRP are found in patients with type II diabetes, glucose intolerance, and Syndrome X, many of whose features are common to the lipodystrophy syndrome seen in AIDS (e.g., excessive abdominal fat, insulin resistance, elevated blood sugar and serum triglycerides, low levels of HDL cholesterol, etc.). It will be critical to learn whether these HIV-associated/ART-associated metabolic aberrations are also found in connection with the traditional markers of chronic inflammation, whether CRP levels are elevated even in AIDS patients in good virologic control, and whether such biochemical abnormalities, if they exist, have the same poor outcomes as seen in other populations of patients.

1.3 Skeletal Complications Associated with HAART

1.31 Bone Mineral Density (BMD)

The role of antiretroviral therapy and other factors in the development of bone disease is poorly understood at this point. Prior to the era of potent antiretroviral therapy, studies indicated that BMD was unaffected or only minimally affected in HIV-infected individuals. Paton et al. reported that 45
HIV-infected subjects had marginally lower BMD at the lumbar spine than seronegative controls ($p = 0.04$) (68). However, the subjects and controls did not differ in total body or hip BMD. None of the subjects had reduced BMD to levels associated with a diagnosis of osteoporosis. On longitudinal follow-up (15 months), a small decrease in total body BMD (-1.6%; $p = 0.02$) was observed, but there was no significant reduction in spine or hip BMD. The authors concluded that, in spite of the many features of HIV infection that might be expected to reduce BMD, such as cytokine activation, decreased physical activity, small bowel disease, hypogonadism, and direct infection of osteogenic cells by HIV, they could not find significant differences in BMD between HIV-infected subjects and controls. Furthermore, the HIV-infected subjects studied did not appear to show excessive loss in BMD over time.

1.32 Measurement of Bone Metabolic Markers in HIV-Infected Individuals

Aukrust et al. analyzed serum markers of bone formation (osteocalcin) and bone resorption (C-telopeptide) in 73 HIV-infected subjects (69). Aukrust et al. also analyzed serum bone metabolic markers in 17 subjects who started potent antiretroviral therapy (zidovudine, lamivudine [3TC], and indinavir [IDV]). There was a marked rise in serum osteocalcin levels together with a profound fall in viral load levels and TNF components and a marked rise in CD4$^+$ T-cell counts. Also, there was a shift from no correlation to a significant correlation between osteocalcin and C-telopeptide levels during such therapy. Their data suggest an increase in markers of bone turnover with the initiation of antiretroviral therapy. Unfortunately, the results of the study are limited because no BMD data were obtained.

Teichmann et al. recently reported data on the influence of HIV infection on the calcitropic hormones and markers of bone metabolism in a cross-sectional study of 100 subjects with proven HIV infection (70). Reduced bone mineral content (BMC) was found among the HIV-infected subjects. Confirming previous studies, Teichmann et al. observed significantly lower osteocalcin concentrations in subjects with advanced disease, indicating a reduced bone formation rate. There was a significant correlation between the severity of reduced bone formation rate and the progressive loss of CD4 helper cells, but severity of reduced bone formation rate was independent of low vitamin D-3 (1,25-dihydroxycholecalciferol) levels and alterations of protein metabolism. Increased urinary excretion of cross-links as an expression of enhanced bone resorption was likewise significantly correlated with the loss of CD4 helper cells and independent of the vitamin D concentration and protein metabolism.
1.33 Osteopenia Associated with Protease Inhibitor (PI)-Containing Therapy

As part of their ongoing studies to characterize body fat redistribution in subjects with HIV infection, Tebas et al. observed a high frequency of relatively low BMD in subjects on potent antiretroviral therapy (71).

A cross-sectional analysis was performed on 112 men. The study enrolled HIV-infected subjects receiving PIs, HIV-infected subjects not on PIs, and healthy adults (HIV-negative controls).

Table 1. Characteristics of 112 Men in the Study by Tebas et al. (71)

<table>
<thead>
<tr>
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<th>HIV+/PI-</th>
<th>Controls</th>
<th>p Values</th>
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<td>Age (years)</td>
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<td>33.7 ± 7</td>
<td>33.3 ± 9</td>
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<td>BMI (kg/m²)</td>
<td>224 ± 4</td>
<td>222 ± 6</td>
<td>223 ± 4</td>
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<tr>
<td>Median lumbar spine BMD</td>
<td>0.9860</td>
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<tr>
<td>Median t-score</td>
<td>-1.005</td>
<td>-0.382</td>
<td>-0.227</td>
<td>0.02</td>
</tr>
<tr>
<td>Median z-score</td>
<td>-0.923</td>
<td>-0.382</td>
<td>0.145</td>
<td>0.04</td>
</tr>
<tr>
<td>% of subjects with (t-score &lt; -1)</td>
<td>550</td>
<td>223</td>
<td>229</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1HIV+/PI+ = HIV-infected subjects on protease inhibitors.
2HIV+/PI- = HIV-infected subjects not on protease inhibitors
3Controls = Healthy, HIV-uninfected subjects
4Body Mass Index

Men receiving PIs had lower median t-scores for the lumbar spine BMD than men did in the other two groups. Median z-scores for the lumbar spine BMD were also lower in PI recipients. Median z-scores for BMD in the trochanter, femoral neck, and Ward’s triangle regions of the proximal femur were significantly lower in PI-treated subjects than in HIV-infected subjects not receiving PIs and in controls.

Using lumbar spine BMD t-scores, 50% of the subjects on PIs were classified as osteopenic or osteoporotic according to the World Health Organization (WHO) classification. The relative risk for osteoporosis in these subjects was 2.19 (95% confidence interval 1.13-4.23) when compared with HIV-infected subjects not receiving PIs. Eleven percent of the subjects receiving PIs had a lumbar spine BMD t-score more than 2.5 standard deviations (SD) lower than the mean values expected for 30 year old men and met the WHO definition of osteoporosis. Only 6% of the
controls and of the HIV-infected subjects not taking PIs were classified as osteoporotic using these same criteria. Subjects with more prolonged use of PIs tended to have more negative t-scores in the lumbar spine (Pearson correlation coefficient = -0.19, p = 0.14), but this correlation did not reach statistical significance. These findings have been confirmed by Cunney, et al., Hoy, et al., and by Tebas, et al. in a second cohort of subjects (72-74). In a recent study of children actively involved in HIV care in Texas, a large proportion were found to be either osteopenic or osteoporotic. There was no difference in mean BMD Z-scores between those on a PI-containing regimen versus those on a PI-sparing regimen. However, there appeared to be a relationship between duration of use of PI-containing HAART and prevalence of osteopenia (75).

1.34 Bone Mineral Metabolism in HIV-Infected Subjects Taking PIs

Tebas and colleagues also performed preliminary studies characterizing multiple bone metabolic parameters in a cohort of 73 HIV-infected subjects taking PI-containing, potent antiretroviral regimens (74). All of these individuals had DEXA scans of the lumbar spine and proximal femur and evaluation of multiple bone metabolic parameters, including measurements of the concentration of several hormones, bone remodeling markers, calcium, and vitamin D metabolites. Forty-three percent of the subjects were osteopenic/osteoporotic according to the WHO definition, confirming the observation by Tebas et al. in the earlier cohort. There was no association of osteopenia with specific PIs or differences in bone metabolic parameters among them.

As a group, a significant proportion of subjects taking potent antiretroviral therapy that included a PI had increased markers of bone resorption, including increased urine pyridinoline and urine deoxypyridinoline. Also, subjects taking PI-containing, potent antiretroviral therapy had increased markers of bone formation, including increased alkaline phosphatase and osteocalcin. This finding is in sharp contrast with the previous observations of low osteocalcin levels in subjects with advanced disease. The levels of bone alkaline phosphatase and pyridinolines in urine correlated with BMD in the lumbar spine and the hip. Testosterone levels and thyroid stimulating hormone (TSH) levels were normal in this population, and they did not correlate with BMD either in the lumbar spine or the hip. More than 50% of the subjects had urinary calcium levels greater than 200 mg/24h, and 25% had levels greater than 300 mg/24h. Bone alkaline phosphatase correlated with t-scores in the lumbar spine and hip: (r = -.324, p = 0.008). Subjects with higher bone alkaline phosphatase tended to have lower BMD in the lumbar spine and hip than subjects with
lower bone alkaline phosphatase values. Markers of bone resorption, urine pyridinoline, and urine deoxypyridinoline also correlated inversely with BMD in the spine and the hip (r = -.367, p = 0.003; r = -.390, p = 0.001, respectively). Markers of bone formation like bone alkaline phosphatase correlated strongly with markers of bone resorption in the urine (r = .581, p < 0.0001).

These findings suggest that subjects receiving PI-containing, potent antiretroviral therapy have a state of low BMD secondary to increased bone remodeling, or alternatively an alteration of bone mineralization that leads to increased urinary calcium loss. Additional studies are necessary to characterize the sequence of events that lead to this abnormal state.

Published reports suggest that decreased BMC and BMD are also seen in children with HIV infection (77, 78). Mora, et al. examined biochemical markers and found that reduced BMD was associated with increased bone turnover, and that bone turnover was higher in children on HAART. On the other hand, Arpadi, et al. found no association between PI use and decreased BMC. Thus, the uncertainty regarding the specific role of ART that has been seen in studies in adults (Tebas, et al., Nolan, et al., Carr, et al., studies in men; Jacobson, et al., Arnsten, et al. studies in women) is also present in children (74, 79-81).

Preliminary reports from more recent studies suggest that the prevalence of osteopenia and osteoporosis in HIV-infected children is alarmingly high. Using data from DEXA scans of the lumbar spine in small groups of children, both Schwarzwald, et al. and McComsey, et al. reported that more than 70% of children studied had osteopenia or osteoporosis (75, 76). Clearly, if these prevalence estimates are confirmed in larger studies, interventions to increase BMD in children would be warranted, regardless of the etiology or questions about the specific role of PI therapy.

1.4 Mitochondrial Abnormalities and Lipodystrophy

Nucleoside analogue reverse transcriptase inhibitors remain the foundation of combination antiretroviral therapy for HIV infection. In addition to the therapeutic effect of these agents through inhibition of viral replication, to varying degrees they also inhibit human mitochondrial DNA polymerase. The inhibition of mitochondrial polymerase may account for many of the major toxicities associated with NRTI therapies. Treatment with nucleoside analogue antiretroviral agents has been associated with lactic acidosis, hepatic steatosis, liver failure, peripheral neuropathy, myopathy, cardiomyopathy, pancreatitis, and possibly lipodystrophy (34, 83-89). A lipodystrophy syndrome associated with
nucleoside analogue therapy has recently been described and is characterized by the onset of fatigue, nausea, peripheral lipoatrophy, abdominal distension with or without ascites, hepatomegaly, and elevated lactic acid and hepatic transaminase levels (65).

Lactic acidosis is perhaps the most dramatic acute manifestation of NRTI-associated mitochondrial dysfunction. Cases of lactic acidosis have been described since the HIV epidemic, but have been increasingly recognized. Prospective data on the incidence are not available, but observational cohorts estimate the incidence in adults at 1.3 per 1000 person-years (65, 90). Although acute lactic acidosis is rare, a milder form of NRTI-associated mitochondrial dysfunction, “symptomatic hyperlactatemia,” has been reported to occur more frequently, with an incidence of 25.6 cases/1000 person years among d4T users (91, 92). Hyperlactatemia is also a serious complication often necessitating at least a transient discontinuation of NRTI, if lactate levels are confirmed to be > 4 X ULN or if lactate levels of >2 X ULN occur in a symptomatic patient.

Recent reports now indicate that although adults with HIV infection experience some increases in lactate levels upon initiation of NRTI therapy, these levels remain stable in most patients, and even occasional blips do not predict subsequent symptomatic hyperlactatemia or lactic acidosis (27, 93). However, the long-term metabolic consequences of subclinical hyperlactatemia are not known, and children may be at increased risk of developing clinically significant hyperlactatemia, given the increased energy demands associated with growth.

The importance of proper collection and processing of specimens for lactate determination is illustrated in a preliminary report indicating that elevations in lactate are found much less frequently when standardized procedures for collection and processing are followed, even in patients with other biochemical or somatic risk factors for hyperlactatemia (94).

An optimal method to detect early evidence of mitochondrial toxicity of nucleoside antiretroviral therapies, which should be minimally invasive, sensitive and specific, has not been identified. Elevated blood lactate has been considered as a potential non-invasive marker for mitochondrial dysfunction. As long as other causes of hyperlactatemia are excluded, an elevated blood lactate (or hyperlactatemia) could in principal be considered strongly suggestive of mitochondrial dysfunction.

1.5 **Rationale**

As previously described, potentially deleterious metabolic effects, including insulin resistance, dyslipidemia, osteopenia, osteoporosis, hyperlactatemia, and lipodystrophy are being associated with life-prolonging HAART in HIV-infected
children. Studies conducted to date present a very wide range in findings, with regard to both prevalence and putative associations with the conditions, and composition and duration of ART therapy. Much larger prospective studies are needed with standardized definitions, anthropometric measurements, and laboratory evaluations.

Recent studies in large cohorts in countries with widespread access to HAART suggest that nearly 50% of HIV-infected adults develop the morphologic features of “lipodystrophy syndrome” (11, 23, 33, 95). Smaller studies have also indicated the presence of both metabolic and morphologic abnormalities in perinatally HIV-infected children (54, 96, 97). However, there have been no studies addressing the prevalence and comprehensive risk assessment of these complications in children.

The skeletal complications of osteopenia and osteoporosis are serious and long-term abnormalities. Since maximum BMD is achieved during adolescence, a metabolic syndrome that reduces that density would be expected to have long-term consequences. As described previously, findings suggest that subjects receiving PI-containing, potent antiretroviral therapy have a state of low BMD secondary to increased bone remodeling, or alternatively an alteration of bone mineralization that leads to increased urinary calcium loss. Whether this is a direct effect of PI-containing, potent antiretroviral therapy on the bone, mediated through interferences in the vitamin D metabolism pathway, a direct effect on the renal excretion of calcium, or a result of other mechanisms needs to be elucidated. Additional studies are necessary to characterize the sequence of events that lead to this abnormal state.

This protocol will take a “staged” approach to characterizing the prevalence of these abnormalities. Initially, only minimal screening tests will be performed (e.g. lipid profiles, CRP, OGTT, DEXA scans, etc.). If abnormalities are found to be prevalent in a particular group or groups, further tests on the repository samples will be performed to further define these abnormalities. For example, if the DEXA scans reveal significant osteopenia, markers of bone turnover will be measured in blood and urine. Since diet, exercise, and family history play a role in the development of lipid and glucose abnormalities, standardized data on these factors will be collected (e.g. Food Frequency Questionnaire, etc.).

1.51 Rationale for Inclusion of all Tanner Stages

In the normal population, insulin sensitivity and bone mineral accrual differ depending on Tanner staging. Little is known about specific changes in these factors in HIV-infected children and youth. This study seeks to determine the presence of abnormalities in glucose metabolism, lipids, body composition and bone density among HIV-infected and uninfected children and youth. Without inclusive data from all Tanner staged groups, significant findings in our HIV-infected subjects may be
missed. We cannot assume that HIV-infected subjects will have "normal" patterns of insulin resistance or bone accrual. Comparison of these findings at different Tanner stages will allow us to determine the prevalence of insulin resistance and osteoporosis and determine when the metabolic causes of these problems begin.

Since the complications associated with antiretroviral therapies may occur in children at different rates or with different emphases during different stages of childhood, it seems very important to evaluate the prevalence of abnormalities over the entire age spectrum to be studied. The youth and youngest child groups may offer the greatest difference in prevalence of the abnormalities to be assessed. However including the intervening age and developmental group may provide evidence that could lead to subsequent more intensive pathophysiologic questions; these questions will target specific developmental stages. The frequencies of abnormalities are to be compared primarily in the different therapy exposure groups and against HIV-uninfected controls and across developmental groups.

1.52 Adolescent Trials Network Collaboration

P1045 will utilize data already being collected as part of the ATN 021 study, “Prevalence of Morphologic and Metabolic Abnormalities in HIV-Infected and Uninfected Young Women” for joint P1045/021 analyses related to shared study objectives, particularly the sub-analysis of horizontally and vertically infected subjects.

2.0 STUDY OBJECTIVES

2.1 Primary

To compare the prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density in:

2.11 vertically HIV-infected children and youth on ART and HIV-uninfected children and youth.

2.12 vertically HIV-infected children and youth who are receiving PI-containing regimens and vertically HIV-infected children and youth who are not receiving PI-containing regimens.

2.13 vertically HIV-infected children and youth on ART and HIV-uninfected children and youth according to Tanner stage.
2.2 Secondary

2.21 To compare the prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density in vertically and horizontally HIV-infected females on ART through collaboration with ATN 021.

2.22 To explore the relationship of duration of exposure to HAART and the specific components of HAART therapy to prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density.

2.23 To explore the relationship between drug and non-drug factors and risk of specific aberrations in the morphologic and metabolic parameters that are being studied.

3.0 STUDY DESIGN

This is a cross-sectional study designed to (1) comprehensively evaluate the prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density in vertically HIV-infected and HIV-uninfected children and youth, (2) evaluate whether exposure to PIs is correlated with the metabolic and morphologic abnormalities among HIV-infected children and youth, and (3) compare the prevalence of abnormalities in this population according to Tanner Stage. These data are critical for designing intervention trials and studies designed to study pathophysiologic mechanisms.

A total of 450 subjects will be enrolled as outlined in Table 2.

Table 2. Stratification According to Tanner Stage, HIV Disease Status, and Exposure to PIs

<table>
<thead>
<tr>
<th>Group</th>
<th>A Tanner 1</th>
<th>B Tanner 2-3</th>
<th>C Tanner ≥4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HIV-uninfected</td>
<td>n=50</td>
<td>n=50</td>
</tr>
<tr>
<td>2</td>
<td>HIV-infected; non PI-containing regimen</td>
<td>n=50</td>
<td>n=50</td>
</tr>
<tr>
<td>3</td>
<td>HIV-infected; PI-containing regimen</td>
<td>n=50</td>
<td>n=50</td>
</tr>
</tbody>
</table>
Screening clinical and laboratory evaluations will be completed at one visit. On-study clinical and laboratory evaluations may be completed at one study visit (Entry) or over the course of up to three study visits (Entry plus a second or third visit). All on-study evaluations must be completed within 30 days of Entry. No additional follow-up visits are required of study subjects. Refer to the Schedule of Evaluations (Appendix I) for specific requirements.

Collaboration with ATN 021

Certain ATN 021 data will be pooled with P1045 data for joint analyses related to shared study objectives, including a sub-analysis of vertically and horizontally infected females. These data include information collected from the medical history, Body Image Questionnaire, Food Frequency Questionnaire, anthropometric measurements, physical assessment, whole body and regional (AP spine and hip) DEXA scans, and metabolic laboratory evaluations.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.11 Age 7 to < 25 years

4.12 HIV Infection Status

4.121 For HIV-uninfected subjects (Group 1):
Confirmed HIV-1 seronegative as defined by one nonreactive result by licensed ELISA at a CLIA certified laboratory.
Perinatally HIV-exposed but uninfected subjects are eligible.

4.122 For HIV-infected subjects (Groups 2 & 3):

a) Vertically acquired HIV infection

b) A confirmed diagnosis of HIV-1 infection as defined by two positive assays from two different samples drawn on different days performed in a CLIA certified laboratory. The two results may be in any combination of the following:

- HIV-1 DNA PCR
- HIV-1 culture
- Plasma HIV RNA value > 10,000 copies/mL
- Neutralizable HIV p24 antigen detection (regular or ICD)
- Licensed ELISA with confirmatory Western Blot
4.13 Antiretroviral therapy requirements (Groups 2 & 3 only)

Subjects enrolled in the PI-containing group must:
• currently be on a PI-containing regimen, and
• have been on the same PI-containing regimen continuously for ≥ 12 months prior to study entry.

Subjects enrolled in the non-PI-containing group cannot:
• currently be on a PI-containing regimen,
• have been on a PI-containing regimen in the 12 months prior to study entry, and
• have ever received a PI for ≥ 2 weeks.

4.14 Accessible medical history and medications history

4.15 Parent, legal guardian or subject (when age appropriate, as defined by the site or by rules for obtaining assent) willing to give written informed consent and willing to comply with study requirements

4.16 Females who have had their first menses must have a negative serum or urine pregnancy test

4.2 Exclusion Criteria

4.21 Receipt of any of the following, currently or within 6 months prior to study entry:

• megestrol acetate (Megase)
• injectable, oral or transdermal anabolic agents, including oxandrolone (Óxandrin), oxymethalone (Anavar), nandrolone (Decadurabolin, Nandroblem), dehydroepiandrosterone (DHEA) and other testosterone derivatives
• anticytokine agents, including thalidomide (Thalomid), pentoxifylline (Trental) and ketotifen (Zaditen)
• systemic ketoconazole (Nizoral) or any systemic glucocorticoids

Note: Subjects receiving stable physiologic glucocorticoid doses (defined as prednisone < 7.5 mg/m²/day or its equivalent) will not be excluded. Subjects receiving a short course (defined as no longer than 2 weeks) of pharmacologic glucocorticoid therapy prior to study entry will not be excluded.
• Treatment for osteoporosis estrogens, bisphosphonates (alendronate [Fosamax] and others).
  
  Note: Physiologic supplementation of calcium and vitamin D is allowed.

4.22 Type II DM and cannot omit DM medication for the 48-hour period prior to OGTT specimen collection.

4.23 Pregnancy within the last 12 months or currently pregnant or breastfeeding.
  
  Note: Women who have undergone therapeutic abortion (TAB) or spontaneous abortion (SAB) for pregnancies that were < 12 weeks may enter the study 6 months post TAB/SAB.

4.24 Any clinically significant diseases (other than HIV infection) or clinically significant findings during the screening medical history or physical examination that, in the investigator’s opinion, would compromise the outcome of this study.

4.25 History of eating disorders (bulimia, anorexia nervosa)

4.3 Disallowed Medications

• Growth hormone

• Megestrol acetate (Megase)

• Injectable, oral, or transdermal anabolic agents including oxandrolone (Oxandrin), oxymethalone (Anavar), nandrolone (Decadurabolin, Nandrobolic), dehydroepiandrosterone (DHEA) and other testosterone derivatives

• Anticytokine agents, including thalidomide (Thalomid), pentoxifylline (Trental) and ketotifen (Zaditen)

• Systemic ketoconazole (Nizoral) or any systemic glucocorticoids
  
  Note: Subjects may receive stable physiologic glucocorticoid doses (defined as prednisone < 7.5 mg/m²/day or its equivalent).

• Treatment for osteoporosis with estrogens, bisphosphonates (alendronate [Fosamax] and others).
4.4 Enrollment Procedures

PACTG sites interested in participating in P1045 must submit a Site Implementation Plan (SIP) to the protocol team. An approved SIP is required to complete the protocol registration process. For each of the nine subgroups, the SIP will inform the protocol team of:

- the estimated number of children and youth seen at the site in a 12 month period who would be potentially eligible for the study,
- the estimated number of potentially eligible children and youth the site has the resources to enroll,
- the estimated percentage of children and youth approached for enrollment that will refuse participation,
- the estimated percentage of males/females, and
- the estimated racial breakdown (White, Black Hispanic, Black non-Hispanic, Other)

Once the SDMC has received this information from those sites approved to participate, site-specific sampling probabilities will be generated for each of the nine subgroups. (Refer to Section 7.3 for details.) Subjects will be pre-screened to determine if they fit into one of the nine subgroups (i.e. determination of HIV infection status for all subjects; type of regimen [PI or non-PI] and therapy duration for HIV-infected subjects). Sites will use the SDMC randomization system to randomly select children and youth who should be approached for study participation, and must agree to approach all identified children and youth. Those children and youth who are approached and consent to study participation will be screened to determine eligibility according to the inclusion/exclusion criteria.

4.5 Co-Enrollment Guidelines

Subjects may co-enroll in other PACTG protocols as long as the eligibility criteria of the co-enrollment protocol are met. All co-enrollments require the assent of the PACTG P1045 Protocol Chair and the Protocol Chair of the co-enrollment study.

5.0 SUBJECT MANAGEMENT

5.1 Adverse Experiences

Adverse experiences must be documented on the appropriate case report form(s), but are not required to be reported to the SAE Office as adverse experiences.
5.2 Criteria for Study Discontinuation

- The subject becomes pregnant.
- The subject or legal guardian refuses further follow-up evaluations.
- The investigator determines that further participation would be detrimental to the subject's health or well-being.
- The subject fails to comply with the study requirements so as to cause harm to himself/herself or seriously interfere with the validity of the study results.

6.0 SERIOUS ADVERSE EXPERIENCE (SAE) REPORTING

Antiretroviral treatment is not provided through this study. Thus Serious Adverse Experiences (SAE) will not be reported to the Regulatory Operations Center SAE Office. However, sites are encouraged to report any SAE related to study procedures or requirements to the FDA via MedWatch.

7.0 STATISTICAL CONSIDERATIONS

7.1 General Design Issues

P1045 is a cross-sectional study designed to determine the prevalence of abnormalities in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density in Tanner stage 1, 2-3, and ≥ 4 HIV-uninfected children and youth, and vertically HIV-infected children and youth who are receiving PI-containing regimens or PI-sparing regimens for at least 12 months. HIV uninfected children of HIV infected women whether or not they were exposed to antiretrovirals in utero or post-partum are eligible. In order to avoid the selection bias than can occur if each site were to choose which subjects to enroll, a stratified sampling design will be used. Enrollment will be stratified by Tanner Stage (1, 2-3, and 4-5), and by HIV status/antiretroviral drug history (HIV infected/uninfected and PI-containing versus PI-sparing regimen) for a total of nine strata, with 50 subjects in each for a total of 450 subjects.

Secondary analyses will investigate drug and non-drug factors related to specific abnormalities in the HIV-infected subjects receiving PI containing or PI sparing regimens compared to HIV-uninfected subjects, including duration of exposure to different components of HAART. In race/ethnicity and Tanner Stage matched analyses, prevalence rates will be compared in vertically (P1045) and horizontally HIV-infected young women enrolled in protocol ATN 021. Because of the relatively
small sample size in the nine strata, secondary analyses will be exploratory and hypothesis-generating in nature.

7.2 Outcome Measures

Abnormal cut-off points in the primary outcomes of glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density are presented in Table 3. Subjects will be classified as abnormal if their value for a given parameter falls in the abnormal range. This dichotomous outcome will be used to calculate the prevalence of abnormalities for each variable in each stratum.

There are no published norms available for body composition, so the abnormal cut-offs quoted in Table 3 will be derived using data from the HIV-uninfected strata. For these outcomes, rather than calculating prevalence of abnormalities, the primary comparisons will use the continuous data and compare the entire distribution of values.
Table 3. Abnormal cut-off points in glucose metabolism, serum lipid levels, body composition, fat deposition and distribution, and bone density by gender and Tanner stage (98-99)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Abnormal cut-off</th>
<th>Abnormal cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Serum Lipid Levels(^98)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Total Cholesterol</td>
<td>mg/dl</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>*LDL Cholesterol</td>
<td>mg/dl</td>
<td>&gt;130</td>
<td>&gt;130</td>
</tr>
<tr>
<td>*HDL Cholesterol</td>
<td>mg/dl</td>
<td>&lt;40</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>mg/dl</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Oral Glucose Tolerance Test(^98)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>mg/dl</td>
<td>&gt;125</td>
<td>&gt;125</td>
</tr>
<tr>
<td>2 Hour Post Glucose: Impaired</td>
<td>mg/dl</td>
<td>&gt;139</td>
<td>&gt;139</td>
</tr>
<tr>
<td>2 Hour Post Glucose: Diabetes</td>
<td>mg/dl</td>
<td>&gt;199</td>
<td>&gt;199</td>
</tr>
<tr>
<td>Plasma Lactate(^98)</td>
<td>mmol/L</td>
<td>&gt;2.1</td>
<td>&gt;2.1</td>
</tr>
<tr>
<td>Serum Insulin(^98, 99)</td>
<td>µU/ml</td>
<td>&gt;13</td>
<td>&gt;13</td>
</tr>
<tr>
<td>Serum Proinsulin(^98, 99)</td>
<td>pm/L</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Body Composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Lean Body Mass</td>
<td>kg or kg/m</td>
<td>&lt; 10(^{th}) percentile of HIV-uninfected distribution</td>
<td>&lt; 10(^{th}) percentile of HIV-uninfected distribution</td>
</tr>
<tr>
<td>Total Body Fat</td>
<td>kg or kg/m</td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
</tr>
<tr>
<td>% fat</td>
<td></td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
</tr>
<tr>
<td>Limb Fat</td>
<td>kg or kg/m</td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
</tr>
<tr>
<td>Trunk Fat</td>
<td>kg or kg/m</td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
<td>&lt; 10(^{th}) or &gt;90(^{th}) percentile of HIV-uninfected distribution</td>
</tr>
<tr>
<td>Bone density (DEXA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteopenia</td>
<td></td>
<td>Z score -1 to -2.4 from hip or spine</td>
<td>Z score -1 to -2.4 from hip or spine</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td></td>
<td>Z score &lt; -2.5 from hip or spine</td>
<td>Z score &lt; -2.5 from hip or spine</td>
</tr>
</tbody>
</table>
7.3 Registration and Stratification

To avoid the selection bias in prevalence estimates that could occur if each site chose which subjects to enroll, and to make the study subject pool as representative as possible of all subjects seen at a site, a stratified sampling design will be implemented. Participating sites must agree to approach all subjects identified for enrollment.

During the study, sites will be required to maintain a log of all HIV-infected children and youth receiving care at the site, as this will provide the sampling frame from which the P1045 subjects will be drawn. Sites will use the SDMC randomization system to randomly select subjects who should be approached for study participation. Site accrual may be capped, depending on projected enrollment and the number of sites agreeing to participate in the study. Sampling probabilities will be adjusted throughout the study as necessary.

It is important that the HIV-uninfected subjects be ‘similar’ to the HIV-infected subjects with respect to gender and race/ethnicity. When the study opens, one HIV-uninfected sibling closest in age to the HIV-infected subject successfully enrolled into the study may simultaneously be offered study participation. General enrollment of HIV-uninfected subjects will be delayed until 100 subjects have been enrolled. At that time, the SDMC will generate reports for the protocol team describing the gender and race/ethnicity of enrolled HIV-infected subjects and HIV-uninfected siblings. Using these reports, the SDMC registration system will be set up to ensure that the gender and race/ethnicity distribution of the HIV-uninfected subjects matches those of the HIV-infected subjects. Enrollment should be completed in two years.

Accrual by stratum and by site will be reviewed monthly by the protocol team. Sampling probabilities may be adjusted every 6 months based on the sites’ abilities to accrue more or less than the site cap, actual accrual, and the number of sites participating.

7.4 Sample Size and Accrual

Based on the numbers obtained from current enrollment to PACTG 219C, and focusing on the nine strata defined by Tanner stage, HIV infection status, and antiretroviral treatment history, the proposed sampling scheme should be feasible (see Table 2). Fifty subjects will be enrolled to each of the nine strata for a total of 450 subjects.
7.41 Confidence Interval Approach

The primary objective of the study is to estimate the prevalence of a variety of conditions, both within strata (n=50) and across strata, eg. HIV-infected on PIs versus HIV-uninfected across Tanner Stage (n=150). Table 4 shows the widths of a 2-sided 95% confidence interval (CI) on estimated binomial proportions for 50, 100, or 150 evaluable subjects.

Table 4. Confidence Intervals for Various Prevalences (n=50, 100, and 150).

<table>
<thead>
<tr>
<th>Prevalence (%)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=50</td>
</tr>
<tr>
<td>0</td>
<td>0, 7</td>
</tr>
<tr>
<td>6</td>
<td>1, 17</td>
</tr>
<tr>
<td>10</td>
<td>3, 22</td>
</tr>
<tr>
<td>16</td>
<td>7, 29</td>
</tr>
<tr>
<td>20</td>
<td>10, 34</td>
</tr>
<tr>
<td>26</td>
<td>15, 40</td>
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<tr>
<td>30</td>
<td>18, 45</td>
</tr>
<tr>
<td>36</td>
<td>23, 51</td>
</tr>
<tr>
<td>40</td>
<td>26, 55</td>
</tr>
<tr>
<td>46</td>
<td>32, 61</td>
</tr>
<tr>
<td>50</td>
<td>36, 64</td>
</tr>
</tbody>
</table>

With n=50 in a stratum and a prevalence of 50%, the CI is at its widest (a width of 28%). However, if the true prevalence of the condition is greater than 6%, it will be possible to conclude that the condition has a prevalence >0% as the lower limit of the 95% CI will be greater than 0.

7.42 Hypothesis Testing Approach

Table 5 shows the exact power of two-sided alpha = 0.05 level tests for comparing two binomial proportions with a sample size of 50 in each cell.
Table 5:  Exact power of two-sided tests for comparing two binomial proportions with an alpha level of 0.05 and a sample size of 50 in each stratum.

<table>
<thead>
<tr>
<th>HIV- % abnormality</th>
<th>HIV+ (PI- or PI+) % Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14%</td>
</tr>
<tr>
<td>Rare</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>6%</td>
</tr>
<tr>
<td>Medium</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>12%</td>
</tr>
<tr>
<td>High</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>20%</td>
</tr>
</tbody>
</table>

The table shows that the power to detect differences between the HIV-infected and uninfected strata is low (≤ 59%) when prevalence of a given condition in the infected group is ≤ 20% and the rate in the uninfected group is ≥ 4%. If the prevalence in the HIV-infected group is ≥ 40%, then the study would have at least 80% power to detect a difference from the uninfected group if their prevalence was ≤ 14%. Hypotheses which collapse across strata (e.g., comparing all HIV-infected children and youth regardless of Tanner stage to all HIV+ subjects on PI-containing regimens, n=150 in each group) will have higher power to detect smaller differences in prevalence.

7.5 Monitoring

Data monitoring reports will be generated by the SDMC and reviewed by the study team monthly. Reports will include accrual by stratum and by site.

Six months after the first subject is enrolled, or when 100 subjects have been enrolled, whichever occurs first, a more detailed accrual report including proportions of subjects refusing to participate by stratum and site will be generated. Site caps and sampling probabilities for the HIV-infected subjects will be updated as necessary. In order to collect a comparable number of gender and race/ethnicity matched HIV-uninfected subjects in each stratum, the proportions by gender and race/ethnicity for the HIV-infected subjects enrolled to that point will be determined and general enrollment of all HIV-uninfected subjects will start. These more detailed reviews of accrual will continue to occur every 6 months. The study team will report any problems in study conduct to the Complications RAC.
An interim analysis will be conducted after 50% of the subjects have been accrued or after 1 year - whichever occurs first. Prevalences by strata for the conditions in Table 3 will be calculated with 95% CIs. If accrual is lagging in any strata, the team will need to reconsider the feasibility of continuing enrollment in those cells. In particular, if enrollment of the HIV-uninfected strata is slow, the team may consider allowing enrollment of more than one uninfected sibling, other household members of the HIV-infected enrollee or other closely related individuals. This could compromise the ‘random’ sampling of the HIV-uninfected controls, but could also prevent serious under-acrual. This interim analysis will be reviewed by a Study Monitoring Committee including the Study Chair, Co-chair, NIAID and NICHD Medical Officers, statisticians, data manager, clinical trials specialist, lab data coordinator and independent representatives from the Complications and Adolescent Research Agenda Committees. The Study Monitoring Committee’s recommendations will be forwarded to the Complications RAC. Formal interim analyses will be conducted yearly as long as the study is open to accrual.

7.6 Analysis

7.61 Primary Objectives

The prevalence of each condition of interest will be calculated by stratum with 95% CIs. For body composition, where there are no published normal values, distributions of continuous measures will be summarized by stratum.

Cross-tabulations of each abnormality with other demographic and disease variables will be conducted. Chi-square and Fisher’s exact tests will be used to test univariate associations. All continuous variable outcome measures including DEXA BMD results, height, weight, BMI, z-scores (height, weight, BMI), hip/waist ratio, mid thigh circumference, and skin-fold means will be summarized within strata and compared across strata using methods for continuous data.

Multiple logistic and linear regression will be used to evaluate whether prevalence rates of each abnormality or distributions vary across the sub-groups of interest. The analysis will include covariates to adjust for patient disease characteristics. Stepwise regression techniques will be used to select variables to include in the most parsimonious model. Weighted prevalence estimates accounting for the sampling design will be used if the data are collapsed across strata. Methods for hierarchical data may be necessary to account for the enrollment of siblings.
7.62 Secondary Objectives

Objective 2.21 will use females with Tanner Stage ≥4 from P1045 (vertically-infected) and comparable subjects enrolled in ATN 021 (horizontally-infected). Prevalence rates will be calculated and compared in the two populations using methods outlined in 7.61. Although the two studies are designed to be as similar as possible, these comparative analyses will need interpreted with care.

Objectives 2.22, and 2.23 aim to explore the relationship of duration of exposure to HAART and specific components of HAART and the relationship between drug and non-drug factors to prevalence of the abnormalities. Similar statistical methods will be used, but it must be recognized that these results will be exploratory and hypothesis-generating in nature.

8.0 HUMAN SUBJECTS

The Division of AIDS has concluded that this protocol does NOT meet Federal requirements governing prisoner participation in clinical trials and should NOT be considered by local IRBs for the recruitment of prisoners.

8.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol, the informed consent document (Appendix X), and any subsequent modifications must be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. Written informed consent must be obtained from the subject (or parents/legal guardians of subjects who cannot consent for themselves, such as those below the legal age). The subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks of the study. The informed consent will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject (or parent/legal guardian).

8.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified only by a coded number to maintain subject confidentiality. All records will be kept in a secured area. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA, the pharmaceutical sponsor, or the NIAID.
8.3 Study Discontinuation

The study may be discontinued at any time by the NIAID.

9.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by PACTG policies. Publication of results involving data collected from both PACTG P1045 and ATN 021 will be governed by PACTG and ATN policies, as outlined in the Memorandum of Agreement between the two groups.

10.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other bloodborne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK mandated by the International Air Transport Association Dangerous Goods Regulations-Packing Instruction 602. Refer to individual carrier guidelines (e.g., Federal Express or Airborne) for specific instructions.
11.0 REFERENCES


42. Wensing, A.M., M. Reedijk, C. Richter, C.A. Boucher, and J.C. Borleffs. 2001. Replacing ritonavir by nelfinavir or nelfinavir/saquinavir as part of highly active antiretroviral therapy leads to an improvement of triglyceride levels. AIDS 15:2191-2193.


98. Quest Diagnostics, Baltimore Maryland.

99. Esoterix Laboratories, Calabasas Hills, CA.
APPENDIX I

SCHEDULE OF EVALUATIONS

<table>
<thead>
<tr>
<th>Event</th>
<th>Screening</th>
<th>Entry/Study Visit(s)</th>
<th>Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLINICAL EVALUATIONS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiretroviral Medications History</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapeutic Agents History</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanner Stage Assessment</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometric Measurements</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>DEXA Scans</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Physical Assessment</td>
<td></td>
<td>X</td>
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</tr>
<tr>
<td>Body Image Questionnaire</td>
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</tr>
<tr>
<td>Food Frequency Questionnaire</td>
<td></td>
<td>X</td>
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<tr>
<td>Dietary Intake Evaluation</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LABORATORY EVALUATIONS</td>
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<td>X&lt;sup&gt;15, 16&lt;/sup&gt;</td>
<td>Site</td>
</tr>
<tr>
<td>HIV Test</td>
<td>X&lt;sup&gt;15&lt;/sup&gt;</td>
<td></td>
<td>Site</td>
</tr>
<tr>
<td>Pregnancy Test</td>
<td>X&lt;sup&gt;15&lt;/sup&gt;</td>
<td>X&lt;sup&gt;15, 16&lt;/sup&gt;</td>
<td>Site</td>
</tr>
<tr>
<td>Fasting Lipid Panel &amp; CRP</td>
<td></td>
<td>4 mL</td>
<td>BRI&lt;sup&gt;17&lt;/sup&gt;</td>
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<td>Oral Glucose Tolerance Test</td>
<td></td>
<td>14 mL</td>
<td>BRI</td>
</tr>
<tr>
<td>Fasting Lactate</td>
<td></td>
<td>3 mL</td>
<td>BRI</td>
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<tr>
<td>Serum BUN &amp; Creatinine</td>
<td></td>
<td>6 mL</td>
<td>Site</td>
</tr>
<tr>
<td>HIV RNA PCR</td>
<td></td>
<td>3 mL</td>
<td>PVCL</td>
</tr>
<tr>
<td>CD4 T Cell Count/Percentage</td>
<td></td>
<td>3-6 mL&lt;sup&gt;15&lt;/sup&gt;</td>
<td>Site</td>
</tr>
<tr>
<td>Urinalysis (dipstick)</td>
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<td></td>
<td>Site</td>
</tr>
<tr>
<td>STORED SPECIMENS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Serum</td>
<td></td>
<td>8.5 mL</td>
<td>BRI</td>
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<tr>
<td>Plasma/PBMCs</td>
<td></td>
<td>7 mL</td>
<td>BRI</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td>X</td>
<td>BRI</td>
</tr>
<tr>
<td>TOTAL BLOOD VOLUME</td>
<td></td>
<td></td>
<td>48.5-51.5 mL</td>
</tr>
</tbody>
</table>
APPENDIX I

1. Screening evaluations must be completed within 30 days prior to study entry.
2. On-study evaluations may be completed at one study visit (Entry), or over the course of up to three study visits (Entry plus a second or third visit). All on-study evaluations must be completed within 30 days of Entry. Refer to Appendix II for fasting and exercise restriction requirements applicable to the study visit during which lab specimens are collected.
3. Antiretroviral medications history (inclusive of ARV history over the subject’s lifetime) and medical history may be abstracted from the subject’s medical chart. Medical history should include whether or not the subject has been immunized against Hepatitis B, and any in utero exposure to antiretrovirals. For subjects on HAART, highest ever HIV-1 RNA value and lowest ever CD4 T cell count must be recorded.
4. Therapeutic agents history (inclusive of non-ARV history over the past year) may be abstracted from the subject’s medical chart. Refer to Appendix IX for instructions.
5. Refer to Appendix VII for instructions on taking anthropometric measurements.
6. Whole body and regional (AP spine and hip) DEXA scans required. Refer to Appendix VIII for guidelines on performing DEXA scans.
7. Food Frequency Questionnaire and Dietary Intake Evaluation should be completed at the study visit during which lab specimens are collected.
8. Only for HIV-uninfected subjects and HIV-infected subjects without appropriate documentation of HIV infection (see Section 4.122 for acceptable assay results).
9. Urine or serum pregnancy test required for all female study subjects who have had their first menses.
10. Lipid panel includes total cholesterol, triglycerides, HDL, and LDL calculated.
11. Includes glucose, insulin, proinsulin, and C-peptide at two time points. Refer to Appendix IV for specific instructions.
12. May use same specimen collected for fasting glucose, if specimen was collected without a tourniquet. Refer to Appendix III for specific instructions.
14. Stored specimens will be processed on site and aliquots will be shipped to the PACTG Specimen Repository. Refer to Appendix V for specific shipment instructions.
15. Blood volume and tube type as recommended by the site laboratory.
16. If DEXA scans are not completed within 30 days of Screening, a second urine or serum pregnancy test is required on the day the DEXA scans are to be performed for all female study subjects who have had their first menses. A negative test result must be documented before proceeding with DEXA scans.
17. Biomedical Research Institute: PACTG Specimen Repository.
APPENDIX II

SPECIMEN COLLECTION AND PROCESSING INSTRUCTIONS:
HIV RNA PCR, LIPID PANEL & C-REACTIVE PROTEIN, AND STORED SPECIMENS

General Comments

All primary specimens and specimen aliquots must be entered into the PACTG Laboratory Data Management System (LDMS) computer. All tubes and specimen aliquots are to be labeled with subject ID#, study ID#, site ID#, visit ID#, date and time of collection, and specimen type (e.g., NaF plasma, Serum, PBMC, etc.). Be sure to record time and dose of all medications, both antiretroviral and non-antiretroviral, taken within 24 hours prior to collection of entry specimens.

Subject Preparation

For the study visit during which specimen collection occurs (specifically, prior to administration of the Oral Glucose Tolerance Test), subjects must be in a FASTING state. Refer to Appendix IV for complete instructions.
APPENDIX II

Plasma HIV-1 RNA Ultrasensitive PCR

1. Collect one (3 mL) purple-top EDTA anticoagulated blood tube.
3. Freeze plasma in three 0.6 mL aliquots and store at minus 70°C.
4. Ship 1 aliquot to the designated PACTG Virology Core Laboratory. Ship the remaining 2 aliquots to the PACTG Specimen Repository as described in Appendix V.
6. Prepare replicate dry cell PBMC pellet aliquots at 1 x 10^6 cells per vial.
7. Freeze dry cell PBMC aliquots at minus 70°C.
8. Ship frozen vials on dry ice on a monthly basis to the PACTG Specimen Repository as described in Appendix V.

Lipid Panel and C-Reactive Protein

1. Collect one (4 mL) Serum Separator Tube (SST).
2. Let blood clot for 30 minutes in a vertical position.
3. Within 1 hour of collection, spin blood tube for 10 minutes in a swinging bucket centrifuge or for 15 minutes in a fixed angle centrifuge at 1100 to 1300 X g.
4. Separate serum from cells and aliquot into 4-5 0.5 mL cryo vials.
5. Freeze serum aliquot vials at minus 70°C within 8 hours of collection.
6. Ship frozen vials on dry ice on a monthly basis to the PACTG Specimen Repository as described in Appendix V.

Serum Storage

1. Collect one (8.5 mL) Serum Separator Tube (SST).
2. Let blood clot for 30 minutes in a vertical position.
3. Within 1 hour of collection, spin blood tube for 10 minutes in a swinging bucket centrifuge or for 15 minutes in a fixed angle centrifuge at 1100 to 1300 X g.
4. Separate serum from cells and aliquot into 8-9 0.5 mL cryo vials.
5. Freeze serum aliquot vials at minus 70°C within 8 hours of collection.
6. Ship frozen vials on dry ice on a monthly basis to the PACTG Specimen Repository as described in Appendix V.
APPENDIX II

Plasma/PBMC Storage

Plasma
1. Collect one (7 mL) purple-top EDTA anticoagulated blood tube.
3. Freeze plasma in 7-8 0.5 mL aliquots and store at minus 70°C.
4. Ship frozen vials on dry ice on a monthly basis to the PACTG Specimen Repository as described in Appendix V.

PBMC
2. Prepare replicate dry cell PBMC pellet aliquots at 1 x 10^6 cells per vial.
3. Freeze dry cell PBMC aliquots at minus 70°C.
4. Ship frozen vials on dry ice on a monthly basis to the PACTG Specimen Repository as described in Appendix V.

Urine Storage

1. Collect random urine (10 mL) in a clean container.
2. Refrigerate urine in transit to the laboratory.
3. Aliquot urine into ten (1 mL) cryovials.
4. Freeze urine aliquots at minus 70°C within 8 hours of collection.
5. Ship frozen vials on dry ice on a monthly basis to the PACTG Specimen Repository as described in Appendix V.
APPENDIX III

SPECIMEN COLLECTION AND PROCESSING INSTRUCTIONS:
LACTATE LEVEL

Venous lactate levels are highly dependent on collection techniques. The procedures listed below should be followed precisely. **Note that the specimen must be collected in a chilled gray-top tube and processed within 30 minutes of collection.**

Lactate can be run on the same specimen collected for the baseline glucose for the OGTT if the specimen was collected without a tourniquet. (Refer to Appendix IV.)

1. The subject MUST be in a fasting state and have avoided strenuous exercise the day of the study visit and the day before the study visit (refer to definitions in Appendix IV).
2. In order to minimize subject discomfort, we urge sites to apply a topical anesthetic (e.g. Emla® Cream) prior to venipuncture.
3. Have subject sit, relaxed for 5 minutes prior to venipuncture.
4. Instruct subject to not clench the fist before or during the procedure and to relax the hand as much as possible.
5. Use of a tourniquet should be avoided, if at all possible. If a tourniquet is necessary, then apply the tourniquet lightly and draw lactate first before the other samples with the tourniquet still in place.
6. Collect one chilled (3 mL) gray-top Sodium Fluoride/Potassium Oxalate anticoagulated tube. Label tube with subject ID#, study ID#, site ID#, visit ID#, date and time of collection, and specimen type BLD/SPO/BLD.
7. Place the specimen immediately on ice, and complete specimen processing Steps 7 & 8 **within 30 minutes of specimen collection.**
8. Centrifuge grey-top tube at 800 to 1,000 x g for 10 minutes.
9. Remove plasma and prepare replicate 0.5 mL plasma aliquots and freeze at minus 70°C.
10. Label aliquots with subject ID#, study ID#, site ID#, visit ID#, date and time of collection, and specimen type BLD/SPO/PL1.
11. Specimens should be batched and sent to the PACTG Specimen Repository on a monthly basis as described in Appendix V.
APPENDIX IV

SPECIMEN COLLECTION AND PROCESSING INSTRUCTIONS:
ORAL GLUCOSE TOLERANCE TEST (OGTT)

Subjects MUST be in a fasting state. Subjects should consume no food or beverage (other than
normal amounts of plain water) for at least 8 hours before collection of entry specimens.
Subjects should be asked whether they have fasted for this minimum length of time prior to
collection of entry specimens. If fasting requirements have not been met, subjects should return
to the clinic on another day in a fasting state. Subjects should also avoid strenuous exercise (i.e.
running, any form of structured resistance exercise, heavy lifting, stair-climbing [more than one
flight]) the day of the study visit and the day before the study visit.

Although subjects must fast prior to collection of entry specimens, they should be specifically
instructed to take all required medications according to their regular schedules and with the usual
amounts of water, to the extent that they can be tolerated in a fasting condition. Exception:
Subjects on Type II DM medications must stop taking these medications 48 hours prior to the
specimen collection for the OGTT.

Glucose
1. Collect one chilled (3 mL) gray-top Sodium Fluoride/Potassium Oxalate anticoagulated tube.
   Label tube with subject ID#, study ID#, site ID#, visit ID#, date and time of collection, and
   specimen type BLD/SPO/BLD.
2. Place the specimen immediately on ice, and complete processing steps 3 & 4 within 30
   minutes of collection.
3. Centrifuge gray-top tube at 800 to 1,000 x g for 10 minutes.
4. Remove plasma and prepare replicate 0.5 mL plasma aliquots and freeze at minus 70°C.
5. Label aliquots with subject ID#, study ID#, site ID#, visit ID#, date and time of collection,
   and specimen type BLD/SPO/PL1.
6. Ship frozen vials on dry ice to the PACTG Specimen Repository on a monthly basis as
described in Appendix V.

Insulin, Proinsulin and C-Peptide
1. Collect one (4 mL) Serum Separator Tube (SST). Label tube with subject ID#, study ID#, site
   ID#, visit ID#, date and time of collection, and specimen type BLD/SST/BLD.
2. Let blood clot for 30 minutes in a vertical position.
APPENDIX IV

3. Within 1 hour of collection, spin blood tube for 10 minutes in a swinging bucket centrifuge or for 15 minutes in a fixed angle centrifuge at 1100 to 1300 X g.
4. Separate serum from cells and aliquot into four 0.5 mL cryo vials.
5. Freeze serum aliquot vials at minus 70°C within 8 hours of collection.
6. Label aliquots with subject ID#, study ID#, site ID#, visit ID#, date and time of collection, and specimen type BLD/SST/SER.
7. Ship frozen vials on dry ice to the PACTG Specimen Repository on a monthly basis as described in Appendix V.

After collection of baseline specimens, the subject will immediately consume 75g of chilled oral dextrose in solution accompanied by up to 200mL of water. T=0 is the time that the consumption of the dextrose solution is completed. The subject will wait for 120 minutes after consumption of dextrose solution. Then specimen collection and processing for glucose, insulin, proinsulin, and c-peptide will be repeated as outlined above.

Caution: The subject should not eat or smoke during the test interval.
APPENDIX V

INSTRUCTIONS FOR SHIPMENT OF REPOSITORY SPECIMENS

REPOSITORY FACILITY

Biomedical Research Institute (BRI)
c/o John C. Ward, Jr.
12264 Wilkins Avenue, Bay F
Rockville, MD 20852
Phone: 301-881-7636
Fax: 301-770-9811
E-mail: BRIRepository@AOL.com
LDMS code: 999

SPECIFICATIONS/INSTRUCTIONS

Please refer to the Repository Guidelines on the PACTG Web Page for more detailed instructions and to determine which specimens are eligible to be stored at the Repository. Send only those protocol specimens approved to be stored at the Repository. 

NOTE: These instructions are for NIAID sites only.

1. Per the Repository Guidelines, Section 2.0, sites should send specimens to the PACTG Specimen Repository at BRI (“Specimen Repository”) once a month following the schedule below. Shipments to the Specimen Repository should be limited to Monday through Wednesday of your designated week. Shipments should be sent via overnight courier. Do not ship the day before a holiday. The Specimen Repository is closed on weekends and holidays and will be unable to receive the specimens. Please call the Specimen Repository around a holiday to determine the available days for shipping.

<table>
<thead>
<tr>
<th>Site Numbers</th>
<th>Week 1*</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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<tr>
<td>2802-4005</td>
<td>4101-4705</td>
<td>6501-6904</td>
<td>7101-8101</td>
<td></td>
</tr>
</tbody>
</table>

*Week 1 = First week that includes a Monday

If a site is unable to ship specimens during their designated week, the site must wait until the following month to ship OR contact the Specimen Repository to determine if there is an alternate shipping date available. Non-compliance with the designated shipping week will be monitored.
APPENDIX V

2. All specimens must be clearly and completely labeled according to the PACTG specifications (PID, date of draw, study number, specimen type, etc) and entered into the LDMS.

3. Ship only full boxes of specimens to the Specimen Repository. For sites that have a low number of subjects and are unable to fill a storage box on a monthly basis, less frequent shipments to the Specimen Repository would be allowed (e.g., every 3 months). Boxes may be filled with specimens from multiple protocols to meet the full box requirement.

4. The 2-inch fiberboard storage boxes with 9x9 or 8x8 arrays are recommended for the 2.0 mL Nunc, Wheaton, and Corning brand cryovials; the 10x10 array should only be used for the 2.0 mL Sarsted cryovials.

5. Laboratories that process and store specimens for both the PACTG and AACTG should not mix Pediatric and Adult specimens in the same box, nor send Pediatric and Adult specimens in the same shipment.

6. Frozen viable cells should not be sent in the same storage box with plasma and serum.

7. Every shipment must be accompanied by a LDMS diskette, manifest, and box map (when preparing the LDMS diskette, the LDMS code for the Specimen Repository is 999). The specimens, manifest, and box map should be QC’d prior to shipping to ensure that they match. Boxes with >10% discordance will be returned to the sender for reconciliation. Specimens sent to the Specimen Repository not accompanied by an LDMS diskette or incorrectly labeled will be returned to the sender. Returned boxes and other shipment problems will be reported to the DMC for submission to the Pediatric Laboratory Steering Committee.

8. Subunits without the LDMS must send specimens to their Main Unit for logging into the LDMS and shipping to the Specimen Repository according to the schedule recommended for that site.

9. At least one week prior to your designated shipping week, the site should order shipping containers from the Specimen Repository using the Shipping Container Order Form on the Web (http://aactg.s-3.com/specrepos.htm).

10. The sites are encouraged to use the shipping containers provided by the Specimen Repository to reduce improper packaging problems. All the components of the shipping containers must be used in order to comply with the regulations. Do not substitute any components from other containers.
11. The shipping diskette and storage boxes must be labeled with the batch number and laboratory or clinic site number. Treat each box as a batch. The LDMS diskette should be inserted into a cardboard diskette mailer and placed on top of the polystyrene insulated package along with the paperwork.

Note: The diskette and paperwork should not be placed on the dry ice.

12. All International Air Transportation (IATA) Dangerous Goods regulations must be followed when packing, labeling, and shipping specimens. Please refer to the ACTG Shipping Guidelines and Shipping Checklist on the Web: http://aactg.s-3.com/specship.htm

13. The site must FAX a notification to the Specimen Repository prior to shipping using the “ACTG Specimen Shipment Notice” document on the Web: http://aactg.s-3.com/specship.htm
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GUIDE TO TANNER STAGING

For the purpose of this protocol, stage of sexual maturation will be determined by Tanner stage of breasts in females and by testicular volume (using an orchidometer) in males. Pubic hair will not be used to determine stage of sexual maturation since the presence or absence of pubic hair may be in part genetically determined, and there is a strong contribution of adrenarche. Sites are encouraged to contact the protocol team @actg.teamp1045@fstrf.org with any questions. Clinicians unfamiliar with using an orchidometer should consult their local endocrinologist or oncologist for guidance on how to accurately and sensitively perform this examination. A nurse and/or the subject’s guardian should be present during the examination.

FEMALE BREAST DEVELOPMENT

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Breasts during childhood. The breasts are flat and show no signs of development.</td>
</tr>
<tr>
<td>2</td>
<td>Breast bud stage. Milk ducts and fat tissue forms a small mound.</td>
</tr>
<tr>
<td>3</td>
<td>Breasts continue to grow. Breasts become rounder and fuller; breast fills like a donut with a hole in the middle.</td>
</tr>
<tr>
<td>4</td>
<td>Nipple and areola form separate small mound; absence of “hole” as in Stage 3. Not all girls go through this stage. Some skip stage 4 and go directly to stage 5.</td>
</tr>
<tr>
<td>5</td>
<td>Breast growth enters final stage. Adult breast is full and round shaped.</td>
</tr>
</tbody>
</table>
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MALE GENITALIA DEVELOPMENT

Stage

1
Testicular volume ≤ 3 cc

2
Testicular volume 4-12 cc

3
Testicular volume = 15 cc

4
Testicular volume > 15-25 cc

5
Testicular volume ≥ 25 cc
APPENDIX VII

INSTRUCTIONS FOR ANTHROPOMETRIC MEASUREMENTS

1.0 Training

Inter-measurer variability and errors related to lack of skills could introduce major errors in data. Therefore, study staff who will be taking anthropometric measurements as part of P1045 must have completed the anthropometric training and certification provided either (1) as part of the BIA/AnthroMe Seminars held at past PACTG Meetings, (2) by site personnel who have become certified through one of the BIA/AnthroMe Seminars. Alternatively, study staff may complete the P1045 Anthropometric Training Session at the 2003 PACTG Meeting. All study staff who will be taking anthropometric measurements as part of P1045 must complete practice measurements on 10 volunteers. Reports of the practice measurements must be sent to the P1045 Clinical Trials Specialist for review by the protocol team. Yearly retraining by certified study staff is recommended.

The training will teach measurers techniques for taking body circumference, skinfold and length/height measurements. As part of the training, data recording methods, equipment calibration, and potential sources of error will be demonstrated. Trainees must accurately complete a written and a practical test. For the practical test, trainees must satisfactorily complete two sets of measurements for each procedure under an instructor’s supervision. The written test may be obtained by contacting the P1045 Clinical Trials Specialist and scored on site by certified site personnel.

2.0 General Instructions

Whenever possible, measurements should be taken by a team of two measurers. One measurer takes the measurements while the other measurer records. The measurer taking the measurements calls out the results to the recorder. The recorder repeats the results and then calls out the name of the next measurement. The measurer keeps the measuring instrument in place until the recorder repeats the number. The recorder checks the examinee’s position during the procedure. The subject's cooperation is extremely important for obtaining accurate measurements.

All measurements are to be recorded on the Anthropometric Measurements Form.

Circumferences and skinfold measurements are made once before repeating them a second time in the same sequence by the same observer. Document measurement conditions (i.e., type of scale used, whether length or height was measured, subject's behavior during procedure, etc.). Always take two measurements in each category. A
third measurement will be needed when the second measurement differs from the first one by one of the following:

- Circumferences, > 0.5 cm.
- Skinfold thickness, > 2 mm for every 10 mm in the first measurement, i.e., 2 mm tolerance for 0- up to 10-mm first measurements; 4-mm tolerance for 10- up to 20-mm first measurements; 6-mm tolerance for 20- up to 30-mm first measurements; 8-mm for 30- up to 40-mm first measurements; and 10-mm tolerance for first measurements > 40 mm.

All measurements are taken on the right side of the subject being measured. If measurements need to be taken on the left side due to abnormalities, be sure to record this on the case report form.

3.0 Equipment

Each center will use the same apparatus for all measurements as described below:

- Circumferences: Fiberglass measuring tapes or paper measuring tapes specific to 1.0 mm
- Skinfolds: The Lange caliper
- Skinfold caliper calibration block
- Grease marking pencil or washable felt-tip marker
- Alcohol wipes for cleaning tapes and calipers
- Anthropometric Measurements Form

4.0 Circumference Measurements

Circumferences should be recorded with the zero end of the tape held by the left hand above the remaining part of the tape held by the right hand. The plane of the tape around the body part should be perpendicular to the long axis of the body part being measured. Care should be taken to ensure that the tape is touching the surface of the skin, but is not altering contours or compressing tissue. Maintenance of a perpendicular plane with the tape touching but not altering contours of the body can be challenging when measuring the obese individual and requires extra care on the part of the examiner.

4.1 Mid-Upper Arm Circumference

To locate the midpoint, the subject’s elbow is flexed to 90 degrees with the palm facing superiorly. The measurer stands above or behind the subject and locates the lateral tip of the acromion by palpating laterally along the superior surface of the spinous process of the scapula. The tape is placed from the acromion process
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to the tip of the olecranon and the midpoint is marked. The arm is now repositioned to hang loosely at the side with the palm facing the thigh. The tape is passed around the arm from left to right, and the free and fixed ends are transferred. Ensuring the tape is at the same level as the mid-upper-arm mark, the measurer tightens the tape so that it touches the skin all around the circumference but does not compress the tissue or alter the contour of the arm. Because the arm in cross-section is not an exact circle but rather oval, some difficulty may be met in ensuring that the tape actually touches the skin on the medial side of the arm. If necessary, the middle finger of the left hand can be used to gently press the tape to the skin. The circumference is then read and recorded twice in centimeters to the nearest millimeter.

4.2 Mid Waist Circumference

Subject should stand during this measurement with feet hip-distance apart. Ask the subject not to try to hold in the stomach during the measurements. All measurements should be made after the subject has exhaled. To ensure reproducibility, locate the level of the umbilicus (belly button) and measure the waist circumference at this level. The tape measure should be perpendicular to the long axis of the body. Perform this measurement twice, recording results in centimeters to the nearest millimeter.

4.3 Maximum Hip Circumference

Subject should stand during this measurement with feet hip-distance apart. Ask the subject not to try to hold in the stomach during the measurements. Viewing the subject from the side, visually identify the widest width of the hip. The widest point is generally where there is maximal protuberance of the buttocks. Measure the circumference at that point, making sure the measuring tape is exactly perpendicular to the long axis of the body. Perform this measurement twice, recording results in centimeters to the nearest millimeter.

4.4 Mid-Thigh (Upper Leg) Circumference

The subject lies down with legs extended. The thigh circumference is measured at the midpoint, located with the subject’s upper leg flexed to 90 degrees in the supine position. The measurer stands alongside the subject and locates the inguinal crease in the midline of the leg. (The midline is also identifiable from the anterior superior iliac spine.) The subject relaxes the leg being measured with knee flexed and foot flat on surface on which the subject is lying. The tape is
placed from this point to the top of the patella, and the midpoint is marked. The tape is passed around the thigh at mid-thigh level. Ensuring that the tape is at the same level as the mid-thigh mark all the way around the leg, the measurer tightens the tape so that it divides the skin all around the circumference but does not compress the tissue or alter the contour of the leg. The circumference is then read. The measurement is made to the nearest 0.1 cm.

4.5 Weight

Use an electronic or beam scale with non-detachable weights. Zero the scale prior to each measure. Use a calibrated scale. Subjects should be weighed while wearing only a hospital gown, underwear, and socks. All other clothing, including shoes, should be removed. Instruct the subject to stand with both feet centered on the scale with arms at the sides. The subject should not move or hold onto anything during the measurement. Allow the scale to stabilize and record the weight in the units provided by the scale (lbs. or kg).

4.6 Height

Height should be measured using a calibrated, wall-mounted stadiometer. For best results, the subject is measured wearing a gown that allows the measurer to visualize the subject's body position. The subject stands with bare feet close together, body and legs straight, arms at sides, relaxed shoulders, and head, back, buttocks, and heels against the wall or shaft of the stadiometer. Instruct the subject to look straight ahead and stand tall, keeping heels on the ground. Bring the headboard down to the top of the subject's head while at eye-to-eye level with the subject and record the height.

5.0 Skinfold Measurements

- Lange calipers will be calibrated with standard metal blocks on the day of each exam. Calipers require calibrating to less than 1.5 mm (i.e., 1.0 mm or less) at each of the 10-, 20-, 30-, 40- and 50-mm test distances. Calipers that do not meet the standards must be removed from service and repaired.
- The fold of skin should be firmly grasped between the left thumb and forefinger (for right-handed observers) and then raised. The fold may be pinched and raised several times to make certain that no musculature is grasped. The skinfold is held firmly with the thumb and index finger, and the calipers are placed below the thumb and finger.
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The grip on the caliper is released completely, allowing the spring to compress the fold. With the fold held, the reading should be taken 3 seconds after caliper jaw pressure is released.

- A firm grip on the skinfold, not exceeding the pain threshold, eliminates or at least substantially reduces the variations in the apparent thickness of skinfold that would result from wide differences in the pulling force of the fingers. It should be noted that the fold is held with the thumb and index finger of the left hand and is not released until the caliper is removed.

- The width of the skin that is enclosed between the fingers cannot be standardized, in its absolute size, for all the sites of the body. With a larger subcutaneous layer, for example, on the thigh, a wider segment of the skin must be “pinched” in order to form a fold compared with areas where the adipose tissue is poorly developed, as it is on the dorsum of the hand. For a given site, the width of the skin should be minimal, still yielding a well-defined fold.

- The depth of the skinfold at which the calipers are placed on the fold also requires comment. The two sides of the fold are not likely to be parallel when the skin is lifted by one hand, being narrower near the crest and larger toward the base. When the calipers are placed at the base, the resulting measurement is too large. The correct distance from the crest of a true fold is obtained when the surfaces are approximately parallel to each other and to the contact surfaces of the calipers.

- It is extremely important to measure skinfolds accurately. Even after extensive practice, it is possible to make errors due to slight misplacement of the calipers or misreading of the dial. To avoid such errors, the following procedure is recommended:
  - Skinfolds should be lifted two or three times to determine the fold to be measured before placing the calipers.
  - Avoid becoming overly anxious to put the calipers in place before determining what is really to be measured.
  - The calipers are placed below the thumb and index finger, and the dial is read. The calipers must be removed and the skinfold released between each measurement.

5.1 Triceps (Arm) Skinfold
The purpose of the Triceps Skinfold measurement is to permit (in conjunction with the Mid-Upper Arm Circumference) an estimation of relative proportions of muscle and fat in the arms.
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The level for the triceps skinfold is the same as that for the midarm circumference, as marked with the felt pen. It is midway between the acromion and the olecranon when the arm is bent at a right angle, and measurement is made at the marked point. (See Section 4.1.) With the subject's arm dropped and hanging loosely, the skinfold is raised from the underlying muscle fascia at this point with a sweeping motion of the fingers to the point at which the observer is holding the fold between the index finger and thumb. The skinfold calipers are then applied to this vertical fold.

5.2 Subscapular Skinfold

The purpose of the Subscapular Skinfold is to estimate the fat depot at the mid back.

The point of measurement is located immediately below the inferior angle of the scapula. The subject stands with his/her back to the observer, with shoulders relaxed and arms hanging loosely at the sides. This posture is most important to prevent movement of the scapulae. The skinfold is picked up, as for the triceps skinfold, by a sweeping motion of the finger and thumb, and the calipers are applied at a slight angle following the natural cleavage of the skin.

5.3 Vertical Umbilical Skinfold

The purpose of the Vertical Umbilical Skinfold measurement is to permit (in conjunction with the Mid Waist Circumference, the Side Skinfold at Umbilical Level, and the Back Skinfold at the Umbilical Level) an estimation of the relationship of the abdominal subcutaneous fat area to the visceral fat area.

The point of measurement is located about 2 cm to the person’s right of his/her umbilicus (belly button). Subject should stand during this measurement with feet hip-distance apart and arms relaxed at the side. Ask the subject not to try to hold in the stomach during the measurements. The skinfold is picked up, as for the triceps skinfold, by a sweeping motion of the finger and thumb, and the calipers are applied vertical to the Mid Waist Circumference. All measurements should be made after the subject has exhaled. Perform this measurement twice, recording results in centimeters to the nearest millimeter.
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5.4 Side Skinfold at Umbilical Level

The purpose of the Side Skinfold at the Umbilical Level measurement is to permit (in conjunction with the Mid Waist Circumference, the Vertical Umbilical Skinfold, and the Back Skinfold at the Umbilical Level) an estimation of the relationship of the abdominal subcutaneous fat area and the visceral fat area.

The point of measurement is located at the mid-axillary line and at the umbilical level. Subject should stand during this measurement with feet hip-distance apart and raise his/her right arm to the side. Ask the subject not to try to hold in the stomach during the measurements. The skinfold is picked up, as for the triceps skinfold, by a sweeping motion of the finger and thumb, and the calipers are applied vertical to the Mid Waist Circumference. All measurements should be ade after the subject has exhaled. Perform this measurement twice, recording results in centimeters to the nearest millimeter.

5.5 Back Skinfold at the Umbilical Level

The purpose of the Back Skinfold at the Umbilical Level measurement is to permit (in conjunction with the Mid Waist Circumference, the Side Skinfold at Umbilical Level, and the Vertical Umbilical Skinfold) an estimation of the relationship of the abdominal subcutaneous fat area and the visceral fat area.

The point of measurement is located about 2 cm to the person’s right of his/her spinal column at the umbilical level. Subject should stand during this measurement with feet hip-distance apart and arms relaxed at the side. Ask the subject not to try to hold in the stomach during the measurements. The skinfold is picked up, as for the triceps skinfold, by a sweeping motion of the finger and thumb, and the calipers are applied vertical to the Mid Waist Circumference. All measurements should be made after the subject has exhaled. Perform this measurement twice, recording results in centimeters to the nearest millimeter.

Note that for some slim children, subcutaneous fat at this site may be too small to obtain a skinfold. Such a situation should be noted on the physical assessment form.
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5.6 Thigh Skinfold

The purpose of the Thigh Skinfold measurement is to permit (in conjunction with the Mid Thigh Circumference) an estimation of relative proportions of muscle and fat in the legs.

The point of measurement is located on the superior surface of the thigh, in the midline at the level of the mid-thigh circumference measurement. The subject lies down with legs positioned as for the mid-thigh circumference measurement. The skinfold is picked up, as for the triceps skinfold measurement, by a sweeping motion of the finger and thumb, and the calipers are applied following the natural cleavage of the skin. Perform this measurement twice, recording results in centimeters to the nearest millimeter.

VERIFICATION PROCEDURES FOR ANTHROPOMETRIC MEASUREMENTS

The measurer completes and records a full set of circumferences and skinfolds. A second complete set is then measured and recorded. If the difference between any first and second measurement is greater than that allowed under the guidelines, the measurer performs a third measurement. All measurements are to be recorded on the Anthropometric Measurements Form.
APPENDIX VIII

AACTG STANDARD OPERATING PROCEDURES: DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA) SCANS

Whole-Body DEXA Scans Performed for Whole Body Composition Measurements and Regional (Spine and Hip) DEXA Scans for Bone Mineral Density (BMD)

1.0 General Instructions

The following is a summary of procedures for whole-body DEXA scans performed for body composition measurements and regional (hip and spine) DEXA scans performed for BMD studies. Please follow the manufacturer’s instructions for operating your instrument. Whole-body and hip and spine scans should be done using standard operating procedures defined by the manufacturer in order to allow comparability between study sites as well as generalizability to subjects outside the study conditions.

Subjects should be scanned on the same instrument using the same version of software throughout the study. The Central Reading Center at Tufts (Jodi Weiner) and the protocol team (actg.teamp1045@fstrf.org) should be notified as soon as possible if an instrument or software change is anticipated, and no scans should be performed without input from the team. The Central Reading Center and the protocol team should also be notified if any adjustments are made to the scanner during service or maintenance.

If the computer is left on continuously, it is necessary to reboot the computer first thing in the morning. This will set the system date and time to the computer’s internal clock and will perform basic checks on the computer system memory.

The subject should be asked about and examined for metal that could be in the scan path. Typical things to look for are earrings, eyeglasses, wristwatches, coins, rings, buttons, buckles, zippers, and support braces. The subject should remove shoes, and it may be necessary to remove skirts, slacks, etc. If in doubt, it is best to remove the object in question. If clothes are removed, a subject gown will be provided and a sheet made available to place over the subject during the scan. Rings that cannot be removed (applicable to the total-body scan only) can be left on, but should be noted and should always be left on in subsequent scans. Small objects removed from the subject should be placed in a small box to keep them together and the box left in the room with the subject. After the scan is performed, the objects should be returned to the subject and the subject queried to make sure he/she has received all objects back.
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1.1 Lunar Systems

- Perform the calibration procedure and quality control checks daily.
- Enter the scan type from the main menu.
- Enter basic subject information in the Lunar Mandatory Information screen:
  - For First Name, enter P1045.
  - For Last Name, enter the subject’s PID number.
  - Enter the subject’s date of birth, height, weight, sex, and ethnic type.

Note: Do not enter the subject's actual name in order to preserve confidentiality.

1.2 Hologic Systems

- The calibration procedure and quality control checks should be performed daily.
- Go to “Patient” in the menu.
  - For First Name, enter P1045.
  - For Last Name, enter the subject’s PID number. Then press Enter.
  - Enter the subject’s date of birth and sex.

Note: Do not enter the subject's actual name in order to preserve confidentiality.

- Press F10, and check that P1045 and the subject’s PID appear at the top of the screen.
- Position the subject as discussed below, then press F3 (Scan), and choose the appropriate scan.
- Press F10, and let the scan complete itself. Never move the subject off the table until the tabletop is centered over the base.

1.3 Phantom Scanning

A special phantom, one used for calibrating your instrument for lean and fat mass, will be sent or brought to each site, one time. It is not necessary to perform this scan before the study starts, but it will need to be done before the completion of the study. The data obtained from scanning this phantom will allow the central reader (Tufts University) to analyze the data from your site with better accuracy.

Before sending the phantom to you, a coordinator from Tufts will contact you to confirm where the phantom should be sent and who will receive it. The instructions for performing a scan of the phantom will be provided by Tufts University. They will be provided to you with the phantom.
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The data from this scan and the phantom itself (if necessary) should be sent to Tufts University by the methods described in the Instructions for Submission of DEXA Scans section of this appendix.

2.0 Radiation Safety

As part of DEXA evaluations, subjects are exposed to radiation. Most academic and private institutions have radiation safety committees that review clinical protocols in which subjects are exposed to any amount of radiation. Each institution has different requirements that need to be met. It is the responsibility of the site to meet all the local requirements to conduct these tests.

At the time of submitting the protocol application, it is important to consider the following issues:

• Review with your radiologist the calculated amount of radiation that the subject will be exposed to as part of the study.
• If more than one radiation center is going to be used, this applies to each one of the different sites where DEXA scans will be performed.
• At the time of calculating the total amount of radiation, remember that DEXA can be whole body or localized, and that some protocols use only one of the tests, neither, or both. Adjust the amount of radiation to the total number and the types of DEXA scans to be done.

As an example, consider the following calculations. These calculations are specific for the individual machine where they were developed and should be adapted to the site-specific situation.

Example: Subject Dose Estimates for a Whole-Body DEXA

DEXA (dual energy x-ray absorptiometry) measurements will be performed with a total body scanner (QDR-2000 Hologic, Waltham, MA) generating x-rays at two energy levels (40 and 70 kVp). Participants will be lying down on the device and the x-ray will pass through the body in a fine beam. A series of transverse scans will be made from head to toe at 1-cm intervals. Data will be collected for approximately 120 pixel elements per transverse, with pixel size being approximately 5 x 10 mm. The total scan area with this instrument is approximately 60 x 200cm. Scan speed is 16 cm/s (8 cm/s if body weight is greater than 70 kg). This gives a maximum scan of approximately 25 minutes. The maximum radiation dose for the total body measurement will be 5 mrem, the amount of radiation a person receives during 5 days of normal background radiation. Percent body fat will be derived from the DEXA using computer algorithms provided by the manufacturer.
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The maximum radiation doses allowed in human research subjects vary by IRB. The guidelines below are based on the Food and Drug Administration's allowable dose limits for radioactive drugs used in research.

Title 21 CFR (Code of Federal Regulations) Part 361.1 (b)(3) “Limit on radiation dose” states:

"The amount of radioactive material to be administered shall be such that the subject receives the smallest radiation dose with which it is practical to perform the study without jeopardizing the benefits to be obtained from the study.

(i) Under no circumstances may the radiation dose to an adult research subject from a single study or cumulatively from a number of studies conducted within 1 year be generally recognized as safe if such doses exceed the following:

Whole body, active blood-forming organs, lens of the eye, and gonads:

- Single dose: 3 Rems (3,000 mRems)
- Annual and total dose commitment: 5 Rems (5,000 mRems)

Other organs:

- Single dose: 5 Rems (5,000 mRems)
- Annual and total dose commitment: 15 Rems (15,000 mRems)

(ii) For a research subject under 18 years of age at his last birthday, the radiation dose shall not exceed 10 percent of that set forth in paragraph (b)(3)(i) of this section.

(iii) All radioactive material included in the drug either as essential material or as a significant contaminant or impurity shall be included when determining the total doses and dose commitments. Radiation doses from x-ray procedures that are part of the research study (i.e. would not have occurred but for the study) shall also be included. The possibility of followup studies shall be considered for inclusion in the dose calculations."

3.0 Subject Positioning Techniques and Scan Acquisition

3.1 Whole Body

1. When prompted by the program, ask the subject to lie down on the scan table.
2. The centerline on the table pad should divide the subject's body in half. Use the lines on the table pad to ensure the subject is lying straight on the table.
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The subject's head should be directly below (about 1") the horizontal line located along the top of the table pad.

3. Have the subject tilt his/her head back slightly during the scan. This makes placement of the head region cut line easier to place.

4. A foam wedge (or pillow) should not be used under the subject's head as it will affect the body composition results.

5. Have the subject place hands palms down alongside the body with fingers together. Do not overlap hands and legs. The subject's entire body should fit within the scan lines on the table pad.

6. If the subject is too wide to fit within the boundary lines, the technician should proceed to the Hemiscan Protocol section of this appendix. For tall subjects, please proceed to the Tall Subjects section of this appendix.

7. The subject's feet should be held together using a Velcro strap, and the subject is asked not to move until directed to do so.

8. Continuing the scan will cause the x-ray tube to ramp up to the appropriate current and voltage. The operator should check to make sure the orange "X-Ray On" light is lit, and remain in the room to check the progress of the scan acquisition as it appears on the screen.

9. The subject's head should appear with a few blank scan lines above it. As the scan proceeds, the total-body image should be in a straight line vertically on the screen. If these conditions are not met, the scan should be stopped. The scan arm will move to the original start position and the localizer light will come on. The subject should be repositioned as needed.

10. When the detector goes past the subject's feet, the auto stop feature then interrupts the scan and closes the shutter. A message appears on the screen, allowing the operator to continue the scan or shut down the system. After the scan ends the shutter closes, the voltage and current ramp down, and a messages appears for the operator to wait. The scan arm moves to the home position and a screen message appears to inform the operator that the scan is over and to remove the subject from the table. The technician should get to the Total-body Scan Options screen, then save the scan file.

11. The operator may now exit the Total-body portion of the program to do another type of scan. If you plan to analyze the scan for local purposes, that’s fine, but please save an unanalyzed scan as well.

IMPORTANT NOTE: MAKE SURE TO SAVE THE SCAN FILE TO THE HARD DRIVE AND TO THE FLOPPY DISK FOR THE CENTRAL READER. SAVE THE DATA IN RAW FORM - DO NOT SEND ANALYZED SCAN FILES, PLEASE.
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3.2 Hemiscan Protocol for Obese Subjects

Overweight subjects may not fit between the normal scanning parameters. This poses an important problem with respect to whole-body DEXA scanning which does not appear when spine or hip scans alone are contemplated.

The recommended method is to scan half the body. In a study of 27 subjects in whom each half of the body was scanned separately, there was no difference in composition based on DEXA between the left and right halves. In order to facilitate subject flow at the scanning centers, and because obese subjects have to be scanned in slow mode (35 minutes), we recommend that subjects who do not fit within the scan lines have a single hemiscan of their left side.

1. The left side of the body is aligned within the left margin of the scanning table, and the subject is scanned as previously described. This allows the patient’s left side to be completely within the cut lines, so that all the “overflow” occurs on the right side. This overflow should be ignored.
2. During the scan analysis at the Central Reading Site, the midline of the body is selected by the technician to demarcate the left half of the body as the region of interest, and a scan report is produced.
3. The regional scan data are multiplied by 2 to give the whole-body estimate of body composition.

3.3 Tall Subjects

If a person is too tall to fit within the lines, prioritize what part to “cut off” using the following list. Go down the list from top to bottom, and cut off as many parts as needed to fit the person on the table. Please note the parts that were cut off on the “technical problems” line of the DEXA Scan Description Form and make sure that follow-up scans are performed in exactly the same manner.

- Top half of the head (to the tops of the ears)
- Lower foot (toes)
- Bottom half of the head (to the jaw)

1. Position against the board, relaxed.
2. Do not use support under the knees for size, only for comfort when absolutely necessary.
3. If necessary, use a head pillow carefully only after the scanner has passed over the head region. When inserting a pillow under the head, please make sure
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that the subject does not move (the neck may have to be analyzed for lipodystrophy).

3.4 Hip and Spine

For PACTG P1045, hip DEXA scans will be conducted on the left side, unless there is a specific reason not to do so (e.g. subject has a pin or rod in the left hip or has undergone hip replacement). For hip and spine scans, the subject-positioning techniques and the scan acquisition procedures should be done using the standard operating procedures defined by the instrument manufacturer.

4.0 Instructions for Submission of DEXA Scans

1. Sites performing whole-body and hip and spine DEXA scans should send the following:
   - The formatted disk containing raw unanalyzed data for each study subject. Data from more than one scan or subject can be sent on a disk, as long as the disk is clearly labeled. Double-check that the disk indeed contains the scans you intend to send!
   - Please label the disk with the following:
     - PACTG P1045
     - Site Number
     - Subject ID Number (PID)
     - Date of scan
     - Scanner type (Hologic or Lunar) and software version (v. 5.76, etc.)
   - Send one hard copy of the subject’s DEXA reports and the DEXA Scan Description Form along with the disk to the contact person below.
   - Notify the contact person listed below by faxing the Fax Notification Form prior to shipping.

NOTE: Before mailing a disk, take it to a computer, put it in the A: drive, and confirm that the file is indeed on the floppy disk!
APPENDIX VIII

2. Scans should be mailed by your preferred carrier (e.g., FedEx, UPS, or Express Mail), using a method that can be traced. Mail your scans to:

Jodi Lee Weiner, B.S.
Senior Research Coordinator/Manager of Tufts Central Reading Center
Tufts University, Jaharis Building
150 Harrison Avenue
Room #211
Boston, MA 02111
Phone: (617) 636-3745
FAX: (617) 636-3662
E-Mail: jodi.weiner@tufts.edu

3) All costs associated with mailing the DEXA scans will be paid for by the study using procedures described by the Central Reading Center at Tufts. Do not send scans “collect” to be billed to the recipient.
APPENDIX VIII

FAX NOTIFICATION FORM:
DEXA Scans Shipped to the Central Reading Center at Tufts

Please Fax This Form Each Time You Send Scans to Tufts

PACTG Protocol Number: __________________________
Date: ______________________

To: Jodi Lee Weiner, B.S.
Senior Research Coordinator/Manager of Tufts Central Reading Center
Tufts University, Jaharis Building
150 Harrison Avenue
Room #211
Boston, MA  02111
Phone: (617) 636-3745
FAX: (617) 636-3662
E-Mail: jodi.weiner@tufts.edu

From: Sender’s name: ___________________________________
Phone number: _________________________________
E-Mail: _________________________________________
Site Name: _______________________________________
Site #: _______________________________________
PID #(#s): _______________________________________
Technician performing scan: __________________________

VIA: Courier: ______________________________________
Airway Bill #: _________________________________
Date to expect: __________________________________

Package Contents:

5 ¼ “ disks: 0 1 2 3 Other _____
3 ½ “ disks: 0 1 2 3 Other _____
APPENDIX VIII

DEXA SCAN DESCRIPTION

Please submit this form with every disk

1. PACTG Protocol Number _____________  PID _____________
   Site Name/Number ___________________________________________
   Technician’s Name/Email ______________________________________
   Date of scan _________
   Instrument Used (circle):   Lunar  Hologic  Other
   Type of Scan (circle):        Whole body          Hip  Spine
   Technical problems? __________________________
   Hemiscan? __________________________

2. PACTG Protocol Number _____________  PID _____________
   Site Name/Number ___________________________________________
   Technician’s Name/Email ______________________________________
   Date of scan _________
   Instrument Used (circle):   Lunar  Hologic  Other
   Type of Scan (circle):        Whole body          Hip  Spine
   Technical problems? __________________________
   Hemiscan? __________________________

3. PACTG Protocol Number _____________  PID _____________
   Site Name/Number ___________________________________________
   Technician’s Name/Email ______________________________________
   Date of scan _________
   Instrument Used (circle):   Lunar  Hologic  Other
   Type of Scan (circle):        Whole body          Hip  Spine
   Technical problems? __________________________
   Hemiscan? __________________________

4. PACTG Protocol Number _____________  PID _____________
   Site Name/Number ___________________________________________
   Technician’s Name/Email ______________________________________
   Date of scan _________
   Instrument Used (circle):   Lunar  Hologic  Other
   Type of Scan (circle):        Whole body          Hip  Spine
   Technical problems? __________________________
   Hemiscan? __________________________
APPENDIX IX

INSTRUCTIONS FOR THERAPEUTIC AGENTS HISTORY

Please record the subject’s use of any of the following medications within one year prior to study entry on the Therapeutic Agents History Form. Medications within the listed categories but not specifically noted should also be recorded.

1. Antidiabetic Agents

   Insulin
   NPH (Humulin N®, Novolin N®), Regular (Humulin R®), Lente (Novo Nordisk®), Lispro (Humalog®)

   Thiazolidinediones (‘‘glitazones’’)
   Troglitazone (Rezulin®), Pioglitazone (Actos®), Rosiglitazone (Avandia®)

   Sulfonylureas
   Acetohexamide (Dymelor®), Chlorpropamide (Diabenase®), Glimepride (Amapryl®), Glipizide (Glucotrol®), Glyburide (Diabeta®, Micronase®) Tolazamide/tolbutamide (Tolinase®)

   Other
   Metformin (Glucophage®), Acarbose (Precose®)

2. Lipid Lowering Agents

   Fibrates
   Gemfibrozil (Lopid®), Fenofibrate (Tricor®), Clofibrate (Atromid®)

   HMG CoA reductase inhibitors (‘‘statins’’)
   Atorvastatin (Lipitor®), Fluvastatin (Lescol®), Lovastatin (Mevacor®), Pravastatin (Pravachol®), Simvastatin (Zocor®)

   Other
   Cholestyramine (Questran®), Colestipol (Colestid®), Niacin (Nicolar®)

3. Hormonal Anabolic Agents

   Growth hormone (Serostim®, Saizen®, Nutropin®, Genotropin®, Biotropin®, Norditropin®, Humatrope CMB®)
   Testosterone (IM injection [Delatestryl®], transdermal patch [Testoderm® or Androderm®] or transdermal gel [Androgel®])
   Methyltestosterone (Oreton Methyl®)
   Nandrolone decanoate (Deca-Durabolin®, Nandrobolic®)
   Oxandrolone (Oxandrin®)
APPENDIX IX

4. Systemic Glucocorticoids
   Prednisone (Orasone®, Deltasone®)
   Methylprednisolone (Solumedrol®)
   Dexamethasone (Decadron®)
   Hydrocortisone (Solucortef®)

5. Inhaled Steroids
   Azmacort®
   Advair Discus
   Flovent
   Pulmicort

6. Appetite Stimulants
   Megestrol acetate (Megace®)
   Dronabinol (Marinol®)
   Cyproheptadine (Periactin®)

7. Over the Counter Agents (sold under a variety of trade names)
   Androstenedione
   DHEA
   Chromium picolinate
   Creatine monohydrate
   Fish oil (n-3 fatty acids)
   GH-releasers (arginine, ornithine, lysine)
   B-hydroxy-B-methylbutyrate (betaHMB)
   L-carnitine
   Alpha lipoic acid
   Shark cartilage

8. Systemic Ketoconazole (Nizoral®)

9. Herbal Medicine
APPENDIX IX

10. Thiazide Diuretics
   Hydrochlorothiazide

11. β-blockers
   Carvedilol
   Metoprolol
   Atenolol

12. Treatment for Osteoporosis
   Estrogens
   Bisphosphonates (Alendronate [Fosamax®] and others)
   Calcitonin
   Fluoride
   PTH

13. Hormonal Contraception

14. Oral Contraceptives/Progestin and Estrogen Combinations

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<tr>
<td>Trivora</td>
<td>Zovia 1/35E</td>
<td>Zovia 1/50E</td>
</tr>
</tbody>
</table>

15. Topical Contraceptives
   Ortho Evra
APPENDIX IX

16. Progestins
   Micronor
   Nor-QD
   Ovrette
   Prometrium
   Provera

17. Injectable Contraceptives/Progestins
   Depo-Provera
APPENDIX X

DIVISION OF AIDS
PEdiatric AIDS CLINICAL TRIALS GROUP (PACTG)
SAMPLE INFORMED CONSENT

For protocol:

SHORT TITLE FOR THE STUDY:

INTRODUCTION

You are/your child is being asked to take part in this research study because:

• you are/your child is infected with the human immunodeficiency virus (HIV), the virus that causes AIDS, or
• you are/your child is not infected with HIV, but we would like to compare your/your child’s study results with the study results of children who are infected with HIV.

This study is sponsored by the National Institutes of Health (NIH). The doctor in charge of this study at this site is: (insert name of Principal Investigator). Before you decide if you want to be/want your child to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree/allow your child to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to look at glucose metabolism (how the body breaks down blood sugar), the amounts of lipids (fats), proteins, and other substances in the blood, the amount of body fat and fat distribution, and how dense (solid) bones are in:
APPENDIX X

- HIV-infected children compared to HIV-uninfected children
- HIV-infected and uninfected children at different stages of sexual development
- HIV-infected children taking a class of anti-HIV drugs called protease inhibitors (PIs) compared to HIV-infected children not taking PIs.

WHAT DO I/DOES MY CHILD HAVE TO DO IF I AM/MY CHILD IS IN THIS STUDY?

Before Starting the Study

Once you agree/allow your child to participate in this study, you/your child will have some basic blood tests done and be asked some questions to be sure you/your child can participate in this study.

- The study staff will ask you some questions about your/your child’s medical history, including immunizations, and use of any past or present anti-HIV drugs. You/your child may be asked for permission to review your/your child’s medical records.
- If you are/your child is not infected with HIV, you/your child will have an HIV test to make sure. About a ½ teaspoon of blood will be taken for this test. You/your child will be informed of the HIV test result as soon as it is available.
- If you are/your child is infected with HIV but your medical records don’t show this, you/your child will have an HIV test. About a ½ teaspoon of blood will be taken for this test. You/your child will be informed of the HIV test result as soon as it is available. Your/your child’s medical chart may be reviewed by the study staff to determine the highest ever level of HIV and the lowest ever number of CD4 cells (cells that fight HIV) in your blood.
- You/your child’s stage of sexual development will be determined. For girls/women, this will be done by looking at how developed the breasts are. For boys/men, this will be done by measuring the size of the testes with a painless instrument called an orchidometer.
- Girls/women who have had their first menstrual period will have a pregnancy test. A small amount of urine or blood (less than 1 teaspoon) will be taken for this test. You/your child will be informed of the test result as soon as it is available. If you are/your child is pregnant, you/your child cannot be in this study.
During the Study

- Some blood (about 3 teaspoons) will be taken to test the levels of glucose (blood sugar), lipids, lactate (a substance found in muscles), insulin (a hormone that regulates blood sugar), and other substances in your blood. You/your child cannot eat or drink anything (except for required medications and normal amounts of plain water) for 8 hours before these blood tests. You/your child must avoid exercise for 1½ days before these blood tests. After this blood is taken, you/your child will be asked to drink a sugar solution. About 2 hours later, 1½ more teaspoons of blood will be taken to retest the levels of glucose and insulin in your/your child’s blood. You/your child will not be allowed to smoke during the 2 hours between blood draws.

- If you are infected with HIV, blood will be taken to determine the amount of HIV and CD4 cells (cells that fight HIV) that are in your/your child’s blood. About 1½ teaspoons of blood will be taken for these tests.

Your/your child’s blood samples will be stored (with usual protectors of identity) in a special laboratory called a repository. Your/your child’s blood samples will not be used for these tests until all of the children needed for the study have been enrolled, which may take 1-2 years. You/your child will not receive the results of these tests unless specifically requested. If you/your child want the results of these tests, you must provide the study staff with current contact information.

- You/your child will be asked to give a urine sample.

- You/your child will have two special x-rays done. These x-rays are called dual energy x-ray absorptiometry or DEXA scans. One of the x-rays will scan the whole body and the other x-ray will scan just the spine and hip. The purpose of the scans is to look at body fat distribution. For these scans, you/your child will be asked to lie down on a table for a total of 25-45 minutes.

  Girls/women may have a second pregnancy test on the day the DEXA scans are to be done. If you are/your child is pregnant, the DEXA scans will not be done and you/your child will be taken off of the study.

- Your/your child’s height and weight will be measured. The distance around your/your child’s waist, hips, arm and thigh will be measured using a measuring tape. Folds of skin will be measured at your/your child’s arm, shoulder, stomach, hip and thigh using calipers, a harmless device that looks like pliers.

- You/your child will be asked to answer some questions about changes in your/your child’s body image and appearance.
APPENDIX X

- You/your child will be asked some questions about the kinds of food you/your child eat normally and how often you/your child eat. You/your child will also be asked specific questions about the food you/your child ate the day before you came to the clinic.

Storage of Blood & Urine Samples

Some of your/your child’s blood and urine will be taken and stored (with usual protectors of identity) and used for future PACTG-approved, HIV-related research. About 4 teaspoons of blood and 2 teaspoons of urine will be taken for this purpose.

Your/your child’s samples will be stored at a special laboratory facility where only approved researchers will have access to them. People who work at the facility will also have access to your/your child’s samples to keep track of them, but these people won’t have information that directly identifies you/your child. Your/your child’s samples will not be sold or directly used to produce commercial products. All proposed research studies using your/your child’s samples will be reviewed by the National Institutes of Health (NIH). There is no time limit on how long your/your child’s samples will be stored.

The researchers do not plan to contact you or your/your child’s regular doctor with the results of studies done using your/you child’s stored samples. This is because research studies are often done with experimental procedures, and results of such studies should not be used to make decisions about your/your child’s medical care. If the researchers decide that the result of a certain study provides important information for your/your child’s medical care, then your/your child’s study doctor will be notified. If you would like to be contacted with this sort of information, you must notify the study staff of any changes in your/your child’s address or phone number.

You may decide that you do not want your/your child’s samples stored for future research studies. You/your child can still participate in this study even if you make this decision.

You may withdraw your consent for the storage and use of your/your child’s samples at any time. If you withdraw your consent, these stored samples will be destroyed.

Please read the following statement carefully and then mark your initials in the appropriate space provided.

_I agree to allow my/my child’s blood and urine samples to be stored for use in future PACTG-approved, HIV-related research studies._

__________ Yes  __________ No  __________ Date
APPENDIX X

HOW MANY CHILDREN WILL TAKE PART IN THIS STUDY?

About 450 children will take part in this study.

HOW LONG WILL I BE/MY CHILD BE IN THIS STUDY?

You/your child will be in this study from the time you agree to participate/allow your child to participate until the study tests and procedures are completed. These tests and procedures may be completed in one study visit (3-4 hours long), or may take two or three study visits to complete (each 1-2 hours long).

WHY WOULD THE DOCTOR TAKE ME/MY CHILD OFF THIS STUDY EARLY?

The study doctor may need to take you/your child off the study early without your permission if:

- the study is cancelled by the National Institutes of Health (NIH), or the site’s Institutional Review Board (IRB). (An IRB is a committee that watches over the safety and rights of research subjects.)
- continuing with study procedures is harmful to you/your child
- you are/your child is not able to attend the study visits as required by the study
- you/your child becomes pregnant

WHAT ARE THE RISKS OF THE STUDY?

Blood Draws

You/your child may feel faint or may feel some discomfort when you are/your child is having blood taken. There may be some swelling, bleeding, or bruising where the needle goes into the skin, or a small blood clot may develop. There is a small risk of infection forming where the needle goes into the skin.

Body Measurements

You/your child may have some discomfort or redness where the folds of skin are measured.
APPENDIX X

Fasting
You/your child cannot eat or drink anything for 8 hours before having blood taken. This may make you/your child feel hungry, lightheaded, irritable, or anxious.

Glucose Tolerance Test
You/your child will have to drink a very sweet, sugary solution before this test. This may make you/your child feel nauseous or vomit.

DEXA Scan
DEXA scans use a small amount of radiation, but are considered safe.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?
You will receive no direct benefit from being in this study. However, you/your child and others may benefit in the future from the information that will be learned from this study.

WHAT OTHER CHOICES DO I/DOES MY CHILD HAVE BESIDES THIS STUDY?
Instead of being in this study, you/your child may continue to be followed by your/your child’s regular doctor.

WHAT ABOUT CONFIDENTIALITY?
To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, the researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects.

People who may review your records include: (insert Name of Site) IRB, National Institutes of Health (NIH), study staff, and study monitors.
APPENDIX X

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about you or your participation in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate of Confidentiality to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without your consent, information that would identify you as a participant in the research project if the study staff learns of possible child abuse and/or a risk of harm to yourself/your baby or others.

WHAT ARE THE COSTS TO ME?

There is no cost to you for study-related tests, procedures or clinic visits. Anti-HIV drugs will not be provided through this study. You may be responsible for the cost of your/your child’s anti-HIV drugs.

Taking part in this study may lead to added costs to you and your insurance company. In some cases it is possible that your insurance company will not pay for these costs because you are/your child is taking part in a research study.

WHAT HAPPENS IF I AM/MY CHILD IS INJURED?

If you are/your child is injured as a result of being in this study, you/your child will be given immediate treatment for your/your child’s injuries. The cost for this treatment will be charged to you or your insurance company. There is no program for compensation either through this institution or the National Institutes of Health (NIH). You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY/MY CHILD’S RIGHTS AS A RESEARCH SUBJECT?

Taking part in this study is completely voluntary. You may choose not to take part/not to allow your child to take part in this study or leave this study/take your child out of the study at any time. You/your child will be treated the same no matter what you decide.
APPENDIX X

We will tell you about new information from this or other studies that may affect your/your child’s health, welfare, or willingness to stay in this study. If you want the results of this study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- name of the investigator or other study staff
- telephone number of above

For questions about your/your child’s rights as a research subject, contact:

- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site
- telephone number of above
**SIGNATURE**

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree/agree to allow your child to take part in this study, please sign your name below.

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<th>Participant’s Signature and Date</th>
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