AIDS Research and Human Retroviruses

DOI: 10.1089/AID.2016.0327

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.
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Insulin-like Growth Factor is Associated with Changes in Body Composition with ART Initiation

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Word Count: Abstract 250; Manuscript 2127

Key words: HIV; obesity; lipodystrophy; antiretroviral therapy, highly active; insulin-like growth factor-1; anthropomorphics

Running head: IGF-1 and ART initiation

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Abstract:

Background: Growth hormone (GH)/insulin-like growth factor (IGF)-1 axis abnormalities have been associated with body composition changes among HIV-infected persons with wasting or lipodystrophy. Little is known of GH/IGF-1 axis alterations with ART initiation or differing ART therapies.

Methods: The AIDS Clinical Trials Group Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) study was a prospective, randomized clinical trial of ART initiation with emtricitabine/tenofovir + efavirenz (FTC/TDF+EFV) vs lamivudine/zidovudine + efavirenz (3TC/ZDV+EFV) in HIV-1-infected individuals from resource-diverse settings. IGF-1 was measured from baseline, week 48, and week 96 stored serum samples. Multivariate models were constructed.

Results: 415 participants were included: 170 (41%) were randomized to FTC/TDF+EFV and 245 (59%) to 3TC/ZDV+EFV. The mean age was 35 years, 60% were black, 42% women. The mean IGF-1 level did not change significantly from baseline to week 96 (-0.65 ng/mL; CI -5.18, 3.87), p=0.78 and there were no differences by treatment arm at week 96, p=0.74. Lower baseline IGF-1 was associated with age, non-white race, greater waist-hip ratio (WHR), low CD4 count and lower baseline albumin (all p<0.01) but not plasma HIV-1 RNA, body mass index (BMI), or treatment arm. Greater change in IGF-1 from baseline to 96 weeks was associated with female sex, smaller WHR change, lower baseline albumin and higher baseline HIV-1 RNA (all p<0.01).

Conclusions: ART initiation with either ZDV or TDF did not significantly impact overall IGF-1 levels. Baseline and on-treatment changes in IGF-1 with ART initiation may be related to the body composition changes that occur after ART initiation.

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Introduction

Initiation of antiretroviral therapy (ART) among HIV-infected individuals is associated with changes in body composition including gains in visceral adiposity, loss or gain of subcutaneous fat, and gains in lean body mass [1,2]. These changes are typically most pronounced among HIV-infected persons with most severe disease (i.e., lowest CD4 lymphocyte counts and highest HIV-1 RNA levels) prior to ART initiation[3,4]. The mechanisms that mediate these body composition changes are unclear, but may be in part regulated by changes in anabolic hormones: Free testosterone levels increase significantly with ART initiation, particularly among HIV-infected individuals with the lowest CD4 lymphocyte counts prior to ART initiation[5]. The role of ART on other anabolic hormones, and the role of other anabolic hormones on these body composition changes are unclear.

The growth hormone (GH) and insulin-like growth hormone (IGF)-1 axis is an important regulator of adiposity, muscle mass, and bone in adults, and multiple studies have demonstrated strong associations between low levels of IGF-1 and obesity, metabolic syndrome, immune dysfunction, cardiovascular disease, physical and cognitive functional impairments, and increased mortality[6-12]. Regulation of the GH/IGF-I axis is complex: hypothalamic GH-releasing hormone (GHRH) secretion triggers pulsatile pituitary secretion of GH, which subsequently results in hepatic and extrahepatic IGF-1 production. A link between HIV infection and abnormalities in the GH/IGF-1 axis was recognized in the early pre-ART era: HIV proteins were present in the hypothalamus of deceased subjects with advanced HIV [13] and injection of the HIV envelope protein (gp120) into the third ventricle of rodents suppressed GH secretion compared to saline[14], suggesting HIV may have direct central effects on GH/IGF-1 dynamics. Subsequent cross-sectional studies have found numerous disruptions including low GH and IGF-1 levels, blunted GH pulsatility, and a relative GH resistant state in prior studies of lipodystrophy and wasting [15-17]. Furthermore, therapies targeting the GH/IGF-1 axis such as the

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growth hormone releasing hormone (GHRH) analogue, tesamorelin, are effective in reversing body composition changes in some but not all lipodystrophic patients[18-20].

No prior studies have assessed changes in IGF-1 with ART initiation, and most studies examining the impact of any ART on IGF-1 are limited to small studies (N<20) in the pre-ART era or with ZDV monotherapy[21]. ZDV use is associated with lipoatrophy, but also achieves high concentrations in the central nervous system[22], thus the impact of ZDV on the GH/IGF-1 axis could be multifactorial. In a preliminary, unpublished analysis, we found significantly higher IGF-1 concentrations (185 ± 6 ng/mL) among participants on ZDV (N=15) compared to participants using non-ZDV based regimens (N=65; 101 ± 4 ng/mL; p<0.001; Erlandson unpublished data). Based on prior studies[17,21], we expected a decreased rather than increased IGF-I level in those taking ZDV. Our preliminary results may have been confounded by ART selection, body composition, gender, comorbidities, physical activity, and other lifestyle factors that were not controlled for in this small sample. Thus, the primary goal of the current study was to compare changes in IGF-1 with randomized ART initiation between a ZDV containing or non-containing regimen, as a potential mechanism in the development of lipodystrophy. Within this large sample, we also wanted to 1) explore clinical factors associated with baseline and change in IGF-1 levels, and 2) determine whether baseline IGF-1 levels were predictive of change in body composition.

Materials and Methods

The AIDS Clinical Trials Group (ACTG) Prospective Evaluation of Antiretrovirals in Resource- Limited Settings (PEARLS) study (ClinicalTrials.gov NCT00084136) was a phase IV, randomized, open-label comparison of the once-daily non-nucleoside reverse transcriptase inhibitor (efavirenz, EFV) with either lamivudine/zidovudine [3TC/ZDV] or emtricitabine/tenofovir disoproxil fumarate [FTC/TDF]. The Data and Safety Monitoring Board recommended stopping

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treatment early with a third regimen (didanosine-EC + FTC + atazanavir) due to inferiority; these participants are not included here. The study included participants from nine countries: Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, United States, and Zimbabwe. As we have previously shown, the two study arms did not differ significantly in virologic, immunologic, or mortality outcomes [23]. The EFV + FTC/TDF arm experienced significantly greater gains in body mass index, waist, arm, and thigh circumference, and significantly fewer cases of lipoatrophy than the EFV + 3TC/ZDV arm [24]. Written informed consent was obtained from all participants, and the human experimentation guidelines of the US Department of Health and Human Services were followed. The study was approved by local Ethics Committees at each participating institution.

Briefly, participants were ≥18 years old, had documented HIV-1 infection, were ART-naïve, and had a CD4 cell count <300 cells/μL within 90 days prior to entry into the study enrollment began in 2005, and participants were followed through May 2010. Participants had received no more than 7 days of cumulative prior ART (prior use of single-dose nevirapine or ZDV for any duration to prevent mother-to-child transmission of HIV was allowed). Body mass index (BMI) was categorized as underweight (<18.5 kg/m^2), normal/overweight (18.5-29.9 kg/m^2), and obese (≥30kg/m^2) [25]. Waist and hip circumference were measured as previously described [24]. IGF-1 levels were measured from stored serum samples using chemiluminescence (Immunodiagnostics Systems, Fountain Hills, AZ) in the Clinical Translational Research Center of the University of Colorado.

Statistical Analyses. Two-sample t-tests were used to describe the differences between baseline, week 48, and week 96 IGF-1 levels respectively, along with baseline to week 48 and baseline to week 96 change in IGF-1 levels, between study treatment arms. Linear regression analysis was used to explore the role of other a priori identified variables and potential confounders in baseline, week 48, week 96 and change in IGF-1 levels. Covariates considered

during model building included study treatment arm, age, race/ethnicity, sex, region (North America, South/Central America, Africa), body mass index, waist-to-hip circumference ratio, CD4 T-lymphocyte count, and plasma HIV-1 RNA concentration; variables with a p value <0.10 were retained in models. Two sensitivity analyses restricted the analyses to participants who remained on the initial randomized ART and to participants who remained virologically suppressed. Additional regression analyses included IGF-1 as a predictor variable and BMI and WHR as outcome measures, adjusted for age, gender, region, treatment group, baseline HIV-1 RNA, and CD4 (< or ≥ 200 cells/μL). Statistical analyses were conducted in SAS v. 9.4 (SAS Institute, Cary, NC) and assumed a two-sided significance level of 0.05. No adjustment was made for multiple comparisons.

Results

Four hundred and fifteen participants of the 1045 individuals who were randomized to receive EFV with either 3TC/ZDV or FTC/TDF had samples available for IGF-1 measurement from baseline, week 48 and week 96. Among participants, 245 (59%) were randomized to EFV + 3TC/ZDV and 170 (41%) to EFV + FTC/TDF. The median age was 35 (IQR 29-43 years), and 42% were women. Between group differences were similar with the exception of race (Table 1).

At baseline, mean IGF-1 level was similar between treatment groups (156.7 ng/mL [95% CI 149.0, 164.4 ng/mL] in 3TC/ZDV+EFV versus 158.5 ng/mL [95% CI 149.1, 168.0 ng/mL] in FTC/TDF+EFV; p=0.77). IGF-1 level was below the age/gender-specific reference range among 32 (8%) participants. The baseline IGF-1 level was significantly lower among underweight participants (131.5 ng/mL [95% CI 109.3, 153.7 ng/mL]) compared to normal/overweight participants (160.9 ng/mL [95% CI 154.4, 167.4 ng/mL], p=0.03), but not significantly different between normal/overweight and obese participants (143.1 ng/mL [95% CI

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123.6, 162.6 ng/mL; p=0.10]). In multivariate models, lower baseline IGF-1 level was significantly associated with increased age, black and other non-white race/ethnicity, greater WHR, lower CD4 count, and lower baseline albumin (all p≤0.02) (Table 2).

Following treatment initiation, mean IGF-1 levels at 96 weeks were not significantly different than baseline (-0.65 ng/mL [95% CI -5.18, 3.87], p=0.78). The mean difference in IGF-1 level from baseline to week 48 was significantly greater in the FTC/TDF+EFV arm (7.0 ng/mL) compared to 3TD/ZDV + EFV (-3.4 ng/mL; p=0.04), but was not significantly different between arms from week 0 to week 96 (0.3 and -1.3 ng/mL, respectively; p=0.74; Figure). In one sensitivity analysis restricted to participants who remained on the initial randomized ART at week 48 (n=376) and week 96 (n=370), these differences were similar (week 48: 6.7 and -3.4 ng/mL, respectively, p=0.05; week 96: -0.3 and -0.7 ng/mL; p=0.93). In another sensitivity analysis restricted to participants who remained virologically suppressed at week 48 (n=376) and week 96 (n=370), these differences were also similar (week 48; 6.5 and -3.5 ng/mL; p=0.06 and week 96; -0.1 and -0.7; p=0.91). In multivariate analyses, female sex, higher baseline HIV-1 RNA, less change in WHR and lower baseline albumin were associated with a greater increase in IGF-1 level from baseline to week 96 (all p<0.01) (Table 2).

To account for known age and gender differences in the expected “normal” range of IGF-1, we investigated whether a baseline IGF-1 below the specific reference range for age and gender might serve as a simple predictor for change in weight with ART initiation. In an unadjusted comparison, participants with low baseline IGF-1 levels had a significantly greater increase in BMI at 96 weeks compared to participants with normal or high baseline IGF-1 (9.9 vs 5.7% increase in BMI, p= 0.03).

Lastly, the ability of IGF-1 to predict changes in BMI or WHR was explored in multivariate models with age, gender, region, treatment group, baseline HIV-1 RNA, CD4 (< or ≥ 200 cells/µL) and baseline albumin level (g/dL). A greater percent increase in BMI from

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baseline to week 96 was associated with lower baseline HIV-1 RNA (<100,000 copies/mL; β 4.07%, SE 1.01, p <0.0001), lower CD4 count (<200 cells/μL; β 4.23%; SE 1.04; p <0.0001), lower baseline albumin level (g/dL; 3.77%; SE 1.06; p=0.0004), assignment to FTC/TDF (β 2.16%; SE 0.99, p=0.03) and younger age (β 0.12%, SE 0.05, p=0.02), but not baseline IGF-1 (continuous). In contrast, in similar multivariate models, greater percent increase in WHR was associated with higher baseline IGF-1 level (β 0.03%, SE 0.01, p=0.01) and assignment to 3TC/ZDV (β2.45%, SE 1.25, p=0.05).

Discussion

Here we have shown the IGF-1 trajectories over 96 weeks of ART initiation, in a cohort of participants from low-, middle- and high-income countries. Although prior literature in the pre-ART era support abnormalities in the GH/IGF-1 axis associated with HIV and ZDV-based regimens[16,21,26], we found no significant changes in IGF-1 with ART initiation, and no significant differences between ZDV-based versus TDF-based regiments. Baseline and change in IGF-1 levels were, however, associated with albumin and WHR.

Due to differing treatment toxicities of the ART-regimens, we anticipated that IGF-1 levels would differ by the two comparative treatment arms in our study. In a small, Eastern European study, IGF-1 was significantly higher among HIV-infected patients on a protease-inhibitor based regimen (N=39) compared to an NNRTI-based regimen (N=17, both regimens with 2 NRTIs)[27]. In contrast, other studies have not found differences: peak growth hormone concentration and growth hormone area under the curve were not significantly different among HIV-infected men and women taking tenofovir, lamivudine or efavirenz[21] . Similarly, growth hormone, IGF-1, and IGFBP-3 concentrations among ZDV-treated patients with AIDS (N=8) or asymptomatic HIV (N=2) were not significantly different than patient not on ZDV (n=6) [17]. To the

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best of our knowledge, this analysis is the first report of differences in IGF-1 in a large cohort with randomized ART, minimizing bias that may influence treatment choice and differences in HIV severity.

The associations between IGF-1, body composition, and the changes in these measures are complex: 1) a low baseline IGF-1 was associated with greater baseline WHR, 2) greater change in IGF-1 from baseline to week 96 was associated with smaller WHR changes, and 3) greater change in WHR was associated with lower baseline IGF-1. How can we make sense of these relationships? First, the low baseline IGF-1 was associated with both greater baseline WHR as well as lower CD4 count, higher HIV-1 viral load, and lower albumin. This combination of findings suggests that low IGF-1 may serve as a marker for HIV disease severity and wasting, with a higher baseline WHR reflecting more loss of hip girdle musculature than abdominal girth. Several prior studies have found a strong association between IGF-1 and AIDS or AIDS wasting: Low IGF-1 has been associated with skeletal muscle wasting among both older adults without HIV infection[28] and with AIDS wasting[29]. Other studies have described AIDS wasting as a growth hormone resistant state, with increased basal growth hormone, but decreased IGF-1 and decreased IGF-binding proteins, resulting in further decreased systemic effects of bioactive IGF-1 [15,30,31]. Lastly, although seldom used in the current ART-era, growth-hormone-based therapies previously demonstrated marked improvement in AIDS wasting [32,33] and an increase in CD4+ T-cells among HIV-infected patients on ART with an incomplete immune response[34].

Second, a greater change in IGF-1 from baseline to week 96 was associated with smaller WHR changes. In contrast to the baseline associations, these findings likely reflect the relationship between adiposity and IGF-1. Among both HIV-infected and uninfected populations, IGF-1 is suppressed in the setting of central abdominal fat or visceral adipose tissue[35-37]. Thus the greater gains in central adiposity following ART initiation[24] may have

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attenuated expected increases in IGF-1 with a “return to health”. This concept is also illustrated in the third point above: greater increases in WHR were associated with both lower baseline IGF-1 and randomized ZDV, independent HIV disease severity. Together, these findings suggest that a low baseline IGF-1 in addition to 3TC/ZDV may be risk factors for the development of WHR- estimated lipodystrophy. Although directionality cannot be assumed with the data, our findings suggest that low baseline IGF-1 and blunted IGF-1 increases with ART are associated with a greater central fat accumulation and/or subcutaneous fat loss.

Several limitations of this study should be noted. Most circulating IGF-1 is bound to one of six different binding proteins which were not assessed in this study [26]. IGF-1 is highly influenced by many different confounders such as nutritional status, alcohol consumption, and physical activity, or by additional comorbidities including hypothyroidism, diabetes, renal function, and liver function. Furthermore, the study population is diverse, with marked differences in nutrition and diet and physical activity, in addition to unmeasured genetic differences. Although we were unable to evaluate many of these additional factors in this setting, we do not expect that confounders such as nutritional status or physical activity would differ significantly by the ART regimen. Ideally, measures of growth hormone stimulation could be assessed to determine the responsiveness of the GH/IGF-1 axis. Anthropomorphic measures such as WHR may not accurately distinguish subcutaneous adiposity and VAT. However, use of measurements such as BMI and WHR allow for translation of findings in the clinical setting where body composition imaging is not routinely available. The study dohas multiple notable strengths: the cohort includes nearly 50% women and a high proportion of persons of African race, populations with very limited data in HIV and IGF-1 previously. We provide the first data on the effect of ART initiation on IGF-1, and the first comparison of two randomized ART, regimens differing only by the NRTI backbone.

In summary, IGF-1 changes did not differ by randomized ART treatment arm. Clinically, an important next question is whether a biomarker, such as IGF-1, prior to or during ART initiation can predict development of body composition changes and inform subsequent clinical care or treatment decisions. The association between IGF-1 and body composition suggest that baseline IGF-1 may be a marker of HIV disease severity, while on-treatment changes in IGF-1 may be associated with the heterogeneity of body composition changes seen following ART initiation. Attention should be directed towards limiting excessive weight gains among underweight and obese persons initiating ART. Lastly, changes in the GH/IGF-1 axis should be investigated with more modern ART regimens, as body composition changes are still common with ART initiation and predict increased morbidity and mortality.

Acknowledgements: We thank the study sites, study participants, and support of the AIDS Clinical Trials Group.

Funding: Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number UM1 AI068634, UM1 AI068636 and UM1 AI106701. KME received support by the National Institute of Aging of the National Institutes of Health under Award Number K23AG050260 and NIH/NCATS Colorado CTSA Grant Number UL1 TR001082, TTB is supported in part by NIH/NIAID K24 AI120834. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The pharmaceutical sponsors (Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, and GlaxoSmithKline) provided study drug and Gilead Sciences provided funding to purchase study drug that was not otherwise available. Bristol Myers Squibb provided atazanavir, didanosine-EC and efavirenz (with consent of Merck); Gilead Sciences, Inc. provided emtricitabine, tenofovir-DF, emtricitabine/tenofovir-DF; GlaxoSmithKline provided lamivudine, zidovudine and lamivudine/zidovudine; and Boehringer Ingelheim Pharmaceuticals, Inc. provided nevirapine. Representatives of the pharmaceutical company sponsors had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest: KME has received research funding from Gilead Sciences and has served as a medical advisor for Theratechnologies. TTB has served as a consultant to Gilead Sciences, Merck, BMS, EMD-Serono, Theratechnologies.

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Insulin-like Growth Factor is Associated with Changes in Body Composition with ART Initiation

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Word Count: Abstract 250; Manuscript 2127

Key words: HIV; obesity; lipodystrophy; antiretroviral therapy, highly active; insulin-like growth factor-1; anthropomorphics

Running head: IGF-1 and ART initiation

Abstract:

Background: Growth hormone (GH)/insulin-like growth factor (IGF)-1 axis abnormalities have been associated with body composition changes among HIV-infected persons with wasting or lipodystrophy. Little is known of GH/IGF-1 axis alterations with ART initiation or differing ART therapies.

Methods: The AIDS Clinical Trials Group Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) study was a prospective, randomized clinical trial of ART initiation with emtricitabine/tenofovir + efavirenz (FTC/TDF+EFV) vs lamivudine/zidovudine + efavirenz (3TC/ZDV+EFV) in HIV-1-infected individuals from resource-diverse settings. IGF-1 was measured from baseline, week 48, and week 96 stored serum samples. Multivariate models were constructed.

Results: 415 participants were included: 170 (41%) were randomized to FTC/TDF+EFV and 245 (59%) to 3TC/ZDV+EFV. The mean age was 35 years, 60% were black, 42% women. The mean IGF-1 level did not change significantly from baseline to week 96 (-0.65 ng/mL; CI -5.18, 3.87), p=0.78 and there were no differences by treatment arm at week 96, p=0.74. Lower

baseline IGF-1 was associated with age, non-white race, greater waist-hip ratio (WHR), low CD4 count and lower baseline albumin (all p<0.01) but not plasma HIV-1 RNA, body mass index (BMI), or treatment arm. Greater change in IGF-1 from baseline to 96 weeks was associated with female sex, smaller WHR change, lower baseline albumin and higher baseline HIV-1 RNA (all p<0.01).

Conclusions: ART initiation with either ZDV or TDF did not significantly impact overall IGF-1 levels. Baseline and on-treatment changes in IGF-1 with ART initiation may be related to the body composition changes that occur after ART initiation.

Introduction

Initiation of antiretroviral therapy (ART) among HIV-infected individuals is associated with changes in body composition including gains in visceral adiposity, loss or gain of subcutaneous fat, and gains in lean body mass [1,2]. These changes are typically most pronounced among HIV-infected persons with most severe disease (i.e., lowest CD4 lymphocyte counts and highest HIV-1 RNA levels) prior to ART initiation[3,4]. The mechanisms that mediate these body composition changes are unclear, but may be in part regulated by changes in anabolic hormones: Free testosterone levels increase significantly with ART initiation, particularly among HIV-infected individuals with the lowest CD4 lymphocyte counts prior to ART initiation[5]. The role of ART on other anabolic hormones, and the role of other anabolic hormones on these body composition changes are unclear.

The growth hormone (GH) and insulin-like growth hormone (IGF)-1 axis is an important regulator of adiposity, muscle mass, and bone in adults, and multiple studies have demonstrated strong associations between low levels of IGF-1 and obesity, metabolic syndrome, immune dysfunction, cardiovascular disease, physical and cognitive functional impairments, and increased mortality[6-12]. Regulation of the GH/IGF-I axis is complex:

hypothalamic GH-releasing hormone (GHRH) secretion triggers pulsatile pituitary secretion of GH, which subsequently results in hepatic and extrahepatic IGF-1 production. A link between HIV infection and abnormalities in the GH/IGF-1 axis was recognized in the early pre-ART era: HIV proteins were present in the hypothalamus of deceased subjects with advanced HIV [13] and injection of the HIV envelope protein (gp120) into the third ventricle of rodents suppressed GH secretion compared to saline[14], suggesting HIV may have direct central effects on GH/IGF-1 dynamics. Subsequent cross-sectional studies have found numerous disruptions including low GH and IGF-1 levels, blunted GH pulsatility, and a relative GH resistant state in prior studies of lipodystrophy and wasting [15-17]. Furthermore, therapies targeting the GH/IGF-1 axis such as the growth hormone releasing hormone (GHRH) analogue, tesamorelin, are effective in reversing body composition changes in some but not all lipodystrophic patients[18-20].

No prior studies have assessed changes in IGF-1 with ART initiation, and most studies examining the impact of any ART on IGF-1 are limited to small studies (N<20) in the pre-ART era or with ZDV monotherapy[21]. ZDV use is associated with lipoatrophy, but also achieves high concentrations in the central nervous system[22], thus the impact of ZDV on the GH/IGF-1 axis could be multifactorial. In a preliminary, unpublished analysis, we found significantly higher IGF-1 concentrations (185 ± 6 ng/mL) among participants on ZDV (N=15) compared to participants using non-ZDV based regimens (N=65; 101 ± 4 ng/mL; p<0.001; Erlandson unpublished data). Based on prior studies[17,21], we expected a decreased rather than increased IGF-I level in those taking ZDV. Our preliminary results may have been confounded by ART selection, body composition, gender, comorbidities, physical activity, and other lifestyle factors that were not controlled for in this small sample. Thus, the primary goal of the current study was to compare changes in IGF-1 with randomized ART initiation between a ZDV containing or non-containing regimen, as a potential mechanism in the development of lipodystrophy. Within this large sample, we also wanted to 1) explore clinical factors associated with baseline and change

in IGF-1 levels, and 2) determine whether baseline IGF-1 levels were predictive of change in body composition.

Materials and Methods

The AIDS Clinical Trials Group (ACTG) Prospective Evaluation of Antiretrovirals in Resource- Limited Settings (PEARLS) study (ClinicalTrials.gov NCT00084136) was a phase IV, randomized, open-label comparison of the once-daily non-nucleoside reverse transcriptase inhibitor (efavirenz, EFV) with either lamivudine/zidovudine [3TC/ZDV] or emtricitabine/tenofovir disoproxil fumarate [FTC/TDF]. The Data and Safety Monitoring Board recommended stopping treatment early with a third regimen (didanosine-EC + FTC + atazanavir) due to inferiority; these participants are not included here. The study included participants from nine countries: Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, United States, and Zimbabwe. As we have previously shown, the two study arms did not differ significantly in virologic, immunologic, or mortality outcomes [23]. The EFV + FTC/TDF arm experienced significantly greater gains in body mass index, waist, arm, and thigh circumference, and significantly fewer cases of lipoatrophy than the EFV + 3TC/ZDV arm [24]. Written informed consent was obtained from all participants, and the human experimentation guidelines of the US Department of Health and Human Services were followed. The study was approved by local Ethics Committees at each participating institution.

Briefly, participants were ≥18 years old, had documented HIV-1 infection, were ART-naïve, and had a CD4 cell count <300 cells/μL within 90 days prior to entry into the study enrollment began in 2005, and participants were followed through May 2010. Participants had received no more than 7 days of cumulative prior ART (prior use of single-dose nevirapine or ZDV for any duration to prevent mother-to-child transmission of HIV was allowed). Body mass index (BMI) was categorized as underweight (<18.5 kg/m^2), normal/overweight (18.5-29.9

kg/m^2), and obese (≥30kg/m^2) [25]. Waist and hip circumference were measured as previously described [24]. IGF-1 levels were measured from stored serum samples using chemiluminescence (Immunodiagnostics Systems, Fountain Hills, AZ) in the Clinical Translational Research Center of the University of Colorado.

Statistical Analyses. Two-sample t-tests were used to describe the differences between baseline, week 48, and week 96 IGF-1 levels respectively, along with baseline to week 48 and baseline to week 96 change in IGF-1 levels, between study treatment arms. Linear regression analysis was used to explore the role of other a priori identified variables and potential confounders in baseline, week 48, week 96 and change in IGF-1 levels. Covariates considered during model building included study treatment arm, age, race/ethnicity, sex, region (North America, South/Central America, Africa), body mass index, waist-to-hip circumference ratio, CD4 T-lymphocyte count, and plasma HIV-1 RNA concentration; variables with a p value <0.10 were retained in models. Two sensitivity analyses restricted the analyses to participants who remained on the initial randomized ART and to participants who remained virologically suppressed. Additional regression analyses included IGF-1 as a predictor variable and BMI and WHR as outcome measures, adjusted for age, gender, region, treatment group, baseline HIV-1 RNA, and CD4 (< or ≥ 200 cells/μL). Statistical analyses were conducted in SAS v. 9.4 (SAS Institute, Cary, NC) and assumed a two-sided significance level of 0.05. No adjustment was made for multiple comparisons.

Results

Four hundred and fifteen participants of the 1045 individuals who were randomized to receive EFV with either 3TC/ZDV or FTC/TDF had samples available for IGF-1 measurement from baseline, week 48 and week 96. Among participants, 245 (59%) were randomized to EFV

+ 3TC/ZDV and 170 (41%) to EFV + FTC/TDF. The median age was 35 (IQR 29-43 years), and 42% were women. Between group differences were similar with the exception of race (Table 1).

At baseline, mean IGF-1 level was similar between treatment groups (156.7 ng/mL [95% CI 149.0, 164.4 ng/mL] in 3TC/ZDV+EFV versus 158.5 ng/mL [95% CI 149.1, 168.0 ng/mL] in FTC/TDF+EFV; p=0.77). IGF-1 level was below the age/gender-specific reference range among 32 (8%) participants. The baseline IGF-1 level was significantly lower among underweight participants (131.5 ng/mL [95% CI 109.3, 153.7 ng/mL]) compared to normal/overweight participants (160.9 ng/mL [95% CI 154.4, 167.4 ng/mL], p=0.03), but not significantly different between normal/overweight and obese participants (143.1 ng/mL [95% CI 123.6, 162.6 ng/mL; p=0.10]). In multivariate models, lower baseline IGF-1 level was significantly associated with increased age, black and other non-white race/ethnicity, greater WHR, lower CD4 count, and lower baseline albumin (all p≤0.02) (Table 2).

Following treatment initiation, mean IGF-1 levels at 96 weeks were not significantly different than baseline (-0.65 ng/mL [95% CI -5.18, 3.87], p=0.78). The mean difference in IGF-1 level from baseline to week 48 was significantly greater in the FTC/TDF+EFV arm (7.0 ng/mL) compared to 3TD/ZDV + EFV (-3.4 ng/mL; p=0.04), but was not significantly different between arms from week 0 to week 96 (0.3 and -1.3 ng/mL, respectively; p=0.74; Figure). In one sensitivity analysis restricted to participants who remained on the initial randomized ART at week 48 (n=376) and week 96 (n=370), these differences were similar (week 48: 6.7 and -3.4 ng/mL, respectively, p=0.05; week 96: -0.3 and -0.7 ng/mL; p=0.93). In another sensitivity analysis restricted to participants who remained virologically suppressed at week 48 (n=376) and week 96 (n=370), these differences were also similar (week 48; 6.5 and -3.5 ng/mL; p=0.06 and week 96; -0.1 and -0.7; p=0.91). In multivariate analyses, female sex, higher baseline HIV-1 RNA, less change in WHR and lower baseline albumin were associated with a greater increase in IGF-1 level from baseline to week 96 (all p<0.01) (Table 2).

To account for known age and gender differences in the expected "normal" range of IGF-1, we investigated whether a baseline IGF-1 below the specific reference range for age and gender might serve as a simple predictor for change in weight with ART initiation. In an unadjusted comparison, participants with low baseline IGF-1 levels had a significantly greater increase in BMI at 96 weeks compared to participants with normal or high baseline IGF-1 (9.9 vs 5.7% increase in BMI, p= 0.03).

Lastly, the ability of IGF-1 to predict changes in BMI or WHR was explored in multivariate models with age, gender, region, treatment group, baseline HIV-1 RNA, CD4 (< or ≥ 200 cells/μL) and baseline albumin level (g/dL). A greater percent increase in BMI from baseline to week 96 was associated with lower baseline HIV-1 RNA (<100,000 copies/mL; β 4.07%, SE 1.01, p <0.0001), lower CD4 count (<200 cells/μL; β 4.23%; SE 1.04; p <0.0001), lower baseline albumin level (g/dL; 3.77%; SE 1.06; p=0.0004), assignment to FTC/TDF (β 2.16%; SE 0.99, p=0.03) and younger age (β 0.12%, SE 0.05, p=0.02), but not baseline IGF-1 (continuous). In contrast, in similar multivariate models, greater percent increase in WHR was associated with higher baseline IGF-1 level (β 0.03%, SE 0.01, p=0.01) and assignment to 3TC/ZDV (β2.45%, SE 1.25, p=0.05).

Discussion

Here we have shown the IGF-1 trajectories over 96 weeks of ART initiation, in a cohort of participants from low-, middle- and high-income countries. Although prior literature in the pre-ART era support abnormalities in the GH/IGF-1 axis associated with HIV and ZDV-based regimens[16,21,26], we found no significant changes in IGF-1 with ART initiation, and no significant differences between ZDV-based versus TDF-based regiments. Baseline and change in IGF-1 levels were, however, associated with albumin and WHR.

Due to differing treatment toxicities of the ART-regimens, we anticipated that IGF-1 levels would differ by the two comparative treatment arms in our study. In a small, Eastern European study, IGF-1 was significantly higher among HIV-infected patients on a protease-inhibitor based regimen (N=39) compared to an NNRTI-based regimen (N=17, both regimens with 2 NRTIs)[27]. In contrast, other studies have not found differences: peak growth hormone concentration and growth hormone area under the curve were not significantly different among HIV-infected men and women taking tenofovir, lamivudine or efavirenz[21] . Similarly, growth hormone, IGF-1, and IGFBP-3 concentrations among ZDV-treated patients with AIDS (N=8) or asymptomatic HIV (N=2) were not significantly different than patient not on ZDV (n=6) [17]. To the best of our knowledge, this analysis is the first report of differences in IGF-1 in a large cohort with randomized ART, minimizing bias that may influence treatment choice and differences in HIV severity.

The associations between IGF-1, body composition, and the changes in these measures are complex: 1) a low baseline IGF-1 was associated with greater baseline WHR, 2) greater change in IGF-1 from baseline to week 96 was associated with smaller WHR changes, and 3) greater change in WHR was associated with lower baseline IGF-1. How can we make sense of these relationships? First, the low baseline IGF-1 was associated with both greater baseline WHR as well as lower CD4 count, higher HIV-1 viral load, and lower albumin. This combination of findings suggests that low IGF-1 may serve as a marker for HIV disease severity and wasting, with a higher baseline WHR reflecting more loss of hip girdle musculature than abdominal girth. Several prior studies have found a strong association between IGF-1 and AIDS or AIDS wasting: Low IGF-1 has been associated with skeletal muscle wasting among both older adults without HIV infection[28] and with AIDS wasting[29]. Other studies have described AIDS wasting as a growth hormone resistant state, with increased basal growth hormone, but decreased IGF-1 and decreased IGF-binding proteins,

resulting in further decreased systemic effects of bioactive IGF-1 [15,30,31]. Lastly, although seldom used in the current ART-era, growth-hormone-based therapies previously demonstrated marked improvement in AIDS wasting [32,33] and an increase in CD4+ T-cells among HIV-infected patients on ART with an incomplete immune response[34].

Second, a greater change in IGF-1 from baseline to week 96 was associated with smaller WHR changes. In contrast to the baseline associations, these findings likely reflect the relationship between adiposity and IGF-1. Among both HIV-infected and uninfected populations, IGF-1 is suppressed in the setting of central abdominal fat or visceral adipose tissue[35-37]. Thus the greater gains in central adiposity following ART initiation[24] may have attenuated expected increases in IGF-1 with a "return to health". This concept is also illustrated in the third point above: greater increases in WHR were associated with both lower baseline IGF-1 and randomized ZDV, independent HIV disease severity. Together, these findings suggest that a low baseline IGF-1 in addition to 3TC/ZDV may be risk factors for the development of WHR- estimated lipodystrophy. Although directionality cannot be assumed with the data, our findings suggest that low baseline IGF-1 and blunted IGF-1 increases with ART are associated with a greater central fat accumulation and/or subcutaneous fat loss.

Several limitations of this study should be noted. Most circulating IGF-1 is bound to one of six different binding proteins which were not assessed in this study [26]. IGF-1 is highly influenced by many different confounders such as nutritional status, alcohol consumption, and physical activity, or by additional comorbidities including hypothyroidism, diabetes, renal function, and liver function. Furthermore, the study population is diverse, with marked differences in nutrition and diet and physical activity, in addition to unmeasured genetic differences. Although we were unable to evaluate many of these additional factors in this setting, we do not expect that confounders such as nutritional status or physical activity would

differ significantly by the ART regimen. Ideally, measures of growth hormone stimulation could be assessed to determine the responsiveness of the GH/IGF-1 axis. Anthropomorphic measures such as WHR may not accurately distinguish subcutaneous adiposity and VAT. However, use of measurements such as BMI and WHR allow for translation of findings in the clinical setting where body composition imaging is not routinely available. The study dohas multiple notable strengths: the cohort includes nearly 50% women and a high proportion of persons of African race, populations with very limited data in HIV and IGF-1 previously. We provide the first data on the effect of ART initiation on IGF-1, and the first comparison of two randomized ART, regimens differing only by the NRTI backbone.

In summary, IGF-1 changes did not differ by randomized ART treatment arm. Clinically, an important next question is whether a biomarker, such as IGF-1, prior to or during ART initiation can predict development of body composition changes and inform subsequent clinical care or treatment decisions. The association between IGF-1 and body composition suggest that baseline IGF-1 may be a marker of HIV disease severity, while on-treatment changes in IGF-1 may be associated with the heterogeneity of body composition changes with ART initiation. Particular attention should be directed towards limiting excessive weight gains among underweight and obese persons initiating ART. Lastly, changes in the GH/IGF-1 axis should be investigated with more modern ART regimens, as body composition changes are still common with ART initiation and predict increased morbidity and mortality.

Acknowledgements: We thank the study sites, study participants, and support of the AIDS Clinical Trials Group.

Funding: Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number UM1 AI068634, UM1 AI068636 and UM1 AI106701. KME received support by the National Institute of Aging of the National Institutes of Health under Award Number K23AG050260 and NIH/NCATS Colorado CTSA Grant Number UL1 TR001082, TTB is supported in part by NIH/NIAID K24 AI120834. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The pharmaceutical sponsors (Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, and GlaxoSmithKline) provided study drug and Gilead Sciences provided funding to purchase study drug that was not otherwise available. Bristol Myers Squibb provided atazanavir, didanosine-EC and efavirenz (with consent of Merck); Gilead Sciences, Inc. provided emtricitabine, tenofovir-DF, emtricitabine/tenofovir-DF; GlaxoSmithKline provided lamivudine, zidovudine and lamivudine/zidovudine; and Boehringer Ingelheim Pharmaceuticals, Inc. provided nevirapine. Representatives of the pharmaceutical company sponsors had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest: KME has received research funding from Gilead Sciences and has served as a medical advisor for Theratechnologies. TTB has served as a consultant to Gilead Sciences, Merck, BMS, EMD-Serono, Theratechnologies.

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Figure.

Treatment arm differences in mean (95% CI) insulin-like growth factor (IGF)-1 levels at weeks 0, 48, and 96. FTC/TDF+EFV, emtricitabine/tenofovir + efavirenz; 3TC/ZDV+EFV, lamivudine/zidovudine + efavirenz

Table 1. Baseline Participant Characteristics

Characteristics	Overall * n=415	3TC/ZDV+EFV n=245	FTC/TDF+EFV n=170	p-value
Age, years (median, IQR)	35 (29 – 43)	35 (29 – 43)	35 (29 – 44)	0.52
Male	239 (57.6)	141 (57.6)	98 (57.7)	0.98
Female	176 (42.4)	104 (42.4)	72 (42.3)	
Race				
White	97 (23.4)	70 (28.6)	27 (15.9)	0.01
Black	247 (59.5)	134 (54.7)	113 (66.5)	
Other	71 (17.1)	41 (16.7)	30 (17.6)	
Region				
United States	71 (17.1)	39 (15.9)	32 (18.8)	0.16
Africa (Malawi, South Africa)	128 (30.8)	69 (28.2)	59 (34.7)	
South American/Carribean (Brazil, Peru, Haiti)	216 (52.1)	137 (55.9)	79 (46.5)	
Body mass index (kg/m^2)	23.2 (20.8 – 26.1)	23.4 (20.8 – 26.0)	22.9 (20.8 – 26.3)	0.36
Waist-to-Hip Ratio	0.88 (0.83 – 0.92)	0.88 (0.83 – 0.93)	0.88 (0.83 – 0.92)	0.97
CD4+ count (cells/μL)	168 (84 – 233)	172 (89 – 244)	164 (83 – 220)	0.35
HIV-1 RNA (log_{10} copies/mL)	5.03 (4.64 – 5.47)	5.05 (4.65 – 5.48)	5.02 (4.61 – 5.44)	0.83
Albumin (g/dL)	4.0 (3.6 – 4.3)	4.0 (3.6 – 4.3)	4.0 (3.6 – 4.3)	0.46

*results presented as n (frequency) or median (intraquartile range).

Table 2. Multivariate analyses of Covariate Associations with IGF-1 serum levels at Baseline and Change in IGF-1 from Baseline to 96 weeks

Baseline IGF-1 Level Associations with Baseline Covariates*			
Covariate	*Coefficient*	*SE*	*p-value*
Age, years	-1.61	0.30	<0.0001
Race (white, reference)	0.00		
Black	-16.77	7.42	0.02
Other race	-28.14	9.03	0.002
Baseline WHR (per 0.1 change)	-11.68	4.17	0.005
CD4 count (>200 cells/μL)	13.47	5.88	0.02
Albumin (g/dL)	24.60	5.51	<0.0001
Baseline to Week 96 Change IGF-1 Level Associations with Baseline and Change Covariates†			
Covariate	*Coefficient*	*SE*	*p-value*
Female sex	13.33	5.16	0.01
Change in WHR (per 0.1 change)	-10.56	3.66	0.004
HIV-1 RNA level (>log5)	-11.39	4.86	0.02
Albumin (g/dL)	-10.80	4.50	0.02

WHR, waist-hip ratio; BMI, body mass index

*Also included sex, HIV-1 RNA, BMI, treatment group.

† Also included: age, race, BMI week 96, CD4+ count week 96, baseline to week 96 change in CD4+ count, baseline to week 96 change in BMI, WHR week 96, and treatment group.