Published in final edited form as:

Pediatr Infect Dis J. 2010 December ; 29(12): 1118–1122. doi:10.1097/INF.0b013e3181ed9f4c.

Associations of Pro-Inflammatory Cytokine Levels with Lipid Profiles, Growth, and Body Composition in HIV-Infected Children Initiating or Changing Antiretroviral Therapy

Joseph S. Cervia, MD¹, Caroline J. Chantry, MD², Michael D. Hughes, PhD³, Carmelita Alvero, MS³, William A. Meyer III, PhD⁴, Janice Hodge, BS⁵, Peggy Borum, PhD⁶, Jack Moye Jr., MD⁷, Stephen A. Spector, MD⁸, and For The Pactg 1010 Team

¹Albert Einstein College of Medicine, Bronx, NY

²University of California, Davis Medical Center, Sacramento, CA

³Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, MA

⁴Quest Diagnostics, Baltimore, MD

⁵Frontier Science & Technology Research Foundation, Amherst, NY

⁶University of Florida, Gainesville, FL

⁷NICHHD, NIH, Bethesda, MD

⁸University of California, San Diego, La Jolla, CA

Keywords

HIV; pro-inflammatory cytokines; children; antiretroviral therapy; lipid profile

Corresponding Author and Address for Reprints: Joseph S. Cervia, M.D., M.B.A., FACP, FAAP, FIDSA, Division of Infectious Diseases, North Shore University Hospital, 300 Community Drive, Manhasset, NY 11030, Telephone: 516-801-9056, Fax: 516-484-8919, cervia@lij.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have no relevant conflicts.

The following sites and individuals have contributed to this study: Howard University: S. Rana, P. Yu, S. Dangol, J. Roa; Bronx Lebanon Hospital Center; St. Jude Children's Hospital: M. Donohoe, K. Knapp, N. Patel, J. Utech; Baylor Texas Children's Hospital: K. Owl, M. Dobmeier, M. Paul, C. Hanson; Children's Hospital of Boston; Harlem Hospital: E. Abrams, D. Calo, M. Fere, S. Champion; North Broward Hospital District; Jacobi Medical Center: A. Wiznia, M. Chin, K. Dorio, J. Abadi; University of Florida: J. Sleasman, R. Lawrence, C. Delany, C. RN; Children Hospital of LA: T. Dunaway, L. Heller; University of Maryland: J. Farley, M. MacFadden; State University of New York at Stony Brook: S. Nachman, M. Davi, C. Seifert, S. Muniz; Metropolitan Hospital Center: M. Bamji, I. Pathak, S. Manwani; Childrens Hospital, Oakland: A. Petru, T. Courville, K. Gold, S. Bessler; Harbor-UCLA Medical Center: M. Keller, K. Zangwill, J. Hayes, A. Gagajena; Columbia Presbyterian Medical Center: A. Higgins, M. Foca; University of Miami: C. Goldberg, M. Bissainthe, C. Mitchell, G. Scott; New York University School of Medicine: T. Hastings, M. Mintor, N. Deygoo, W. Borkowsky; University of Illinois: K. Rich; K. Hayani, J. Camacho; Children's Hospital University of Colorado, Denver: E. McFarland, M. Levin, C. Salbenblatt, E. Barr; Medical College of Georgia: W. Foshee, C. Mani, C. White, B. Kiernan; Johns Hopkins University: S. Marvin, A. Ruff; Duke University: R. McKinney, Y. Choi, L. Ferguson, J. Swetnam; Children's National Medical Center; San Juan City Hospital: M. Acevedo, M. Gonzales, C. Martinez Betancoult, F. Pabon; Yale University School of Medicine: D. Schroeder, S. Romano, M.J. Aquino-de Jesus; Los Angeles County Medical Center: J. Homans, Y. Rodriguez, A. Kovacs; University of Puerto Rico: I. Febo Rodriguez, L. Lugo, I. Heyer, C. Martinez; University of Massachusetts Medical School.

Introduction

Elevated levels of pro-inflammatory cytokines (PIC) TNF- α , IL-1 β and IL-6 have been detected in the blood of HIV-infected individuals. In infected children PIC have been implicated in loss of lean body mass¹ and growth impairment.² Decreases in TNF- α levels have been associated with initiation of effective combination antiretroviral therapy (ART)³ while persistent TNF- α activation has been associated with virologic and immunologic treatment failure in HIV-infected adults.⁴ However, little information is available on the association of PIC in HIV-infected children receiving ART.⁵

Poor growth is a common manifestation of HIV infection in children^{6,7,8,9,10} and predicts survival, regardless of plasma viral load (VL), and CD4⁺ lymphocyte count.¹¹ Yet, the pathophysiology of growth impairment in HIV-infection remains poorly understood as are the relationships between PIC and growth, markers of growth hormone activity, VL, CD4⁺, and ART. In addition, little is understood regarding associations of these factors with serum lipid levels, which have been reported to be elevated in children receiving ART.¹²

The weight of scientific evidence to date, almost exclusively from adults, suggests that PIC are up-regulated in HIV-infection, and are associated with increased HIV replication and adverse metabolic consequences. We undertook this study to address the dearth of knowledge regarding associations of PIC with measures of disease progression, growth, body composition and metabolism in HIV-infected children. We hypothesized that children responding to ART with favorable virologic and immunologic response measures would exhibit PIC reductions associated with improved growth, body composition, and lipid profiles.

Methods

Subjects and Design

Pediatric AIDS Clinical Trials Group Protocol 1010 (P1010) was a multi-site, prospective 48 week cohort study of HIV-infected, pre-pubertal children aged 1 month to <13 years who were beginning or changing ART. Subjects were required to be Tanner Stage I at study entry; however, there was no requirement to exclude children who may have progressed in the relatively brief 48 weeks of study follow-up. The ART criteria for inclusion were one of the following: a) beginning any ART if ART naïve, b) beginning protease inhibitor (PI) based ART if PI naïve, or c) changing ART with at least two new drugs in response to virologic failure. Exclusion criteria have been previously described.¹³ Concurrent acute illness or fever in the 14 days prior to study entry was an exclusion criterion; however, there was no requirement to exclude enrolled subjects with intercurrent illnesses from follow-up visits.

Ethics committee approval was obtained from each participating institution. Written informed consent from the parent or legal guardian and assent from the child when appropriate were also obtained. Accrual began in June 2000 and continued until March 2004. This study focused on the 49 subjects who had sufficient sample available for evaluation of cytokine levels (IL-1 β , IL-6 and TNF- α) evaluated by ultrasensitive ELISA assays. These subjects constituted the study sample.

The following evaluations were performed by trained staff at study entry (within 72 hours prior to ART initiation or change) and 48 weeks thereafter: interim history and physical examination including Tanner staging; anthropometry [weight, height, circumferences (waist, hip and limb) and skinfold thicknesses (triceps, thigh and sub-scapular)]; single frequency tetrapolar bioelectrical impedance analysis [(BIA) 50 KHz, UniQuest-SEAC

BIM4 instrument; UniQuest Limited, Brisbane, Australia] of total body impedance, resistance, reactance, and phase angle; plasma VL (HIV-1 RNA by PCR) and CD4+ T-lymphocyte count and percentage. BIA measures were used to calculate total body water (TBW, L), fat free mass (FFM, kg), and fat mass (FM, kg) using equations previously validated in HIV-infected and uninfected children.¹⁴ For children <8 years of age, the resistance index (H^2/R) was utilized as a measure of total body water.¹⁵

Laboratory Analyses

Laboratories with approved performance in the NIAID Division of AIDS Virology and Immunology Quality Assurance Programs conducted VL by RNA PCR and CD4⁺ lymphocyte count by flow cytometric measurements. TNF- α , IL-1 β , and IL-6 were measured in serum by ELISAs at baseline and 48 weeks with a limit of detection of 0.1 pg/ml (R&D Systems, Minneapolis, MN). PIC specimen aliquots were held at -80°C prior to batch testing at a single laboratory. Fasting lipid panel, apolipoprotein AI and B, glucose, insulin, IGF-1, IGFBP-1 and -3 testing was conducted centrally by Quest Diagnostics.

Statistical Analyses

For the cytokine data, statistical analyses were performed using $\log_{10}$ transformed values. Values less than or equal to the $\log_{10}$ of the assay detection limits were considered as censored values. Linear regression (for censored data if necessary) was used to evaluate associations. At baseline, bivariate analyses evaluated the associations of $\log_{10}$ PIC levels with measures of disease status, prior antiretroviral therapy and z-scores for body composition, lipid and metabolic parameters with reference to children matched for age, gender, and race-ethnicity from the National Health and Nutrition Evaluation Survey (NHANES). Where z-scores were not available or were only available for a restricted age range because the parameters were not measured or were measured only in certain age groups in NHANES, we also evaluated bivariate associations between the $\log_{10}$ PIC levels and these parameters but only report associations which persisted in analyses adjusted for age, sex and race/ethnicity. These parameters were log transformed in these analyses as this gave better satisfaction of assumptions in regression models. Similar analyses were undertaken to evaluate associations between changes in z-scores or changes in log parameters and changes in log PIC levels.

Bivariate associations of changes in $\log_{10}$ PIC levels and changes in CD4% and suppression of VL to <400 copies/ml at week 48 were analyzed in a similar manner. To evaluate whether type of antiretroviral treatment was associated with changes in PIC levels independent of changes in CD4% and suppression of VL, covariates for the broad classes of treatment received during follow-up were included in a model which already included these variables. McNemar's test was used to compare the proportion of children having IL1- β levels which changed from undetectable to detectable or vice versa between baseline and week 48.

Results

Demographic and clinical characteristics of the study population are summarized in Supplemental Digital Content 1 (table). The mean (standard deviation) age at entry was 7.0 (3.7) years with 57% female, and 41% CDC Clinical Class A or N; mean CD4+ % was 25% (14%) and mean plasma VL was 4.46 (0.88) $\log_{10}$ copies/ml corresponding to a geometric mean of 28,926 copies/ml. Twenty-nine percent of subjects were ART naïve at study entry; 22% had received ART but were PI naïve and the remaining 49% had received ART including a PI. By 48 weeks the proportion of children with VL<400 copies/ml had increased from 2% to 55% in response to either initiation or change in ART.

Baseline PIC associations

Baseline PIC levels were detectable (>0.1 pg/ml) for IL-1 β in 28 (58%) of 48 children with measurements, and for TNF- α and IL-6 in all 49 children. Geometric mean baseline levels were 0.19, 4.7 and 2.1 pg/ml, respectively. Baseline PIC levels were not significantly associated with gender or race/ethnicity. TNF- α levels, but not IL-1 β or IL-6 levels, declined significantly with age ($p<0.001$).

At baseline, IL-6 levels were significantly higher among children with lower CD4% ($p=0.010$), but were not associated with CDC Category ($p=0.72$) or VL ($p=0.14$). On average, IL-6 levels increased 28% with each 10% lower CD4% such that the estimated geometric mean IL-6 level for children with CD4% of 45%, 25% and 5% were 1.34, 2.19 and 3.58 pg/ml, respectively. In multivariate analysis of the association between IL-6 and CD4%, CDC Category and VL, only the association with CD4% was significant. There were no significant associations between IL-1 β or TNF- α levels and CD4%, CDC Category or viral load at baseline ($p>0.2$ for all associations).

Baseline IL-1 β levels, but not IL-6 or TNF- α levels, were significantly associated with type of prior therapy (PI-exposed versus PI-naïve/ART-exposed versus ART-naïve; $p=0.046$). This primarily reflected higher levels among PI-exposed children (geometric mean: 0.56 pg/ml) than PI-naïve/ART-exposed (0.26 pg/ml) or ART-naïve children (0.21 pg/ml).

Children with higher baseline IL-6 levels had lower z-scores for TBW ($p=0.014$), FFM ($p=0.014$; Figure 1), body fat percentage ($p=0.052$), and total cholesterol ($p=0.006$); this analysis was, however, restricted to children aged 8 years or older (3 years or older for cholesterol) as NHANES only measured these parameters in this age range. Children with higher IL-6 levels also had lower apolipoprotein A-I levels (Figure 2a) and higher IGFBP-1 levels (Figure 2b), which persisted when adjusted for age, sex and race/ethnicity ($p=0.003$ and $p=0.017$, respectively). Children with higher IL-1 β levels had higher IGF-1 levels and higher IGFBP-3 levels, though both associations were of borderline significance when adjusted for age, sex and race/ethnicity ($p=0.029$ and $p=0.062$, respectively). There were no other significant associations between IL-1 β or TNF- α levels and body composition, lipid or metabolic parameters at baseline.

Changes in PIC between baseline and week 48

Interim cytokine levels at 24 weeks after starting or changing ART were detectable for IL-1 β in 30 of 39 subjects (77%), and for both IL-6 and TNF- α in 40 of 40 (100%) of subjects. Forty-one (40 for IL-1 β) of the 49 subjects had PIC measurements available at 48 weeks after starting or changing ART; one subject was lost to follow-up before week 48 and 7 subjects had insufficient samples available for testing. IL-1 β levels showed little change at week 48 from baseline: 5 of 16 subjects (31%) had increases from below to above the limit of detection whereas 6 of 24 subjects (25%) had decreases from above to below ($p=0.76$), and geometric mean levels at baseline and week 48 were similar among the 40 subjects with measurements at both times (0.21 and 0.18 pg/ml, respectively). All 41 subjects had detectable values of IL-6 and TNF- α at week 48. IL-6 levels decreased on average by 29% from a geometric mean of 2.1 pg/ml at baseline to 1.5 pg/ml at week 48 ($p=0.065$) and TNF- α levels by 33% from 4.7 to 3.1 pg/ml ($p<0.001$).

Changes in TNF- α levels between baseline and week 48 were strongly associated with suppression of VL and improvements in CD4% following initiation or change in antiretroviral treatment. The 26 subjects who achieved VL <400 copies/ml at week 48 had an average decline in TNF- α levels between baseline and week 48 of 45%; whereas, the 15 subjects who had VL ≥ 400 copies/ml at week 48 had an average decline of 5% ($p=0.009$). In addition, each 10% improvement in CD4% between baseline and week 48 was associated

with a 35% decline in TNF- α level ($p < 0.001$; Figure 3). These associations persisted in multivariate analysis which adjusted for baseline levels of TNF- α , VL and CD4%, as well as for age, sex and race/ethnicity: Subjects with VL < 400 copies/ml had TNF- α levels at week 48, which were on average 32% lower than those with VL ≥ 400 copies/ml ($p = 0.018$); and, each 10% improvement in CD4% was associated with a 26% lower TNF- α level at week 48 ($p = 0.002$).

Changes in IL-6 levels between baseline and week 48 were not significantly associated in bivariate analyses with suppression of VL ($p = 0.57$) or with improvements in CD4% ($p = 0.11$) following initiation or change in antiretroviral treatment. However, in multivariate analysis which adjusted for baseline levels of IL-6, VL and CD4%, as well as for age, sex and race/ethnicity and suppression of VL at week 48, there was a significant association between change in IL-6 and change in CD4%: each 10% improvement in CD4% was associated with a 31% lower IL-6 level at week 48 ($p = 0.016$). The increased statistical significance in the multivariate analysis compared with the bivariate analysis primarily reflected the adjustment for baseline CD4% which, as noted above, was associated with baseline IL-6 level. No significant association of changes in IL-1 β at week 48 and changes in CD4% or VL was found.

When the multivariate analyses were extended to include a variable for whether or not antiretroviral therapy included a protease inhibitor, or whether or not it included a non-nucleoside reverse transcriptase inhibitor, and so evaluate whether there was an effect of drug class independent of effects of drugs on VL suppression or CD4% change on change in IL-1 β , TNF- α , or IL-6, no significant effect of drug class was identified.

There was only one association between PIC and IGF-1, growth or changes in lean body mass, body fat distribution, or percent body fat that achieved significance, i.e. $p < 0.05$. This was for the association between IL-1 β at week 48 and waist/height ratio, adjusted for baseline level, age, sex and race/ethnicity ($p = 0.049$). However, the large number of associations evaluated and the fact that an association between IL-1 β at week 48 and z-score for waist/height ratio was not found ($p = 0.33$) suggests that this likely reflects chance variation. However, greater reductions in TNF- α , but not in IL-1 β or IL-6, were significantly associated with greater increases from baseline to week 48 in total ($p = 0.004$), LDL ($p = 0.025$), and HDL ($p < 0.001$) cholesterol levels, but reduction in total/HDL cholesterol ratio ($p = 0.021$; Figure 4). These associations persisted in multivariate analyses, which adjusted for the baseline lipid level and baseline TNF- α , as well as age, sex, and race/ethnicity. Specifically, a 10-fold reduction in TNF- α between baseline and week 48 was associated with an average 62% increase in total cholesterol ($p < 0.001$), an 82% increase in LDL ($p = 0.005$), and a 131% increase in HDL ($p < 0.001$), but a 16% decrease in total/HDL cholesterol ratio ($p = 0.003$). No statistically significant association between decline in TNF- α from week 0 to week 48 and apolipoprotein AI/B ratio was demonstrated.

Discussion

The current study represents to our knowledge the first effort to systematically and prospectively evaluate the association of PIC with virologic and immunologic responses, serum lipids, and measures of growth and body composition in HIV-infected children beginning or changing combination ART. It was observed that PIC were elevated at baseline in this sample of children with mild to moderate disease who were either treatment-naïve or required a change in ART due to poor virologic control. Furthermore, among those who achieved generally favorable virologic and immunologic responses to initiating or changing ART, PIC and particularly TNF- α , were reduced over 48 weeks. This finding is particularly noteworthy as TNF- α levels have been demonstrated to increase with age in healthy

children.¹⁶ Interestingly, although levels of TNF- α were not significantly associated with CD4+% at baseline, TNF- α reductions over 48 weeks were significantly associated with increases in CD4+%. One explanation for this observation may be that at baseline, regardless of its degree of progression as measured by CD4+%, subjects were universally experiencing PIC elevation as a result of HIV-related immune dysregulation. This was likely ameliorated in many subjects by successful ART, for which an increase in CD4+% served as a marker. Another interesting observation was that higher baseline levels of IL-6 were measured in children with more pronounced immunosuppression, more limited body cell mass and lower apolipoprotein AI levels, as well as higher adjusted IGFBP-1 levels. We previously demonstrated that both lean body mass and apolipoprotein AI levels in these children were associated with greater IGF-1 levels both at baseline and over time.^{11,17} Together these findings suggest a causal relationship between IL-6 elevation and lower body cell mass and apolipoprotein AI, which is mediated by growth hormone resistance.

Perhaps most importantly, better virologic and immunologic responses to ART were associated with greater reductions in TNF- α and IL-6. In addition, with competing concerns that have been raised concerning the potential impact of unchecked HIV infection per se versus that of ART upon serum lipid levels, it is somewhat reassuring that observed reductions in TNF- α levels with successful ART were also associated with an improved lipid profile as reflected by reduced total/HDL cholesterol ratio. This latter finding supports the hypothesis that inflammation related to immunologic dysregulation in uncontrolled or poorly controlled HIV infection is, at least in part, responsible for the increase in cardiovascular risk in infected individuals.¹⁸ This premise is also supported by a recent report of dyslipidemia and elevated inflammatory markers in HIV-1 subtype C-infected, treatment-naïve individuals.¹⁹ There are several limitations of this study, including the relatively small number of children for whom sufficient blood was available for cytokine testing, as well as the relatively limited duration of clinical follow-up. Though noteworthy changes in PIC related to successful ART were noted within this time frame, it is very possible that the full impact of these changes upon lipids, growth, and body composition may be fully recognized only with more sustained virologic and immunologic improvement. Nevertheless, as demonstrated in Supplemental Digital Content 1 (table), the study population was well characterized, and not at all atypical of pre-pubertal HIV-infected children undergoing treatment with ART. In addition, as suggested earlier, a number of minor findings in this analysis, including the associations of: baseline IL-1 β with prior PI-containing ART, IL-6 with body fat percentage, IL-1 β with IGF-1 and IGFBP-3, and IL-1 β with waist/height ratio were of borderline statistical significance, and should be interpreted with caution. Finally, there exists little information on normal values for PIC in healthy children to establish a basis for comparison.²⁴

Notwithstanding the complexities of PIC regulation and measurement, these data support the assertion that PIC do appear to persist at reduced though systemically detectable levels in HIV-infected children despite generally favorable virologic and immunologic responses to ART. Insofar as reductions in PIC (especially TNF- α) levels do appear to be associated with more favorable metabolic indicators (i.e. serum lipids, and likely insulin and growth hormone sensitivity) and possibly virologic and immunologic outcomes as well, further studies, possibly including immune-based therapies that could hold the promise of augmenting currently available ART are warranted.

Acknowledgments

The authors would like to acknowledge the children who participated in this study and their families, the entire protocol 1010 team for their contributions and support. Overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID) [U01 AI068632], the Eunice Kennedy Shriver National Institute of Child Health and

Human Development (NICHD), and the National Institute of Mental Health (NIMH) [AI068632]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work was supported by the Statistical and Data Analysis Center at Harvard School of Public Health, under the National Institute of Allergy and Infectious Diseases cooperative agreement #5 U01 AI41110 with the Pediatric AIDS Clinical Trials Group (PACTG) and #1 U01 AI068616 with the IMPAACT Group. Support of the sites was provided by the National Institute of Allergy and Infectious Diseases (NIAID) the NICHD International and Domestic Pediatric and Maternal HIV Clinical Trials Network funded by NICHD (contract number N01-DK-9-001/HHSN267200800001C).

References

1. Cunningham-Rundles S, Kim S, Dnistrian A, Noroski L, Mendez-Botet C, Grassey C, Hinds G, Cervia J. Micronutrient and cytokine interaction in congenital pediatric HIV infection. *J Nutr* 1996;126:2674S–2679S. [PubMed: 8861931]
2. Johann-Liang R, O'Neill L, Cervia J, Haller I, Giunta Y, Licholai T, Noel G. Energy balance, viral burden, insulin-like growth factor-1, interleukin-6 and growth impairment in children infected with human immunodeficiency virus. *AIDS* 2000;14(6):683–690. [PubMed: 10807191]
3. Brazille P, Dereuddre-Bosquet N, Leport C, Clay Boyer P, Vilde J-L, Dormont D, Benveniste O. Decreases on plasma TNF-alpha level and IFN-gamma mRNA level in mononuclear cells (PBMC) and an increase in IL-2 mRNA in PBMC are associated with highly active antiretroviral therapy in HIV-infected patients. *Clin Exp Immunol* 2003;131(2):304–311. [PubMed: 12562393]
4. Aukrust P, Muller F, Lien E, Nordoy I, Liabakk N-B, Kvale D, Espevik T, Froland S. Tumor Necrosis Factor (TNF) system levels in human immunodeficiency virus-infected patients during highly active antiretroviral therapy: Persistent TNF activation is associated with virologic and immunologic treatment failure. *J Infect Dis* 1999;179:74–82. [PubMed: 9841825]
5. Jones B, Chiu S, Wong W, Lim W, Lau Y. Cytokine profiles in human immunodeficiency virus-infected children treated with highly active antiretroviral therapy. *Med Gen Med* 2005;7(2):71.
6. McKinney R, Robertson W. Effect of human immunodeficiency virus infection on the growth of young children. *J Pediatr* 1993;123:579–582. [PubMed: 8410511]
7. Miller T, Evans S, Orav E, Morris V, McIntosh K, Winter H. Growth and body composition in children with human immunodeficiency virus-1 infection. *Am J Clin Nutr* 1993;57:588–592. [PubMed: 8460616]
8. Moya J, Rich K, Kalish L, Sheon A, Diaz C, Cooper E, Pitt J, Handelsman E. Women and Infants Transmission Study Group. Natural history of somatic growth in infants born to women infected by human immunodeficiency virus. *J Pediatr* 1996;128(1):58–69. [PubMed: 8551422]
9. Saavedra J, Henderson R, Perman J, Hutton N, Livingston R, Yolken R. Longitudinal assessment of growth in children born to mothers with human immunodeficiency virus infection. *Arch Pediatr Adolesc Med* 1995;149:497–502. [PubMed: 7735401]
10. Lindegren M, Steinberg S, Byers R. Epidemiology of HIV/AIDS in children. *Pediatr Clin N Amer* 2000;47:1–20.
11. Chantry C, Byrd R, Englund J, Baker C, McKinney R. PACTG 152 Team. Growth, survival and viral load in childhood HIV infection. *Pediatr Infect Dis J* 2003;22(12):1033–1039. [PubMed: 14688560]
12. Farley J, Gona P, Crain M, Cervia J, Oleske J, Seage G, Lindsey J. Pediatric AIDS Clinical Trials Group Study 219C Team. Prevalence of elevated cholesterol and associated risk factors among perinatally HIV-infected children (4–19 years old) in Pediatric AIDS Clinical Trials Group 219C. *J Acquir Immune Defic Syndr* 2005;38(4):480–487. [PubMed: 15764965]
13. Chantry C, Hughes M, Alvero C, Cervia J, Meyer W, Hodge J, Borum P, Moya J. for the PACTG 1010 Team. Lipid and Glucose Alterations in HIV-Infected Children Beginning or Changing Antiretroviral Therapy. *Pediatr* 2008;122(1):e1–e10.
14. Horlick M, Arpad S, Bethel J, Wang J, Moya J, Cuff P, Pierson R, Kotler D. Bioelectrical impedance analysis models for prediction of total body water and fat-free mass in healthy and HIV-infected children and adolescents. *Am J Clin Nutr* 2002;76(5):991–999. [PubMed: 12399270]
15. Kushner R, Schoeller D, Fjeld C, Danford L. Is the impedance index (ht²/R) significant in predicting total body water? *Am J Clin Nutr* 1992;56(5):835–839. [PubMed: 1415001]

16. Hoffmann F, Albert MH, Arenz S, Bidlingmaier C, Berkowicz N, Sedlacek S, Till H, Pawlita I, Renner ED, Weiss M, Belohradsky BH. Intracellular T-cell cytokine levels are age-dependent in healthy children and adults. *Eur Cytokine Netw* 2005;16(4):283–288. [PubMed: 16464742]
17. Chantry C, Hughes M, Alvero C, Cervia J, Hodge J, Borum P, Moye J Jr. PACTG 1010 Team. Insulin-like growth factor-1 and lean body mass in HIV-infected children. *J Acquir Immune Defic Syndr* 2008;48(4):437–443. [PubMed: 18614924]
18. Crowe SM, Westhorpe CL, Mukhamedova N, Jaworowski A, Sviridov D, Bukrinsky M. The macrophage: the intersection between HIV infection and atherosclerosis. *J Leukoc Biol.* 2009 Dec 1; [Epub ahead of print].
19. Fourie CM, Van Rooyen JM, Kruger A, Schutte AE. Lipid Abnormalities in a Never-Treated HIV-1 Subtype C-Infected African Population. *Lipids.* 2009 Nov 15; [Epub ahead of print].

Figure 1.

Association of fat-free mass (FFM) z-scores with IL6-beta levels at baseline (N=24 children aged 8 years or older as NHANES only measured FFM in this age range). Line is the estimated linear regression ($p=0.014$).

Figure 2. Associations of apolipoprotein AI and IGFBP-1 levels with IL6-beta levels at baseline (N=35 and 27 children, respectively, with data available). Lines are the estimated linear regression ($p=0.062$ and $p=0.005$ in unadjusted analysis; $p=0.017$ and $p=0.003$ with adjustment for age, sex and race/ethnicity).

Figure 3.
Association of change in $\log_{10}$ TNF- α and change in CD4 percentage between baseline and week 48 (N=35 with data available).

Figure 4.
Association of change in log10 total/HDL cholesterol ratio and change in log 10 TNF- α between baseline and week 48 (N=30 with data available).