Published in final edited form as:
Antivir Ther. 2013 ; 18(5): 729–733. doi:10.3851/IMP2645.

An Association Between Adiposity and Serum Levels of Macrophage Inflammatory Protein-1α and Soluble CD14: Results from a Cross-Sectional Study

John R. Koethe, MD, MS[1], **Aihua Bian, MS**[2], **Ayumi K. Shintani, PhD, MPH**[2], **C. William Wester, MD, MPH**[1,3], **Husamettin Erdem**[1], and **Todd Hulgan, MD, MPH**[1]

[1]Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA

[2]Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA

[3]Department of Immunology & Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts, 02115, USA

Abstract

Background—Greater adipose tissue is associated with increased circulating high-sensitivity C-reactive protein (hsCRP) levels in HIV-infected adults on antiretroviral therapy (ART), but the relationship between adiposity and other inflammation biomarkers is not well characterized.

Methods—We measured total and regional adipose tissue deposits using dual energy X-ray absorptiometry (DXA) and serum levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) receptor 1 & 2, macrophage inflammatory protein-1α (MIP-1α), macrophage chemotactic protein-1 (MCP-1), soluble CD14, and hsCRP in a cohort of adults on long-term ART. Regression models were adjusted for age, sex, CD4+ count, smoking status, PI use, and daily use of either NSAIDs or aspirin.

Results—The majority (77%) of the 85 study participants were male, median CD4+ cell count was 500 cells/μl (IQR 315, 734) and median BMI was 25.1 kg/m^2 (IQR 22.7, 28.1). DXA measurements of total fat mass were positively associated with serum hsCRP (β=1.82, $p<0.01$) and MIP-1α (β=1.36, $p<0.01$), but negatively associated with soluble CD14 (β=0.90, $p<0.01$). Results were similar for trunk fat, limb fat, and serum leptin level. The positive relationship between DXA measurements and TNF-α receptor 1 approached significance ($p\leq0.07$ for all). There was no consistent relationship between adiposity and serum IL-6, TNF-α receptor 2, or MCP-1 levels.

Conclusions—Total and regional adiposity was associated with serum hsCRP, but not other inflammatory cytokines shown to predict morbidity and mortality in treated HIV. Greater adiposity is associated with higher MIP-1α and lower soluble CD14 levels possibly reflecting an important role for cells of the monocyte/macrophage lineage.

Keywords

HIV; antiretroviral therapy; inflammation; obesity; adipose tissue; nutrition

Corresponding author (same address for requests for reprints): John R. Koethe, MD, MS, Assistant Professor, Division of Infectious Diseases, Vanderbilt University Medical Center, A2200-MCN, 1161 21st Avenue South, Nashville, TN 37232-2582, USA, Phone: +1 (615) 322-2035, Fax: +1 (615) 343-6160, john.r.koethe@vanderbilt.edu.

Conflict of Interest:
TH has received research funding from Merck and Co. None of the other authors of this manuscript report a conflict of interest.

Introduction

At present, the tissue origin of the persistent inflammatory state observed in virally suppressed ART-treated adults is not well understood. The etiology is likely multifactorial, but prior studies found increased serum highly-sensitive C-reactive protein (hsCRP) levels in persons with greater adiposity, suggesting that adipose tissue-derived proinflammatory biomolecules may contribute to inflammation in treated HIV [1–3]. In this study we sought to characterize the relationship of total and regional adiposity to circulating levels of several inflammatory biomarkers associated with increased risk of mortality or the development of metabolic and cardiovascular disease in HIV-infected individuals [4–8]. To this end, we measured serum hsCRP, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) receptor 1 and 2, macrophage inflammatory protein-1α (MIP-1α) and macrophage chemotactic protein-1 (MCP-1; two markers of monocyte/macrophage activation), and soluble CD14 (a marker of monocyte/macrophage activation and an acute-phase reactant) in a cohort of chronically HIV-infected adults on stable ART.

Methods

We obtained dual energy X-ray absorptiometry (DXA) measurements of whole body, trunk and limb adipose tissue mass, serum samples for laboratory assays, and a detailed clinical history from a prospective cohort of chronically suppressed, HIV-infected adults at the Vanderbilt Comprehensive Care Clinic, which has been previously described [9]. Briefly, the inclusion criteria included age >18 years, ≥24 weeks of continuous combination ART treatment with a regimen containing ≥2 nucleoside reverse transcriptase inhibitors (NRTI) and either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI), and a plasma HIV-1 RNA ≤10,000 copies/ml within 180 days of enrollment. Patients with diabetes mellitus not controlled by diet and/or a history of myocardial infarction were excluded. The current ART regimen and most recent CD4+ cell count and HIV-1 RNA level (within 3 months of enrollment) were obtained from the medical record. Current smoking status and self-reported use of routine non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin were recorded.

Whole body DXA measurements of total fat, trunk fat, and limb fat were performed using a Lunar Prodigy bone densitometer (General Electric Healthcare, Wauwatosa, WI, USA). Serum samples were assayed for hsCRP using a commercial laboratory (Laboratory Corporation of America, Birmingham, AL, USA). Averaged duplicate measurements of plasma leptin were measured using the Luminex multi-plex immunoassay system (Millipore, Billerica, MA, USA). Serum levels of IL-6, TNF-α receptor 1 and 2, MCP-1, and MIP-1α were measured using cytometric bead array (BD Biosciences, San Jose, California, USA). Soluble CD14 was measured using ELISA (R&D Systems, Minneapolis, MN, USA).

We assessed the relationship between DXA measurements or serum leptin levels with each inflammation biomarker using separate multivariable linear regression models adjusted for for age, sex, CD4+ lymphocyte count, smoking status, PI use, and daily use of either NSAIDs or aspirin. As a sensitivity analysis, we further adjusted the multivariable models for race (white versus non-white), HIV-1 RNA level, and the inclusion of an older generation NRTI (zidovudine, stavudine, or didanosine) in the treatment regimen. P-values were not adjusted for multiple comparisons.

All serum biomarkers were natural-log transformed to normalize residuals. To aid interpretation, the regression coefficients from multivariable linear regression were back transformed and expressed as a fold-change in the dependent variable (e.g., serum hsCRP) for an interquartile range (IQR) increase in the independent variable (e.g., DXA total fat).

Analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA) and R 2.15.1 (http://www.r-project.org). Analysis scripts are posted at: http://biostat.mc.vanderbilt.edu/wiki/pub/Main/ArchivedAnalyses/JohnRoCytokine.R. The study was approved by the Vanderbilt University Institutional Review Board. All participants provided written informed consent.

Results

Eighty-five participants were included in the study (Table 1). Fifty-six (65%) had a plasma HIV-1 RNA level <50 copies/ml and among the remainder the median viral load was 190 copies/ml (IQR 100, 345). There was no statistically significant difference between sexes according to age, race, CD4+ cell count, smoking status, HIV-1 RNA level, PI or NSAID usage, or any of the inflammation biomarkers. Compared to men, women had higher mean BMI (29.7 vs. 25.0 kg/m^2), serum leptin levels (1558 vs. 348 ng/ml), and total body (30.7 vs. 18.0 kg), trunk (16.9 vs. 11.1 kg), and limb fat (5.9 vs. 4.1 kg, $p<0.01$ for all). None of the participants were taking a HMG CoA reductase inhibitor (statin) at the time of the assessment.

In the primary analysis we assessed the relationship between each inflammation biomarker and the DXA measurements of total fat, trunk fat, and limb fat, in addition to serum leptin level (table 2). For data presentation purposes, we grouped biomarkers associated with at least 3 of the 4 body composition variables (i.e., with a p-value <0.05) together. Serum hsCRP and MIP-1α were positively associated with all four measurements of adipose tissue mass. Soluble CD14 demonstrated a consistent, statistically significant negative association with all three DXA measurements (i.e., the fold-change in soluble CD14 was less than 1.0), but it was not significantly associated with serum leptin level. TNF-α receptor 1 was closely associated with serum leptin level ($p<0.01$), but less strongly for the three DXA measurements ($p\leq0.07$ for all). Serum IL-6, TNF-α receptor 2 and MCP-1 were not significantly associated with any of the DXA measurements.

The associations between fat mass and hsCRP, MIP-1α and CD14 remained significant, and the results for IL-6 and other biomarkers remained non-significant, when the models were further adjusted for race/ethnicity, plasma HIV-1 RNA level, and the inclusion of zidovudine, stavudine, or didanosine in the treatment regimen (71% of participants were receiving one of these medications). The results of a sensitivity analysis using multiple imputation to account for missing leptin (n=2) and IL-6 (n=4) levels were comparable to the analysis with case-wise deletion (i.e., no change in statistically significant associations).

Discussion

In a cohort of HIV-infected adults on effective ART, greater adipose tissue mass was associated with higher serum hsCRP levels, a general marker of systemic inflammation, but not associated with serum IL-6 levels, a stronger predictor of morbidity and mortality in recent studies [4, 10]. However, greater adiposity was associated with higher MIP-1α and lower soluble CD14 levels, which may indicate an important biological interaction between adipose tissue and cells of the monocyte/macrophage lineage with implications for the inflammatory state in treated HIV. Additional prospective studies are needed to confirm these findings and evaluate the underlying biological mechanisms.

Our analysis was limited by the cross-sectional design, the inability to differentiate subcutaneous from visceral fat, and a small sample size which reduced the number of covariates in the statistical models and may have underestimated the effects of concomitant anti-inflammatory medications and/or clinical/demographic characteristics. Lastly, patients with a history of diabetes mellitus or myocardial infarction were excluded from enrollment

and none of the participants were taking a HMG CoA reductase inhibitor (statin) at the time of the study. However, the importance of primary prevention of cardiovascular disease in HIV-infected individuals is increasingly recognized, and further studies are needed to assess whether HMG CoA reductase inhibitors might affect our observed relationship between adiposity and inflammation.

Our finding that greater adiposity was accompanied by higher MIP-1α, but lower soluble CD14, was unexpected given that both MIP-1α and soluble CD14 are produced by cells of the monocyte/macrophage lineage. Adipocyte hypertrophy results in disproportionate increases in MIP-1α, which promote the macrophage migration and accumulation in adipose tissue observed in diet-induced obesity, and further MIP-1α is produced by adipose-resident macrophages [11–14]. Conversely, monocyte/macrophage expression of CD14 is upregulated in the presence of bacterial endotoxin and a variety of non-infectious inflammatory conditions, and prior studies have posited soluble CD14 to be a marker of bacterial translocation in treated HIV [15, 16]. We believe the discordant statistical relationships between MIP-1α and soluble CD14 reflect the contribution of adipocyte-resident macrophage activation to the systemic inflammatory state, independent of bacterial endotoxin exposure, and support the hypothesis that *in situ* adipose tissue inflammation contributes to circulating cytokine levels in treated HIV.

Further studies are needed both to confirm our CD14 findings and to investigate the underlying mechanisms. Hepatocytes are a second major source of circulating soluble CD14, which has a range of functions beyond LPS signaling and may have a role in modulating both inflammation and cellular and humoral immune responses by interacting directly with T and B cells [15–19]. The statisitically significant, negative association between adiposity and CD14 we observed may result from a complex immune regulatory process which our study was not designed to evaluate.

In conclusion, our findings suggest that increased macrophage recruitment and activation accompanying greater adiposity may be reflected in higher serum levels of MIP-1α in treated HIV, but additional studies are needed to understand the etiology underlying lower soluble CD14 levels among those with greater fat mass. We observed an increase in serum hsCRP as has been previously reported, but we did not detect a significant effect of adiposity on other well-described predictors of metabolic and cardiovascular disease in treated HIV, namely serum IL-6 or TNF-α receptor 1 levels. Further prospective studies are needed to characterize the causal pathways linking adipose accumulation and changes in circulating inflammatory mediators, and to understand whether these findings have clinical implications for health outcomes.

Acknowledgments

The authors thank Dr. Michael Rock, Director of the Vanderbilt University Immunology Core Laboratory, for his assistance in completing the laboratory assays and Valerie Mitchell of the Vanderbilt Comprehensive Care Center for her assistance with patient recruitment and sample collection.

Sources of Support: This work was supported by a NIH/NIAID Career Development Award [grant number K23 100700] to JK; a NIH/NCCAM Career Development Award [grant number K23 AT002508] to TH; the Vanderbilt Meharry Center for AIDS Research [grant number AI54999], a Vanderbilt Diabetes Research and Training Center Pilot and Feasibility Award [supported by NIH grant number P60 DK020593], and the National Center for Advancing Translational Sciences [grant number UL1 TR000445]. Additional support for statistical analyses was provided by an award to the Vanderbilt Multidisciplinary Clinical Research Center [NIH grant number P60 AR056116].The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Reingold J, Wanke C, Kotler D, et al. Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study. J Acquir Immune Defic Syndr. 2008; 48:142–148. [PubMed: 18344877]
2. Koethe JR, Bian A, Shintani AK, et al. Serum leptin level mediates the association of body composition and serum C-reactive protein in HIV-infected persons on antiretroviral therapy. AIDS Res Hum Retroviruses. 2012; 28:552–557. [PubMed: 22145933]
3. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med. 2011; 62:141–155. [PubMed: 21090961]
4. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008; 5:e203. [PubMed: 18942885]
5. Shikuma CM, Ribaudo HJ, Zheng Y, et al. Change in High-Sensitivity C-Reactive Protein Levels Following Initiation of Efavirenz-Based Antiretroviral Regimens in HIV-Infected Individuals. AIDS Res Hum Retroviruses. 2011; 27:461–468. [PubMed: 20863238]
6. Ford ES, Greenwald JH, Richterman AG, et al. Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection. AIDS. 2010; 24:1509–1517. [PubMed: 20505494]
7. Brown TT, Tassiopoulos K, Bosch RJ, Shikuma C, McComsey GA. Association between systemic inflammation and incident diabetes in HIV-infected patients after initiation of antiretroviral therapy. Diabetes Care. 2010; 33:2244–2249. [PubMed: 20664016]
8. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr. 2009; 51:268–273. [PubMed: 19387353]
9. Boger MS, Shintani A, Redhage LA, et al. Highly sensitive C-reactive protein, body mass index, and serum lipids in HIV-infected persons receiving antiretroviral therapy: a longitudinal study. J Acquir Immune Defic Syndr. 2009; 52:480–487. [PubMed: 19911471]
10. Duprez DA, Neuhaus J, Kuller LH, et al. Inflammation, Coagulation and Cardiovascular Disease in HIV-Infected Individuals. PLoS One. 2012; 7:e44454. [PubMed: 22970224]
11. Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J Clin Endocrinol Metab. 2005; 90:2282–2289. [PubMed: 15671098]
12. Lee YH, Nair S, Rousseau E, et al. Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes. Diabetologia. 2005; 48:1776–1783. [PubMed: 16059715]
13. Skurk T, Alberti-Huber C, Herder CHauner H. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab. 2007; 92:1023–1033. [PubMed: 17164304]
14. Weisberg SP, McCann D, Desai M, et al. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112:1796–1808. [PubMed: 14679176]
15. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006; 12:1365–1371. [PubMed: 17115046]
16. Bas S, Gauthier BR, Spenato U, Stingelin SGabay C. CD14 is an acute-phase protein. J Immunol. 2004; 172:4470–4479. [PubMed: 15034063]
17. Landmann R, Knopf HP, Link S, et al. Human monocyte CD14 is upregulated by lipopolysaccharide. Infect Immun. 1996; 64:1762–1769. [PubMed: 8613389]
18. Arias MA, Rey Nores JE, Vita N, et al. Cutting edge: human B cell function is regulated by interaction with soluble CD14: opposite effects on IgG1 and IgE production. J Immunol. 2000; 164:3480–3486. [PubMed: 10725700]
19. Rey Nores JE, Bensussan A, Vita N, et al. Soluble CD14 acts as a negative regulator of human T cell activation and function. Eur J Immunol. 1999; 29:265–276. [PubMed: 9933108]

Table 1

Demographic and clinical characteristics of the study subjects (n=85).

Parameter	
Age, median years (interquartile range)	47 (41, 52)
Male, n (%)	65 (77%)
Non-white race, n (%)	37 (44%)
Active tobacco use, n (%)	44 (52%)
Routine NSAID use, n (%)	37 (44%)
Routine aspirin use, n (%)	23 (27%)
CD4+ cell count, cells/ul	500 (315, 734)
Plasma HIV-1 viral load, copies/ml	50 (50, 100)
Protease inhibitor in treatment regimen, n (%)	50 (59%)
Body mass index, kg/m^2	25.1 (22.7, 28.1)
Leptin, ng/ml	252 (114, 900)
DXA measurements	
Total body fat, kg	17.9 (11.8, 25.5)
Total body % fat	26.4 (18.6, 33.9)
Trunk fat, kg	11.1 (7.7, 16.1)
Trunk % fat	30.7 (22.6, 37.8)
Limb fat, kg	7.0 (3.9, 9.8)
Limb, % fat	26.4 (18.6, 34.7)
Serum inflammation biomarkers	
hsCRP, mg/l	3.2 (0.7, 5.5)
Il-6, pg/ml	2.7 (1.8, 3.9)
TNF-α receptor 1, pg/ml	198 (139, 253)
TNF-α receptor 2, pg/ml	2983 (2364, 3877)
MCP-1, pg/ml	46 (33, 67)
MIP-1α, pg/ml	3.9 (3.0, 5.3)
Soluble CD14, mg/ml	2.2 (1.8, 2.6)

Data are presented as N (%) or median (interquartile range).

Table 2

Relationship between adipose tissue and inflammation biomarkers (n=85)

Biomarker	DXA total fat†	DXA trunk fat†	DXA limb fat	Serum leptin
	regression coefficient (95% confidence interval), p-value			
Biomarkers with three or more significant relationships (p<0.05)				
hsCRP	1.82 (1.21, 2.73) p<0.01	1.98 (1.31, 3.00) p<0.01	1.58 (1.04, 2.39) p=0.04	2.57 (1.49, 4.42) p<0.01
MIP-1α	1.36 (1.11, 1.65) p<0.01	1.36 (1.10, 1.67) p<0.01	1.33 (1.09, 1.63) p<0.01	1.42 (1.07, 1.88) p=0.02
Soluble CD14	0.90 (0.84, 0.97) p<0.01	0.91 (0.84, 0.98) p=0.02	0.89 (0.83, 0.96) p<0.01	0.96 (0.86, 1.07) p=0.45
Biomarkers not consistently associated with adipose tissue mass				
IL-6	1.12 (0.95, 1.33) p=0.17	1.10 (0.93, 1.31) p=0.29	1.15 (0.97, 1.36) p=0.10	1.27 (1.01, 1.60) p=0.04
TNF-α receptor 1	1.19 (1.00, 1.42) p=0.05	1.19 (1.00, 1.43) p=0.06	1.18 (0.99, 1.41) p=0.07	1.70 (1.38, 2.10) p<0.01
TNF-α receptor 2	1.06 (0.97, 1.17) p=0.21	1.06 (0.96, 1.17) p=0.24	1.06 (0.97, 1.17) p=0.22	1.18 (1.05, 1.34) p=0.01
MCP-1	1.01 (0.84, 1.20) p=0.94	1.01 (0.85, 1.21) p=0.90	1.00 (0.84, 1.19) p=1.00	1.19 (0.94, 1.52) p=0.15

Model adjusted for age, sex, CD4+ lymphocyte count, smoking status, protease inhibitor use, and daily non-steroidal anti-inflammatory drug or aspirin use. The coefficient (β) represents the fold-change in each biomarker accompanying a one interquartile change in the independent variable.

† Results were similar when DXA-derived percent total body fat was substituted for total fat weight, and when percent trunk fat was substituted for trunk fat weight.