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Soluble Insulin Receptor as a Source of Insulin Resistance and Cognitive Impairment in HIV-seropositive Women

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CONFLICT OF INTEREST:

Authors declare that they have no conflict of interest.

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Abstract

Background—Insulin resistance occurs in HIV-infected individuals and is associated with HIV-associated neurocognitive disorders (HAND). However, the mechanisms involved are not well understood. Previously we showed a correlation between soluble insulin receptor (sIR) and HAND. Here we investigated if binding of free insulin to sIR and soluble insulin-like growth factor-1 receptor (sIGF1-R) levels are associated with sIR in HAND.

Methods—Thirty-four (34) HIV-seropositive women stratified by cognitive status and 5 HIV-seronegative women were evaluated. In a subgroup of 20 HIV positive and 5 donors, binding of plasma insulin to sIR was determined by ELISA assay of residual insulin levels in plasma immuno-depleted with anti-IR-monoclonal antibody-Sepharose beads. sIR and sIGF1-R levels were determined by ELISA. Nonparametric statistics were used.

Results—Higher percentages of insulin bound to sIR significantly correlated with sIR levels and were associated with HAND status. Higher levels of plasma sIGF1-R had a positive correlation with sIR levels ($p=0.011$) and were associated with HAND ($p=0.006$). No correlations were observed with age, viral-immune profile, antiretroviral therapy, or TNF.

Conclusion—This study suggests that changes in sIGF1-R levels and insulin binding to sIR may contribute to HAND.

Keywords

soluble insulin receptor; soluble insulin-like growth factor-1 receptor; insulin binding; HIV-associated neurocognitive disorder; women

INTRODUCTION

There is an urgent need to better understand age-related co-morbidities in people living with HIV/AIDS (PLHA) (Cahill and Valadez 2013). With the use of combined antiretroviral treatment (cART), PLHA are living longer and may present with premature aging either by HIV infection, the long-term effects of cART, or both. Blood glucose metabolism abnormalities such as insulin resistance and diabetes are one set of age-related co-morbidities that have been associated with cognitive impairment in PLHA (Valcour et al. 2005 & 2006). These metabolic abnormalities have been linked to the use of cART specially protease inhibitors in several cohorts (Lee 2004; Brown et al. 2005; Tebas 2008; Lo et al. 2008; Nachega et al. 2009), but not in others (Howard et al. 2005; Danoff et al. 2005; Tien et al. 2007; Sobieszczyk et al. 2008). Hence, there is a need to identify other factors that could contribute the development of insulin resistance in this population. One such factor is insulin receptor dysfunction. We previously showed that soluble insulin receptor (sIR) levels are increased in the plasma of HIV-seropositive women, and that in these women increased sIR levels are associated with cognitive impairment (Gerena et al. 2012). Thus, IR

dysfunction may play a role in HIV-associated neurocognitive disorder (HAND), and could represent a biomarker for the presence and severity of HAND.

The insulin receptor and the insulin-like growth factor receptor-1 (IGF1-R) have essential roles in glucose metabolism, energy homeostasis, neuronal growth and survival, and synaptic plasticity (Bateman and McNeill 2006; Schulingkamp et al. 2000; Chiu and Cline 2010; Huang et al. 2010). IR and IGF1-R bind the structurally homologous proteins insulin and IGF1, respectively. In the CNS, insulin and IGF1 are responsible for cell proliferation, differentiation, and survival during development (de Pablo and de la Rosa 1995; O'Kusky et al. 2000) and in adulthood (Brooker et al. 2000; Aberg et al. 2003). The IR and IGF1-R are closely related receptor tyrosine kinases (van der Geer et al. 1994) and show similar structural and ligand binding mechanisms (Alvino and Ong 2011). A decrease in the tyrosine phosphorylation levels of the cellular IRS-1 can contribute to the development of insulin resistance and diabetes in humans (Carvalho et al. 1999). The Soluble Insulin Receptor Group indicated that any form of soluble insulin receptor may contribute to insulin resistance by sequestering free plasma insulin (The Soluble Insulin Receptor Group 2007). Indeed, in our previous study we found a significant decrease in IRS-1 tyrosine phosphorylation in HIV-seropositive women with symptomatic cognitive impairment compared to those with normal cognition (Gerena et al. 2012).

Based on these observations, we investigated if changes in binding of free plasma insulin to sIR were associated with the presence and severity of HAND in a cohort of HIV-seropositive women. In addition, we quantified the levels of sIGF1-R and how they might correlate with the severity of HAND in these women.

METHODS

Participants and study design

This is a retrospective cross-sectional study nested in the Hispanic/Latino Longitudinal Cohort of Women, and used that patient database information and sample repository. This study was approved by the University of Puerto Rico Medical Sciences Campus (UPR, MSC) Institutional Review Board and all participants signed a written informed consent. This cohort is part of the NeuroAIDS Specialized Neuroscience Research Program (SNRP) at the UPR, MSC. This is a unique cohort of Hispanic HIV-seropositive women characterized longitudinally with viral and immune profiles, neurological exams, and neuropsychological tests. Thirty-four (34) HIV-seropositive women without history of diabetes and 5 HIV-seronegative controls were evaluated as described previously (Wojna et al. 2006; Gerena et al. 2012).

Description of Procedures undertaken

Ethics, Participant evaluations, and Neurocognitive testing—After giving their consent to take part in this IRB-approved research project, individual participants were asked to provide demographic and medical history information, given a macroneurological exam, and had blood samples drawn for laboratory analysis. The information included age, most likely mode of HIV transmission, nadir and current CD4 cell count, and actual

antiretroviral treatment. Plasma viral load was determined using an Ultrasensitive RNA Roche Amplicor at an AIDS Clinical Trials Group (ACTG)-Certified Laboratory. Neurocognitive testing was performed as described previously (Wojna et al. 2006; Gerena et al. 2012). HIV-seropositive women were grouped according to their cognitive performance into normal cognition (NC, n=11), asymptomatic impairment (AI, n=8), and symptomatic impairment (SI, mild cognitive motor disorder [MCMD] plus HIV-associated dementia [HAD], n=15).

Characteristics of HIV-seropositive women—When the HIV-seropositive women were grouped by cognitive performance, no significant differences were observed between groups with regard to age, current CD4 cell count, plasma viral load, body mass index (BMI), co-infection with hepatitis C virus, or toxicology ($p>0.05$). Nadir CD4 cell count was significantly higher in the AI group ($p=0.04$) and the Beck Depression Index (BDI, $p<0.001$) was significantly higher in the SI group. Most of the HIV-seropositive women were using cART (94%, or 32 of the 34 women). There were no significant differences among groups with regard to specific cART regimens, use of protease inhibitors, or CNS penetration effect (CPE, Letendre et al. 2010) (Table 1).

Blood sample preparation—Fresh blood samples were drawn from each patient and collected in acid citrate dextrose (ACD) tubes. Plasma samples were obtained after centrifuging the blood samples twice at room temperature for 10 minutes at $355 \times g$. A plasma aliquot of 700 μL was used for viral load determination, and aliquots of 500 μL were used to determine sIR full-length levels, binding of free plasma insulin to sIR full-length, and levels of sIGF1-R.

Soluble insulin receptor full-length (sIR- $\alpha\beta$, intact) Assay—Plasma sIR full-length levels were previously determined and published in Gerena *et al.*, 2012. Briefly, levels were determined by incubating with an anti-IR ectodomain antibody (1:1000) (Abcam, Cambridge, MA) for 2 hours at 20°C, and a FITC-secondary antibody (Abcam, Cambridge, MA). Samples were analyzed in a Cytofluor 4000 (Applied Biosystems, CA) using 485/530nm excitation/emission filters.

Soluble insulin-like growth factor-1 receptor (sIGF1-R) assay—Plasma total sIGF1-R levels were measured using a commercial ELISA kit (Abcam, Cambridge, MA) following the manufacturer's instructions. In brief, 100 μL of each plasma sample, standards, and positive and negative controls were loaded into wells of a 96-well plate coated with an antibody against human IGF1-R. After addition of reagents, incubations and washes, the optical density of each well was measured using a microplate reader set to 450 nm. All samples were analyzed in triplicate and measurements repeated twice. Standard curves were run in duplicate in each assay.

Plasma insulin binding to sIR—Aliquots (50 μL) of plasma samples were diluted with 100 μL insulin-binding buffer (50 mmol/l HEPES-NaOH, pH 7.4, 150 mmol/l NaCl, 1% BSA, 0.1% Tween 20) and immuno-depleted with anti-hIR β -specific monoclonal antibody-protein A-Sepharose beads for 16 h at 4°C with gentle agitation. The levels of unbound

insulin to the sIR were measured using an insulin ELISA kit (R & D Systems) in depleted and undepleted samples.

Determination of plasma TNF-alpha—Higher plasma tumor necrotic factor alpha (TNF- α) levels have been associated with decreased IRS-1 phosphorylation (Hotamisligil 2006). The levels of TNF- α were measured as previously described (Gerena et al. 2012).

Statistical analyses

We compared sIR and sIGF1-R levels in plasma between three groups: HIV-seropositive women stratified by cognitive impairment into NC, AI, and SI. Because the data was not normally distributed, these comparisons were made using non-parametric correlations (Spearman's) and associations (Kruskal-Wallis or Mann Whitney tests). Using a similar statistical technique, we compared levels of plasma insulin that were unbound and bound to the sIR between the HIV-seropositive women stratified by cognitive performance. All statistical analyses were performed with SPSS version 13 (IBM SPSS Statistics) and graphs with Graph Pad Prism version 5. A $p > 0.05$ was considered significant.

RESULTS

Soluble insulin receptor (sIR) full-length levels

These results were previously published in Gerena *et al.*, 2012. Briefly, among HIV-seropositive women stratified by HAND status, higher levels of plasma sIR full-length were associated with worse cognitive performance.

Soluble insulin-like growth factor-1 receptor (sIGF1-R) levels

Among HIV-seropositive women stratified by HAND status, there was higher levels of plasma sIGF1-R were associated with worse cognitive performance ($p = 0.006$, Table 1, Figure 1[A]). There was no significant difference in sIGF1-R levels between controls and HIV-seropositive women with NC. However, significant differences were seen between the NC and AI groups of HIV-seropositive women ($p = 0.001$) and between the NC and SI groups ($p = 0.027$, Table 1, Figure 1[A]). These findings are similar to those we previously observed for sIR full length (Gerena 2012).

A positive correlation between plasma sIR and sIGF1-R levels was observed ($\rho = 0.475$, $p = 0.011$, $n = 28$, Figure 1 [B]). No correlations were observed between TNF α , sIGF1-R, and sIR and between plasma HIV RNA, sIGF1-R, and sIR.

Plasma insulin binding to sIR full-length

When HIV-seropositive women were stratified by HAND status, a higher percentage of plasma insulin bound to sIR full-length was associated with worse cognitive performance ($p < 0.001$). Conversely, a decreased percentage of unbound insulin correlated with worse cognitive performance ($p < 0.001$, Table 1, Figure 1[B]). No significant differences in bound and unbound insulin levels were seen between controls and HIV-seropositive women with NC. However, significant differences were observed between controls and HIV-seropositive women with AI and SI ($p = 0.004$ and 0.002 respectively). Among HIV-seropositive women,

significant differences were observed between those with NC and those with AI and SI ($p=0.005$ and 0.013 respectively, Figure 1[C], Table 1). No correlations were observed between binding percentage and age, hepatitis C virus infection, plasma HIV RNA levels, CD4 cell count, antiretroviral therapy, or TNF levels.

When analyzing for the percentage of insulin unbound to sIR, the findings are the same but in the opposite direction (Table 1, Figure 1[C]).

DISCUSSION

Insulin and IGF1 together control many aspects of glucose metabolism and growth in a wide range of mammalian tissues through the activation of both insulin and IGF1 receptors. We previously showed that higher levels of plasma sIR full-length were associated with worse cognitive performance among HIV-seropositive women stratified by HAND status. In addition, our cohort showed a significant decrease in IRS-1 tyrosine phosphorylation between HIV-seropositive women with normal cognition and symptomatic impairment (Gerena et al. 2012). Decreased IRS-1 levels and tyrosine phosphorylation are good biomarkers for insulin resistance, but no studies to date have evaluated plasma sIR as a measure of and contributor to insulin resistance in HIV infected patients. Because the sIGF1-R shows close similarities to the IR in structure, ligand binding, and signaling pathways, we decided to quantify sIGF1-R levels in the plasma of HIV-seropositive women, and learn how they might correlate with plasma sIR levels and the presence and severity of HAND in these patients.

In this study we observed a significant positive association between sIGF1-R full-length levels and the presence and severity of HAND, similar to the association we previously observed between sIR full-length levels and HAND (Gerena et al. 2012). In addition, we observed a positive correlation between sIR and sIGF1-R levels in the plasma of these patients ($\rho=0.475$, $p=0.011$, Figure 1 [A and B]). These findings may suggest that similar or coordinated cellular mechanisms are responsible for the secretion of both receptors to the plasma of these patients. Soluble receptors can be generated by several mechanisms such as alternative splicing of the mRNA transcripts, exosome release of full-length receptors, virally encoded soluble receptors, damaged tissues, and the proteolytic cleavage or shedding of cell-surface receptor ectodomains (Levine 2004 & 2008). However, our previous studies revealed no significant association between plasma sIR α ectodomain levels and the presence and severity of HAND (Gerena 2012). We also explored the possibility that the HIV can synthesize a sIR homologue by the mechanism of virally encoded soluble receptors. However, after aligning the gene sequences between HIV and sIR we found no homology (data not shown). Thus, the other aforementioned mechanisms may be responsible for the secretion of soluble full-length insulin receptors to the plasma of these patients. Presently there are no treatment options for patients with HAND, therefore further studies are warranted to better understand the mechanisms involved and their contribution to the development and progression of insulin resistance and cognitive impairment (Buch 2014).

Previous studies have shown that plasma insulin can be sequestered by sIR subunits in patients with diabetes (Soluble Insulin Receptor Group 2007). In our study, we observed a significantly increased binding of plasma insulin to sIR full-length in patients with worse cognitive performance. Significant amounts (>60–80%) of insulin were bound to the plasma sIR (Figure 1 [C]) of HIV-seropositive patients with symptomatic impairment. As we know, both receptors are activated through the binding of insulin or IGF-1 to their extracellular alpha domains in the membrane of the target tissue cells. Since the two receptors bind insulin with similar affinities (Alvino and Ong 2011), we expect that binding of insulin to the sIR and sIGF1-R may contribute to the insulin resistance observed in HIV-seropositive patients. One of the limitations of this study is that we were not able to evaluate blood sugar metabolism, since we used stored plasma samples from patients who had not been given relevant tests (e.g., the oral glucose tolerance test, glycosylated hemoglobin assay, or homeostasis model assessment). However, the participants had no history of diabetes or use of hypoglycemic medications and glucose tests performed for other reasons shown normal levels (Gerena et al. 2012). Nevertheless, we previously observed in these patients that increased levels of plasma sIR correlated with a significant decrease in IRS-1 tyrosine phosphorylation in CSF leukocytes, a finding that is consistent with insulin resistance (Gerena et al. 2012; Sesti 2006; Petersen and Shulman 2006).

This study provides evidence that sIR and sIGF1-R are increased in HIV-seropositive women, and is associated with cognitive impairment. In addition, we observe that the sIR full-length serves as a scavenger receptor since it binds free plasma insulin in HIV-seropositive patients. Our findings suggest that changes in plasma sIR and sIGF1-R could be associated with asymptomatic glucose derangements in HIV-seropositive women using cART, and may contribute to the development and progression of HAND. Since early intervention may help prevent the progression of insulin resistance and therefore HAND, it is vital to identify the mechanisms and contributing factors for these co-morbidities in HIV-seropositive patients.

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REFERENCES

1. Aberg MA1, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skwirut C, Bang P, Rosengren LE, Olsson T, Gage FH, Eriksson PS. IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. *Mol Cell Neurosci*. 2003; 24:23–40. [PubMed: 14550766]
2. Alvino CL, Ong SC, et al. Understanding the mechanism of insulin and insulin-like growth factor (IGF) receptor activation by IGF-II. *PloS one*. 2011; 6(11):e27488. [PubMed: 22140443]
3. Bateman JM, McNeill H. Insulin/IGF signalling in neurogenesis. *Cell Mol Life Sci*. 2006; 63:1701–1705. [PubMed: 16786222]
4. Brooker GJ, Kalloniatis M, Russo VC, Murphy M, Werther GA, Bartlett PF. Endogenous IGF-1 regulates the neuronal differentiation of adult stem cells. *J Neurosci Res*. 2000; 59:332–341. [PubMed: 10679768]

5. Brown TT, Cole SR, Li X, Kingsley LA, Palella FJ, Riddler SA, Visscher BR, Margolick JB, Dobs AS. Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Arch Intern Med.* 2005; 165:1179–1184. [PubMed: 15911733]
6. Buch S. Growth factor signaling: implications for disease and therapeutics. *J Neuroimmune Pharmacol.* 2014; 9:65–68. [PubMed: 24610034]
7. Cahill S, Valadez R. Growing older with HIV/AIDS: new public health challenges. *Am J Public Health.* 2013; 103:e7–e15. [PubMed: 23327276]
8. Carvalho E, Jansson PA, Axelsen M, Eriksson JW, Huang X, Groop L, Rondinone C, Sjostrom L, Smith U. Low cellular IRS 1 gene and protein expression predict insulin resistance and NIDDM. *Faseb J.* 1999; 13:2173–2178. [PubMed: 10593864]
9. Chiu SL, Cline HT. Insulin receptor signaling in the development of neuronal structure and function. *Neural Dev.* 2010; 5:7. [PubMed: 20230616]
10. Danoff A, Shi Q, Justman J, Mulligan K, Hessol N, Robison E, Lu D, Williams T, Wichienkuer P, Anastos K. Oral glucose tolerance and insulin sensitivity are unaffected by HIV infection or antiretroviral therapy in overweight women. *J Acquir Immune Defic Syndr.* 2005; 39:55–62. [PubMed: 15851914]
11. de Pablo F, de la Rosa EJ. The developing CNS: a scenario for the action of proinsulin, insulin and insulin-like growth factors. *Trends Neurosci.* 1995; 18:143–150. [PubMed: 7754526]
12. Gerena Y, Skolasky RL, Velez JM, Toro-Nieves D, Mayo R, Nath A, Wojna V. Soluble and cell-associated insulin receptor dysfunction correlates with severity of HAND in HIV-infected women. *PLoS One.* 2012; 7:e37358. [PubMed: 22629383]
13. Hotamisligil GS. Inflammation and metabolic disorders. *Nature.* 2006; 444:860–867. [PubMed: 17167474]
14. Howard AA, Floris-Moore M, Arnsten JH, Santoro N, Fleischer N, Lo Y, Schoenbaum EE. Disorders of glucose metabolism among HIV-infected women. *Clin Infect Dis.* 2005; 40:1492–1499. [PubMed: 15844072]
15. Huang CC, Lee CC, Hsu KS. The role of insulin receptor signaling in synaptic plasticity and cognitive function. *Chang Gung Med J.* 2010; 33:115–125. [PubMed: 20438663]
16. Lawrence MC, McKern NM, Ward CW. Insulin receptor structure and its implications for the IGF-1 receptor. *Curr Opin Struct Biol.* 2007; 17:699–705. [PubMed: 17851071]
17. Lee J. Altered Glucose Metabolism in HIV-infected Patients Treated with HAART. *Journal of Pharmacy Practice.* 2004; 17:80–86.
18. Letendre SL, Ellis RJ, Ances BM, McCutchan JA. Neurologic complications of HIV disease and their treatment. *Top HIV Med.* 2010; 18:45–55. [PubMed: 20516524]
19. Lo YC, Chen MY, Sheng WH, Hsieh SM, Sun HY, Liu WC, Wu PY, Wu CH, Hung CC, Chang SC. Risk factors for incident diabetes mellitus among HIV-infected patients receiving combination antiretroviral therapy in Taiwan: a case-control study. *HIV Med.* 2009; 10:302–309. [PubMed: 19220492]
20. Nachega JB, Trotta MP, Nelson M, Ammassari A. Impact of metabolic complications on antiretroviral treatment adherence: clinical and public health implications. *Curr HIV/AIDS Rep.* 2009; 6:121–129. [PubMed: 19589297]
21. O'Kusky JR, Ye P, D'Ercole AJ. Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. *J Neurosci.* 2000; 20:8435–8442. [PubMed: 11069951]
22. Petersen KF, Shulman GI. Etiology of insulin resistance. *Am J Med.* 2006; 119:S10–S16. [PubMed: 16563942]
23. Schulingkamp RJ, Pagano TC, Hung D, Raffa RB. Insulin receptors and insulin action in the brain: review and clinical implications. *Neurosci Biobehav Rev.* 2000; 24:855–872. [PubMed: 11118610]
24. Sesti G. Pathophysiology of insulin resistance. *Best Pract Res Clin Endocrinol Metab.* 2006; 20:665–679. [PubMed: 17161338]
25. Sobieszczyk ME, Hoover DR, Anastos K, Mulligan K, Tan T, Shi Q, Gao W, Hyman C, Cohen MH, Cole SR, Plankey MW, Levine AM, Justman J. Prevalence and predictors of metabolic

- syndrome among HIV-infected and HIV-uninfected women in the Women's Interagency HIV Study. *J Acquir Immune Defic Syndr*. 2008; 48:272–280. [PubMed: 18545157]
26. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol*. 2006; 7:85–96. [PubMed: 16493415]
27. Tebas P. Insulin resistance and diabetes mellitus associated with antiretroviral use in HIV-infected patients: pathogenesis, prevention, and treatment options. *J Acquir Immune Defic Syndr*. 2008; 49(Suppl 2):S86–S92. [PubMed: 18725817]
28. The soluble insulin receptor study group. Soluble insulin receptor ectodomain is elevated in the plasma of patients with diabetes. *Diabetes*. 2007; 56:2028–2035. [PubMed: 17563065]
29. Tien PC, Schneider MF, Cole SR, Levine AM, Cohen M, DeHovitz J, Young M, Justman JE. Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study. *Aids*. 2007; 21:1739–1745. [PubMed: 17690572]
30. Valcour VG, Sacktor NC, Paul RH, Watters MR, Selnes OA, Shiramizu BT, Williams AE, Shikuma CM. Insulin resistance is associated with cognition among HIV-1-infected patients: the Hawaii Aging With HIV cohort. *J Acquir Immune Defic Syndr*. 2006; 43:405–410. [PubMed: 17099311]
31. Valcour VG, Shikuma CM, Shiramizu BT, Williams AE, Watters MR, Poff PW, Grove JS, Selnes OA, Sacktor NC. Diabetes, insulin resistance, and dementia among HIV-1-infected patients. *J Acquir Immune Defic Syndr*. 2005; 38:31–36. [PubMed: 15608521]
32. van der Geer PI, Hunter T, Lindberg RA. Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu Rev Cell Biol*. 1994; 10:251–337. [PubMed: 7888178]
33. Wojna V, Skolasky RL, Hechavarria R, Mayo R, Selnes O, McArthur JC, Melendez LM, Maldonado E, Zorrilla CD, Garcia H, Kraiselburd E, Nath A. Prevalence of human immunodeficiency virus-associated cognitive impairment in a group of Hispanic women at risk for neurological impairment. *J Neurovirol*. 2006; 12:356–364. [PubMed: 17065128]

Figure 1. Plasma soluble insulin-like growth factor-1 receptor (sIGF1-R) levels and insulin binding to soluble insulin receptor (sIR) full-length in HAND patients

(A) The sIGF1-R was measured by ELISA in plasma of HIV-seropositive women (HIV+) ($n = 34$) stratified by HAND into normal cognition (NC, $n = 11$), asymptomatic impairment (AI, $n = 8$), and symptomatic impairment (SI, $n = 15$); and 5 HIV-negative controls (HIV-). Among HIV-seropositive women stratified by HAND status, higher levels of plasma sIGF1-R were associated with worse cognitive performance ($p = 0.006$). There was no significant difference in sIGF1-R levels between controls and HIV-seropositive women with NC. However, significant differences were seen between the NC and AI groups of HIV-seropositive women ($p = 0.001$) and between the NC and SI groups ($p = 0.027$). (B) A positive correlation was observed between the levels of sIR and sIGF1-R in the plasma of these patients ($\rho = 0.475$, $p = 0.011$, $n = 28$). (C) The percentages of insulin bound to the sIR (light grey) were significantly increased between HIV-negative controls ($n = 5$) and HIV-seropositive women ($n = 20$) with the presence and severity of HAND (NC [$n = 6$]; AI [$n = 6$]; SI [$n = 8$]) (see p values between group comparisons at the right). HIV+ patients

with SI had significantly higher percentage of insulin bound to the sIR than HIV+ NC and AI. The inverse relationship was observed with the percent of insulin unbound to the sIR (dark grey).

Association between plasma soluble insulin growth factor 1 receptor (sIGF1-R) and binding of plasma insulin to soluble insulin receptor (sIR) full-length stratified by cognitive impairment and the correlation between plasma sIGF1-R and sIR levels.

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Subject Characteristics

Table 1

	HIV (-) (n=5)	HIV (+)			p value (*p<0.05)
	Normal Cognition (NC, n= 11)	Asymptomatic Impairment (AI, n= 8)	Symptomatic Impairment (SI, n= 15)		
GENERAL CHARACTERISTICS					
Age (years)	39 (28, 48)	44 (39, 47)	42 (35, 45)	43 (41, 48)	0.437
BDI ^a		3.0 (3.0, 12.0)	1.5 (0.3, 6.8)	15.0 (11.0, 22.0)	0.003*
BMI ^b		26.5 (25.6, 30.4)	29.9 (22.7, 39.4)	25.6 (23.1, 28.2)	0.420
VIRAL-IMMUNE PROFILE					
CD4 cell count (cells/mm ³)		397.0 (275.0, 459.0)	521.00 (330.8, 735.3)	423.0 (291.0, 613.0)	0.503
Nadir CD4 cell count (cells/mm ³)		177.0 (14.0, 384.0)	439.0 (262.5, 521.3)	191.0 (105.8, 399.0)	0.044*
Plasma HIV RNA (log 10) ^c		1.7 (1.7, 3.28) 5/9 ND	2.875 (1.978, 3.71) 1/6 ND	1.7 (1.7, 2.295) 7/12 ND	0.095
SOLUBLE RECEPTORS LEVELS & BINDING					
Plasma sIGF1-R (pg/ml) ^{d,e}	3.16 (2.77, 3.76) (n = 5)	2.92 (2.52, 3.49) (n = 8)	5.03 (4.65, 10.91) (n = 6)	4.79 (3.07, 7.8) (n = 10)	0.006*
Plasma sIR (MFI) ^f	9692 (9230, 9307) (n = 5)	9900 (9707, 9989) (n = 11)	10181 (9844.5, 10373) (n = 8)	10510 (10330, 10649) (n = 15)	<0.001*
Insulin bound to sIR (%) ^g	3.3 (0.0, 14.2) (n = 5)	7.0 (0.0, 36.0) (n = 6)	31.3 (23.7, 59.8) (n = 6)	74.2 (54.9, 87.4) (n = 8)	0.001*
Insulin unbound to sIR (%) ^h	96.7 (85.8, 100.0) (n = 5)	93.0 (64.0, 100.0) (n = 6)	68.7 (40.2, 76.3) (n = 6)	25.8 (12.7, 45.2) (n = 8)	0.001*
TNF alpha	0.34 (0.19, 0.825)	0.73 (0.11, 1.69)	1.51 (0.43, 3.38)	0.90 (0.32, 2.51)	0.244

Values presented as median (25th, 75th percentiles);

^aBDI: Beck's Depression Index;

^bBMI: Body Mass Index;

^cND: not detected,

^dsIGF1-R=soluble Insulin-like growth factor receptor,

^esignificant association was observed between NC and AI and NC and SI (p=0.001 and 0.027 respectively; figure 1A),

^fsIR=soluble insulin receptor, previously determined and published in Gerena et al., 2012, Table 3,

^gsignificant association was observed between donors and AI (p=0.004) and SI (p=0.002) and between NC and AI (p=0.005) and SI (p=0.013),

same findings as with insulin bound to sIR (%) but with an inverse direction (see figure 1C).

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