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Tiffany R Butterfield, *University of the West Indies*  
David B Hanna, *Albert Einstein College of Medicine*  
Robert C Kaplan, *Albert Einstein College of Medicine*  
Jorge R Kizer, *Albert Einstein College of Medicine*  
Helen G Durkin, *Suny Downstate Medical Center*  
Mary A Young, *Georgetown University*  
Marek J Nowicki, *University of Southern California*  
Phyllis C Tien, *University of California San Francisco*  
Elizabeth T Golub, *Johns Hopkins University*  
Michelle A Floris-Moore, *University of North Carolina*

*Only first 10 authors above; see publication for full author list.*

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## Increased Glucose Transporter-1 Expression on Intermediate Monocytes from HIV-Infected Women with Subclinical Cardiovascular Disease

Tiffany R Butterfield<sup>1</sup>, David B Hanna<sup>2</sup>, Robert C Kaplan<sup>2</sup>, Jorge R Kizer<sup>2,3</sup>, Helen G Durkin<sup>4</sup>, Mary A Young<sup>5</sup>, Marek J Nowicki<sup>6</sup>, Phyllis C Tien<sup>7</sup>, Elizabeth T Golub<sup>8</sup>, Michelle A Floris-Moore<sup>9</sup>, Kehmia Titanji<sup>10</sup>, Margaret A Fischl<sup>11</sup>, Sonya L Heath<sup>12</sup>, Jefferey Martinson<sup>14</sup>, Suzanne M Crowe<sup>13</sup>, Clovis S Palmer<sup>13</sup>, Alan L Landay<sup>14</sup>, and Joshua J Anzinger<sup>1</sup>

<sup>1</sup>Department of Microbiology, University of the West Indies – Mona, Kingston

<sup>2</sup>Department of Epidemiology and Population Health, Albert Einstein College of Medicine, New York

<sup>3</sup>Department of Medicine, Albert Einstein College of Medicine, New York

<sup>4</sup>Department of Pathology, SUNY Downstate Medical Center, New York

<sup>5</sup>Division of Infectious Diseases, Georgetown University, Washington, D.C

<sup>6</sup>Department of Medicine, University of Southern California, Los Angeles

<sup>7</sup>Department of Medicine, University of California, San Francisco

<sup>8</sup>Department of Epidemiology, Johns Hopkins University, Baltimore

<sup>9</sup>Division of Infectious Diseases, University of North Carolina, Chapel Hill

<sup>10</sup>Department of Medicine, Emory University, Atlanta

<sup>11</sup>Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami

<sup>12</sup>Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham

<sup>13</sup>Centre for Biomedical Research, Burnet Institute, Melbourne

<sup>14</sup>Department of Immunology-Microbiology, Rush University Medical Center, Chicago

### Abstract

**Objective**—People living with HIV (PLWH) have chronic immune activation and increased cardiovascular disease (CVD) risk. Activation of monocytes and T lymphocytes causes up-regulation of glucose transporter-1 (GLUT1) for efficient function. PLWH have an increased percentage of GLUT1-expressing monocytes and T lymphocytes, but it is unclear if these cells are

Corresponding Author: Joshua Anzinger, University of the West Indies – Mona, Department of Microbiology, joshua.anzinger@uwimona.edu.jm.

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associated with CVD. We evaluated expression of GLUT1 and CD38 on monocyte and T lymphocyte populations from HIV-infected women with subclinical CVD.

**Methods**—Participants with >75<sup>th</sup> percentile (n=15) and <25<sup>th</sup> percentile (n=15) age-adjusted intima-media thickness (IMT) at the right common carotid artery and bifurcation were identified from the Women's Interagency HIV Study. Groups were matched by age, race/ethnicity, smoking status, and CD4 count. All women were receiving suppressive antiretroviral therapy except for one high and one low IMT participant. Monocyte and T lymphocyte populations were evaluated for GLUT1 and CD38 expression using flow cytometry.

**Results**—Intermediate monocytes from high IMT women had significantly increased expression of GLUT1 (310 MFI vs. 210 MFI,  $p=0.024$ ) (66.4% vs. 48.5%,  $p=0.031$ ) and CD38 (339 MFI vs. 211 MFI,  $p=0.002$ ) (10.5% vs. 3.8%,  $p=0.0002$ ) compared to women with low IMT. High and low IMT participants showed no differences in GLUT1 or CD38 expression on classical monocytes, non-classical monocytes, CD4+ and CD8+ T lymphocytes.

**Conclusion**—GLUT1-expressing intermediate monocytes are elevated in HIV-infected women with subclinical CVD. These cells may contribute to development of CVD in PLWH and could be a novel target to limit inflammation.

### Keywords

HIV; monocyte; T cell; GLUT1; cardiovascular disease

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### Introduction

Persons living with HIV (PLWH) can be effectively treated with combination antiretroviral therapy (cART); yet their lifespans may still be shorter than those of HIV-uninfected persons [1]. In both high and lower income settings the causes of death among PLWH are shifting from AIDS-related to non-AIDS-related diseases such as cardiovascular disease (CVD) [1,2].

CVD is a metabolic and inflammatory disease associated with activated monocytes and T lymphocytes [3,4]. The metabolism of monocytes and T lymphocytes can change dramatically upon activation, with both activated cell types upregulating surface expression of glucose transporter-1 (GLUT1) for efficient function [5–8]. The percentage of GLUT1-expressing monocytes and CD4 T lymphocytes is elevated in both untreated and cART-treated PLWH when compared to uninfected persons [5,6]. This increase in GLUT1-expressing monocytes and T lymphocytes could be an important source of inflammation leading to CVD as GLUT1-expressing mononuclear phagocytes have been shown to express proinflammatory cytokines [9]. It remains unclear if these GLUT1-expressing cells are associated with CVD in PLWH.

Monocytes are composed of several populations that can be characterized by the differential expression of CD14 and CD16 surface markers [10]. Classical monocytes (CD14<sup>++</sup>CD16<sup>-</sup>) express high levels of CD14, lack CD16, and represent the majority of monocytes in the blood. CD16-expressing monocytes are associated with increased production of TNF- $\alpha$  and are considered to be inflammatory. CD16-expressing monocytes are further subdivided into

non-classical monocytes (CD14<sup>+</sup>CD16<sup>++</sup>) and intermediate monocytes (CD14<sup>++</sup>CD16<sup>+</sup>). The intermediate monocyte population, while only recently considered to be a distinct subset of monocytes, has been increasingly associated with inflammation as it relates to the increased risk of CVD [11]; increased levels of TNF- $\alpha$  [12], IL-1 $\beta$  [13], and D-dimer [14]; and a distinct role in antigen presentation and processing [15].

Both activated CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes have been pathogenetically implicated in HIV –infected people with CVD. Activated CD8<sup>+</sup> T lymphocytes have previously been linked to markers of CVD inflammation [16] and Kaplan *et. al.* identified an association between activated CD4<sup>+</sup> T lymphocytes and the presence of carotid artery plaques [17]. However, other reports have shown that T lymphocyte activation was not associated with serious non-AIDS events, including CVD outcomes [18].

The aim of this study was to evaluate the expression of GLUT1 on monocyte and T lymphocyte populations in HIV-infected women with subclinical CVD to explore if it could contribute to CVD in PLWH.

## Methods

High and low IMT groups were identified from the Women's Interagency HIV Study (WIHS), a longitudinal cohort study of women with HIV and a risk-set matched control group. The high IMT group was defined as having >75<sup>th</sup> percentile intima-media thickness (IMT) measured at the right common carotid artery (CCA) and bifurcation (BIF) (CCA-IMT range 0.779-1.488 mm, BIF-IMT range 0.920-1.621 mm) and low IMT group was defined as having <25<sup>th</sup> percentile intima-media thickness at the CCA and BIF (CCA-IMT 0.591-0.704 mm, BIF-IMT 0.614-0.772 mm). IMT was measured by high resolution B-mode carotid artery ultrasound of the far wall of the right common carotid artery (CCA) and bifurcation (BIF) according to the procedure of Hodis *et al.* [19]. IMT values were first age-adjusted because IMT and age are correlated. Groups were then matched by age, race/ethnicity, smoking status, and CD4 count. Thereafter, 15 pairs were identified, giving a total of thirty (30) participants. All participants except one case and one control were virally suppressed on cART.

Blood samples were collected in the WIHS at semiannual visits [20]. Twenty-seven (27) participants were fasting at the time of sample collection. The samples used in this study were collected between April 2012 and March 2015. Peripheral blood mononuclear cells (PBMCs) were processed and stored in liquid nitrogen at a minimum 6 million cells per aliquot [20]. Plasma (never previously thawed) was also collected from women at these visits and stored at -20°C.

Cryopreserved PBMCs were rested for 2 hours at 37°C prior to staining in RPMI-1640 medium (Corning CellGro) containing 10% fetal bovine serum (Gemini Bio-Products), 200 IU penicillin/200  $\mu$ g/mL streptomycin (Sigma Aldrich) and 2 mM L-glutamine (Sigma Aldrich). PBMCs were stained on ice for LIVE/DEAD Fixable Aqua Stain (Life Technologies; catalog L34957), CD3–BV421 (BD Biosciences; catalog 562426), CD4–PE-CF594 (BD Biosciences; catalog 562281), CD8–APC-H7 (BD Biosciences; catalog

560179), CD38–APC (BD Biosciences; catalog 555462), HLA-DR–PE–Cy7 (BD Biosciences; catalog 560651), CD14–BV786 (BD Biosciences; catalog 563698), CD16–BV650 (BD Biosciences; catalog 563692), CD19–AF700 (BD Biosciences; catalog 557921), CD56–AF700 (BD Biosciences; catalog 557919) and GLUT1–PE (R&D Systems; catalog FAB1418P). Antibody concentrations were selected according to the manufacturer's recommendation. Cells were acquired on an LSRFortessa II (BD Biosciences) and analyzed using FlowJo Software (Tree Star Inc.). Samples were run daily with matched samples from the high and low IMT group on each day. SPHERO™ rainbow calibration particles (Spherotech Inc.; catalog RCP-30-5A) were used each day to check the sensitivity and linearity of the flow cytometer. The cryopreservation and thawing process was previously shown to have no effect on the expression of GLUT1 on cells [5].

T lymphocyte and monocyte subpopulations were identified by flow cytometry (Supplemental Figure 1). Briefly, scatter properties were used to determine initial monocyte and lymphocyte gates which were then gated to select live cells. CD3, CD4 and CD8 expression were used to distinguish T lymphocyte populations, while CD3, CD56 and CD19 were used to exclude T lymphocytes, NK cells and B cells, respectively, from the initial monocyte gate. Monocyte subpopulations were then distinguished using CD14 and CD16 expression. CD38 was used as an activation marker of T lymphocytes and monocytes [21,22]. Histograms depicting the expression of GLUT1 and CD38 on monocyte populations are shown in Supplemental Figure 1.

For cytokine detection, plasma was thawed and ELISA was performed according to the manufacturer's instructions for IL-6 (Human IL-6 Quantikine HS ELISA Kit, catalog HS600B; MDD < 0.70 pg/mL), sCD14 (Human sCD14 Quantikine ELISA Kit, catalog DC140; MDD = 125pg/mL) and sCD163 (Human CD163 Quantikine ELISA Kit, catalog DC1630; MDD = 0.177 ng/mL). All ELISA kits were obtained from R&D Systems.

The non-parametric Mann Whitney U test was used for comparison of unpaired data. The Spearman rank test was used to test for correlation. The Fisher's exact test was used for comparison of categorical data. P values less than 0.05 were considered significant. All analyses were conducted using GraphPad Prism (Graph Pad Software, version 6.07).

## Results

Demographic and clinical characteristics of participants are summarized in Table 1. High IMT participants had significantly greater CCA-IMT (0.893 mm vs. 0.653 mm,  $p<0.0001$ ) and BIF-IMT (0.984 mm vs. 0.701 mm,  $p<0.0001$ ) than participants with low IMT. Participants with high IMT had higher hemoglobin A1c (6.0% vs. 5.5%,  $p=0.022$ ), serum insulin (19.5 mIU/mL vs. 13.0 mIU/mL,  $p=0.039$ ), triglycerides (138.5 mg/dL vs. 91.5 mg/dL,  $p=0.043$ ), systolic blood pressure (126 mmHg vs. 114 mmHg,  $p=0.036$ ) and lower HDL levels (50.0 mg/dL vs. 60.5 mg/dL,  $p=0.037$ ) than women with low IMT. Plasma levels of sCD14, IL-6 and sCD163 showed no difference between women with high or low IMT.

The percentage of intermediate monocytes expressing GLUT1 (66.4% vs. 48.5%,  $p=0.031$ ) was higher in women with high IMT compared to those with low IMT while there were no

differences between groups for non-classical monocytes, classical monocytes, and CD4 and CD8 T lymphocytes (Fig. 1a & b). Also, the expression of GLUT1 on intermediate monocytes was higher in women with high IMT (310 MFI vs. 210 MFI,  $p=0.024$ ) with no differences in GLUT1 expression between groups for other monocyte subsets and CD4 and CD8 T lymphocytes (Fig. 1c & d). When assessing all samples, IMT correlated with both the percentage of GLUT1-expressing intermediate monocytes ( $r=0.390$ ,  $p=0.037$ ) and expression of GLUT1 on intermediate monocytes ( $r=0.398$ ,  $p=0.032$ ). There were no differences in the proportions of classical, non-classical and intermediate monocytes and CD4 and CD8 T lymphocytes between the two groups. The percentage of CD38 expressing intermediate monocytes was significantly elevated when comparing the high and low IMT groups (10.5% vs. 3.8%,  $p=0.0002$ ) (Fig. 1e). There were no differences in the percentages of CD4 and CD8 T lymphocytes expressing CD38 when comparing high and low IMT groups (Fig. 1f). Similar to CD38 expression, the percentage of intermediate monocytes expressing both CD38 and GLUT1 was significantly higher in the high IMT group (15.5% vs. 4.5%,  $p=0.0008$ ) (Fig. 1g), with no differences observed between groups for T lymphocytes (Fig. 1h). These data suggest an immunometabolic basis for monocyte activation.

## Discussion

GLUT1 expression has been previously shown to be associated with activation of T lymphocytes and monocytes in treated and untreated HIV infection [5,6]. For CD4 T lymphocytes, the percentage of cells expressing GLUT1 is increased during untreated HIV infection; however, during treated HIV infection these levels are significantly decreased compared to untreated persons [5]. In contrast, the percentage of GLUT1-expressing monocytes is similar between untreated and treated HIV infection, indicating that even during treated HIV infection monocytes remain activated [6]. Consistent with these studies, we found that GLUT1-expressing intermediate monocytes, but not GLUT1-expressing T lymphocyte populations, are associated with subclinical CVD in a predominantly cART-treated population of HIV-infected women. Our results are also consistent with several recent reports showing that markers of activated monocytes are associated with CVD in PLWH [18,23,24].

The percentages of pro-inflammatory intermediate and non-classical monocyte subpopulations are similar between PLWH with uncontrolled viremia and HIV-uninfected persons with acute coronary syndrome [25], indicating that PLWH may have an inflammatory monocyte profile that contributes to a pro-atherogenic state. This is consistent with the data presented here demonstrating a higher percentage of GLUT1-expressing intermediate monocytes from HIV-infected women with high IMT compared to those with low IMT. These proinflammatory GLUT1-expressing intermediate monocytes are activated, as GLUT1 expression correlated with the activation marker CD38. This correlation between GLUT1 and CD38 suggests an interrelationship between metabolism and monocyte activation.

Immune stimuli present during HIV infection, such as the microbial translocation product LPS, can activate monocytes [26], causing an upregulation of GLUT1 [26,27]. Although we



did not examine the cause of differential intermediate monocyte GLUT1 expression for the two IMT groups in this study, it is possible that increased levels of monocyte stimuli in some HIV-infected persons could lead to increased monocyte activation and GLUT1 expression [28,29]. We previously showed that monocytes stimulated with LPS upregulate GLUT1 expression and produce increased levels of TNF- $\alpha$  compared to unstimulated monocytes [6]. Similarly, Freemerman *et al.* indicated that GLUT1 expression on mononuclear phagocytes facilitated their activation and was essential for proinflammatory cytokine production [9]. It is possible that these GLUT1-expressing intermediate monocytes contribute to an inflammatory state that increases CVD risk in PLWH.

While intermediate monocytes have only recently been defined as a subset of human monocytes [15,30], it has become increasingly apparent that this monocyte subset is linked to inflammation. A number of inflammatory diseases are selectively associated with the intermediate monocyte subset [11,31–33], and recently, intermediate monocytes were shown to be the major monocyte subset responsive to low level LPS injection in humans [34]. A high level of inflammatory and activation gene expression was identified in the intermediate monocyte subset [15], which may explain why this monocyte subset has been linked to many inflammatory diseases. However, mechanistic studies are lacking to explain why intermediate monocytes can be selectively associated with inflammatory disease.

We did not identify a statistical difference in GLUT1-expressing CD4+ or CD8+ T lymphocytes between HIV-infected women with high or low IMT. These results differ from some studies examining PLWH that showed an association between CVD and activated T lymphocytes [16,17]. However, GLUT1 expression was not examined in those studies. Also, our study population consisted of women with lower IMT levels than the noted previous studies, which could explain why activated T lymphocytes did not differ between IMT groups.

sCD14, sCD163 and IL-6 can be produced by activated monocytes and have been associated with IMT in some studies, but most studies show no association between these markers and IMT in HIV-infected persons [35]. In our study we found no differences in these markers between high and low IMT groups and no correlation was observed between any of the markers and GLUT1 intermediate monocytes. It is unclear why sCD14, sCD163 and IL-6 have only been associated with IMT in a small number of studies. Different study populations may explain the discrepant results. It is surprising that GLUT1 expressing intermediate monocytes are not correlated with sCD14, sCD163, or IL-6 as GLUT1 is associated with monocyte activation. It is possible that monocyte production of sCD14, sCD163 and IL-6 occurs through a pathway(s) independently of GLUT1 expression.

In summary, our data suggest a role for GLUT1-expressing intermediate monocytes in subclinical CVD observed during HIV infection. Pending replication, GLUT1-expressing intermediate monocytes could provide a way to predict CVD events in PLWH and may be a useful target for therapy to limit chronic immune activation and inflammation observed during HIV infection.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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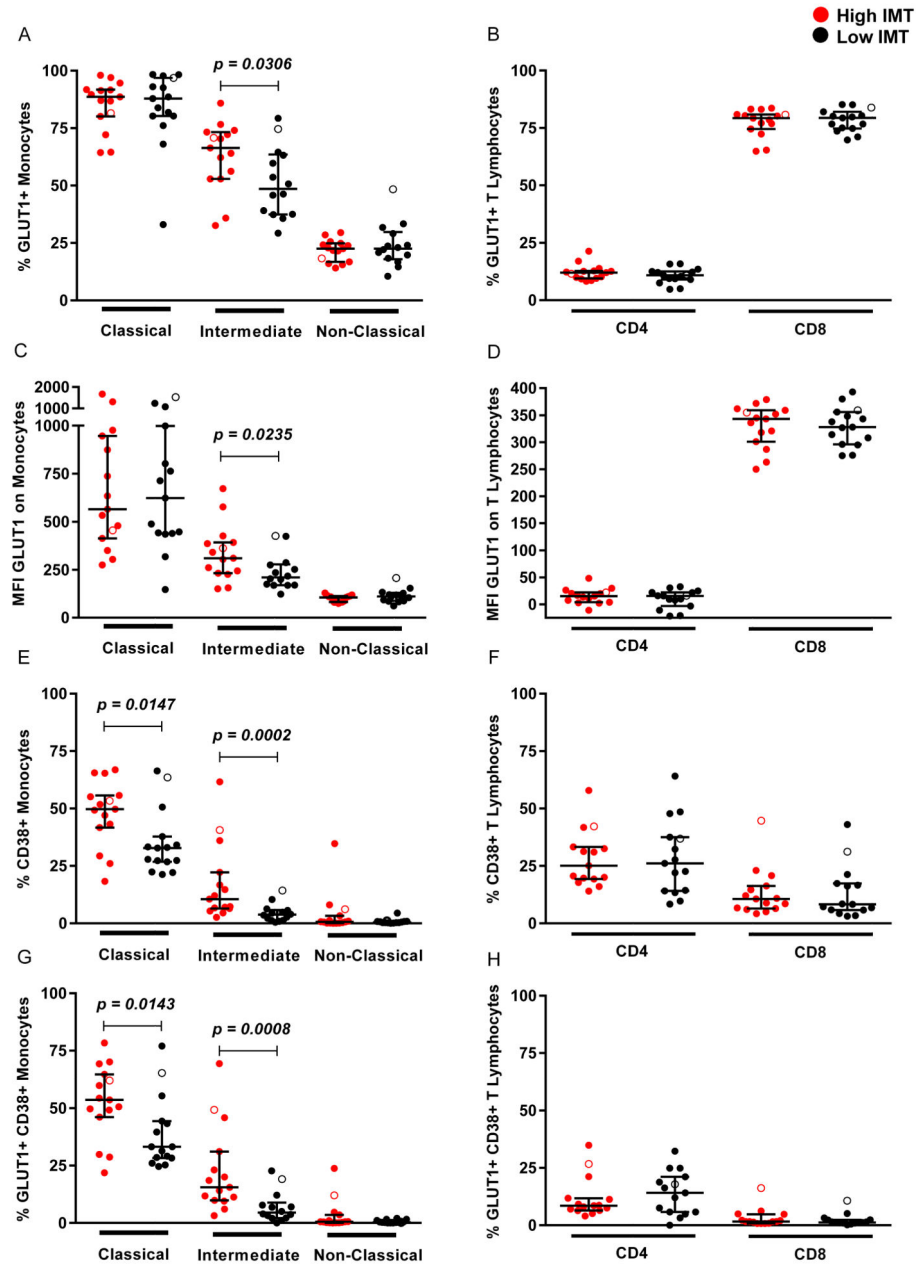
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**Figure 1. Monocyte and T lymphocyte expression of GLUT1 and CD38 from women with high IMT and low IMT**

Monocyte and T lymphocyte populations were identified by flow cytometry as described in the Methods. The percentage of (A) monocyte and (B) T lymphocyte populations expressing GLUT1. The MFI of GLUT1 on the cell surface of (C) monocytes and (D) T lymphocytes. The percentage of (E) monocyte and (F) T lymphocyte populations expressing CD38. The percentage of (G) monocyte and (H) T lymphocyte populations expressing GLUT1 and CD38. Open circles represent women not on antiretroviral therapy at the time of sample collection.

**Table 1**  
**Clinical Characteristics of Study Groups**

Variables	N	Groups		p
		High IMT	Low IMT	
Mean Age $\pm$ SD, years	30	51.48 $\pm$ 6.14	51.68 $\pm$ 6.97	0.934
Race (Black) %	30	60	60	
Median CD4 Count (IQR), cells/mm <sup>3</sup>	30	638 (244 - 793)	630 (441 - 917)	0.675
Median CD8 Count (IQR), cells/mm <sup>3</sup>	30	764 (550 - 1108)	719 (592 - 1122)	0.488
Median CD4/CD8 Ratio (IQR)	30	0.6 (0.3 - 1.2)	0.9 (0.5 - 1.3)	0.272
Viral Load (< 50 copies/mL), %	28	100	73	0.102
Smoking (Current Smokers) %	30	60	60	
Median BIF-IMT (IQR), mm	30	0.984 (0.941 - 1.184)	0.701 (0.643 - 0.740)	< <b>0.0001</b>
Median CCA-IMT (IQR), mm	30	0.893 (0.843 - 1.001)	0.653 (0.642 - 0.692)	< <b>0.0001</b>
Median Systolic BP (IQR), mmHg	30	126 (113 - 140)	114 (105 - 122)	<b>0.036</b>
Median Diastolic BP (IQR), mmHg	30	72 (66 - 80)	71 (69 - 76)	0.472
Median Triglycerides (IQR), mg/dL	28	138.5 (101.5 - 164.5)	91.5 (86.25 - 149.5)	<b>0.043</b>
Median Cholesterol (IQR), mg/dL	28	191.5 (162.0 - 202.5)	184.5 (135.5 - 237.0)	0.473
Median HDL (IQR), mg/dL	28	50.0 (42.3 - 59.5)	60.5 (47.8 - 82.8)	<b>0.037</b>
Median LDL (IQR), mg/dL	28	110.0 (79.5 - 126.8)	110.0 (65.5 - 136.3)	0.424
Median Plasma Glucose (IQR), mg/dL	26	90.50 (87.75 - 107.00)	95.00 (85.75 - 97.75)	0.485
Median Hemoglobin A1c (IQR), %	29	6.00 (5.50 - 6.20)	5.45 (5.28 - 5.80)	<b>0.022</b>
Median Serum Insulin (IQR), mIU/mL	19	19.50 (10.75 - 25.50)	13.00 (6.00 - 16.00)	<b>0.039</b>
Median IL-6 (IQR), pg/mL	30	1.005 (0.599 - 1.223)	0.917 (0.609 - 1.485)	0.479
Median sCD14 (IQR), ng/mL	30	2330 (2075 - 3035)	2286 (1968 - 2508)	0.360
Median sCD163 (IQR), ng/mL	30	756.4 (403.2 - 1201)	732.0 (474.6 - 1206)	0.403