Lipidome abnormalities may contribute to altered macrophage phenotype and vascular inflammation in people with HIV

Emily R Bowman[1], Cheryl Cameron[2], Brian Richardson[2], Manjusha Kulkarni[1], Janelle Gabriel[1], Aaren Kettelhut[1], Morgan Cichon[1], Ken Reidl[1], Subha Raman[1], Susan Koletar[1], Mark Cameron[2], Nicholas T Funderburg[1]

[1]The Ohio State University, Columbus, OH, USA; [2]Case Western Reserve University, Cleveland, OH, USA

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

ABSTRACT

Background: HIV infection and antiretroviral therapy (ART) are associated with dyslipidemia and increased cardiovascular disease (CVD) risk. Macrophages accumulate in arterial walls and produce factors that contribute to vascular inflammation. The relationships among lipids and macrophage phenotype in people with HIV (PWH) are unclear.

Methods: Coronary artery calcification (CAC) in people with (n=40) and without (n=15) HIV was quantified by computed tomography scanning. PBMCs from HIV+ ART+ (n=20) and HIV- donors (n=20) were cultured for 5 days in medium containing 20% autologous serum to generate monocyte derived macrophages (MDMs). Concentration and composition of serum lipids (~1200 lipid species) was measured by mass spectrometry. MDM transcriptomes and differential gene expression (DGE) were analyzed using our R Bioconductor pipeline. Foam cell formation was assessed by Bodipy staining. Immune activation was assessed by flow cytometry.

Results: PWH (ages 27-67) had significantly increased CAC scores compared to people without HIV (ages 25-70) (CAC=367 v 25, p=0.01). Traditional risk assessments categorize individuals with CAC scores <100 at low risk, and >400 at high risk for CVD events. Older (over 55) PWH (n=17) had an average CAC score of 423, compared to a score of 71 in older people without HIV (n=7). PWH had increased serum levels of free fatty acids (FFAs), with enrichment of saturated fatty acids (SaFAs) and a reduction in polyunsaturated fatty acids (PUFAs). DGE analysis of MDMs from participants with and without HIV identified alterations in immune signaling, DNA damage repair, mitochondrial dysfunction, and lipid processing pathways. Levels of SaFA and PUFA lipid species correlated with unique DGE signatures and altered metabolic pathway activation in MDMs. Bodipy staining indicated greater lipid accumulation. MDMs from PWH also produced more TNFa, IL-6, and ROS, and had increased HLA-DR surface expression. SaFA levels were directly related, whereas PUFAs were inversely related to HLA-DR expression on MDMs from PWH. HIV- MDMs exposed to HIV+ pooled serum displayed greater intracellular lipid accumulation and DGE than cells exposed to HIV- pooled serum.

Conclusions: Lipid abnormalities in HIV infection may contribute to a pro-atherogenic MDM phenotype. MDMs from PWH readily form foam cells, have altered transcriptional profiles, and produce mediators of vascular inflammation, which may enhance CVD risk, particularly in the aging HIV population.

RESULTS

PWH have significantly increased coronary artery calcification compared to the general population

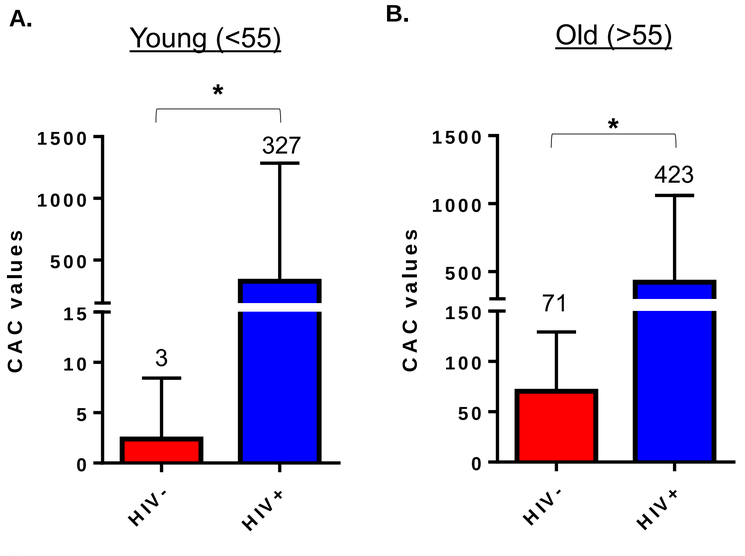


Figure 1. Coronary artery calcification values were quantified by computed tomography scanning in people with (N=40) and without HIV (N=20), and separated into A) young (<55 years) B) and old (>55 years) age groups. (*p<0.05)

RESULTS

The lipidomes of PWH display an enrichment of saturated fatty acids and a reduction of polyunsaturated fatty acids

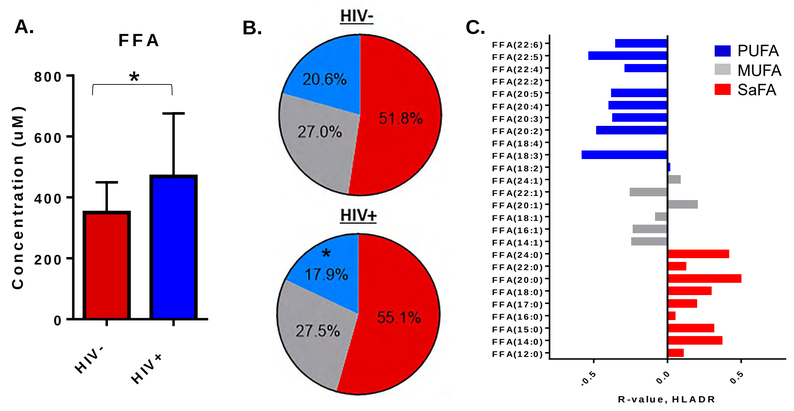


Figure 2. A) Circulating FFAs are increased in PWH. B) Proportions of SaFAs (red) and PUFAs (blue) are altered in PWH. C) SaFAs tend to be positively associated, and PUFAs tend to be negatively associated with HLADR expression on MDMS.

MDMs from people with and without HIV display differential patterns of gene expression and activation marker expression

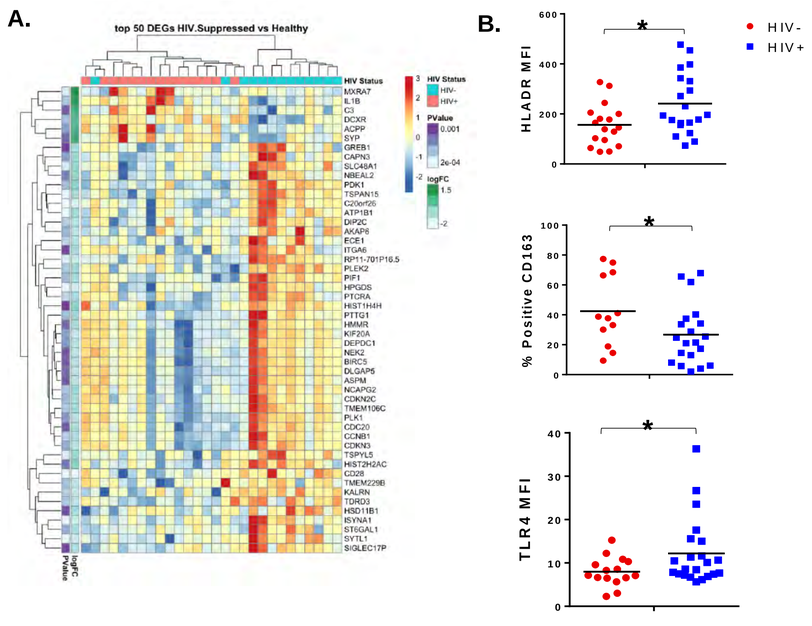


Figure 3. A) Transcriptome analysis identified systematic gene expression differences between MDMs from people with and without HIV cultured in autologous serum alone (811 DGEs, p<0.05). B) Cell surface marker expression was evaluated by flow cytometry. Activation markers, HLA-DR, CD163, and TLR4, were increased on MDMs from PWH.

Cells from PWH display decreased mitochondrial function

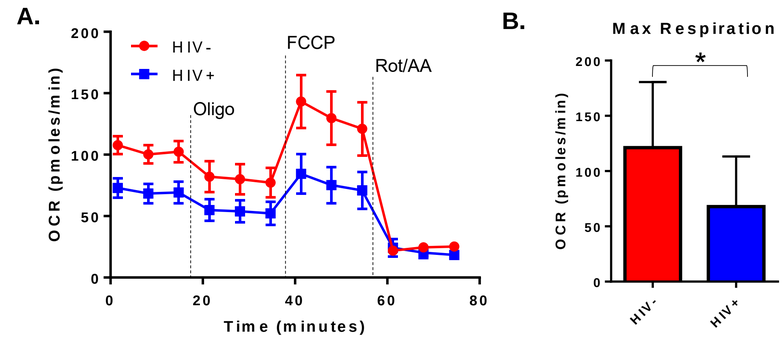


Figure 4. Cryopreserved PBMCs were thawed and rested overnight. Oxygen consumption rate (OCR) kinetics were measured on an Agilent XFp analyzer (HIV- N=8; HIV+ N=10). Subsequent injections of oligomycin, FCCP, and rotenone/antimycin A were used to assess mitochondrial basal and maximum respiration.

Macrophages obtained from PWH form foam cells more readily than macrophages from people without HIV

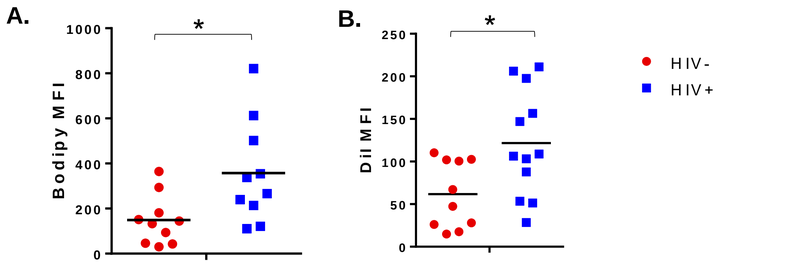


Figure 5. A) Intracellular lipid accumulation was measured using Bodipy (493/503) stain and flow cytometry. B) Lipid uptake was measured by flow cytometry following exposure (4 h) of MDMs to DiI-OxLDL. (p<0.05)

Serum lipids associated with CVD correlate with unique DGE signatures in MDMs from PWH

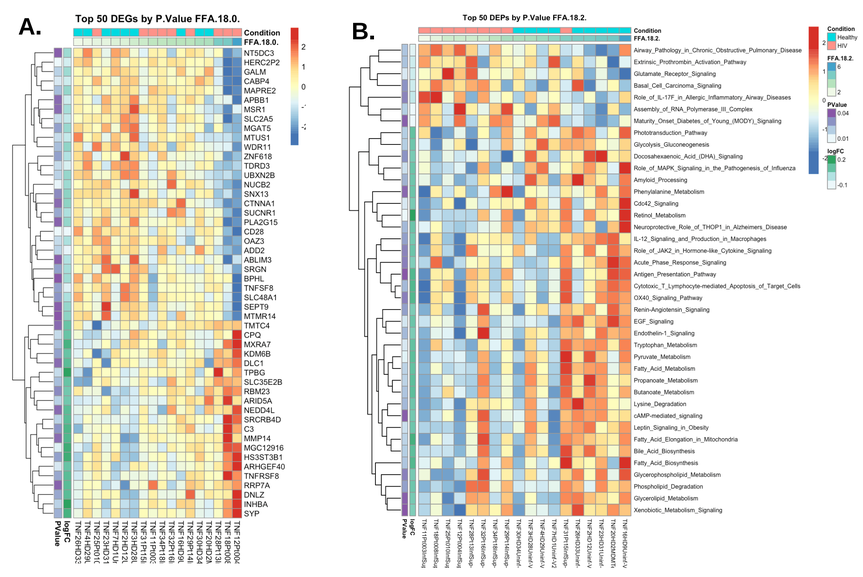


Figure 6. MDM transcript data was stratified based on serum levels of A) stearic acid (18:0) and B) linoleic acid (18:2). Increased inflammatory gene expression was associated with higher levels of SaFA and reduced levels of PUFA.

Exposure to pooled serum from PWH is sufficient to alter macrophage phenotype

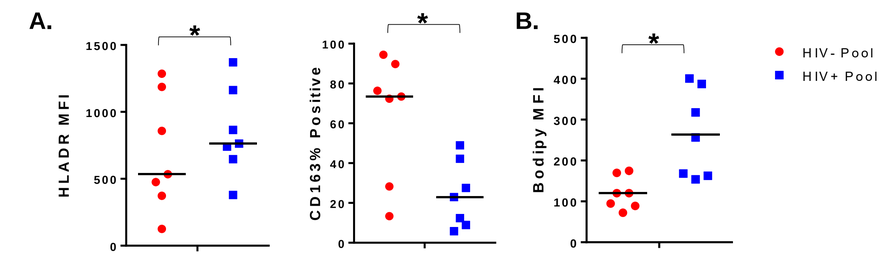


Figure 7. MDMs from HIV- donors were cultured in the presence of serum pooled from HIV- (N=6) or HIV+ (N=6) donors. A) Activation markers, HLADR and CD163, were measured on MDMs by flow cytometry. B) Intracellular lipid accumulation was measured by Bodipy staining (p<0.05).

CONCLUSIONS

- We observed increased coronary artery calcification and altered lipidome composition in PWH.
- MDMs from PWH display broad changes in transcriptome and signaling pathway activation, and increased foam cell formation.
- Pooled serum from PHW is sufficient to alter macrophage phenotype and gene expression (2675 DGEs, p<0.05).
- Lipid levels are associated with markers of immune activation and inflammatory MDM gene signatures, and may play a role in driving a pro-atherogenic macrophage phenotype in PWH.