Background: Dyslipidaemia in untreated HIV infection is characterised by reduced high density lipoprotein cholesterol (HDL) and increased risk of cardiovascular disease (CVD). In vitro, HIV impairs monocyte cholesterol efflux (MCE) onto apolipoprotein A1 (ApoA1) via the ATP-binding cassette transporter A1 (ABCA1) potentially explaining lower HDL. We aimed to determine if MCE was inhibited in untreated HIV in vivo.
Methods: Using a novel, dynamic ex vivo assay we compared MCE in HIV positive (HIVpos) subjects not on antiretroviral therapy (ART) and HIV negative (HIVneg) controls matched for age, gender, race, smoking and hepatitis C status. Monocytes were isolated from fasting blood and monocyte intracellular cholesterol (MIC) was measured by florescence and corrected for total cell count before and after cholesterol loading (T=0, 2, 4, 6, 24 hours post loading). MCE was calculated as a ratio of extracellular (supernatant) cholesterol to MIC (EC_T: MIC_T) with an additional 24 hr measure in the presence of ApoA1 (EC_a: MIC_a) figure 1. Changes in MCE were correlated with lipids and carotid intima-media thickness (CIMT). Data are median [IQR]. Comparisons were made using non-parametric analyses.
Results: We recruited 50 HIVpos subjects (52% homosexual, 36% heterosexual; CD4+ 410[268, 588] cells/mm^3; log HIV RNA 4.0[3.52, 4.78] copies/ml) and 50 matched controls. The HIVpos group had significantly lower total, low density lipoprotein (LDL) and HDL cholesterol but similar triglycerides and CIMT (table 1). There was no significant between-group difference in fasting MIC (HIVpos 2.0[1.6, 2.4] versus HIVneg 1.8[1.6, 2.4] pg/cell, p=0.53) or post cholesterol loaded MIC (HIVpos 7.1[5.5, 9.6] versus HIVneg 6.7[5.1, 9.7], p=0.39). However, MCE was significantly and consistently greater in the HIVpos group over time (table 1). The addition of ApoA1 increased EC_{24}/MIC_{24} in both groups, with no between-group difference observed. Higher HDL correlated with lower EC_T: MIC_T ratio (T_2 r=-0.41, T_4 r=-0.34, T_6 r=-0.28, T_{24} r=-0.29, all p≤0.005). Neither CIMT nor HIV RNA correlated with MCE.
Conclusions: These data suggests that untreated HIV is characterised by enhanced rather than decreased MCE, with higher MCE correlating with lower HDL. This unexpected finding may reflect up-regulation of MCE pathways compensating for any potential negative effect of HIV on ABCA1-medicated cholesterol efflux. Further research into the pathways involved, the effects of ART and the impact of these findings on CVD pathogenesis is required.

Enhanced, not inhibited monocyte cholesterol efflux characterises untreated HIV

Poster #732

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Introduction

Dyslipidaemia in untreated HIV infection is characterised by reduced high density lipoprotein cholesterol (HDL) and increased risk of cardiovascular disease (CVD)[1]. *In vitro*, HIV impairs monocyte/macrophage cholesterol efflux (MCE) onto apolipoprotein A1 (ApoA1) via the ATP-binding cassette transporter A1 (ABCA1), potentially explaining low HDL, with data suggesting a cell-based disturbance mediated by HIV *nef* [2, 3]. Current MCE assays test how efficiently a subjects plasma facilitates cholesterol efflux from cultured cells. These plasma-based assays cannot detect cell based disturbances in MCE.

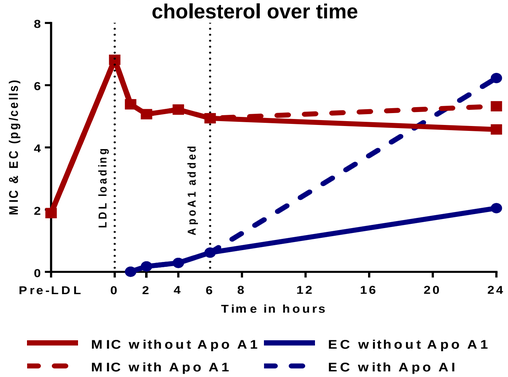
Objective

To determine the effects of HIV on MCE *in vivo* using a novel cell-based MCE assay

Methods

Using a novel, dynamic *ex vivo* assay we compared MCE in HIV positive (HIVpos) subjects not on antiretroviral therapy (ART) and HIV negative (HIVneg) controls matched for age, gender, race, smoking and hepatitis C status enrolled in the Monocyte Intracellular Cholesterol (MONIC) study. Monocytes were isolated from fasting blood and total monocyte intracellular cholesterol (MIC) was measured by fluorescence corrected for total cell count before and after cholesterol (LDL) loading (T=0, 2, 4, 6, 24 hours post loading). MCE was calculated as a ratio of extracellular (supernatant) cholesterol to MIC (EC_T: MIC_T) with an additional 24 hr measure in the presence of ApoA1 (EC_a: MIC_a) figure 1. Changes in MCE were correlated with lipids and carotid intima-media thickness (CIMT). Data are median [IQR]. Comparisons were made using non-parametric analyses.

Figure 1. Monocyte intracellular cholesterol & extracellular cholesterol over time



Results

100 subjects were recruited, (median age 35 [30, 43] years, 79% male, 76% Caucasian), 50 HIVpos and 50 HIVneg matched controls. The HIVpos group had significantly lower total, low density lipoprotein (LDL) and HDL cholesterol but similar triglycerides and CIMT (table 1). A low HDL (<1.05mmol/L (<40mg/dL) for males and <1.30mmol/L (<50mg/dL) for females) was present in 32 (64%) of the HIVpos group compared with only 5 (10%) of the HIVneg group p<0.001).

Table 1. Baseline demographics and laboratory parameters

Overall demographic and laboratory parameters				HIV parameters	
	HIV negative (n=50)	HIV positive (n=50)	p=	HIV positive (n=50)	
Age (years)	34.5 (30, 43)	35 (29, 41)	0.99	HIV acquisition risk	
Male gender (n, %)	39 (78)	40 (80)	0.81	Heterosexual (n, %)	18 (36)
Caucasian (n, %)	38 (76)	38 (76)	1.0	MSM (n, %)	26 (52)
Current smoker (n, %)	14 (28)	12 (24)	0.65	IVDU (n, %)	4 (8)
Cholesterol (mmol/L)	5.0 (4.6, 5.7)	4.3 (3.5, 4.7)	<0.001	Other (n, %)	2 (4)
HDL (mmol/L)	1.29 (1.15, 1.52)	0.96 (0.82, 1.21)	<0.001	AIDS (n, %)	12 (24)
LDL (mmol/L)	3.2 (2.6, 3.8)	2.5 (2.1, 3.0)	<0.001	CD4+ T cell count (cells/mm^3)	410 (268, 588)
Triglycerides (mmol/L)	1.08 (0.78, 1.33)	1.25 (0.87, 1.70)	0.09	CD4+ T cell count %	24 (13, 30)
Total: HDL cholesterol ratio	3.8 (3.3, 4.4)	4.4 (3.6, 5.3)	0.01	Log HIV RNA	4.01 (3.52, 4.78)
CIMT (mm)	0.77 (0.70, 0.87)	0.78 (0.69, 0.90)	0.94		

Data are median (IQR) unless stated; HDL, high density lipoprotein; LDL, low density lipoprotein; CIMT, carotid intima-media thickness; MSM, men who have sex with men; IVDU, intravenous drug user.

There was no significant between-group difference in fasting MIC (HIVpos 2.0[1.6, 2.4] versus HIVneg 1.8[1.6, 2.4] pg/cell, p=0.53) or post cholesterol loaded MIC (HIVpos 7.1[5.5, 9.6] versus HIVneg 6.7[5.1, 9.7] pg/cell p=0.39, figure 2). However, MCE was significantly and consistently greater in the HIVpos group over time (figure 3). The addition of ApoA1 significantly increased the EC_{24}/MIC_{24} in both groups (HIVneg 0.42 [0.32, 0.56] versus 1.13 [0.92, 1.35] p<0.001; HIVpos 0.53 [0.40, 0.69] versus 1.27 [1.06,1.50] p<0.001). Even with addition of ApoA1, the observed greater MCE in the HIVpos group persisted, albeit of borderline significance (p=0.054).

Figure 2. Monocyte intracellular cholesterol at baseline and post cholesterol loading

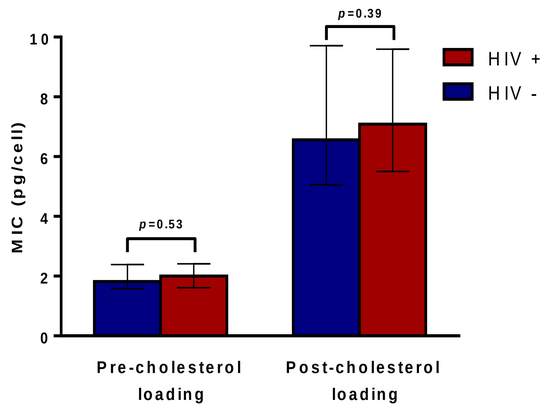
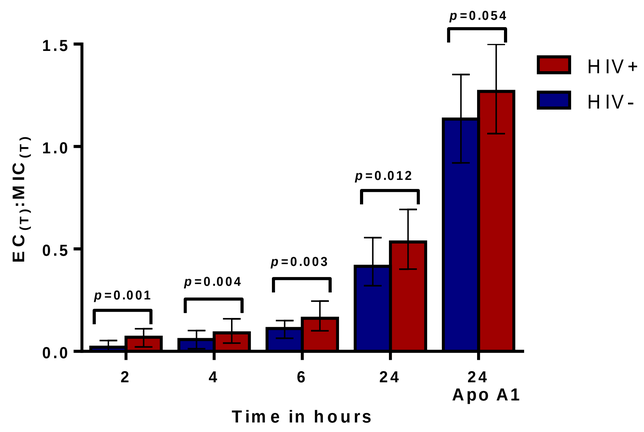


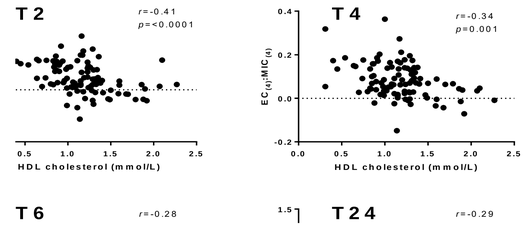
Figure 3. Monocyte cholesterol efflux over time



Results continued

Although, higher HDL correlated with lower MCE at all time points (T_2 r=-0.41, T_4 r=-0.34, T_6 r=-0.28, T_{24} r=-0.29, all p≤0.005, figure 4), MCE did not correlate with either CIMT or HIV RNA.

Figure 4. Correlation between monocyte cholesterol efflux and HDL across time points



Conclusions

- These data suggests that untreated HIV is characterised by enhanced, rather than inhibited MCE.
- Increased MCE has been previously observed in a HIVneg angiographic cohort using plasma-based efflux assays and was associated with increased incident cardiovascular events[4].
- However, results have not been consistent, with another cohort of subjects free from CVD finding an inverse correlation between MCE and incident CVD[5].
- In our study, the up-regulation of MCE may reflect a compensatory effect overcoming the inhibition of HIV on ABCA1-mediated cholesterol efflux.
- Further research is needed to:
  1. clarify if increased MCE contributes to the increased levels of dysfunctional HDL observed in HIV[6].
  2. explore pathways involved, the effects of ART and the impact of these findings on CVD pathogenesis.

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