Background: In vitro and ex vivo, untreated HIV is associated with accumulation of intracellular cholesterol in monocyte/macrophages (M/M) and impaired cholesterol efflux, both of which may impact on atherogenesis and cardiovascular disease (CVD) risk. We aimed to prospectively examine the effect of antiretroviral therapy (ART) initiation on monocyte cholesterol metabolism in vivo.
Methods: In a multi-centre, prospective study, 28 genes involved in regulation of monocyte cholesterol metabolism (sensing, uptake, endogenous cholesterol synthesis and efflux), inflammation and mitochondrial function were measured using quantitative PCR arrays in RNA extracted from monocytes derived from ART-naive adults at baseline and at 4 and 12 weeks post ART initiation. Data are presented as median (IQR), with genes expressed as a ratio to three housekeeping genes (ACTB, TBP, RPL13). Within- and between-group (PI/non-PI) differences were compared using Wilcoxon signed rank and Mann-Whitney U tests respectively.
Results: Of 85 HIV-positive subjects, (median (IQR) age 37 (31, 44) years, 70 (82%) male, 62 (73%) Caucasian, CD4+ T cell count 369 (297,547) cells/mm3, HIV RNA 42,181 (19,474, 107,593) copies/ml), 30 (35%) initiated protease inhibitor (PI) based regimens. ART initiation was accompanied by a coordinated downregulation of cholesterol sensing (SREBF1, SREBF2), uptake (LDLR) and endogenous cholesterol synthesis (HMGCR, PMVK, ACAT2) genes at weeks 4 and 12 consistent with appropriate intracellular responses to increased availability of intracellular cholesterol (table 1). However, cholesterol efflux pathways were dysregulated, with expression of ABCA1 and its regulators (NR1H3 and PPARα) downregulated while SR-B1 gene expression, indicative of an alternative efflux pathway, was upregulated (table 1). ART initiation was accompanied by an expected downregulation of inflammatory pathway genes (TLR4, NFκB1 and NLRP3, all p<0.05) and increased expression of mitochondrial RNA (MT-CTB, p<0.01). There was no significant difference in the pattern of gene expression changes with initiation of PI versus non-PI based ART.
Conclusions: This, the first study to prospectively examine M/M gene expression, demonstrates a molecular signature consistent with further increased availability of intracellular cholesterol and disrupted cholesterol efflux pathways with ART initiation despite expected appropriate downregulation of inflammatory pathways. How these changes impact on CVD risk remains to be determined.

Dysregulated Monocyte Cholesterol Metabolism Gene Expression with ART Initiation

Poster #665

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Introduction

- *In vitro* and *ex vivo*, untreated HIV is associated with accumulation of intracellular cholesterol in monocyte/macrophages (M/M) and impaired cholesterol efflux, both of which may impact on atherogenesis and cardiovascular disease (CVD) risk[1,2].
- Monocyte cholesterol metabolism is regulated by sterol regulatory element binding proteins (SREBPs) and liver X receptors (LXRs).
- When intracellular cholesterol levels decrease, SREPBs upregulate transcription of cholesterol uptake genes such as low-density lipoprotein receptor (LDLR) and the cholesterol synthesis gene HMG co-A reductase (HMGCR) to increase intracellular cholesterol content.
- In contrast, when intracellular cholesterol levels increase, LXRs upregulate the ATP-binding cassette transporter (ABCA1 and ABCG1) pathways promoting cholesterol efflux.

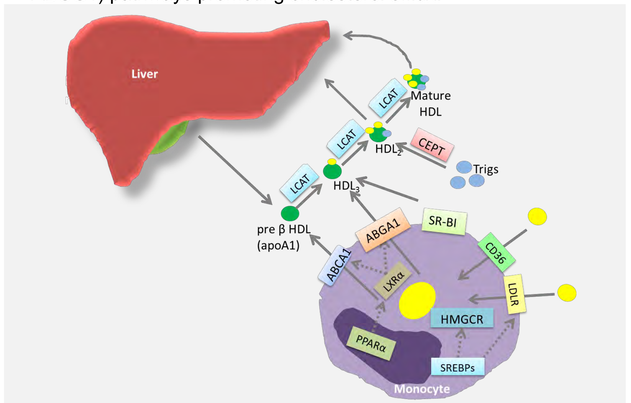


Figure 1. Monocyte cholesterol metabolism. LCAT, Lecithin cholesterol acyltransferase; SR-BI, scavenger receptor class B1; CEPT, cholesteryl ester transfer protein; HDL, High-density lipoprotein; apoA1, apolipoprotein A1

Objective

To prospectively examine the effect of antiretroviral therapy (ART) initiation on monocyte cholesterol metabolism in vivo.

Methods

- The Reverse Cholesterol Transport Study (RCTS) was a multi-centre, prospective study that examined monocyte cholesterol metabolism in HIV-1 positive adults at baseline and at 4 and 12 weeks post ART initiation.
- A panel of 28 genes (table 1) was selected to examine changes in cholesterol gene expression in RNA extracted from monocytes using quantitative PCR.
- Data are presented as median (IQR), with genes expressed as a ratio to three housekeeping genes (ACTB, TBP, RPL13). Within- and between-group (PI/non-PI) differences were compared using Wilcoxon signed rank and Mann-Whitney U tests respectively.

Results

Table 1. Gene targets and their functional category

Cholesterol sensing	Cholesterol synthesis	Inflammation
SREBF1	HMGCR	TLR4
SREBF2	ACAT2	NLRP3
SCAP	PMVK	NFκB1
LXRα		IL1β
LPL	**Cholesterol efflux**	IKBKB
PPARα	ABCA1	TNFα
PPARγ	ABCG1	
MBTPS1	SR-B1	**Mitochondrial toxicity**
MBTPS2		NRF1
		SOD1
Cholesterol uptake		PPARCG1A
LDLR		TFAM
CD36		MTCYB

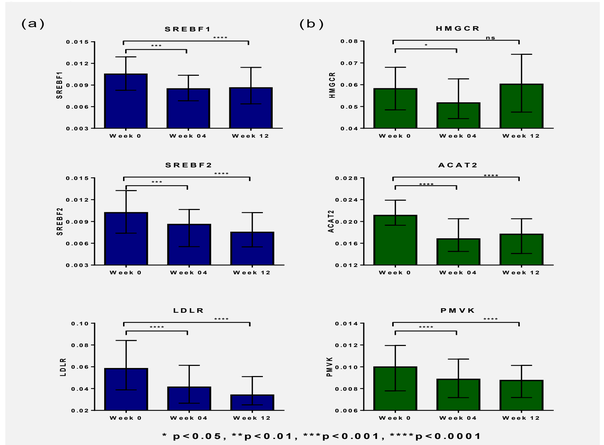
85 subjects were enrolled and subject characteristics are outlines in table 2.

Table 2. Demographics and laboratory parameters

Baseline demographics			
Age in years			37 (31, 44)
Male gender (n (%))			70 (82)
Caucasian (n (%))			62 (73)
MSM acquisition			60 (71)
ART initiation			
Nucleoside reverse transcriptase inhibitor (n (%))			76 (89)
ABC/3TC (n (%))			15 (20)
TDF/FTC (n (%))			61 (80)
Non nucleoside reverse transcriptase inhibitor (n (%))			55 (65)
Protease inhibitor (n (%))			30 (35)
Darunavir/ritonavir (n (%))			18 (60)
Laboratory parameters	**Week 0**	**Week 4**	**Week 12**
CD4+ T cells (cell/mm^3)	369 (297, 547)	438 (353, 601)*	489 (364, 647)‡
Log HIVRNA (copies/ml)	4.6 (4.3, 5.0)	2.4 (2.1, 2.9)*	1.6 (1.6, 1.9)‡
Cholesterol (mmol/L)	4.2 (3.7, 4.8)	4.0 (3.6, 5.0)	4.5 (4.1, 5.4)‡
HDL-c (mmol/L)	1.05 (0.9, 1.3)	1.07 (0.9, 1.3)*	1.16 (1.0, 1.4)‡
LDL-c (mmol/L)	2.6 (2.2, 3.1)	2.7 (2.2, 3.4)*	2.8 (2.3, 3.5)‡
Triglycerides	0.96 (0.8, 1.3)	1.1 (0.9,1.5)	1.2 (0.9, 1.5)‡

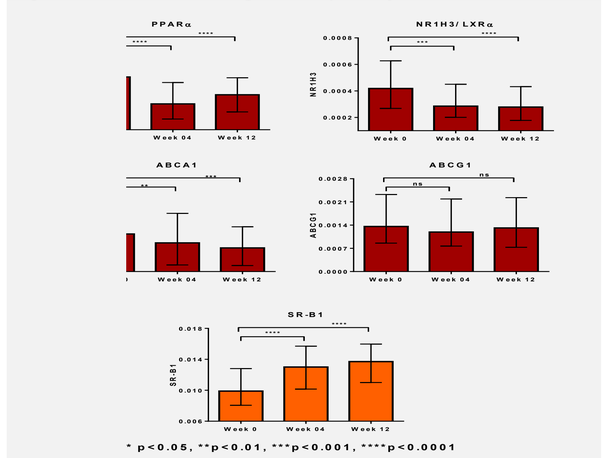
* represents p<0.05 at week 4; ‡ represents p<0.05 at week 12

Figure 2 (a) Cholesterol sensing & uptake and (b) endogenous synthesis pathways



ART initiation was accompanied by a coordinated downregulation of cholesterol sensing (SREBF1, SREBF2), uptake (LDLR) and endogenous cholesterol synthesis (HMGCR, PMVK, ACAT2) genes at weeks 4 and 12 consistent with an appropriate intracellular responses to increased availability of intracellular cholesterol (fig 2).

Figure 3. Cholesterol sensing & efflux pathways



However, cholesterol efflux pathways were dysregulated, with expression of ABCA1 and its regulators (NR1H3 and PPARα) downregulated (figure 3), while SR-B1 gene expression, indicative of an alternative efflux pathway was upregulated (fig 3)

Results continued

ART initiation was accompanied by an expected downregulation of inflammatory pathway genes (TLR4, NFκB1 and NLRP3, all p<0.05) and increased expression of mitochondrial RNA (MTCTB, p<0.01) (table 3).

There was no significant difference in the pattern of gene expression changes with initiation of PI versus non-PI based ART.

Table 3. Change in inflammatory pathway & mitochondrial toxicity genes post ART initiation

Gene	Week 4		Week 12	
	% change	*P* value	% change	*P* value
Inflammation				
TLR4	-9.6 (-22.8, 15.5)	**0.007**	-10.7 (-29.9, 4.9)	**0.002**
NLRP3	-6.8 (-25.9, 22.6)	**0.047**	-6.2 (-31.3, 14.5)	**0.04**
NFκB1	-3.1 (-21.4, 12.4)	**0.043**	-12.0 (-27.2, 4.7)	**<0.0001**
IL1β	-15.9 (-57.7, 79.8)	0.49	-24.1 (-60.3, 77.70	0.34
IKBKB	-6.6 (-26.6, 7.3)	**0.003**	-3.8 (-19.3, 7.0)	**0.04**
TNF	-8.5 (-34.8, 39.1)	0.37	-27.1 (-45.2, 18.2)	**0.001**
Mitochondrial toxicity				
NRF1	-10.3 (-26.8, 13.2)	**0.005**	0.9 (-20.9, 29.1)	0.87
SOD1	-1.6 (-9.4, 11.9)	0.94	1.0 (-14.3, 18.4)	0.72
TFAM	1.1 (-10.3, 12.1)	0.80	-0.9 (-14.6, 12.1)	0.62
MTCYB	29.9 (-5,3, 61.2)	**<0.0001**	9.8 (-9.9, 45.6)	**0.005**

Conclusions

This study, which prospectively examined monocyte cholesterol gene expression, demonstrates a molecular signature consistent with increased availability of intracellular cholesterol and disrupted cholesterol efflux pathways with ART initiation despite expected appropriate downregulation of inflammatory pathways.

How these changes impact on CVD risk remains to be determined.

References

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