Greater adipose tissue mitochondrial toxicity with initiation of AZT/NNRTI based antiretroviral therapy in comparison to AZT/PI

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Introduction

- Thymidine analogue nucleoside reverse transcriptase inhibitors (tNRTIs) including zidovudine (AZT) are associated with the development of mitochondrial toxicity in subcutaneous adipose tissue (SAT) leading to lipoatrophy [1].
- The relative contribution of the choice of third agent (protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI) or integrase strand transfer inhibitor (INSTI)) to SAT toxicity is poorly defined.
- Previously, greater limb fat loss was observed with NNRTI in comparison to PI when used with a tNRTI [2]. However, the underlying mechanism remains unclear.

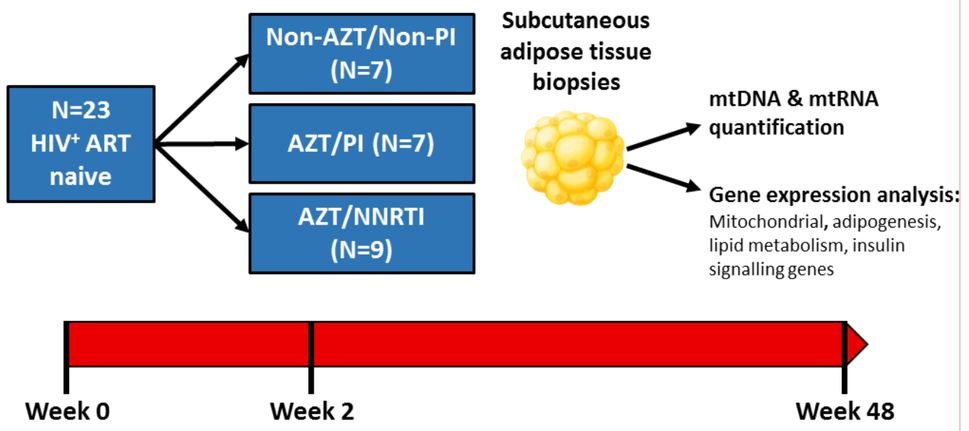
Study Objective

- To explore the relative contribution of tNRTI and the choice of third agent (PI or NNRTI) to the development of SAT toxicity in ART-naïve patients initiating first-line ART.

Methods

- The HIV Infection and Metabolic Abnormalities study (HAMA001) was a prospective cohort study, in which ART-naïve HIV$^+$ adults initiating ART containing AZT/PI, AZT/NNRTI or a tNRTI-sparing regimen containing neither AZT nor PI (non-AZT/non-PI) were followed for 48 weeks.
- Limb and trunk fat was assessed by DXA at weeks 0, 12, 24 and 48.
- Fasting subjects underwent week 0, 2 and 48 fasting SAT biopsies for quantification of mitochondrial DNA (mtDNA) and mitochondrial RNA (mtRNA) levels by quantitative PCR (qPCR) as well as for the gene expression analysis of 55 key adipocyte genes by qPCR array. Target gene categories are listed in Figure 1.
- Time-weighted changes in fat mass and mtDNA content in the AZT groups were compared to those in the non-AZT/non-PI group using longitudinal marginal models.
- Changes in mtRNA levels were assessed by Wilcoxon signed rank tests. qPCR array analysis was carried out using the LIMMA package in R.
- Data are presented as median (IQR), P<0.05 was considered significant

Figure 1: Study Design Overview



Results

- Of the 23 subjects recruited, 7, 7 and 9 initiated non-AZT/non-PI, AZT/PI and AZT/NNRTI based ART respectively.
- Study groups were comparable at baseline for gender, age, ethnicity and measures of body composition but viral load and total cholesterol differed.

Table 1: Baseline Characteristics

	Non-AZT/Non-PI		AZT/PI		AZT/NNRTI		*P*
Number	7		7		9		
Male Gender (%)	6	(86%)	7	(100%)	8	(88%)	0.56
Caucasian (%)	6	(86%)	5	(100%)	9	(100%)	0.16
Age	36	(34, 46)	41	(38, 42)	39	(29, 48)	0.97
CD4$^+$ (cells/mm^3)	66	(40, 325)	210	(98, 268)	126	(60, 140)	0.55
Log HIV RNA	5.6	(5.3, 5.8)	4.4	(4.3, 4.8)	4.9	(4.6, 5.4)	**0.04**
BMI (kg/m^2)	22	(20.5, 23.5)	22	(21, 24.5)	23	(22, 23)	0.65
Trunk Fat (kg)	6	(3.5, 7.5)	10.6	(7.3, 12.8)	7.6	(5.8, 10.8)	0.35
Limb Fat (kg)	3.4	(3.0, 4.0)	6.1	(4.3, 7.2)	6.6	(4.6, 6.7)	0.25
Trunk:Limb fat ratio	1.6	(1.4, 1.7)	1.7	(1.6, 1.8)	1.4	(1.1, 1.6)	0.16
HOMA-IR	0.7	(0.6, 1)	1.4	(1.2, 2.2)	1.1	(0.8, 1.4)	0.27
Total Cholesterol (mmol/L)	3.5	(3, 3.9)	4.7	(4.1, 4.8)	4.9	(4.2, 5.1)	**0.01**
Triglycerides (mmol/L)	1.8	(1, 2)	1.4	(1, 1.8)	1.2	(1, 1.3)	0.97

- Over 48 weeks, increases in trunk and limb fat expected with ART initiation [3] were observed in the non-AZT/non-PI group but not in the AZT groups.

Adipose tissue mtDNA and mtRNA

- mtDNA content was unchanged in the non-AZT/non-PI and AZT/PI groups (Fig. 2A,B) but decreased in the AZT/NNRTI group (Fig. 2B).
- The ratio of distal:proximal mtDNA decreased significantly in the AZT/NNRTI group (Fig. 2C,D) indicating a decrease in mitochondrial genome integrity.

- In keeping with decreased mtDNA in the AZT/NNRTI group, the expression of *MT-CO1* mtRNA was also significantly reduced in this group by week 48 (Fig. 3) while expression was variable in the other groups with no significant changes observed.

Figure 2: Decreases in SAT mtDNA with AZT/NNRTI

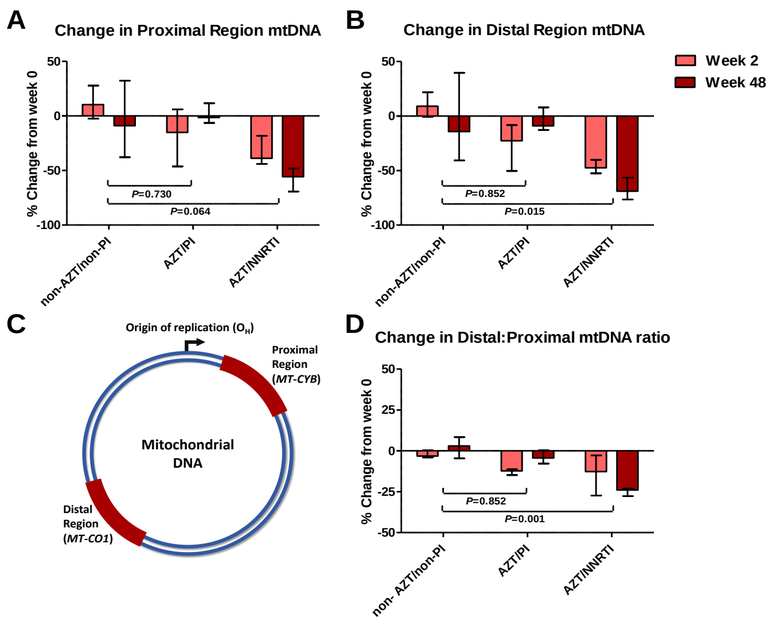
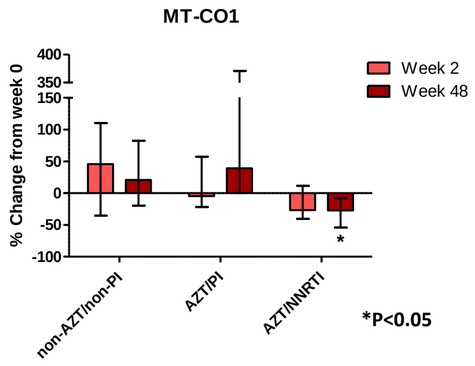


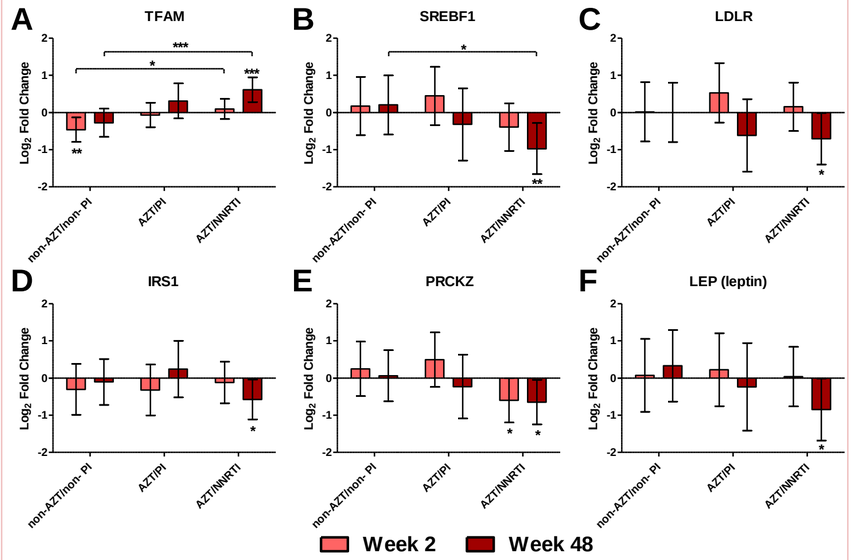
Figure 3: Decreases in SAT mtRNA with AZT/NNRTI



Adipose tissue gene expression

- Gene expression analysis revealed that the AZT/NNRTI group also had compensatory increases in the expression of the transcription factor *TFAM,* required for mitochondrial biogenesis (Fig. 4A).
- Additionally, AZT/NNRTI resulted in decreases in a number of key adipocyte genes involved in lipid metabolism and insulin signalling (Fig. 4 B-F), changes which did not occur in other groups.
- Most notably, the expression of *SREBF1* and its transcriptional target *LDLR* decreased significantly at week 48 in the AZT/NNRTI group (Fig. 4B,C) consistent with the development of lipodystrophy [4]

Figure 4: Increased expression of *TFAM* and decreases in key metabolic genes in SAT with AZT/NNRTI



Bars represent Log_2 fold change (95% C.I.) from week 0 levels, *P≤0.05, **P≤0.01, ***P≤0.005

Conclusions

- In this study, AZT/NNRTI but not AZT/PI led to significant mitochondrial toxicity in SAT together with reduced adipogenic and metabolic gene expression
- The findings of this study suggest that NNRTI may enhance tNRTI-mediated mitochondrial toxicity in SAT.

References & Acknowledgements

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This work was funded by the Wellcome Trust (097424/Z/11/Z).