

# Nº 984 Acute inhibition of mitochondrial respiration by efavirenz induces changes in lipid metabolism.

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## ABSTRACT

**Background.** Recent evidence suggests that the NNRTI efavirenz (EFV) contributes to changes in lipid and body fat composition implicated in lipodystrophy, but the mechanisms responsible are unknown. We have evaluated *in vitro* the effects of EFV on mitochondrial respiration and cellular lipid metabolism.

**Methods.** O<sub>2</sub> consumption was measured with a Clark-type O<sub>2</sub> electrode in non-HIV infected Hep3B cells. Following incubation (1h) with EFV (10, 25 or 50 µM) intracellular ATP was measured by fluorescence. Western blotting was used to evaluate (1h later) AMP-activated protein kinase (AMPK), the enzyme responsible for changes in cellular energetics following the metabolic stress induced by ATP depletion. The activity of the enzyme responsible for the entry of fatty acids in the mitochondria, carnityl palmitoyl transferase 1 (CPT-1) was analyzed spectrophotometrically. To reproduce inflammatory conditions, some experiments were performed in cells incubated (4h) with TNF-α (25 ng/ml). Data (n≥3) were analyzed by one-way ANOVA (p-value vs control <0.05\*, <0.01\*\*, <0.001\*\*\*).

**Results.** EFV induced an rapid, significant and dose-dependent inhibition of mitochondrial O<sub>2</sub> consumption (% reduction vs control; EFV 10µM 26.5 ± 5.7%\*\*\*, 25µM 50.6 ± 8.1\*\*\*, 50 µM 54.7 ± 1.4\*\*\*), which was accompanied by a reduction of intracellular ATP (control 17 ± 2.4 nmoles ATP/mg protein; EFV 10µM 13.7 ± 2.9%, 25µM 9.4 ± 2\*, 50µM 4.8 ± 1\*\*\*) and augmentation in activated AMPK (% of control; EFV 10µM 117.6 ± 31%, 25µM 173.6 ± 37.2, 50 µM 316.7 ± 93.1). This was followed by an increment in activated CPT-1 (% of control; EFV 10µM 112.1 ± 8%, 25µM 268.3 ± 52.5\*\*, 50µM 288.8 ± 28.6\*\*). In cells pre-treated with TNF-α, the effect on ATP dismutation was more pronounced (control 13.2 ± 1.2 nmoles ATP/mg protein, EFV 10µM 10 ± 1.6, TNF-α 9 ± 1.2, EFV 10µM+TNF-α 6.7 ± 0.8\*).

**Discussion.** Our results show that clinically used concentrations of EFV produce an immediate decrease of mitochondrial respiration and intracellular ATP levels. This effect seems to be the consequence of acute interference with mitochondrial functions, since it is too rapid to result from inhibition of mtDNA replication. This metabolic stress leads to activation of the AMPK signalling pathways and promotes lipid catabolism, as evident by the activation of CPT-1. Simulation of inflammatory conditions with TNF-α, exacerbates the changes produced by EFV. These mechanisms could be involved in lipid alterations present in lipodystrophy.

## MATERIALS AND METHODS

Non HIV infected Hep3B cells, cultured in Minimum Essential Medium, were placed in gas-tight chambers and O<sub>2</sub> consumption was measured with a Clark-type O<sub>2</sub> electrode after addition of Efavirenz (EFV), Ritonavir (RT), Atazanavir (ATZ) and Lamivudine (LAM) were used as comparison.

Following incubation with EFV (1h) the intracellular ATP was measured by fluorescence (ATP Bioluminescence Assay Kit HSII, Roche). Phosphorylation of AMP-activated protein kinase (AMPK) after 1h incubation with EFV was evaluated by Western blot and quantified by densitometry, whereas the activity of CPT-1 was analyzed with a spectrophotometer.

To reproduce inflammatory conditions, some experiments were performed in cells incubated (4h) with TNF-α (25ng/mL).

Data (n≥3) were analyzed using one-way ANOVA.

\* p<0.05, \*\* p<0.01, \*\*\* p<0.001 (vs control)

## INTRODUCTION

Lipodystrophy and associated metabolic alterations are the most prevalent adverse effect in patients taking HAART; the drugs and mechanisms involved in its appearance are still unclear.

Recent clinical evidence suggests that the NNRTI Efavirenz (EFV) contributes to changes in lipid composition. We have evaluated the acute effect of this NNRTI on several mitochondrial parameters, in order to determine an acute effect of this drug on the mitochondria, independent of any effect on mtDNA, which could result in changes in important cellular bioenergetic parameters.

AMP-activated protein kinase (AMPK) is a master switch for regulation of cellular energetics in health and disease. AMPK is activated by various forms of stress that compromise cellular ATP levels and, once phosphorylated, AMPK-P promotes catabolic pathways, including fatty acid entry in mitochondria by CPT-1, to restore energy homeostasis.

Inhibition of mitochondrial respiration could lead to activation of AMPK and, thus, to changes in lipid metabolism.

## RESULTS

Fig.1a



Gas-tight chambers in a Clark electrode

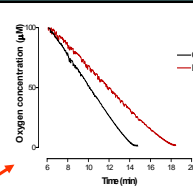


Fig.1b

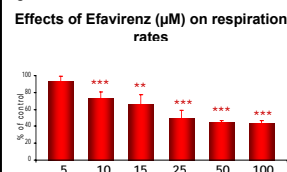


Fig.3

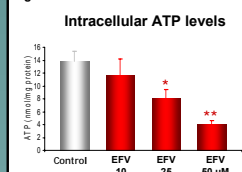


Fig.4

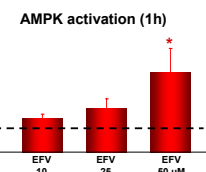


Fig.5

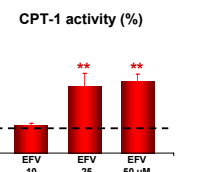


Fig.2a

The Clark electrode measures oxygen concentration versus time in order to establish the respiratory ability of cells.

Fig.2a

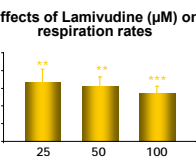


Fig.2b



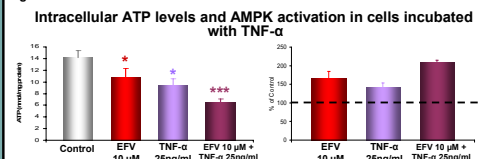
Efavirenz induces a significant and dose-dependent inhibition of mitochondrial O<sub>2</sub> consumption (Fig.1b), which was noticeable immediately after addition of the drug (Fig.1a) This effect was also present with Lamivudine but not with Ritonavir (Fig.2).

Inhibition of mitochondrial function resulted in a dose-dependent reduction of intracellular ATP following 1h incubation with Efavirenz (µM) (Fig. 3).

Expression of AMPK-P increases in Hep3B after treatment with Efavirenz (Fig.4), leading to an augmented activity of CPT-1 (Fig.5).

These effects are more profound when cells are pre-treated with TNF-α (Fig.6).

Fig.6



## CONCLUSIONS

Clinically used concentrations of EFV acutely reduce mitochondrial function in hepatic cells, whereas Lamivudine produce a similar effect only at higher concentrations.

EFV induces a reduction in ATP levels, leading to a bioenergetic stress as shown by the activation of AMP-activated protein kinase. This activation results in a significant increase in CPT-1 activity.

Simulation of inflammatory conditions with TNF-α, exacerbates the changes produced by EFV.

These changes could modify cellular lipid metabolism and could thus be involved in lipid alterations present in HIV therapy.

## REFERENCES

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