# Mitochondrial dysfunction induces mobile lipids and lipid droplet formation: role of lipid and glucose metabolism monitored by 1H and 13C-edited 1H MRS in intact HuT 78 lymphoblastoid cells

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## Introduction

Several studies have reported the detection of narrow mobile lipids (ML) signal in 1H MR spectra of intact cells under different physiological conditions, such as lymphocyte activation, differentiation and apoptosis.

Combined $^{1}H$ and $^{13}C$ MR spectroscopy (MRS) provides powerful methods to elucidate biochemical pathways and monitor metabolic fluxes in biological systems.

In this work we used *a)* $^{1}H$ MRS to investigate the biochemical nature of ML formation in a lymphoblastoid cell line after treatment with the complex III inhibitor of mitochondrial electron transport antimycin A (AMC-A) and *b)* a *J*-editing approach for the indirect detection of $^{13}C$ nuclei, to monitor alterations of $^{13}C$ label fluxes from [1-$^{13}C$]-glucose to glycolytic intermediates and to ML formed after AMC-A-induced mitochondrial impairment.

## Methods

HuT 78 cells were exposed to AMC-A (5 μg/ml) either in complete medium or in medium containing 5mM [1-$^{13}C$]-glucose. Exposure to AMC-A (24 h) did not alter cell viability nor induced apoptosis (<5%). MRS experiments were performed on intact cells and their extracts (organic and aqueous phases) on a Bruker Avance 400 spectrometer using a $^{1}H$-X multinuclear inverse probehead. Samples were analysed by both $^{1}H$ MRS and a double resonance {$^{13}C$}-$^{1}H$ technique which allows the detection of the only protons *J*-coupled to $^{13}C$ nuclei by a sequence called T-Sedor [Casieri et al. *Chem Phys Lett* 338: 137-141, 2001]. This technique combines good sensitivity and chemical resolution which allow short measurement time to monitor the $^{13}C$-glucose metabolism *in vivo*.

Lipid bodies were detected by trasmission electron microscopy (TEM) in cells. Lipid analyses were performed by thin layer chromatography on cell extracts.

## Results and discussion

After 24h AMC-A treatment, $^{1}H$ MR spectra of intact cells showed an over 10-fold increase in the ML $(CH_2)_n$ signal at 1.29 ppm and a well visible CH=CH signal at 5.35 ppm. TLC and TEM analyses confirmed a strong accumulation of neutral lipids assembled in intracellular lipid bodies. Analyses on organic phase extracts confirmed a strong accumulation of neutral lipids, while spectra of aqueous extracts showed an increase in glycerophosphorylcholine, likely due to phospholipase(s)' activity. Cells treated with Amc-A maintained capability of ATP production, attributed to enhanced glycolytic activity.

T-Sedor spectra of AMC-A treated cells showed incorporation of $^{13}C$ label into $(CH_2)_n$ and $CH_3$ of ML signals, a 3-fold increase in the [3-$^{13}C$]-lactate resonance and in {$^{13}C$}-$^{1}H$ signals in the region between 3.5 and 4.0 ppm. Analyses of cell extracts discriminated in AMC-A treated cells the labelling of glycero-3-phosphate, phospholipid and β-lyso-phospholipid (C-α)-glycerol in the 3.5÷4.0 ppm region (confirming an increased glycolitic activity). The [4-$^{13}C$]-glutamate signal was present only in control cells where the mitochondrial oxidative capacity was not compromised.

Conclusion: MRS of intact cells allows detection of multiple inter-linked biosynthetic and catabolic pathways involved in the retrograde communication between mitochondria and other cell compartments. In conclusion, the capability of T-Sedor to select $^{1}H$-$^{13}C$ bonds from different molecules offers novel tools to monitor $^{13}C$ fluxes from glycolysis to ML formation pathways in intact cells.

These results may open new perspectives to the development of non-invasive methods for in vivo monitoring the effects of mitochondrial impairment in a number of human diseases.