NMR-based stable isotope profiling [U-13C] acetate incorporation into HepG2 lipidic metabolism

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1. INTRODUCTION

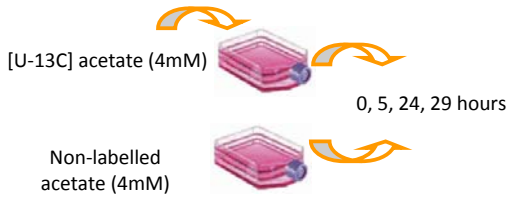
- Metabolomics can provide us a static views of metabolite rearrangements produced upon a certain effect.
- Stable isotope labeling based studies allow a dynamic view of changes and transformations of metabolites
- Isotopomer distribution information related to the incorporation of different labeled positions is an advantage of NMR stable isotope tracing studies.

2. GOALS

This work is aimed to study hepatocyte lipidic metabolism using NMR and [U-13C] acetate tracing of the de-novo lipidic synthesis in HepG2 cell cultures.

3. EXPERIMENTAL DESIGN

A NMR metabolic profiling of lipid cell extracts obtained at different time points: 0, 5, 24, 29 hours after [U-13C] and non-labelled acetate administration.



4. MATERIALS AND METHODS

4.1 Cell obtention

1)Cells were incubated at different time points with [U-13] or non-labelled acetate (4mM)

2) Tripsinization
3) Cells were counted (Neubauer chamber)
4) Cetrifugated (5 min 1000 rpm)
5) Medium was aspirated
6) Cells were cleaned with PBS
7) Centrifugated (5 min 1000 rpm)
8) PBS was aspirated
9) Cell pellet was obtained

4.2 Cell extraction

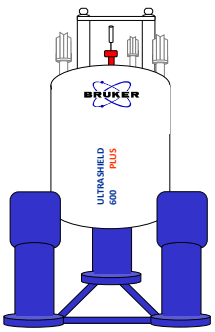
1)Homogenize in 2mL CH_3Cl/CH_3OH (2/1 v/v)
2) Ultrasonicated for 3 minutes
3) Added 2 mL of H_2O
4) Mixed and centrifugated (30min 4ºC 4500rpm)

PELLET:
Lipidic Extract
Reconstitute in CH3Cl:CH3OH (2:1)
N_2 stream and reconstitute in $CD_3Cl:CD_3OD$ (2:1) + TMS (0.01)

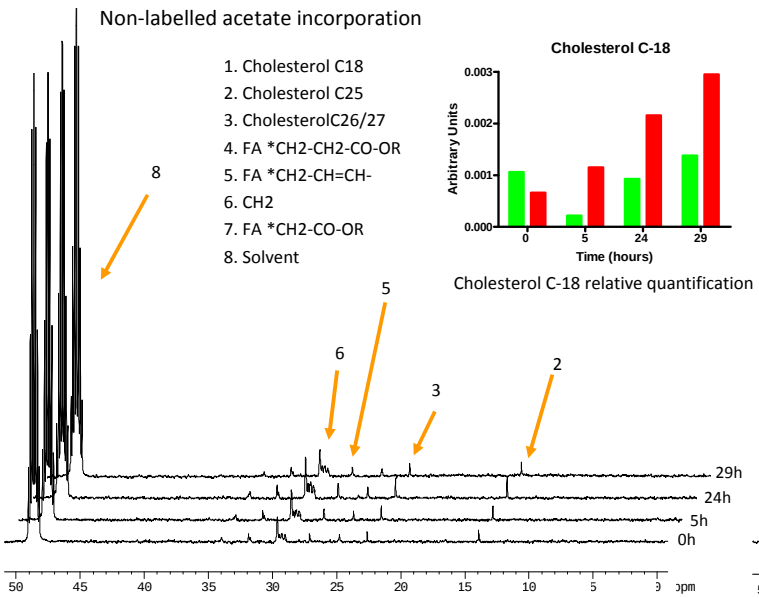
5. NMR ADQUISITION

NMR CONDITIONS

1D NMR spectra were recorded on cell extracts at 310 K on a Bruker Avance III 600 spectrometer® operating at a proton frequency of 600.20 MHz using a 5 mm CPTCI triple resonance (1H, 13C, 31P). Besides, 2-D NMR editing experiments 1H{13C}-HSQC, 1H{13C}-HMBC, 1H{13C}-HMQC and 13C–13C INADEQUATE were also acquired.

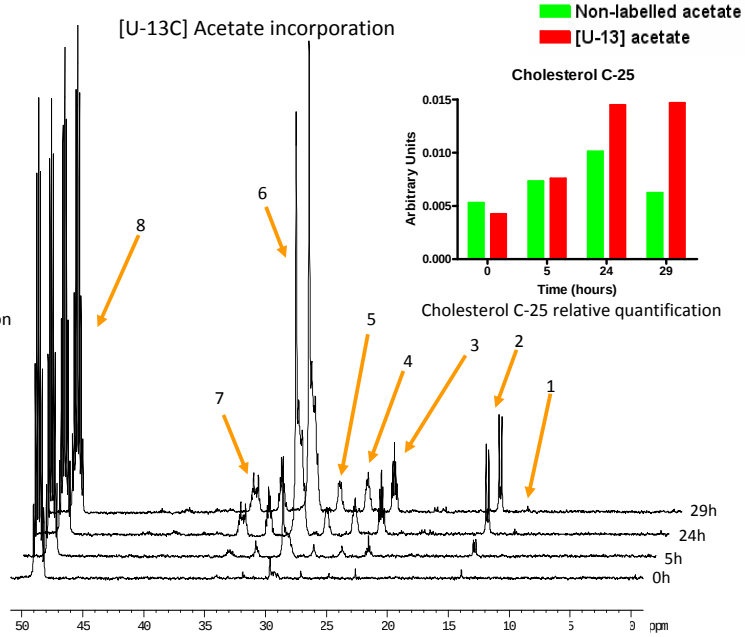


6. RESULTS



Cholesterol C-18 relative quantification

1D ^{13}C NMR lipidic extraction of non-labelled acetate incorporation showed the natural abundance of ^{13}C in a lipidic cell extraction at 0, 5, 24 and 29 hours after non-labelled acetate administration.



Cholesterol C-25 relative quantification

1D ^{13}C NMR lipidic extraction of [U-13C] acetate incorporation showed the metabolites in which labelled acetate was transformed in a lipidic cell extraction at 0, 5, 24 and 29 hours after [U-13C] acetate administration.

8. CONCLUSIONS

- A preliminary study of 1D ^{13}C NMR-lipidic pattern demonstrates not only the incorporation of [U-13C] acetate to fatty acids but also to cholesterol.
- Further work is in progress to elucidate and determine positional isotopomers distribution of metabolites identified in the 2D NMR experiments.
- This would form the basis to metabolic flux modeling.

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