# A FULL QUANTITATIVE NMR METABOLOMICS LIVER PROFILING ON GENETICALLY MODIFIED MICE: ASSESSING METABOLIC DISARRANGEMENTS IN HEPATIC INFLAMMATION

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## 1. INTRODUCTION

Dysfunctions in metabolism are a common finding in clinical medicine. We report the use of $^1$H-NMR-based full quantitative metabolomics liver profiling to assess the metabolic disarrangements in the progression of steatosis to Nonalcoholic Steatohepatitis (NASH).

## 2. GOALS

Using NMR based quantitative metabolomics, it was our goal in the present study (i) to establish and validate NMR based metabolic differences between LDLr$^{-/-}$ fed chow diet and LDLr$^{-/-}$ fed high fat diet supplemented with cholesterol (iii) to identify metabolic pathways related to progression of steatosis towards NASH.

## 3. EXPERIMENTAL DESIGN

We conducted all experiments in male (C57BL/6J) rendered hyperlipidemic by the ablation of LDL receptor (LDLr$^{-/-}$).

| AGE (weeks) | DIET | REPLICATES |
|---|---|---|
| 10 | CD | ×4 |
| 16 | CD | ×4 |
| | HF | ×4 |
| | HF-25 | ×4 |
| 32 | CD | ×4 |
| | HF | ×4 |
| | HF-25 | ×4 |

## 4. LIVER EXTRACTION/$^1$H-NMR / IDENTIFICATION & QUANTIFICATION

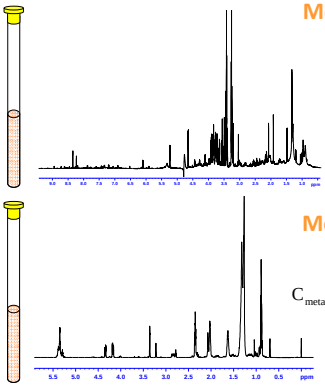
50 mg left lateral lobe

1) Homgenize in 1 ml $CH_3CN/H_2O$ (1/1)

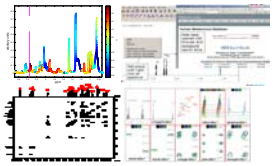
2) Centrifuge 5000g 15 min, T=4ºC

❑ SUPERNATANT:
**Aqueous extract:**
$N_2$/lyophilise
Reconstitute in $D_2O$+TSP (0.01%)

❑ PELLET:
**Lipidic extract:**
N2/ lyophilise
Reconstitute in $CD_3Cl$:$CD_3OD$ (2/1) TMS (0.01%)



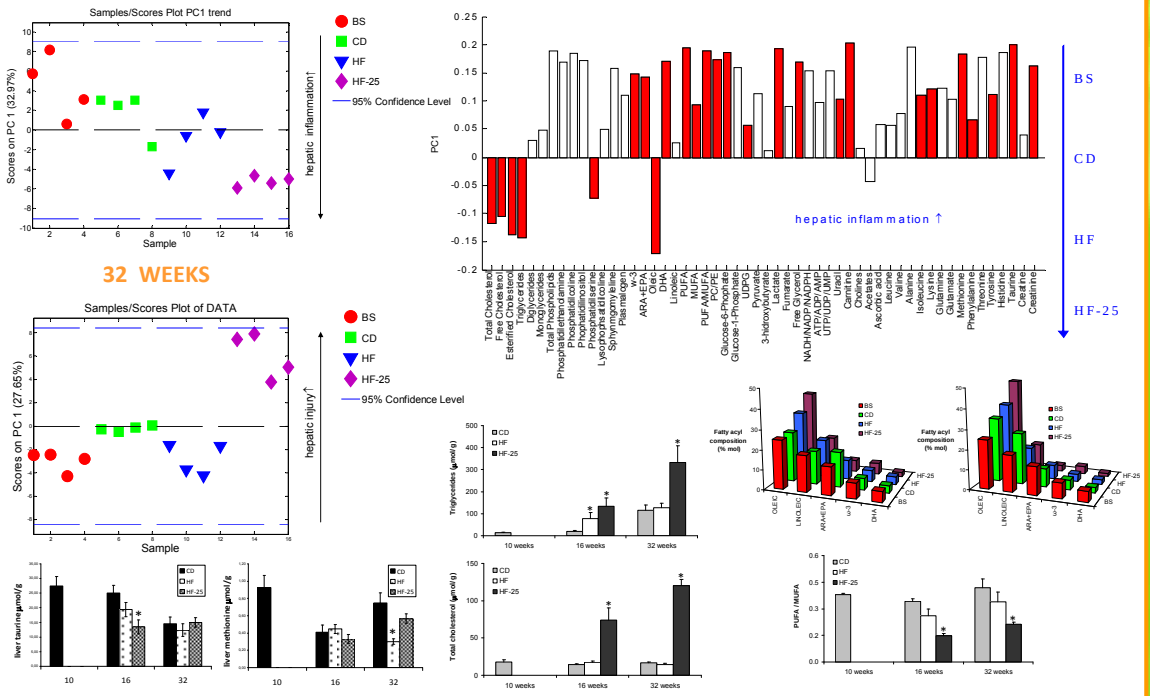
**Metabolites identification**



**Metabolites quantification**

$$C_{metabolite} = \frac{\frac{I_{metabolite}}{N_{metabolite}} \times C_{(TSP\ or\ TMS)}}{\frac{I_{(TSP\ or\ TMS)}}{N_{(TSP\ or\ TMS)}}} \times \frac{V_{extract}}{M_{tissue}}$$

## 5. RESULTS




## 6. CONCLUSIONS

- The full quantitave profiling approach we report allows for the quantification of up to 54 different metabolic markers.
- Evaluation of water-soluble and lipid-soluble extract metabolites is performed simultaneously in the same analysis.
- This metabolomic analysis proved to be fast, simple, reproducible and informative, revealing a suggestive relationship of the occurrence of SAMe depletion at the early stages of NASH development and a causal role of impairment of hepatic transsulfuration reactions.
- Our data suggests an intensive role of dietary cholesterol as a risk factor to the progression to hepatic inflammation in diet-induced NASH.

## REFERENCES

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