NMR BASED METABOLOMICS STUDY OF PTP1B DEFICIENCY INFLUENCE ON HEPATIC REGENERATION IN MICE

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1. INTRODUCTION

- Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of tyrosine kinase growth factor signaling.
- Levels of PTB1B may exert a pivotal role in maintaining the balance between survival and death in hepatocytes.[1]
- Recently, it has been proposed that PTP1B deficiency accelerates hepatic regeneration in mice.[2]

2. GOALS

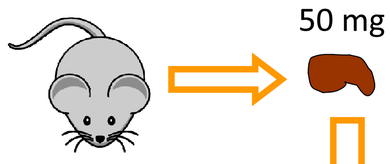
This work is aimed to analyze the differences between PTP1B$^{-/-}$ and WT mice in the early metabolic events produced upon partial hepatectomy (PH) and liver regeneration.

3. EXPERIMENTAL DESIGN

A NMR metabolic profiling of lipid and aqueous liver extracts obtained from PTP1B-/- and WT mice at different time points (24h and 36h) after PH.

Time	KO	Replicates
0 hours after PH	WT	n=4
	PTPB1$^{-/-}$	n=4
24 hours after PH	WT	n=5
	PTPB1$^{-/-}$	n=4
36 hours after PH	WT	n=4
	PTPB1$^{-/-}$	n=4

4. LIVER EXTRACTION



50 mg

1)Homogenize in 1mL CH3CN/H2O (1/1)
2)Centrifugue 5000g 15min 4ºC

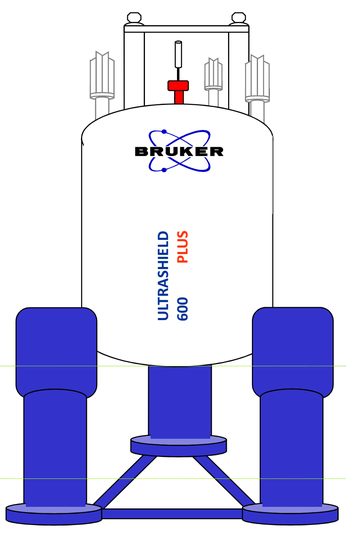
SUPRENATANT:
Aqueous Extract
Lyophilise
Reconstitute in D2O +TSP (0.01%)

PELLET:
Lipidic Extract
Reconstitute in CH3Cl:CH3OH (2:1)
N2 stream and reconstitute in CD3Cl:CD3OD (2:1) + TMS (0.01)

5. NMR ADQUISITION

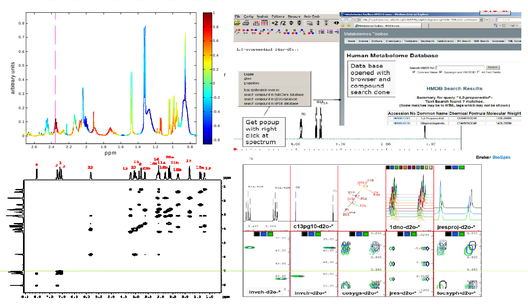
1H-NMR CONDITIONS

Bruker Avance DRX-600 operating at 600.20 MHz
T=283 K for lipidic extracts
T=300K for aqueous extracts.
Eretic signal calibration was introduced at 11 ppm and integrated with respect to 2 mmol sucrose sample (aquous) or 0.1% EB sample (lipidic).



5. DATA ANALYSIS

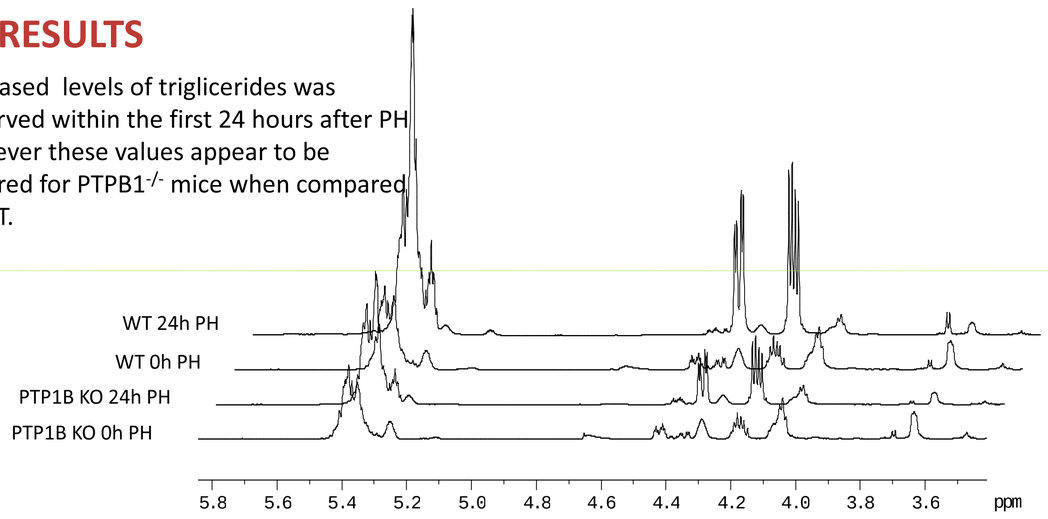
Metabolites ID were based on previous reported literature, BRUKER database, 2D-spectra and STOCSY

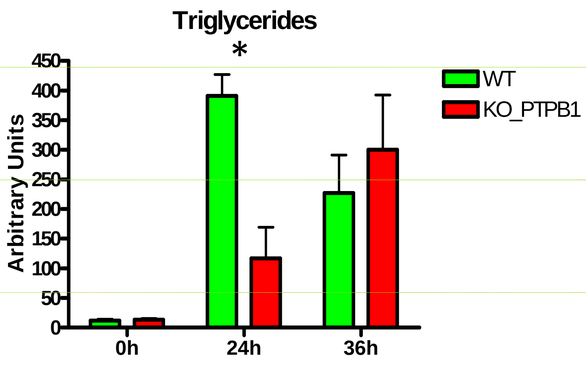


After metabolite identification, metabolite regions were integrated using in-house Matlab scripts. Selected regions were scaled to ERETIC signal and further on to mg of liver tissue.

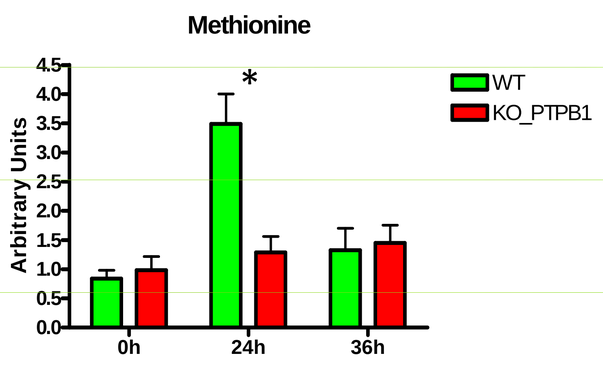
7. RESULTS

Increased levels of triglicerides was observed within the first 24 hours after PH However these values appear to be lowered for PTPB1$^{-/-}$ mice when compared to WT.

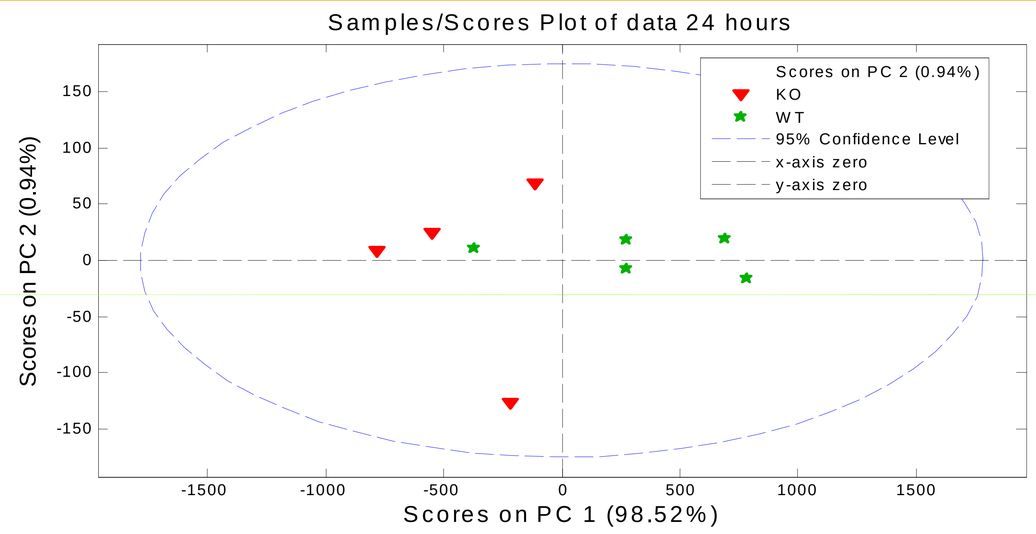


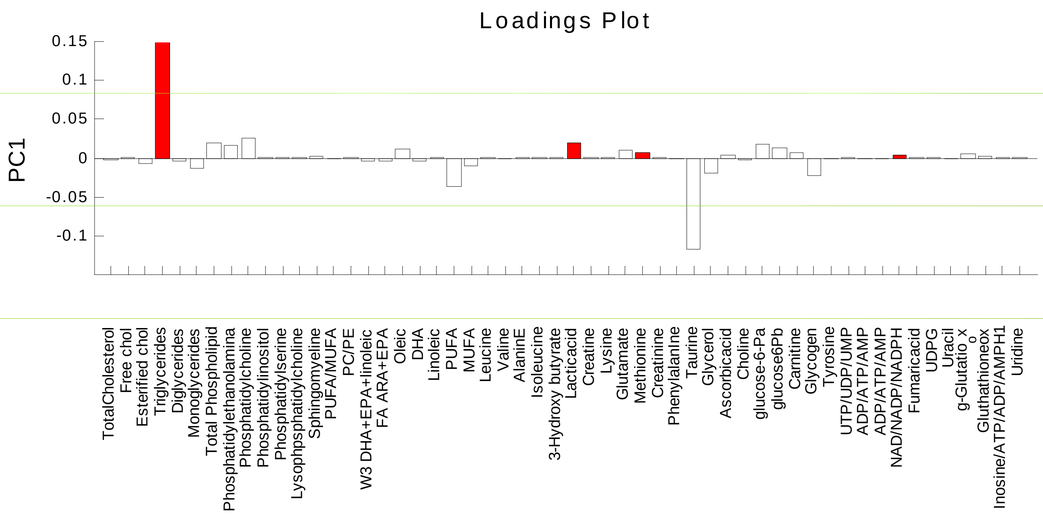


* p-value<0.05 (2-factor ANOVA)



* p-value<0.05 (2-factor ANOVA)





8. CONCLUSIONS

- Methionine levels in WT appears increased after 24 h, but no changes are observed in PTP1B$^{-/-}$. Lowered levels of methionine in PTP1B$^{-/-}$ mice might be indicative of lowered levels of SAM (S-adenosine methionine). A drop in SAM levels is required for the sensitization of liver cells to hepatocyte growth factor (HGF), a key mitogenic signaling molecule in the regeneration process.[3, 4] Such evidence supports the thesis that PTPB1 deficiency accelerates hepatic regeneration.
- After 24 hours PH WT showed rised levels of triglycerides, however PTP1B$^{-/-}$ showed triglycerides peaking after 36h.
- Taken together these findings demonstrated that a coordinated pattern of biochemical changes occur with and after hepatic regeneration.
- Further work is in progress with this dataset in order to gain insight in liver metabolism 36h after hepatectomy.

REFERENCES

1. González-Rodriguez, Á.; Escribano, Ó.; Alba, J.; Rondinone, C. M.; Benito, M.; Valverde, Á. M., Levels of Protein Tyrosine Phosphatase 1B Determine Susceptibility to Apoptosis in Serum-Deprived Hepatocytes. *Journal of Cellular Physiology* **2007,** *212*, 76–88.
2. Revuelta, J.; González, Á.; Miranda, M. S.; Valverde, Á. M. In *PTP1B deficiency accelerates hepatic regeneration in mice* CIBERDEM 2nd Annual Meeting, Arnes, 2009; Arnes, 2009.
3. Bollard, M. E.; Contel, N. R.; Ebbels, T. M. D.; Smith, L.; Beckonert, O.; Cantor, G. H.; Lehman-McKeeman, L.; Holmes, E. C.; Lindon, J. C.; Nicholson, J. K.; Keun, H. C., NMR-Based Metabolic Profiling Identifies Biomarkers of Liver Regeneration Following Partial Hepatectomy in the Rat. *Journal of Proteome Research* **2010,** *9*, 59-69.
4. L.Chen; Zeng, Y.; Yang, H.; Lee, T.; SWFrench; Corrales, F.; García-Trevijano, E.; Avila, M.; Mato, J.; Lu, S., Impaired liver regeneration in mice lacking methionine adenosyltransferase 1A. *FASEB J.* **2004,** *18*(7), 914-6.

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