PXL770, a Novel Direct AMP-activated Protein Kinase Activator, Improves Hepatic Mitochondrial Function in a Rodent NASH Model

Pierre-Axel Monternier, Pascale Gluais-Dagorn, David E. Moller and Sophie Hallakou-Bozec, Poxel, Lyon, France

BACKGROUND and OBJECTIVES

Non-Alcoholic SteatoHepatitis (NASH) is a metabolic disease characterized by several components of liver pathology such as steatosis, inflammation and fibrosis. Hepatic mitochondria play a critical role in the development and pathogenesis of steatosis and NASH. PXL770 is a direct AMP-activated protein kinase (AMPK) activator which successfully completed a Phase 2a clinical trial in NASH. AMPK, a master metabolic regulator, has been described to enhance mitochondrial health by regulating various aspects of mitochondrial homeostasis.

OBJECTIVES : evaluate PXL770 effects on mitochondria in a diet induced obese biopsy proven-NASH mouse model

METHODS

Mice were fed with High Fat, High Fructose diet for 34 weeks. Mice with biopsy-proven NASH were then treated for 8 weeks with PXL770 (75mg/kg, PO/BID). The NAFLD activity score was evaluated by histology in addition to liver steatosis markers. Mitochondrial function was investigated through protein expression and enzyme activities measured in samples of liver and brown adipose tissue (BAT).

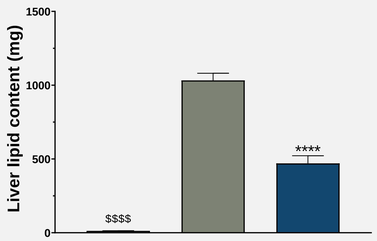
➢ **PXL 770 improved NAFLD score activity by decreasing liver steatosis, liver inflammation and ballooning**

PXL770 improved NASH hallmarks in the Liver while improving mitochondrial function

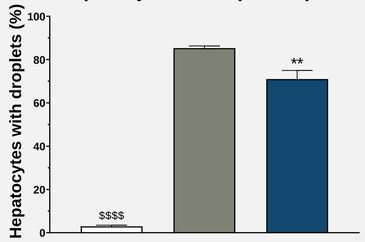
❑ **PXL 770 Increased: mitochondrial biogenesis (PGC-1α) Mitophagy (ULK-1) & fusion (Mitofusin-2)**
➔ **Improved Mitochondria Network and Structure**

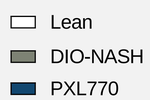
❑ **PXL 770 Improved Liver Steatosis**

Liver Lipid Content

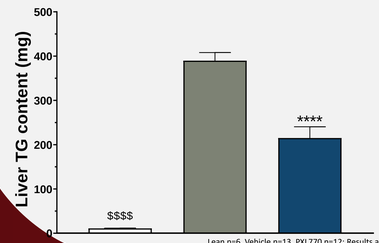


Hepatocytes with lipid droplets

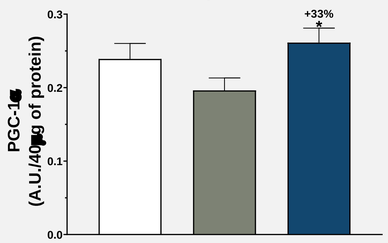




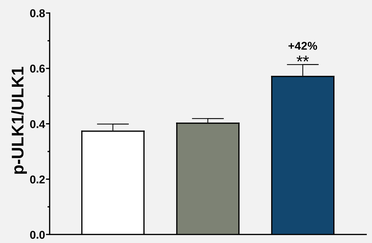
Liver Triglyceride Content



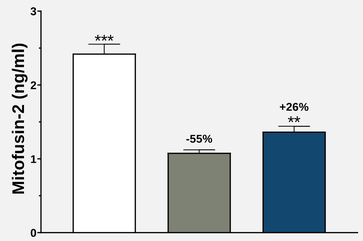
PGC-1α - protein levels



p-ULK1/ULK1 - protein levels

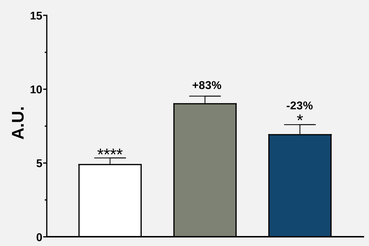


Mitofusin-2 - protein levels

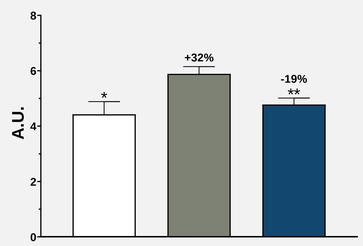


❑ **PXL 770 Decreased Mitochondrial Pro-Apoptotic Markers**
➔ **Decreased cell death**

Bak1 - mRNA levels



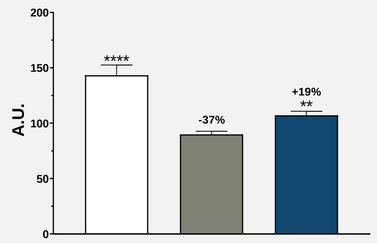
Bax - mRNA levels



Lean n=6, Vehicle n=13, PXL770 n=12; Results are mean ±SEM.
Model characterization: student t-test lean *vs* DIO with $$$$ *p*≤ 0.0001 PXL770 characterization: One Way ANOVA – Dunnett's multiple comparison test
*p<0,05, **P<0.01, ***P<0.001, ****p<0.0001 *vs* DIO

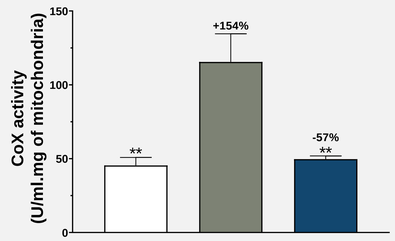
❑ **PXL 770 Increased Succinate DeHydrogenase**
➔ **Decreased Mitochondrial Oxidative Stress**

Succinate DH - mRNA levels



❑ **PXL 770 Restored Mitochondrial Oxidation**
➔ **Improved Bioenergetics**

Cytochrome c Oxidase activity

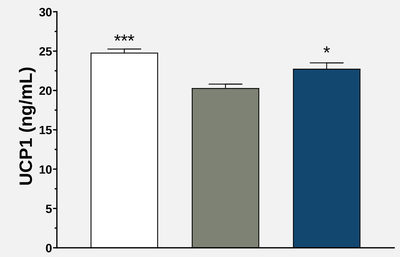


mRNA levels data, n=8 for all groups
Lean n=6, DIO n=13, PXL770 n=12
One-way ANOVA - Dunnett's multiple comparison test
*p<0,05, **P<0.01, ***P<0.001, ****p<0.0001 *vs* DIO-NASH

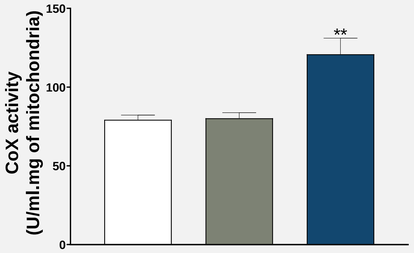
PXL770 benefits in Brown Adipose Tissue

❑ **PXL 770 increased mitochondrial uncoupling and oxidation**
➔ **Increased Metabolic Activity**

UCP-1 Protein Expression



Cytochrome c Oxidase activity



Lean n=6, DIO n=13, PXL770 n=12
One-way ANOVA - Dunnett's multiple comparison test
*p<0,05, **P<0.01, ***P<0.001, ****p<0.0001 *vs* DIO-NASH

➢ CONCLUSION

- PXL770 improved mitochondrial homeostasis in the Liver thus increasing the pool of functional mitochondria
- PXL770 increased mitochondrial activity in the BAT thus increasing substrate turnover, potentially lipid oxidation
- PXL770 overall effect on mitochondria may contribute to the beneficial effect seen on NASH hallmarks particularly on liver steatosis

Acknowledgments:
-Gubra – Danemark
-Metabrain Research - France