2273

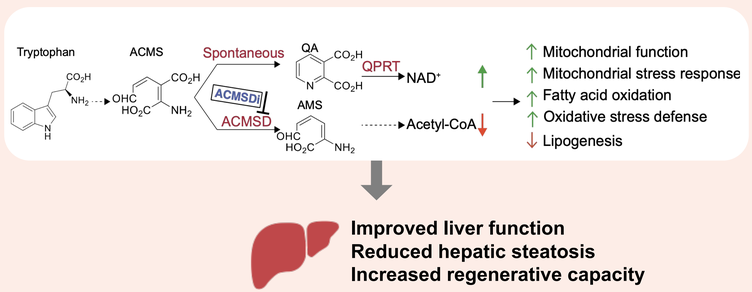
ACMSD inhibition enhances de novo NAD+ biosynthesis and reverses NAFLD/NASH

Yasmine J. Liu[1], Masaki Kimura[2], Xiaoxu Li[1], Sandra Rodriguez Lopez[1], Jonathan Sulc[1], Qi Wang[1], Angelique M.L. Scantlebery[4], Keno Strotjohann[1], Archana Vijayakumar[3], Robert P. Myers[3], Riekelt H. Houtkooper[4], Maroun Bou Sleiman[1], G. Mani Subramanian[3], Takanori Takebe[2], Johan Auwerx[1]

[1]Laboratory of Integrative and Systems Physiology, Interfaculty Institute of Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [3]OrsoBio, Inc., Menlo Park, CA, USA; [4] Laboratory Genetic Metabolic Diseases, Amsterdam UMC, Amsterdam, The Netherlands

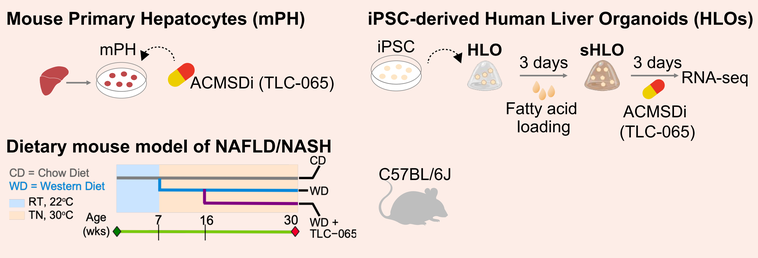
Introduction

- Nicotinamide adenine dinucleotide (NAD+) is critical to maintaining cellular redox, signaling (e.g., via sirtuins), and energy homeostasis
- Advanced liver disease (e.g., nonalcoholic steatohepatitis [NASH]) is characterized by decreased NAD+ levels and impaired mitochondrial function
- Approaches to replenish NAD+ levels hold promise for treatment of liver diseases



- α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) decarboxylase (ACMSD), an enzyme involved in de novo NAD+ biosynthesis from tryptophan, is highly expressed in liver and kidney
- The effects of de novo NAD+ modulation on liver inflammation and fibrosis are poorly understood[1]
- TLC-065 is a potent, small molecule inhibitor of ACMSD that increased NAD+, reduced oxidative stress, and improved mitochondrial function and metabolism in mouse primary hepatocytes (mPH) and in induced-pluripotent stem cell (iPSC)-derived human liver organoids (HLOs)[2-4]
- **Here, we further characterized the activity of TLC-065 in mPH, a dietary mouse model of NAFLD/NASH, and in steatotic HLOs**

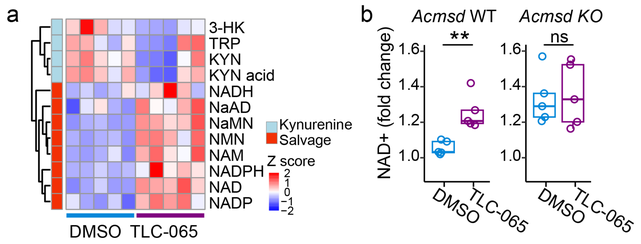
Methods



- mPH were isolated by liberase perfusion method, metabolites were measured by metabolomics, ROS by dichlorodihydrofluorescein diacetate (DCFDA), caspase 3/7 by Caspase-Glo assay, and gene expression by qPCR
- C57BL/6J mice fed a western diet (WD) or chow were housed at thermoneutrality (TN; 30°C). TLC-065 (25 mg/kg) was dosed in diet for 14 weeks
- HLOs were treated with 300 μM oleic acid for 3 days to generate steatotic HLOs (sHLOs)[3] and 10 μM TLC-065 for 3 additional days for treatment effects
- Statistical significance: 2-tailed unpaired t-test and one-way ANOVA (*p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001)

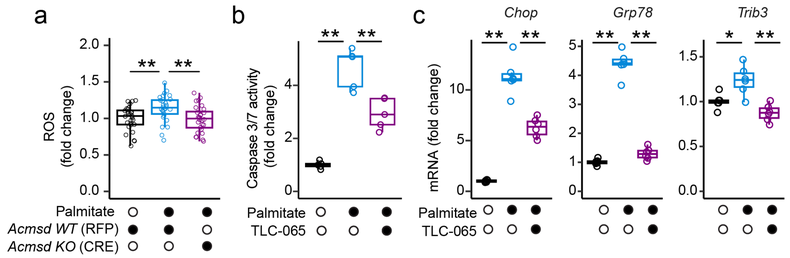
Results

ACMSD inhibition boosts de novo NAD+ synthesis in mPH



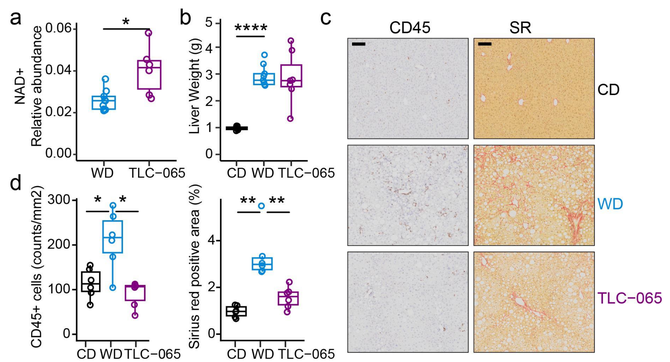
- TLC-065 (0.5 μM) enhanced the NAD+ metabolome in mPH
- TLC-065 (0.5 μM) increased NAD+ levels in wild-type (WT), but not ACMSD knock-out (KO) mPH

ACMSD inhibition improves oxidative stress defense in mPH



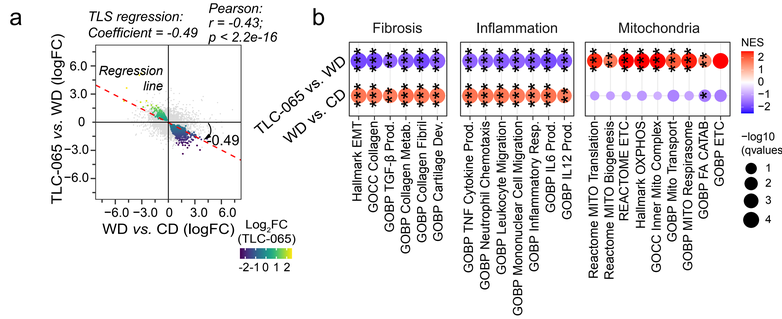
- In mPH, TLC-065 reduced ROS production, apoptosis, and ER stress induced by palmitate

ACMSD inhibition prevents NAFLD/NASH progression



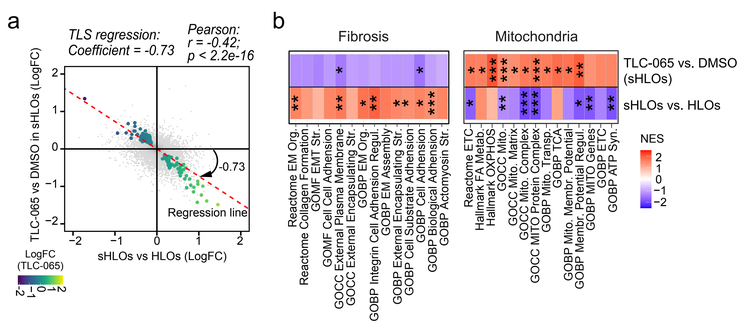
- TLC-065 increased hepatic NAD+ content, and reduced hepatic inflammation and fibrosis, but did not affect hepatic steatosis in WD-fed mice at TN

ACMSD inhibition reverses NAFLD/NASH-associated molecular dysregulation



- WD/TN upregulated fibrosis and inflammation-related genes and downregulated genes associated with mitochondrial function
- TLC-065 reversed hepatic transcriptomic changes induced by WD/TN

Effects of ACMSD inhibition are recapitulated in steatotic HLOs



- Fatty acid loading induced a steatohepatitis-like phenotype in HLOs including upregulation of fibrosis-related genes and downregulation of genes involved in mitochondrial functions
- In sHLOs, TLC-065 reversed gene signatures for fibrosis and mitochondrial dysfunction induced by steatosis

Conclusions

- ACMSD inhibition with TLC-065 improved the NAD+ metabolome and oxidative defense in mPH
- In NAFLD/NASH mice, ACMSD inhibition increased hepatic NAD+ and significantly reduced inflammation and fibrosis
- Gene signatures for reduced fibrosis and enhanced mitochondrial function observed with ACMSD inhibition in NAFLD/NASH mice were recapitulated in iPSC-derived sHLOs
- Our data support the development of novel ACMSD inhibitors, such as TLC-065, to treat liver disease due to NASH and other etiologies

References: 1. Katsyuba E. Nature 2018; 2. Y.J. Liu, et al. AASLD 2022 (Abstract #2441); 3. Ouchi R. Cell Metabolism 2019; 4. Kimura M. Cell 2022. **Disclosures:** YJ Liu, M Kimura: nothing to disclose; A Vijayakumar, E Murakami, T Takebe, RP Myers, GM Subramanian: OrsoBio; J Auwerx: OrsoBio, MitoBridge/Astellas, MetroBiotech, Amazentis, NOV Metapharma. **Acknowledgments:** We thank Auwerx lab members for technical assistance and discussion. Work by the Auwerx team is supported by grants from EPFL, European Research Council (ERC-AdG-787702), Swiss National Science Foundation (SNSF 31003A_179435), and OrsoBio, Inc. Work by the Takebe team is supported by grants from NIH (DP2 DK128799, UH3 DK119982). YL is supported by a postdoctoral fellowship from EMBO (ALTF 1161-2021).

Corresponding authors: Yasmine Liu (yasmine.liu@epfl.ch); Archana Vijayakumar (archana@orsobio.com)

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