Research Article

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# Inhibiting poly ADP-ribosylation increases fatty acid oxidation and protects against fatty liver disease

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**Background & Aims**: To date, no pharmacological therapy has been approved for non-alcoholic fatty liver disease (NAFLD). The aim of the present study was to evaluate the therapeutic potential of poly ADP-ribose polymerase (PARP) inhibitors in mouse models of NAFLD.

**Methods**: As poly ADP-ribosylation (PARylation) of proteins by PARPs consumes nicotinamide adenine dinucleotide ($NAD^+$), we hypothesized that overactivation of PARPs drives $NAD^+$ depletion in NAFLD. Therefore, we assessed the effectiveness of PARP inhibition to replenish $NAD^+$ and activate $NAD^+$-dependent sirtuins, hence improving hepatic fatty acid oxidation. To do this, we examined the preventive and therapeutic benefits of the PARP inhibitor (PARPi), olaparib, in different models of NAFLD.

**Results**: The induction of NAFLD in C57BL/6J mice using a high-fat high-sucrose (HFHS)-diet increased PARylation of proteins by PARPs. As such, increased PARylation was associated with reduced $NAD^+$ levels and mitochondrial function and content, which was concurrent with elevated hepatic lipid content. HFHS diet supplemented with PARPi reversed NAFLD through repletion of $NAD^+$, increasing mitochondrial biogenesis and β-oxidation in liver. Furthermore, PARPi reduced reactive oxygen species, endoplasmic reticulum stress and fibrosis. The benefits of PARPi treatment were confirmed in mice fed with a methionine- and choline-deficient diet and in mice with lipopolysaccharide-induced hepatitis; PARP activation was attenuated and the development of hepatic injury was delayed in both models. Using $Sirt1^{hep-/-}$ mice, the beneficial effects of a PARPi-supplemented HFHS diet were found to be Sirt1-dependent.

**Conclusions**: Our study provides a novel and practical pharmacological approach for treating NAFLD, fueling optimism for potential clinical studies.

**Lay summary**: Non-alcoholic fatty liver disease (NAFLD) is now considered to be the most common liver disease in the Western world and has no approved pharmacological therapy. PARP inhibitors given as a treatment in two different mouse models of NAFLD confer a protection against its development. PARP inhibitors may therefore represent a novel and practical pharmacological approach for treating NAFLD.



## Introduction

Non-alcoholic fatty liver disease (NAFLD) is now considered to be the most common liver disease in the Western world [1]. NAFLD is quickly becoming an important public health concern due to the rising incidence of obesity in both children and adults [2]. The disease spectrum for NAFLD ranges from simple fatty liver (steatosis), to non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma, and finally it may result in liver-related death [3,4]. Moreover, as the prevalence of NAFLD escalates, NASH-related cirrhosis and hepatocellular carcinoma will increasingly become a major health care problem and a primary indication for liver transplantation [5,6]. Currently, the principal treatment for NAFLD/NASH is lifestyle modification by diet and exercise [7,8]. However, pharmacological therapy is indispensable as obese patients with NAFLD often have difficulty in maintaining improved lifestyles. In this context, farnesoid X receptor or peroxisome proliferator-activated receptor-α activators are currently being tested in clinical trials, but they are not yet approved [9].

Keywords: Non-alcoholic fatty liver disease; Poly ADP-ribosylation; Sirtuin; PARP inhibitor; PARylation; NAD.

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In the early stages of NAFLD, liver-specific metabolic adaptations lead to increased liver fat load from the periphery [10], which may promote mitochondrial dysfunction upon chronic fat accumulation [11]. Studies performed in humans and rodents have demonstrated that enzymatic activities involved in oxidative phosphorylation (OXPHOS) are reduced in NAFLD [10,12,13]. The major consequence of reduced OXPHOS activity is a reduction in ATP generation [12]. Another aspect associated with mitochondrial dysfunction in NAFLD is an increase in reactive oxygen species (ROS) production. This phenomenon is often associated with elevated expression of several cytokines, promoting an inflammatory state, which has been shown to be deleterious in NAFLD [14,15]. ROS has also been reported to impair OXPHOS, thereby inhibiting β-oxidation and perpetuating the continued progression of NAFLD [16].

Several recent studies indicate the potential involvement of poly ADP-ribose polymerase (PARP) activity in promoting metabolic dysfunction [17–20]. Interestingly, various metabolic disorders have been associated with elevated oxidative stress and DNA damage, which can subsequently induce PARP activity [21]. The hyperactivation of PARPs can initiate the programmed cell death pathway causing both ATP and nicotinamide adenine dinucleotide ($NAD^+$) depletion [22], which reduces mitochondrial function and β-oxidation of fats [18,19,21,23]. Interestingly, this correlation was also found in the liver, as demonstrated by the negative correlation between *PARP1* and β-oxidation gene transcripts in normal human liver datasets [11].

Being an important substrate for PARP activity, $NAD^+$ is also a critical component for various metabolic reactions in cells [24]. For example, the sirtuin protein, Sirt1, regulates many metabolic pathways, in adaptation to nutritional status. When Sirt1 is active, during a state of energy deficit, it uses $NAD^+$ as a co-substrate for the deacetylation of proteins [25]. As $NAD^+$ is rate-limiting for Sirt1, the consumption of $NAD^+$ by PARPs can lead to reduced sirtuin activity [19,24,26]. We have previously shown that $NAD^+$ levels progressively decline with the development of NAFLD and postulated that this may be due to enhanced PARP activity and enhanced competition with Sirt1 for available $NAD^+$ [11]. Given the lower $K_m$ for $NAD^+$ by PARP1 compared to Sirt1 this is a very likely scenario [24]. Based on these observations, the inhibition of PARP activity may represent a promising approach to counteract the development of NAFLD by increasing $NAD^+$ levels and activating Sirt1-directed mitochondrial biogenesis and β-oxidation of fatty acids.

In this study, we demonstrate that a long-term high-fat high-sucrose (HFHS) diet can induce hepatic PARylation, via increased PARP activity, having a deleterious effect on $NAD^+$/Sirtuin-directed metabolism, resulting in the development of NAFLD. By treating mice with a PARP inhibitor (PARPi) in a preventive and therapeutic manner, we were able to arrest or reverse the development of NAFLD. Moreover, using a liver-specific *Sirt1* knockout mouse line ($Sirt1^{hep-/-}$), we demonstrated that the PARPi-mediated protection against NAFLD development was dependent on hepatic Sirt1 expression. This protective effect of PARP inhibition against NAFLD was also confirmed using a methionine- and choline-deficient (MCD) diet, as another mouse model of NAFLD. Finally, we showed the benefit of PARP inhibition against lipopolysaccharide (LPS)-induced acute hepatitis in mice.

## Materials and methods

### *Animal experiments*

All animal experiments were performed according to Swiss, South Korean and EU ethical guidelines and approved by the local animal experimentation committee of the Canton de Vaud under license #2868 and the Committee on the Ethics of Animal Experiments of Chungnam National University Graduate School of Medicine (CNUH-015-A0001). Detailed experimental protocols are provided in the Supplementary Materials and Methods.

### *Generation of $Sirt1^{hep-/-}$ mice*

Generation of $Sirt1^{L2/L2}$ mice has been previously described [27]. Liver-specific *Sirt1* knockout mice were generated by breeding $Sirt1^{L2/L2}$ mice with mouse albumin (Alb)-Cre mice ($Albcre^{Tg/0}$) [28], both of which have been backcrossed to C57BL/6J mice for 10 generations. These mice lines were then further intercrossed to generate mutant $Albcre^{Tg/0}/Sirt1^{L2/L2}$ mice, which were termed $Sirt1^{hep-/-}$ mice. $Sirt1^{hep-/-}$ and $Sirt1^{L2/L2}$ mice were fed with HFHS pellets containing vehicle (DMSO) or olaparib (PARP inhibitor, GP0126, Glentham Life Sciences, 50 mg/kg/day) for 18 weeks.

### *Histology and liver function*

Preparation of histological tissue sections, staining procedures for hematoxylin and eosin (H&E), Oil Red O, Sirius Red, cytochrome c oxidase activity, succinate dehydrogenase activity and CD45 are described in the Supplementary Materials and Methods. Mitochondrial function in fresh liver tissue was evaluated with Western blotting, high-resolution respirometry [29], and BN-PAGE analysis [30], as described in the Supplementary Materials and Methods. Evaluation of global PARylation in fresh liver tissue was performed as previously described [19].

### *Quantification of $NAD^+$ and adenosine triphosphate levels*

$NAD^+$ was isolated using acidic then alkaline extraction methods. Tissue $NAD^+$ was analysed with mass spectrometry, as previously described [11,31]. Total adenosine triphosphate (ATP) content was measured by the CellTiter-Glo luminescent cell viability assays (Promega). Typically, luminescence was recorded with a Victor X4 plate reader (PerkinElmer, Waltham, MA), and values were normalized by the total protein concentration, determined using a Bradford assay.

### *Liver triglyceride, cholesterol, and lipid peroxidation measurements*

Hepatic lipids were extracted as described previously [32]. Triglyceride (TG) and cholesterol contents in hepatic lipid fractions were quantified using enzymatic assays (Roche). The by-product of lipid peroxidation (LPO) and a marker of oxidative stress, 4-hydroxynonenal (HNE), was measured following the manufacturer's protocol of the OxiSelect HNE-His Adduct enzyme-linked immunosorbent assay Kit (Cell Biolabs Inc., San Diego, CA).

### *Bioinformatic analyses*

All raw transcriptomic data are publicly available on Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) under the accession numbers GSE48452, GSE24031, GSE28619 and GSE50579 or on GeneNetwork (www.genenetwork.org). Heatmaps were built using GENE-E (The Broad institute, www.broadinstitute.org/cancer/software/GENE-E/) Principal component analysis, corrgram and correlation network were accomplished using functions and packages in R (www.r-project.org). The depth of the shading at the correlation matrices (corrgram) indicates the magnitude of the correlation (Pearson's r). Positive and negative correlations within the corrgram and correlation network are represented in blue and red, respectively. Only correlations with a $p < 1 \times 10^{-5}$ are displayed in the correlation network.

### *Statistical analysis*

Statistical analysis was performed with Prism software (version 6.0; GraphPad Software Inc.). The significance of differences between two groups was determined by unpaired two-tailed Student *t* test. For comparison of multiple groups, we applied a one-way analysis of variance (ANOVA) with a post-hoc Bonferroni