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BASIC AND TRANSLATIONAL—LIVER

Hepatic SIRT1 Attenuates Hepatic Steatosis and Controls Energy Balance in Mice by Inducing Fibroblast Growth Factor 21

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BACKGROUND & AIMS: The hepatocyte-derived hormone fibroblast growth factor 21 (FGF21) is a hormone-like regulator of metabolism. The nicotinamide adenine dinucleotide–dependent deacetylase SIRT1 regulates fatty acid metabolism through multiple nutrient sensors. Hepatic overexpression of SIRT1 reduces steatosis and glucose intolerance in obese mice. We investigated mechanisms by which SIRT1 controls hepatic steatosis in mice. **METHODS:** Liver-specific SIRT1 knockout (SIRT1 LKO) mice and their wild-type littermates (controls) were divided into groups that were placed on a normal chow diet, fasted for 24 hours, or fasted for 24 hours and then fed for 6 hours. Liver tissues were collected and analyzed by histologic examination, gene expression profiling, and real-time polymerase chain reaction assays. Human HepG2 cells were incubated with pharmacologic activators of SIRT1 (resveratrol or SRT1720) and mitochondrion oxidation consumption rate and immunoblot analyses were performed. FGF21 was overexpressed in SIRT1 LKO mice using an adenoviral vector. Energy expenditure was assessed by indirect calorimetry. **RESULTS:** Prolonged fasting induced lipid deposition in livers of control mice, but severe hepatic steatosis in SIRT1 LKO mice. Gene expression analysis showed that fasting upregulated FGF21 in livers of control mice but not in SIRT1 LKO mice. Decreased hepatic and circulating levels of FGF21 in fasted SIRT1 LKO mice were associated with reduced hepatic expression of genes involved in fatty acid oxidation and ketogenesis, and increased expression of genes that control lipogenesis, compared with fasted control mice. Resveratrol or SRT1720 each increased the transcriptional activity of the *FGF21* promoter (−2070/+117) and levels of FGF21 messenger RNA and protein in HepG2 cells. Surprisingly, SIRT1 LKO mice developed late-onset obesity with impaired whole-body energy expenditure. Hepatic overexpression of FGF21 in SIRT1 LKO mice increased the expression of genes that regulate fatty acid oxidation, decreased fasting-induced steatosis, reduced obesity, increased energy expenditure, and promoted browning of white adipose tissue. **CONCLUSIONS:** SIRT1-mediated activation of FGF21 prevents liver steatosis caused by fasting. This hepatocyte-derived endocrine signaling appears to regulate expression of genes that control a brown fat-like program in white adipose tissue, energy expenditure, and adiposity. Strategies to activate SIRT1 or FGF21 could be used to treat fatty liver disease and obesity.

Keywords: Liver-Specific Disruption of *Sirt1*; Hepatocyte-Derived Hormone; Metabolic Homeostasis; Obesity.

Deregulation of lipid metabolic homeostasis is a common characteristic of metabolic disorders such as fatty liver disease, obesity, and diabetes. The liver functions as a major metabolic buffering system for metabolic homeostasis, allowing extrahepatic tissues such as the brain and heart to function normally under nutrient stress and deprivation.[1] An important element in the transcriptional response to nutrient deprivation is the nicotinamide adenine dinucleotide (NAD)-dependent deacetylase SIRT1, which tightly regulates fatty acid metabolism through multiple nutrient sensors such as AMP-activated protein kinase (AMPK), sterol regulatory element binding protein-1 (SREBP-1), and peroxisome proliferator-activated receptor-gamma coactivator 1 α (PGC-1α).[2–4] Polyphenolic SIRT1 activators, including resveratrol and the synthetic polyphenol S17834, prevent hepatic steatosis and hyperlipidemia in mice with type 1 and type 2 diabetes.[5,6] Hepatic overexpression of SIRT1 ameliorates hepatic steatosis and glucose intolerance in obese mice.[7] However, the underlying mechanism of hepatic SIRT1 actions remains incompletely understood. In our effort to identify a novel downstream regulator of SIRT1, gene expression chip assays show fibroblast growth factor 21 (FGF21), the hepatocyte-derived

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Abbreviations used in this paper: **Ad, adenovirus; BAT, brown adipose tissue; CPT1α, carnitine palmitoyltransferase 1α; FAS, fatty acid synthase; FGF21, fibroblast growth factor 21; MCAD, medium-chain acyl-CoA dehydrogenase; MEF, mouse embryonic fibroblast; mRNA, messenger RNA; NAD, nicotinamide adenine dinucleotide; PPARα, peroxisome proliferator activated receptorα; SIRT1, NAD-dependent protein deacetylase sirtuin-1; SIRT1 LKO mice, liver-specific SIRT1 knockout mice; SREBP-1, sterol regulatory element binding protein-1; Vco_2, carbon dioxide production; Vo_2, oxygen consumption; WAT, white adipose tissue; WT, wild-type.**

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hormone, as the most markedly down-regulated gene in liver-specific SIRT1 knockout (SIRT1 LKO) mice.

FGF21, a fasting-induced hepatokine, rapidly is gaining interest as a metabolic regulator,[8,9] although the mechanism of the nutrient regulation of FGF21 is unclear. FGF21 is expressed predominantly in the liver, adipose tissue, and pancreas, with most circulating FGF21 originating from the liver.[10] The autocrine/paracrine and endocrine actions of FGF21 hormone are mediated through FGF receptors complexed with β-klotho.[11] Both pharmacologic administration of FGF21 and transgenic overexpression of FGF21 in mice protect against body weight gain and metabolic dysfunction in diabetic rodents, monkeys, and human beings.[8,12–16] Previous studies have reported that SIRT1 regulates FGF21 expression in hepatocytes in vitro and in vivo.[17,18] However, the relative contribution of FGF21 to SIRT1's effects on overall energy metabolism has not been investigated. The present study characterizes FGF21 as a critical downstream regulator of SIRT1 that protects against hepatic steatosis, enhances expression of brown fat-like genes in white adipose tissue, and increases whole-body energy expenditure. Our in vivo and in vitro studies illustrate the following: (1) hepatic SIRT1 is required for fasting-induced production and secretion of FGF21 in the liver; (2) defective FGF21 caused by hepatic SIRT1 ablation exacerbates fasting-induced hepatic steatosis by impairing fatty acid oxidation and increasing lipogenesis; (3) FGF21 is essential for SIRT1 to stimulate hepatocyte fatty acid oxidation; and (4) hepatic overexpression of FGF21 enhances systemic energy expenditure and ameliorates obesity.

Materials and Methods

Animals

Hepatocyte-specific deletion of the *SIRT1* gene in mice (SIRT1 LKO) was achieved by crossing albumin-Cre recombinase transgenic mice with floxed SIRT1$^{\Delta ex4}$ mice containing the deleted SIRT1 exon 4, which encodes 51 amino acids of the conserved SIRT1 catalytic domain, as described previously.[19] The protocol for this study was approved by the Boston University Medical Center Institutional Animal Care and Use Committee.

Animal Fasting and Refeeding Experiments

SIRT1 LKO mice and their WT littermates were divided into 3 groups: fed, fasted, and re-fed. The fed group was placed on a normal chow diet, the fasted group was fasted for 24 hours, and the re-fed group was fasted for 24 hours and then fed for 6 hours.

In Vivo Adenoviral Gene Transfer

The adenovirus producing full-length FGF21 was generated and purified as described previously.[6,20] Adenovirus-mediated overexpression of FGF21 in vivo was accomplished via tail vein injection as described previously.[2,7,20]

Statistical Analysis

Values are expressed as the mean ± SEM. Statistical significance was evaluated using an unpaired 2-tailed *t* test or a 1-way analysis of variance (ANOVA) for more than 2 groups. Differences were considered significant at a *P* level of less than .05.

Results

Hepatocyte-Specific Deletion of SIRT1 in Mice Increases the Susceptibility to Fasting-Induced Fatty Liver

To explore whether liver-tissue–specific SIRT1 is a driving force in maintaining lipid homeostasis, metabolic phenotypes of hepatic SIRT1 LKO mice were characterized. As shown in Figure 1, blood glucose and plasma insulin levels appeared comparable between wild-type (WT) and SIRT1 LKO mice in both fed and fasted states. In the fed state, plasma cholesterol and triglyceride levels were slightly higher in SIRT1 LKO mice compared with those in the WT littermates. In the fasted state, plasma lipids were similar between WT and SIRT1 LKO mice. In liver, prolonged fasting induced lipid deposition in WT mice, as shown by Oil Red O staining and direct triglyceride measurements. Strikingly, pronounced hepatic steatosis was observed in fasted SIRT1 LKO mice, as evidenced by increased stained areas and increased hepatic triglyceride content. Hepatic cholesterol content was comparable among groups. These data indicate that hepatic SIRT1 may play a critical role in regulating lipid metabolism under fasting conditions.

Loss of SIRT1 in the Liver Suppresses Fasting-Induced Gene Expression, Production, and Secretion of Hepatic FGF21

As part of a directed screen to identify a novel secreted protein that could be modulated by genetic manipulation of hepatic SIRT1, gene expression chip analysis showed that FGF21 was reduced by 83% in livers of SIRT1 LKO mice compared with those of WT mice under feeding conditions. Prolonged fasting resulted in an 11.7-fold induction of FGF21 in livers of WT mice. Hepatic SIRT1 deletion ablated approximately 60% of the FGF21 induction under fasting conditions (Supplementary Table 2). Peroxisome proliferator activated receptor (PPAR)α gene expression was increased approximately 2-fold in WT mice in response to fasting and decreased 40% in SIRT1 LKO mice. However, gene expression profiles of proinflammatory mediators, cytokines, growth factors, oxidative stress, and redox modulators appeared comparable between both genotypes of mice under nutrient stress conditions (Supplementary Table 2).

We next determined whether hepatic SIRT1 deficiency might interfere with fasting-induced production and release of hepatic FGF21. As shown in Figure 2, protein expression of hepatic SIRT1 was induced by nutrient deprivation and repressed by nutrient availability. Similar to the gene chip data (Supplementary Table 2), messenger RNA (mRNA) levels of FGF21 were increased robustly 15-fold upon fasting and decreased nearly 50% upon refeeding as observed previously.[9] Consistently, FGF21 protein production was increased 5-fold in WT mice after a 24-hour fast, and this induction was decreased approximately 40% in SIRT1 LKO