Fenofibrate, a Peroxisome Proliferator-Activated Receptor α Agonist, Improves Hepatic Microcirculatory Patency and Oxygen Availability in a High-Fat-Diet-Induced Fatty Liver in Mice

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Abstract Nonalcoholic fatty liver disease (NAFLD) is a common disease of chronic liver diseases. Peroxisome proliferator-activated receptor α (PPARα) has been implicated to play important roles in the development of the disease. Beyond its effects on lipid metabolisms, PPARα activation in the vascular system has emerged as an attractive therapeutic potential for NAFLD, although its actions in the microcirculatory system are not fully understood. In this study, we investigated the effects of fenofibrate, a PPARα synthetic agonist, on hepatic microcirculation in a high-fat diet (HFD)-induced fatty liver in mice. In vivo imaging analysis revealed the adverse effects of HFD on hepatic vasculature with narrowing of hepatic sinusoids and hepatic microcirculatory perfusion. Oxygen tension was significantly decreased in portal venules, while NADH autofluorescence in hepatocytes was greatly elevated. Fenofibrate treatment remarkably improved microvascular patency, tissue oxygenation and redox states in the affected liver. These results suggest beneficial roles of PPARα activated by fenofibrate on the regulation of both lipid metabolisms and microvascular environments of oxygen metabolism in HFD-induced fatty liver.

1 Introduction

Nonalcoholic fatty liver disease (NAFLD) represents a large spectrum of chronic liver disease ranging from simple hepatic steatosis to nonalcoholic steatohepatitis and cirrhosis. NAFLD is strongly associated with obesity,

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type II diabetes and hypertriglyceridemia [5]. Insulin resistance appears to be a primary factor for the development of NAFLD, as it is associated with increased lipolysis and reduced utilization of fatty acids in adipose tissues, leading to enhanced influx of fatty acids to the liver and accumulation of fatty acids as triglyceride (TG) in hepatocytes. These metabolic alterations evoke not only functional defects in the affected hepatocytes, but also distortion of hepatic microcirculation with a marked reduction in sinusoidal spaces and a decrease in the number of functional sinusoids. Such microvascular changes would limit oxygen supply for hepatocytes to oxidize fatty acids, accelerating progression to severer fatty liver diseases.

Peroxisome proliferator-activated receptor α (PPARα) is activated by binding with ligands including fibrate drugs [3]. A growing body of evidence suggests that PPARα is implicated in the pathogenesis and treatment of NAFLD [5]. A high-fat diet (HFD) feeding in mice has been reported to enhance deposit of TG in the liver in conjunction with reduced activity of PPARα. Loss of PPARα in mice has shown to cause severer hepatic steatosis in response to a high-fat diet feeding [2]. Thus, PPARα plays critical roles in the prevention of fat accumulation in the liver by stimulating fatty acid oxidation, for which microvascular blood supply should be ensured to meet the oxygen demands of hepatocytes. However, little is known about the roles of PPARα in the regulation of hepatic microcirculation and tissue oxygenation. To determine the effects of fenofibrate on hepatic microcirculation and tissue oxygenation in NAFLD, we investigated changes in sizes of sinusoids, blood oxygen tension and hepatic NADH autofluorescence in HFD-induced fatty liver in mice. Our present study demonstrated that treatment with fenofibrate remarkably improved microvascular patency, tissue oxygenation and redox states in the liver of HFD-fed mice. These results suggest beneficial roles of PPARα activated by fenofibrate on the regulation of both lipid metabolisms and microvascular environments of oxygen metabolism in the HFD-induced fatty liver.

2 Materials and Methods

Male C57BL/6 mice (5-week-old, Japan CLEA, Tokyo, Japan) were fed a control chow (CE-2, Japan CLEA) or a high-fat diet (HFD, D12327; 20% protein, 40% carbohydrate, and 40% fat, Research Diets Inc., New Brunswick, NJ) for 8 weeks. One experimental group treated with HFD was administered orally fenofibrate (Laboratoires Fournier S.A., Dijon, France) once a day at the dose of 30 mg/kg body weight/day for the last 3 weeks. As controls, mice fed control or HFD chow received 0.5% of carboxymethylcellulose (10 ml/kg body weight/day, Wako Pure Chemical Industries, Japan). Plasma and hepatic TG obtained from over-night fasted mice at the end of experiments were determined by automatic analyzer (TBA-120FR, Toshiba Medical Systems, Inc., Tokyo). For histochemical analysis, frozen sections with 10 μm-thickness from mice at

the end of experiments were stained with hematoxylin and eosin (HE, Wako Pure Chemical Industries). For intravital fluorescence microscopical analysis, surface of left lateral liver lobe was observed with an inverted intravital microscopy (Eclipse TE2000-U, Nikon Inc, Tokyo, Japan) assisted by a charge coupled device camera (JK-TU52H, Toshiba Inc, Tokyo, Japan). After a trans-illuminational image of the liver was captured, size of the sinusoids was measured at 10 points per sinusoid of five randomly selected sinusoids which were located at regular intervals between portal and central venule. After eliminating intrinsic vitamin A autofluorescence in fat-storing Ito cells, NADH autofluorescence was recorded in five randomly selected lobuli at 30 s after re-exposing to epi-illumination and was densitometrically assessed by computer-assisted gray level determination (Win ROOF Version 5.5, Tech Jam, Inc, Osaka, Japan). Oxygen tension in portal and central venule was measured by injecting Pd-meso-tetra-(4-carboxyphenyl)-porphyrin (Pd-TCPP, Porphyrin Products Inc., Logan, UT, 30 mg/kg body weight) intravenously, as described in detail previously [8]. All experiments were approved by the Animal Care and Utilization Committee of Keio University School of Medicine. Statistical analyses were carried out by Student's *t*-test for all experiments. P values less than 0.05 were considered significant.

3 Results

HFD feeding displayed a significant elevation of TG in plasma compared to control chow (67.7 ± 13.2 vs. 34.9 ± 2.6 mg/dl). In mice treated with both HFD and fenofibrate, these values were reduced notably to the levels observed in mice fed control chow (26.1 ± 3.9 mg/dl). Histochemical analysis revealed that HFD feeding elicited accumulation of various sizes of fat droplets predominantly around portal venules, whereas treatment with fenofibrate greatly prevented these fat infiltrations (Fig. 1). Consistent with these findings, the amounts of hepatic TG were significantly higher in HFD-fed mice than those in the control chow-fed group (338.7 ± 37.1 vs. 266.0 ± 43.0 μg/mg protein),

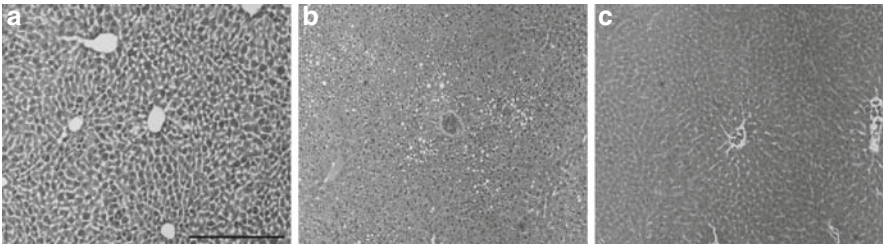

Fig. 1 Fenofibrate treatment reduces fat deposition in mice fed HFD. Shown are representative HE stained sections collected from mice fed control chow (**a**), HFD (**b**) or HFD plus fenofibrate (**c**). Bar, 300 μm

whereas this induction was completely abolished by fenofibrate treatment (178.0 ± 22.7 μg/mg protein).

To determine the impacts of HFD feeding on hepatic microcirculation, in vivo microscopic analysis was performed. In response to a HFD feeding, hepatic sinusoidal perfusion was notably reduced with blood flow being sluggish in some sinusoids, presumably by hepatocytes swollen with accumulated lipids, which narrow the lumen of sinusoids and damage sinusoidal linings. The mean sizes of hepatic sinusoids significantly decreased by 33% in HFD-fed mice compared to control chow-fed mice (Fig. 2a). These morphological alterations were completely abolished by treatment with fenofibrate. Oxygen tension in portal venule was notably lower in HFD-fed mice than that in control mice, whereas fenofibrate treatment partially but significantly restored microvascular oxygen levels (Fig. 2b). To further determine whether fenofibrate improves tissue oxygenation in the affected liver, we measured NADH autofluorescence intensity upon UV epi-illumination. A substantial increase in NADH fluorescence intensity in hepatocytes around both portal and central venules was observed upon HFD exposure (Fig. 2c). Fenofibrate completely abolished these alterations and further decreased the fluorescence intensity below the values of control chow-fed mice.

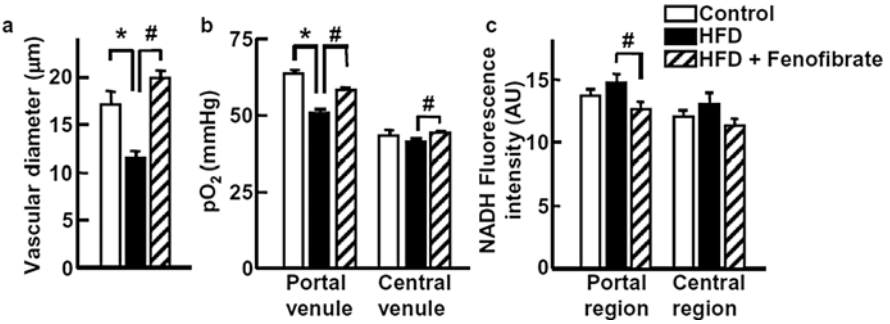

Fig. 2 Fenofibrate treatment improves hepatic microcirculation and oxygenation in mice fed HFD. Changes in size of sinusoids (**a**), oxygen tension (**b**) and NADH fluorescence (**c**) were investigated by using intravital microscopy. Data are expressed as mean ± S.E. of 5 mice per treatment group. $^{*}P < 0.05$ HFD vs. control. $^{\#}P < 0.05$ HFD + fenofibrate vs. HFD

4 Discussion

In the present study, we have shown that fenofibrate, a synthetic PPARα agonist, reduces HFD-induced deposition of TG in the liver and corrects systemic lipid abnormalities. Beyond the effects on lipid metabolism, fenofibrate not only increased the size of sinusoidal vessels, but also restored tissue oxygenation and redox state in the affected liver.

PPARα has been reported to control lipid metabolism in metabolically active organs such as liver by regulating expression of genes involved in mitochondrial and peroxisomal fatty acid oxidation [3, 6]. PPARα activation also increases lipoprotein lipase activity and uptake of fatty acids by peripheral tissues. Considering these biological functions of PPARα, our results showing improvement of hepatic and systemic lipid abnormalities by fenofibrate are most likely explained by the direct effects of PPARα activation in the body. These metabolic alterations lead to morphological changes in hepatocytes with a reduction of both sizes and numbers of fat droplets in cytoplasm. Consequently, physical oppression of sinusoids by fat-swollen hepatocytes is released, and sinusoidal vasodilation occurs [7]. In fact, these microvascular changes were observed in HFD-fed mice treated with fenofibrate, and were associated with an increase in blood flow in hepatic sinusoids. Although the recovery of hepatic sinusoidal perfusion most likely results from morphological changes in hepatic microvasculature by lipid-lowering effects of fenofibrate, direct effects of the drug on hepatic microcirculation can be considered to be involved. Fenofibrate has been reported to show vasoprotective effects by either stimulating production of nitric oxide (NO) [4] or inhibiting endothelin-1 (ET-1) production [1]. Since these vasoactive molecules can regulate vascular tone at the sinusoidal levels, improvement of hepatic microcirculation by the treatment of fenofibrate can be accounted for, at least in part, by eNOS activation and/or ET-1 inhibition. Further investigations are needed to determine the extent to which fenofibrate improves hepatic microcirculation through acting directly on vascular beds in the diet-induced fatty liver.

HFD feeding evoked a decrease and an increase in oxygen tension in portal venule and NADH autofluorescence of hepatocytes in the liver, respectively. In addition, we observed that the difference in oxygen tension between portal and central venule was greatly attenuated in HFD-treated liver, suggesting oxygen extraction (utilization) by the liver was impaired by the diet. On the other hand, treatment of HFD-fed mice with fenofibrate greatly reversed levels of both oxygen tension and NADH. Although hepatic sinusoids seem to be crucial sites responsible for fenofibrate treatment, our present results showing a great reduction of oxygen tension in portal venule may indicate that proximal portal veins or venules rather than sinusoids also are target points of fenofibrate to regulate oxygen supply and availability in liver microcirculation. Considering that both oxygen and NADH serve as important regulators to determine the balance between fatty acid oxidation and TG synthesis in the liver, the adequate tissue oxygenation with increased PPARα-target gene expressions are a prerequisite for hepatocytes to oxidize fatty acids effectively, leading to prevention and treatment of hepatic steatosis.

In conclusion, fenofibrate regulates both lipid metabolism and vascular functions in liver in a coordinated manner, and shows a promising therapeutic potential for NAFLD.

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