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An SOD1 deficiency enhances lipid droplet accumulation in the fasted mouse liver by aborting lipophagy

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ABSTRACT

Under normal feeding conditions, oxidative stress stimulates lipid droplets accumulation in hepatocytes. We found that, despite the low visceral fat in *Sod1*-knockout (KO) mouse, lipid droplets accumulate in the liver to a greater extent than for the wild-type mouse upon fasting. Liver damage became evident in the KO mice. While fasting caused substantial endoplasmic reticulum stress in KO mice, the expression of genes involved in fatty acid production was suppressed. LC3-II, which is essential for the dynamic process of autophagosome formation, was activated in the wild-type mouse and enhanced in the KO mouse. However, the p62, an adapter protein with the ubiquitin- and LC3-binding activity, accumulated abnormally in the livers of KO mice, implying an abortive lipophagic process as the cause for the impaired lipid metabolism and the hepatic damage that occurs upon fasting.

1. Introduction

The liver plays a primary role in the metabolic control of sugars, amino acids, and lipids in blood and hence regulates the energy balance in the entire body. A malfunction of the liver therefore causes multiple metabolic diseases, notably the metabolic syndrome. Among the various deleterious factors, lipids readily accumulate in the liver, causing a fatty liver, which may develop into steatohepatitis. The formation of lipid droplet in the liver is induced by an excess intake of food and pathological conditions such as abnormal lipid metabolism [1]. Among the deleterious factors, oxidative stress, characterized by elevated levels of reactive oxygen species (ROS) is an established cause for liver injury.

Superoxide dismutase (SOD) converts superoxide, a primary ROS produced by the one electron reduction of a oxygen molecule, to the less toxic hydrogen peroxide, and a deficiency in this enzyme can led to elevated oxidative stress [2]. While lipids accumulate more in the liver of KO mice than WT mice, visceral fat levels are lower in KO mice [3–5]. This abnormal lipid metabolism appears to be caused by the suppression of lipoprotein secretion from the liver [3] and intestine [4].

In addition to oxidative stress, endoplasmic reticulum (ER) stress also reportedly induces lipid droplet accumulation in cells under culture conditions [6] and in mice [7]. Oxidative stress impairs oxidative protein folding in the ER, leading to ER stress [8]. ER stress then stimulates lipogenesis [9] since the sterol regulatory element binding protein (SREBP1) is proteolytically activated by the Site-1 protease (S1P) and Site-2 protease (S2P) that are localized on Golgi membranes and also involved in the activation of ATF6 in response to ER stress [10]. We recently found that the lipid droplet accumulation is accelerated in SOD1-deficient hepatocytes under primary culture conditions [11]. Because the genes required for fatty acid synthesis under regulation by SREBP1 are elevated in this situation, the de novo synthesis of fatty acids appears to be the primary cause of lipid droplet formation under conditions an adequate diet or the culture medium.

Upon fasting, the degradation of lipid droplets is stimulated by selective autophagy, referred to as lipophagy [12]. It has recently been suggested that mitochondrial fusion is involved with lipid

Abbreviations: ACC, acetyl CoA-carboxylase; ALT, alanine aminotransferase; apoB, apolipoprotein B; BSA, bovine serum albumin; ER, endoplasmic reticulum; FAS, fatty acid synthase; GLU, glucose; H&E, hematoxylin and eosin; KO, *Sod1* knockout; MTP, microsomal transfer protein; PBS, phosphate-buffered saline; PRDX-SO_3, hyperoxidized peroxiredoxin; ROS, reactive oxygen species; SCD1, stearoyl CoA desaturase 1; S1P, Site-1 protease; S2P, Site-2 protease; SOD, superoxide dismutase; SREBP1, sterol regulatory element binding protein; TBS, tris-buffered saline; TBST, TBS containing 0.1% Tween-20; TG, triacylglycerides; UPR, unfolded protein response; WT, wild type.

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droplet in the lipolytic process, thus representing an alternative pathway [13]. On the other hand, accumulating evidence indicates that lipid droplet formation is stimulated in the liver by fasting [14,15]. Because the glucose supply is limited under conditions of fasting, free fatty acids originated from triglycerides in adipose tissues appear to be a major carbon source for triglyceride formation [16]. It would be intriguing to know the status of lipid droplets in KO livers under fasting conditions because such mice have minimal amounts of visceral fat [4]. The findings reported herein show that, unexpectedly, the lipid droplets in the liver of KO mice are elevated, leading us to propose a tentative mechanism for this peculiar response to fasting.

2. Materials and methods

2.1. Animals

C57B/6 *Sod1*$^{-/-}$ mice under C57BL/6 background were used as described previously [4,17]. The animal room was maintained under specific pathogen-free conditions at a constant temperature of 20–22 °C with a 12-hr alternating light–dark cycle. Animal experiments were performed in accordance with the Declaration of Helsinki under the protocol approved by the Animal Research Committee at our institution.

2.2. Blood test

Blood was collected from the tail vein or the heart under ether anesthesia in the presence of ethylenediaminetetraacetic acid. After centrifugation at 800× g for 5 min, the levels of triglycerides (TG), glucose (GLU), and alanine leucine transaminase (ALT) in blood plasma were determined using Fuji Dry-chem slides on Fuji Drichem 3500V (Fuji film).

2.3. Histological and immunohistochemical analyses of liver

Dissected livers were fixed in 15% buffered formalin followed by embedding in paraffin. Longitudinal sections that were 4 μm thick were subjected to H&E staining and immunohistochemical analyses were carried out, as previously described [18]. For

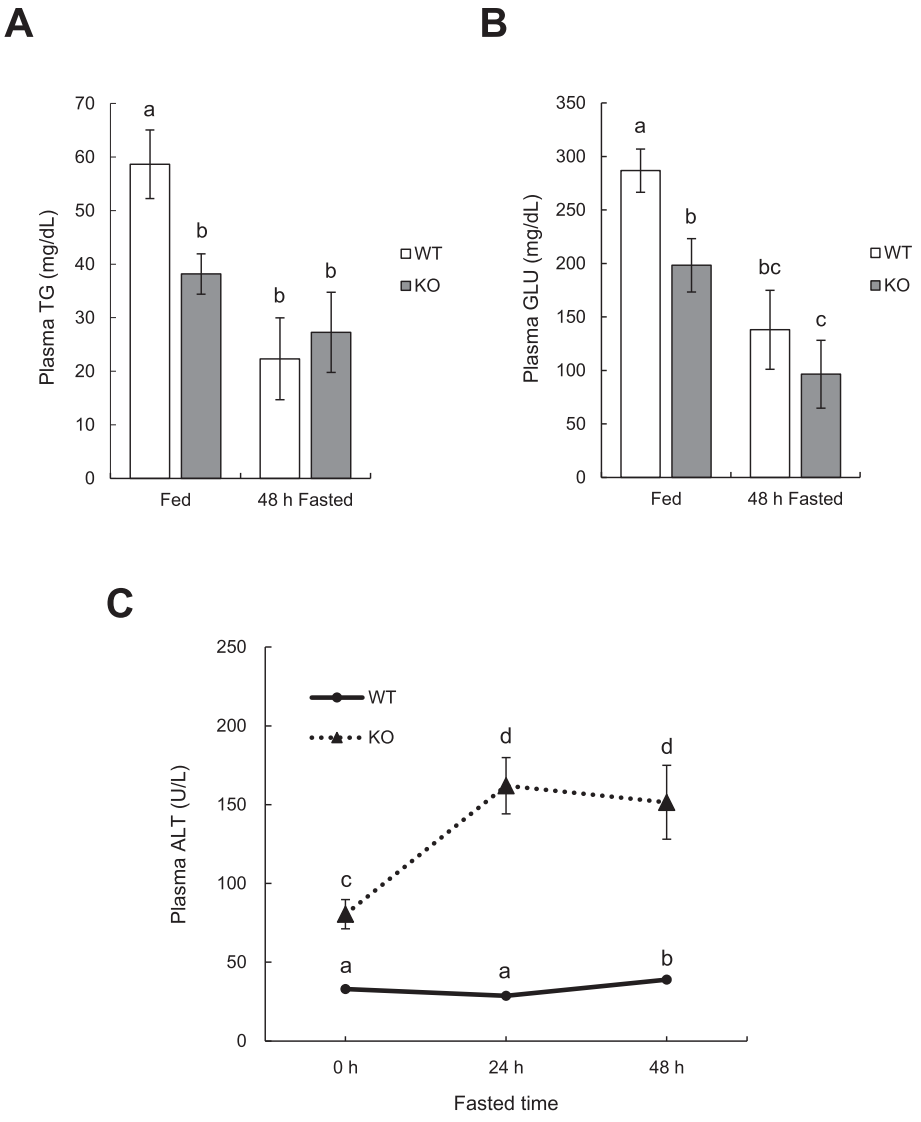

Fig. 1. Effect of fasting on plasma TG, GLU and ALT in WT and KO mice. Plasma TG (A), GLU (B), and ALT (C) were measured at each time point after the start of fasting. (n = 3–6) Statistical analyses were performed using the Student *t* test or one-way ANOVA, followed by the Tukey-Kramer test for multiple groups. Different letters above the columns indicate a statistical difference ($P < 0.05$).