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# The role of glycerol-3-phosphate dehydrogenase 1 in the progression of fatty liver after acute ethanol administration in mice

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ABSTRACT

Acute ethanol consumption leads to the accumulation of triglycerides (TGs) in hepatocytes. The increase in lipogenesis and reduction of fatty acid oxidation are implicated as the mechanisms underlying ethanol-induced hepatic TG accumulation. Although glycerol-3-phosphate (Gro3P), formed by glycerol kinase (GYK) or glycerol-3-phosphate dehydrogenase 1 (GPD1), is also required for TG synthesis, the roles of GYK and GPD1 have been the subject of some debate. In this study, we examine (1) the expression of genes involved in Gro3P production in the liver of C57BL/6J mice in the context of hepatic TG accumulation after acute ethanol intake, and (2) the role of GPD1 in the progression of ethanol-induced fatty liver using *GPD1* null mice. As a result, in C57BL/6J mice, ethanol-induced hepatic TG accumulation began within 2 h and was 1.7-fold greater than that observed in the control group after 6 h. The up-regulation of GPD1 began 2 h after administering ethanol, and significantly increased 6 h later with the concomitant escalation in the glycolytic gene expression. The incorporation of $^{14}$C-labelled glucose into TG glycerol moieties increased during the same period. On the other hand, in *GPD1* null mice carrying normal GYK activity, no significant increase in hepatic TG level was observed after acute ethanol intake. In conclusion, GPD1 and glycolytic gene expression is up-regulated by ethanol, and GPD1-mediated incorporation of glucose into TG glycerol moieties together with increased lipogenesis, is suggested to play an important role in ethanol-induced hepatic TG accumulation.



## 1. Introduction

Acute and chronic ethanol consumption leads to the accumulation of triglycerides (TGs) in the hepatocytes of experimental animals [1]. Continued consumption of ethanol may cause steatosis to progress to hepatitis and fibrosis, which may further lead to liver cirrhosis. Reducing or preventing the accumulation of TGs within the liver in response to ethanol consumption may block the progression of fatty liver to hepatitis and fibrosis. Therefore, it is important to understand the biochemical and molecular mechanisms responsible for ethanol-induced metabolic changes that modulate the accumulation of TGs in the liver.

Early studies indicated that ethanol consumption increased the ratio of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide ($NAD^+$) in hepatocytes as a result of ethanol oxidation by alcohol dehydrogenase and aldehyde dehydrogenase. This altered ratio could affect intermediary metabolism in a number of ways, increasing the hepatic TGs content [2,3]. On the other hand, it has been shown that the TGs accumulation in the liver occurs independently of the changes in the hepatocellular redox-state ([NADH]/[$NAD^+$]) observed after ethanol administration [4,5]. Recent studies indicate that ethanol exposure regulates lipid metabolism-associated transcription factors, such as sterol regulatory element-binding protein-1c (SREBP-1c) and peroxisome proliferator-activated receptor alpha (PPAR-alpha); this stimulates lipogenesis and inhibits fatty acid oxidation [6–18]. We recently reported that acute ethanol administration increased liver TGs content by activating SREBP-1c and carbohydrate

*Abbreviations:* ChREBP, carbohydrate response element-binding protein; DGAT, acyl-CoA:diacylglycerol acyltransferase; DHAP, dehydroxyacetone phosphate; FAS, fatty acid synthase; Foxo1, forkhead box O1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPD1, glycerol-3-phosphate dehydrogenase 1; Gro3P, glycerol-3-phosphate; GYK, glycerol kinase; NADH, reduced nicotinamide adenine dinucleotide; $NAD^+$, oxidized nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; PEPCK, phosphoenolpyruvate carboxykinase; PFKL, liver phosphofructokinase; PGC-1 alpha, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PPAR, peroxisome proliferator-activated receptor; PYGL, liver glycogen phosphorylase; SREBP-1c, sterol regulatory element-binding protein-1c; TG, triglyceride.

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response element-binding protein (ChREBP), which promote de novo lipogenesis; and increases in expression of PPAR gamma and acyl-CoA:diacylglycerol acyltransferase (DGAT), which promote TGs synthesis [19].

Because glycerol-3-phosphate (Gro3P) and fatty acyl-CoAs are the substrates for TG synthesis, Gro3P is required for ethanol-induced hepatic TG accumulation. In fact, the availability of Gro3P has been considered a possible regulatory factor in the synthesis of TG [20]. Gro3P can be formed by glycerol phosphorylation via glycerol kinase (GYK) or by the reduction of dihydroxyacetone phosphate (DHAP) via glycerol-3-phosphate dehydrogenase 1 (GPD1). GYK-mediated production of Gro3P may be increased after acute ethanol consumption because ethanol enhances the incorporation of intraperitoneally administered glycerol into hepatic neutral glycerolipids [1]. Recently, Potter et al. reported that acute exposure of hepatocytes to acetaldehyde causes a rapid increase in the uptake of glycerol via aquaporin 9, and ethanol increased the activities of GYK and phosphoenolpyruvate carboxykinase (PEPCK) leading to increased formation of Gro3P [21]. On the other hand, chronic ethanol-treated rats exhibited lower accumulation of Gro3P after glycerol loading, with a slower rate of glycerol phosphorylation [22]. Inhibition of lipolysis by plasma acetate, one of the metabolites of ethanol, is also observed 1–2 h after consumption of ethanol in humans, suggesting the *in vivo* reduction of the main source of plasma glycerol [23]. Although the availability of Gro3P is considered to increase after acute ethanol consumption by shifting the cytosolic redox-pair [DHAP]/[Gro3P] towards the reduced state via GPD1 [24], it is not clear whether GYK or GPD1 is the predominant enzyme for the production of Gro3P and TG glycerol moieties in acute ethanol-induced fatty liver. In addition, because acute ethanol administration increases ChREBP expression [19], the genes involved in carbohydrate metabolism, including Gro3P production, may be altered. In this study, we examined whether acute ethanol administration affects the expression of genes involved in Gro3P production and the role of GPD1 in the progression of ethanol-induced fatty liver.

## 2. Materials and methods

### 2.1. Experimental animals

Eight-week-old C57BL/6J and BALB/cBy mice were obtained from Japan SLC (Hamamatsu, Japan) and Japan CLEA (Tokyo, Japan), respectively. The origins of the BALB/cHeA mice and their breeding conditions have been previously described [25]. The mice were fed a normal laboratory diet (MF, Oriental Yeast, Tokyo, Japan) for 1 week to stabilize their metabolic conditions and were maintained on a 12:12-h light-dark cycle at constant temperature (22 °C). The mice were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals and our institutional guidelines. All animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee of the University of Shizuoka (No. 135036) and the Osaka Prefecture University (No. 25–28).

### 2.2. Ethanol administration

Mice were starved from 7 to 9 AM before intragastrically administering a 40% ethanol solution in water at a dosage of 3 g ethanol/kg body weight (150 μL/mouse). Control mice received a 40% glucose (w/v) solution of the same caloric value. The mice remained under fasting conditions and were killed at 11 AM or 3 PM, with the exception of those mice that were examined for the incorporation of glucose into TG glycerol moieties, which were killed at 1 PM.

### 2.3. Liver glycogen and TGs

The glycogen content in the liver was determined as glycosyl units after acid hydrolysis [26]. The lipids in the liver were extracted quantitatively with ice-cold 2:1 chloroform–methanol (v/v) as described by Folch et al. [27]. The TGs concentrations in the liver homogenates were measured by enzymatic colorimetric methods utilizing TG E tests (Wako Pure Chemical Industries, Osaka, Japan).

### 2.4. Quantitative real-time RT-PCR

The preparation of RNA and quantitative real-time RT-PCR assays were performed as previously described [28]. The mouse-specific primer pairs used are shown in Table S1.

### 2.5. Incorporation of glucose into TG glycerol moieties in the liver

To examine the incorporation of glucose into TG glycerol moieties in the liver, a piece of liver was incubated for 60 min after placing in a 20-mL glass reaction vial containing 2.5 mL of warmed (30 °C), pregassed (95% $O_2$, 5% $CO_2$, pH 7.4), modified Krebs-Henseleit buffer containing 3% fatty acid-free bovine serum albumin (Sigma Chemical, St. Louis, MO), 0.2 μCi/mL [U–$^{14}$C]-glucose (GE Healthcare Life Sciences, Buckinghamshire, UK), 3.5 mM glucose, and 2 mM sodium acetate. The liver was transferred to a microtube containing ice-cold 2:1 chloroform-methanol (v/v) and homogenized with a polytron. After homogenization, the samples were centrifuged at 2000*g* for 10 min, and the supernatant was transferred to a clean centrifuge tube with a glass Pasteur pipette. After the addition of distilled water, the samples were shaken for 10 min and centrifuged to separate the aqueous and lipophilic phases. The chloroform phase containing the total lipids was gently evaporated under a stream of $N_2$, and the residue was redissolved in ethanolic KOH (0.5 N) and incubated at 70 °C for 1 h. To convert the carboxylate salts to free fatty acids, the samples were acidified by the addition of 6 N HCl. The fatty acids were extracted with petroleum ether, and the aqueous phase was quantified by liquid scintillation counting to determine the amount of incorporated $^{14}$C-glycerol [29].

### 2.6. Other assays

Plasma samples were separated by centrifugation in the presence of EDTA and snap frozen at −80 °C until analysis. Plasma glucose, free fatty acids, glycerol, and ketone bodies were analyzed with the Glucose CII-test, NEFA C-test (Wako Pure Chemical Industries), Free Glycerol Colorimetric/Fluorometric Assay Kit (Bio Vision, Milpitas, CA), and the Wako Autokit Total Ketone Bodies (Wako Pure Chemical Industries), respectively.

### 2.7. Statistical analysis

All values are represented as the mean ± standard error of the mean. Data were analyzed by one-way or two-way analyses of variance. Where differences were significant, each group was compared with the other by a Student *t* test or by a Tukey–Kramer HSD test (JMP 5.1.2; SAS, Cary, NC). Statistical significance was defined as $P < 0.05$.

## 3. Results

### 3.1. Increased TGs levels in the liver following acute ethanol administration

The liver TGs levels were evaluated in fasted C57BL/6J mice at 0 (control), 2, and 4 h after injection of a single dose of ethanol or