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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

Table 1
Body and tissue weight and plasma analyses of C57BL/6J mice after acute ethanol administration.

		Glucose	Ethanol
Number of mice (*n*)	Control	5	
	2 h	5	6
	6 h	5	5
Weight (g)			
Final body weight	Control	23.9 ± 0.8	
	2 h	23.2 ± 0.3	23.3 ± 0.7
	6 h	23.0 ± 0.2	22.5 ± 0.5
Liver	Control	1.23 ± 0.03	
	2 h	1.33 ± 0.05*	1.19 ± 0.05*
	6 h	1.17 ± 0.02	1.03 ± 0.03*,†
Liver triglycerides (mg/g liver)	Control	5.14 ± 0.28	
	2 h	4.24 ± 0.40	7.10 ± 0.97*
	6 h	5.80 ± 1.01	8.68 ± 0.89*,††
Liver glycogen (mg/g liver)	Control	43.6 ± 4.2	
	2 h	64.8 ± 3.1††	36.1 ± 5.7***
	6 h	44.8 ± 2.3	17.8 ± 4.3***,†††
Plasma analyses			
Glucose (mg/dL)	Control	207 ± 11	
	2 h	259 ± 32	195 ± 9*
	6 h	218 ± 30	158 ± 6*
Free fatty acid (mEq/L)	Control	0.79 ± 0.05	
	2 h	0.66 ± 0.05†	0.64 ± 0.05
	6 h	0.68 ± 0.05†	0.89 ± 0.07*
Glycerol (mg/mL)	Control	0.20 ± 0.04	
	2 h	0.16 ± 0.01	0.15 ± 0.01
	6 h	0.12 ± 0.02	0.13 ± 0.03
Ketone body (μmol/L)	Control	451 ± 84	
	2 h	185 ± 84†	189 ± 67†
	6 h	106 ± 55††	667 ± 72***

C57BL/6J mice were fed regular chow for 1 week prior to the experiment. After fasting for 2 h, ethanol (3 mg/g body weight) or a glucose solution of the same caloric value was administered to the mice. Samples were obtained under fasting conditions at 0 (control), 2, and 6 h after administration. Values are means ± SEM. Data were analyzed by one-way analysis of variance. Where differences were significant, each group was compared with the other by a Student *t* test.
* $P < 0.05$ versus glucose.
*** $P < 0.001$ versus glucose.
† $P < 0.05$ versus control.
†† $P < 0.01$ versus control.
††† $P < 0.001$ versus control.

glucose. The body and liver weights, liver TGs and glycogen levels, and blood plasma analyses data are shown in Table 1. The mice that received ethanol showed 1.4 (2 h) and 1.7-fold (6 h) increases in hepatic TGs concentrations and decreased liver glycogen concentrations of 83% (2 h) and 41% (6 h). The increase in hepatic TG concentrations and decrease in liver glycogen concentrations were not observed in the mice that received glucose. An ethanol-induced decrease in plasma glucose levels was observed at both time points. Although there was a significant increase in the plasma free fatty acid level at 6 h after ethanol administration, the plasma glycerol level was unchanged, suggesting that ethanol-induced stimulation of lipolysis had not occurred.

3.2. The expression of mRNA in the liver for genes related to Gro3P production following ethanol administration

The expression of mRNA in the liver for transcription factors and genes related to Gro3P production, lipogenesis and TG synthesis were determined by quantitative real-time RT-PCR (Fig. 1). Although no significant differences in GYK expression were observed between the 2 groups, GPD1 expression in ethanol-exposed mice increased by 3.3- and 4.5-fold 2 and 6 h later, respectively. The expression of genes involved in glycolysis, such as liver phosphofructokinase (*PFKL*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), was significantly increased in the ethanol-injected mice at 6 h. On the other hand, there were no significant differences among the mice that were administered ethanol and glucose for the expression of genes, such as liver glycogen phosphorylase (*PYGL*) and *PEPCK*, that are involved in glycogenolysis and gluconeogenesis, respectively. The expression of fatty acid synthase (*FAS*) and *DGAT2*, which encode enzymes involved in lipogenesis and TGs synthesis, also increased in response to the ethanol. Of the transcription factors examined, only *SREBP-1c* gene expression increased in the mice that received ethanol.

3.3. Ethanol induced increase in incorporation of glucose into TG glycerol moieties

To examine whether glucose was incorporated into TG glycerol moieties in the liver at an increased rate, livers were excised from control and experimental mice and incubated with [^{14}C]-glucose. The radioactivity of the fraction containing [^{14}C]-glycerol (Fig. 2) was measured, and the incorporation of glucose into TG glycerol moieties had increased 1.3-fold in the mice that received ethanol when compared to the control mice or the mice administered glucose.

3.4. Involvement of GPD1 in the development of acute ethanol-induced fatty liver

To examine the role of GPD1 on the accumulation of TGs in the liver after ingesting ethanol, the ethanol-induced changes in hepatic TGs levels were measured in BALB/cHeA mice that lacked GPD1 activity, with BALB/cBy mice acting as controls (Table 2). Only residual hepatic GPD1 activity was present in the BALB/cHeA mice used in this experiment (data not shown). Two and six hours after administering the ethanol, increases in the hepatic TGs levels were observed in the BALB/cBy mice. In contrast, those increases were not observed in the BALB/cHeA mice. These results suggest that hepatic GPD1 activity was involved in the progression of acute ethanol-induced fatty liver. The liver glycogen and plasma glucose levels were decreased in both mouse strains in response to the ethanol. Stimulating lipolysis usually increases both free fatty acid and glycerol levels in the plasma. Although neither mouse strain had a change in plasma glycerol levels in response to the ethanol, an increase in free fatty-acid levels in the plasma was observed in the BALB/cHeA mice, suggesting that production of glycerol and free fatty acid by lipolysis in the adipose tissue might not be responsible for the ethanol-induced increase in free fatty-acid concentration in the plasma.

4. Discussion

In this study, we examined the effects of acute ethanol administration on the expression of genes involved in Gro3P production in the liver, and the role of GPD1 in the progression of ethanol-induced fatty liver. We found that acute ethanol administration increased the TG content in the liver in conjunction with an increase in the mRNA expression of glycolytic enzyme and *GPD1*. Glucose incorporation into TG glycerol moieties was increased in the ethanol-injected mice. In addition, no increase in hepatic TG levels was observed in ethanol-injected *GPD1* null mice (BALB/cHeA). These data suggest that ethanol-induced hepatic TG accumulation is partly due to the enhancement of GPD1-mediated incorporation of glucose into TG glycerol moieties.

Recent studies indicate that ethanol exposure directly or indirectly regulates lipid metabolism-associated transcription factors such as SREBP-1c and PPAR alpha, which stimulate lipogenesis and inhibit fatty acid oxidation, respectively [6–18]. In this study, we observed ethanol-induced *GPD1* expression in conjunction with an increase in *SREBP-1c* mRNA levels. In a study utilizing 3T3-

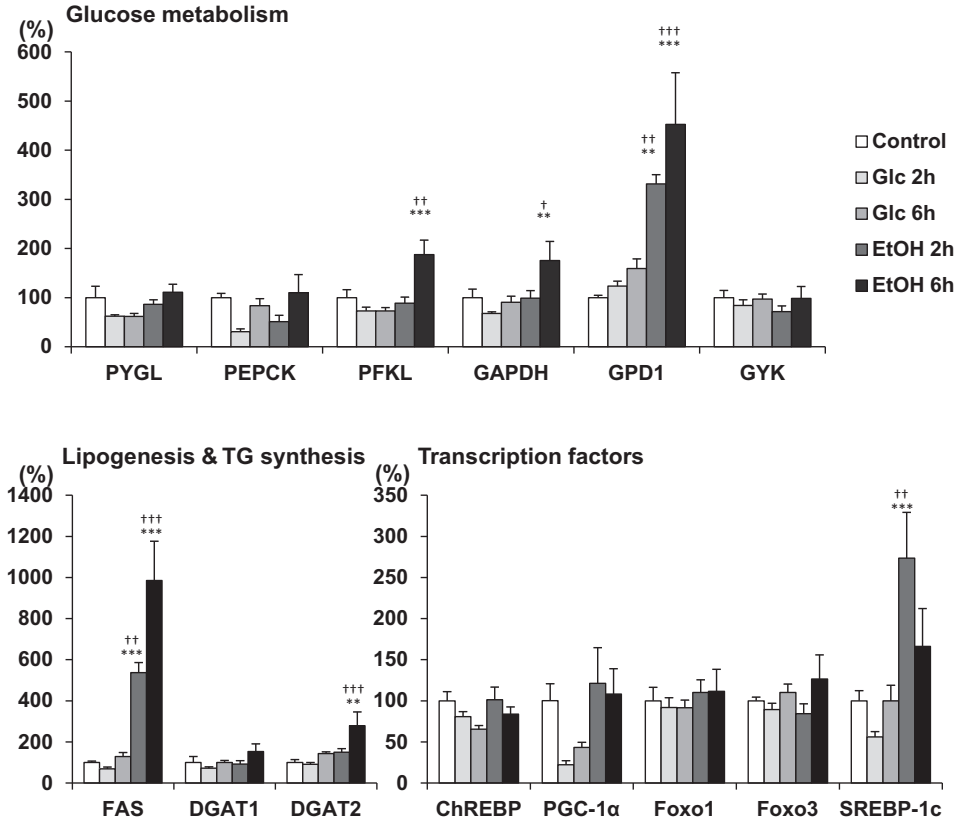

Fig. 1. The expression of mRNA related to glucose metabolism in the liver after acute ethanol administration. C57BL/6J mice were fed regular chow for 1 week prior to the experiment. After fasting for 2 h, ethanol (EtOH, 3 mg/g body weight) or a glucose (Glc) solution of the same caloric value was administered to the mice. Liver samples were obtained under fasting conditions at 0 (Control), 2, and 6 h after administration. The results shown are for the quantitative RT-PCR analysis of transcripts in the liver encoding proteins that are transcription factors or are involved in glucose metabolism, lipogenesis, or triglyceride (TG) synthesis. The results are displayed as a percentage of the level of mRNA expressed in the control mice. Values are means ± SEM ($n = 5–6$). $^{**}P < 0.01$; $^{***}P < 0.001$ versus the same time point of the glucose control. $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.001$ versus the 0 h control. PYGL, liver glycogen phosphorylase; PEPCK, phosphoenolpyruvate carboxykinase; PFKL, liver phosphofructokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPD1 glycerol-3-phosphate dehydrogenase 1; GYK, glycerol kinase; FAS, fatty acid synthase; DGAT, acyl-CoA:diacylglycerol acyltransferase; ChREBP, carbohydrate response element-binding protein; PGC-1 alpha, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; Foxo, forkhead box O; SREBP-1c, sterol regulatory element-binding protein-1c.

F442A cells, overexpression of a dominant-positive form of SREBP-1c prevented the antidepressant phenelzine from inducing inhibition of GPD1 activity [30]. In contrast, a genome-wide analysis of SREBP-1 binding in mouse liver chromatin did not find the *GPD1* gene to be a target of SREBP-1 [31]. *GPD1* was identified as one of the target genes of ChREBP by chromatin immunoprecipitation-sequencing and gene expression analysis [32], with ChREBP up-regulating GPD1. Although acute ethanol administration has been reported to increase the expression level of ChREBP [19], we did not observe any changes. Therefore, transcription factors other than SREBP-1c and ChREBP may be involved in the ethanol-induced expression of GPD1.

The production of Gro3P is catalyzed by different enzymes, including GPD1 and GYK. GPD1 acts by modifying the [NADH]/[NAD$^+$] ratio, and an increase in this ratio increases the production of Gro3P. Because acute ethanol administration increases the [NADH]/[NAD$^+$] ratio, the catalysis of Gro3P production by GPD1 may be stimulated for the ethanol-induced accumulation of TGs in the liver [24]. In addition, we found that expression of GPD1 and the incorporation of glucose into glycerol TG moieties in the liver were stimulated by the ethanol injection. Therefore, we examined whether GPD1 is required for acute ethanol-induced TG accumulation in the liver using a BALB/c sub-line of mice (BALB/cHeA) that are *GPD1* null mutants lacking GPD1 activity [33] with decreased and increased GroP3 and DHAP concentrations in the livers, respectively, in comparison with control mice [34].

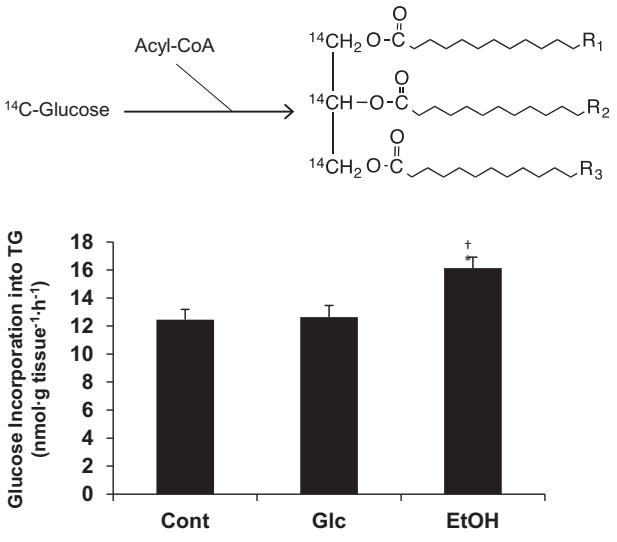

Fig. 2. Increase in the incorporation of glucose into triglyceride glycerol moieties in the liver. C57BL/6J mice were fed regular chow for 1 week prior to the experiment. After 2 h fasting, ethanol (EtOH, 3 mg/g body weight) or a glucose (Glc) solution of the same caloric value was administered to the mice. The incorporation of glucose into triglyceride (TG) glycerol moieties was measured in livers obtained under fasting conditions at 0 (control) and 4 h after administration. Values are means ± SEM ($n = 4–5$). $^{*}P < 0.05$ versus the same time point of the glucose control. $^{\dagger}P < 0.05$ versus the 0 h control.

Table 2
Body and tissue weight and plasma analyses of BALB/cBy and BALB/cHeA mice after acute ethanol administration.

		BALB/cBy		BALB/cHeA		Two-way ANOVA *P* value		
		Glucose	Ethanol	Glucose	Ethanol	Mouse	Ethanol	Mouse × ethanol
Number of mice (*n*)	Control	5		4				
	2 h	4	4	4	4			
	6 h	5	4	4	4			
Weight (g)								
Final body weight	Control	21.3 ± 1.1		20.7 ± 2.4				
	2 h	21.5 ± 1.4	22.4 ± 1.6	21.1 ± 1.6	21.8 ± 2.1	0.802	0.652	0.960
	6 h	22.5 ± 1.3	21.7 ± 1.3	21.9 ± 2.1	23.5 ± 1.7	0.733	0.815	0.457
Liver	Control	1.16 ± 0.07		1.18 ± 0.16				
	2 h	1.17 ± 0.11	1.17 ± 0.08	1.22 ± 0.13	1.21 ± 0.21	0.749	0.966	0.952
	6 h	1.17 ± 0.08	1.07 ± 0.08	1.18 ± 0.17	1.24 ± 0.09	0.415	0.861	0.503
Liver triglycerides (mg/g liver)	Control	18.4 ± 1.6^{bc}		12.8 ± 3.0^{c}				
	2 h	17.3 ± 1.8^{bc}	25.3 ± 3.7^{ab}	11.2 ± 0.9^{c}	13.5 ± 1.0^{c}	<0.01	<0.05	0.206
	6 h	15.9 ± 1.8^{bc}	30.0 ± 4.3^{a}	14.4 ± 1.9^{bc}	14.4 ± 1.5^{bc}	<0.01	<0.05	<0.05
Liver glycogen (mg/g liver)	Control	23.1 ± 3.7^{abc}		37.8 ± 3.4^{a}				
	2 h	32.1 ± 3.7^{ab}	9.4 ± 1.3^{cd}	37.8 ± 2.7^{a}	16.1 ± 5.8^{bcd}	0.121	<0.001	0.888
	6 h	12.8 ± 5.0^{cd}	0.8 ± 0.7^{d}	11.1 ± 4.1^{cd}	2.6 ± 1.7^{d}	0.985	<0.05	0.634
Plasma analyses								
Glucose (mg/dL)	Control	119 ± 5^{a}		100 ± 10^{ab}				
	2 h	117 ± 12^{a}	119 ± 12^{a}	93 ± 12^{ab}	110 ± 12^{a}	0.204	0.434	0.557
	6 h	106 ± 6^{a}	82 ± 7^{ab}	107 ± 20^{a}	51 ± 12^{b}	0.253	<0.01	0.212
Free fatty acid (mEq/L)	Control	0.77 ± 0.07^{b}		0.50 ± 0.06^{b}				
	2 h	0.69 ± 0.07^{b}	0.71 ± 0.08^{b}	0.45 ± 0.02^{b}	0.85 ± 0.09^{b}	0.494	<0.05	<0.05
	6 h	0.72 ± 0.13^{b}	0.95 ± 0.07^{b}	0.85 ± 0.08^{b}	1.80 ± 0.37^{a}	<0.05	<0.05	0.092
Glycerol (mg/mL)	Control	0.14 ± 0.02		0.15 ± 0.02				
	2 h	0.16 ± 0.03	0.17 ± 0.03	0.18 ± 0.03	0.15 ± 0.03	0.967	0.770	0.533
	6 h	0.20 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.25 ± 0.03	0.492	0.163	0.187
Ketone body (μmol/L)	Control	632 ± 68^{a}		312 ± 87^{ab}				
	2 h	522 ± 35^{ab}	615 ± 112^{ab}	187 ± 57^{b}	469 ± 49^{ab}	<0.01	<0.05	0.200
	6 h	527 ± 51^{ab}	728 ± 91^{a}	525 ± 118^{ab}	690 ± 176^{a}	0.861	0.127	0.874

BALB/cBy and BALB/cHeA mice were fed regular chow prior to the experiment. After fasting for 2 h, ethanol (3 mg/g body weight) or a glucose solution of the same caloric value was administered to the mice. Samples were obtained under fasting conditions at 0 (control), 2, and 6 h after administration. Values are means ± SEM. Data for groups of the same time period were analyzed by a two-way analysis of variance (ANOVA). All groups were analyzed by a one-way ANOVA. Where differences were significant, each group was compared with the other by a Tukey–Kramer HSD test. Means in a row without a common letter differ significantly ($P < 0.05$).

Our results with the BALB/cHeA mice demonstrated that a lack of GPD1 activity diminished TG accumulation in the liver after acute ethanol administration, suggesting that GPD1 plays an important role in the development of acute ethanol-induced fatty liver. The role of GYK in acute ethanol-induced fatty liver has been controversial [1,21–23]. In the present study, no increase in the plasma glycerol concentration or GYK expression was observed after administering ethanol. Furthermore, acute ethanol-induced TG accumulation was inhibited in the BALB/cHeA mice, even though these mice have normal GYK activity [34]. These results suggest that GYK might not be required for acute ethanol-induced hepatic TG accumulation, and the glycerol moieties of hepatic TGs are likely derived from glycerol-6-phosphate via GPD1 rather than glycerol after acute ethanol administration at least in the mice model used in the present study.

In conclusion, increase in glucose incorporation into glycerol TG moieties via GPD1 involves in accumulation of TG in the liver after ethanol administration in addition to enhancement of lipogenesis and inhibition of fatty acid oxidation. Ethanol-induced GPD1 expression may be involved in the accumulation of TGs in the liver by increasing Gro3P production in conjunction with altering the [NADH]/[NAD^+] ratio.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.096.

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