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Glycerol-3-phosphate dehydrogenase 1 deficiency induces compensatory amino acid metabolism during fasting in mice

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ABSTRACT

Background. Glucose is used as an energy source in many organs and obtained from dietary carbohydrates. However, when the external energy supply is interrupted, e.g., during fasting, carbohydrates preserved in the liver and glycogenic precursors derived from other organs are used to maintain blood glucose levels. Glycerol and glycogenic amino acids derived from adipocytes and skeletal muscles are utilized as glycogenic precursors. Glycerol-3-phosphate dehydrogenase 1 (GPD1), an NAD^+/NADH-dependent enzyme present in the cytosol, catalyzes the reversible conversion of glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate (DHAP). Since G3P is one of the substrates utilized for gluconeogenesis in the liver, the conversion of G3P to DHAP by GPD1 is essential for maintaining blood glucose levels during fasting. We focused on GPD1 and examined its roles in gluconeogenesis during fasting.

Methods. Using GPD1 null model BALB/cHeA mice (HeA mice), we measured gluconeogenesis from glycerol and the change of blood glucose levels under fasting conditions. We also measured gene expression related to gluconeogenesis in the liver and protein metabolism in skeletal muscle. BALB/cBy mice (By mice) were used as a control.

Results. The blood glucose levels in the HeA mice were lower than that in the By mice after glycerol administration. Although lack of GPD1 inhibited gluconeogenesis from glycerol, blood glucose levels in the HeA mice after 1–4 h of fasting were significantly higher than that in the By mice. Muscle protein synthesis in HeA mice was significantly lower than that in the By mice. Moreover, blood alanine levels and usage of alanine for gluconeogenesis in the liver were significantly higher in the HeA mice than that in the By mice.

Conclusions. Although these data indicate that a lack of GPD1 inhibits gluconeogenesis from glycerol, chronic GPD1 deficiency may induce an adaptation that enhances gluconeogenesis from glycogenic amino acids.

Abbreviations: ALT, alanine amino transferase; ATGL, adipocyte triglyceride lipase; BCAA, branched chain amino acids; DHAP, dihydroxyacetone phosphate; FoxO, forkhead box O; GPD, glycerol-3-phosphate dehydrogenase; GYK, glycerol kinase; G3P, glycerol-3-phosphate; G6Pase, glucose-6-phosphatase; HSL, hormone-sensitive lipase; MuRF, muscle RING finger protein-1; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; SUnSET, SUrface SEnsing of Translation; TCA, tricarboxylic acid; 4E-BP, eukaryotic initiation factor 4E-binding protein.

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1. Introduction

During nutrient deprivation, hepatic gluconeogenesis is stimulated to maintain blood glucose levels within a normal range, and supply sufficient energy for glucose-dependent tissues. In gluconeogenesis, several glycogenic precursors are utilized, including glycogenic amino acids, lactate, and glycerol derived from adipose tissue. These glycogenic precursors are converted to glucose by different glycogenic pathways [1–6].

Under fasting conditions, glycogenic amino acids such as alanine, serine and glycine are converted to pyruvate in the liver [7,8]. Other glycogenic amino acids are converted to metabolites in the tricarboxylic acid (TCA) cycle [9–11]. On the other hand, glycerol derived from adipocytes is phosphorylated by glycerol kinase (GYK) and converted to glycerol-3-phosphate (G3P) in the liver. Subsequently, G3P is oxidized to dihydroxyacetone phosphate (DHAP) and utilized for gluconeogenesis. This reaction is catalyzed by glycerol-3-phosphate dehydrogenase (GPD). There are two isoforms of GPD, GPD1 is an NAD^+/NADH dependent enzyme in the cytosol, and GPD2 is a FAD^+ dependent mitochondrial enzyme [12]. Both enzymes metabolize G3P to DHAP. Moreover, GPD1 and GPD2 play important roles as glycerol phosphate shuttles, transporting reducing equivalents to the mitochondria and improving the NAD^+/NADH ratio in the cytosol and the mitochondria [12].

A previous study showed that the hepatic glycerol phosphate levels in GPD1 null mice (BALB/cHeA) were 38% lower than those in control mice [13]. Moreover, the levels of DHAP and fructose bisphosphate were 2.9, and 1.6-fold higher than those in the control, respectively [13]. This study also suggested that the lack of GPD1 in skeletal muscles induces dysregulation of glycolysis owing to an impaired glycerol phosphate shuttle. These data indicate that GPD1 deficiency influences glucose metabolism and may induce the repression of gluconeogenesis in the livers of fasting mice.

In this study, we used a GPD1 null model, BALB/cHeA (HeA) mice, to examine whether GPD1 deficiency induces inhibition of gluconeogenesis from glycerol and hypoglycemia under fasting conditions.

2. Materials and Methods

2.1. Experimental Animals

BALB/cBy (By) mice were obtained from Japan CLEA (Tokyo, Japan). The origins of HeA mice and their breeding conditions have been described previously [14,15]. The mice were fed a normal laboratory diet (MF diet, Oriental Yeast, Tokyo, Japan) for a week to stabilize their metabolism, and maintained under a 12-h light–dark cycle at constant temperature (22 °C). Male mice aged 10–12 weeks were used in each experiment. 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).

2.2. Intraperitoneal Glycerol Tolerance Test

After 4 h of fasting, glycerol (cat. #075-00616, Wako Pure Chemical Industries, Osaka, Japan) (2 g/kg body weight) was injected intraperitoneally into the mice and blood was collected from the tail vein before injection and 30, 60, 90, and 120 min after injection. The blood glucose was measured using Breeze2 (cat. #R147, BAYER, Leverkusen, Germany).

2.3. Measurement of Blood Glucose Levels Under Fasting Conditions

Mice were transferred to a new cage for fasting. Blood was collected from the tail vein before fasting and 1–6 h after the start of fasting. Blood glucose was measured using Breeze2.

2.4. Quantitative Real-Time RT-PCR

RNA preparation and quantitative real-time RT-PCR (qRT-PCR) were performed as described previously [16]. The mouse-specific primer pairs are shown in Table 1.

2.5. Liver Glycogen

Glycogen content in the liver was measured in glycosyl units after acid hydrolysis as described previously [17].

2.6. Liquid Chromatography/Mass Spectrometry (LC/MS)

Water-soluble metabolites were extracted from the blood, liver and skeletal muscle with chloroform (cat. #038-02603, Wako Pure Chemical Industries, Osaka, Japan)/methanol (cat. #131-01826, Wako Pure Chemical Industries, Osaka, Japan). (2:1, υ/v with 10 nmol/L 2-(N-morpholino)ethanesulfonic acid (cat. #341-01622, Dojindo, Kumamoto, Japan)). Aqueous fractions were evaporated to dryness under vacuum. Samples were reconstituted with 100 μL distilled H_2O. The samples (10 μL) were then injected into the LC/MS system.

Water-soluble metabolite analysis was performed using a LCMS-8040 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan), in both positive and negative ionization modes equipped with an electrospray source ionization probe, LC-30AD binary pump (Shimadzu), SIL-30AC auto sampler (Shimadzu), and CTO-20AC column oven (Shimadzu). For HPLC analysis, a Discovery HS F5-3 (cat. #567503-U, 3 μm, 2.1 mm × 150 mm, Sigma-Aldrich, St Louis, MO) was used. Mobile phase A consisted of 100% water and mobile phase B consisted of 100% acetonitrile (cat. #018-19853, Wako Pure Chemical Industries, Osaka, Japan). Both mobile phases A and B were supplemented with 0.1% formic acid (cat. #067-04531, Wako Pure Chemical Industries, Osaka, Japan). The flow rate was 0.25 mL/min. The gradient was as follows: 0% B at 0 min, 0% B at 2 min, 25% B at 5 min, 65% B at 11 min, 95% B at 15 min, 95% B at 20 min, 100% B at 20.1 min and 100% B at 25 min. For MS analysis, the spray voltage was set to 3.5 kV, and the capillary and heater temperatures were set to 400 °C. The nebulizer gas flow rate was set to 2.0 L/min, and the drying gas flow rate was set to 15.0 L/min. The relative peak area for each species was normalized by the peak area of the internal standard.