Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Deletion of PHGDH in adipocytes improves glucose intolerance in diet-induced obese mice

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ARTICLE INFO

*Article history:*
Received 20 August 2018
Accepted 28 August 2018
Available online xxx

*Keywords:*
Serine
PHGDH
Adipocyte
Obesity
Glucose intolerance

ABSTRACT

Serine is a nonessential amino acid and plays an important role in cellular metabolism. In mammalian serine biosynthesis, 3-phosphoglycerate dehydrogenase (PHGDH) is considered a rate-limiting enzyme and is required for normal development. Although the biological functions of PHGHD in the nervous system have been intensively studied, its function in adipose tissue is unknown. In this study, we found that PHGDH is abundantly expressed in mature adipocytes of white adipose tissue. We generated an adipocyte-specific PHGDH knockout mouse (PHGDH FKO) and used it to investigate the role of serine biosynthesis in adipose tissues. Although PHGDH FKO mice had no apparent defects in adipose tissue development, these mice ameliorated glucose intolerance upon diet-induced obesity. Additionally, we found that the serine levels increase drastically in the adipose tissues of obese wild type mice, whereas no significant rise was observed in PHGDH FKO mice. Furthermore, wild type mice fed a serine-deficient diet also exhibited better glucose tolerance. These results suggest that PHGDH-mediated serine biosynthesis has important roles in adipose tissue glucose metabolism and could be a therapeutic target for diabetes in humans.



## 1. Introduction

Serine is a nonessential amino acid that plays an important role in cellular metabolism, including gluconeogenesis, one-carbon metabolism, and phospholipid synthesis [1,2]. Also, it has been shown that serine has diverse biological functions as a signaling molecule. For instance, D-serine, an enantiomer of L-serine, acts as an endogenous agonist of the N-methyl-D-aspartate (NMND) receptor and mediates neuronal excitation [3,4]. L-serine has been shown to be a natural ligand and allosteric activator of pyruvate kinase M2 and supports the proliferation of cancer cells [5]. It has also been reported that serine starvation induces p53-dependent metabolic remodeling in cancer cells and promotes cell survival [6].

In mammals, serine is synthesized from 3-phosphoglycerate through a three-step enzymatic reactions mediated by 3-phosphoglycerate dehydrogenase (PHGDH), phosphohydroxythreonine aminotransferase (PSAT1), and phosphoserine phosphatase (PSPH) [1,2]. Serine is also generated by reversible conversion of glycine by serine hydroxymethyltransferase (SHMT) [1,2]. Among these enzymes, PHGDH is considered the rate-limiting enzyme in the serine biosynthesis pathway, and its deficiency in human leads to severe neurological symptoms such as congenital microcephaly, severe psychomotor retardation, and intractable seizures [7]. Accordingly, the systemic deletion of PHGDH in mice results in the developmental abnormalities of the brain and in embryonic lethality [8]. Thus, the biological significance of PHGDH in the nervous system has been elucidated. Although PHGDH is expressed in other tissues, including liver, kidney, and adipose tissue [9–11], the biological importance of serine biosynthesis in

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https://doi.org/10.1016/j.bbrc.2018.08.180
0006-291X/

these tissues is poorly understood. In particular, the biological function of PHGDH in adipose tissue is unknown.

Several studies have demonstrated that blood concentrations of branched amino acids, including leucine, isoleucine, valine, tyrosine, and phenylalanine, are tightly associated with future diabetes development in human subjects [12,13]. Another study also indicated that serum levels of glycine are associated with insulin resistance [14]. However, the relationship between serine metabolism and diabetes remains unclear. In this study, we generated an adipocyte-specific PHGDH knockout mouse and used it to investigate the role of serine biosynthesis in adipose tissues.

## 2. Materials and methods

### 2.1. Mice

Adipocyte-specific PHGDH knockout (FKO) mice were generated by mating PHGDH flox mice [15] with Adiponectin-Cre mice [16]. All mice were maintained under a standard light cycle (12 h light/dark) and were allowed free access to water and food. For diet-induced obesity experiments, mice were fed a high-fat high-sucrose containing diet (HFHSD) (Research Diets: D12327) for 6 weeks. Serine-deficient diet (Research Diets: A05080213) was fed to 7-weeks old female C57BL/6 mice for 8 weeks. All of the animals care policies and procedures for the experiments were approved by the animal experiment committee at the University of Toyama.

### 2.2. Glucose tolerance test (GTT)

For the GTT experiments, the mice were fasted for 16 h and then injected with glucose (1 g/Kg body weight) intraperitoneally. The blood glucose concentration was measured using an automatic blood glucose meter (NOVA Biomedical).

### 2.3. Western blot analysis

Tissues were harvested from PHGDH WT and FKO mice. vWAT, sWAT, and BAT were prepared from gonadal, inguinal, and interscapular region, respectively. Harvested tissues were immediately frozen in liquid nitrogen and preserved in −80 °C until utilization. Frozen tissues were grinded by multibeads shocker (Yasui Kikai) with lysis buffer (10 mM Tris HCl, 2 mM EDTA, 0.1% Nonidet P-40, 150 mM NaCl) and centrifuged at 13000×*g* for 10 min at 4 °C. Then, supernatants were subjected to western blot analysis. SVF preparation was performed according to the method described elsewhere [17]. Primary antibodies used for western blot analysis were anti-PHGDH (Atlas Antibodies), anti-PSAT1 (Santa Cruz), anti-pan actin (Chemicon), anti-β-Tubulin (Cell Signaling), and anti-β-actin (Cell Signaling).

### 2.4. Real-time quantitative PCR

Total RNAs were extracted from adipose tissues and 3T3-L1 cells using RNEasy Lipid Tissue Mini Kit (QIAGEN) and TRI Reagent (Molecular Research Center, Inc.), respectively. cDNA was prepared using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Japan) according to the supplier's protocol. Real-time PCR was carried out using THUNDERBIRD SYBR qPCR Mix (Toyobo) on Thermal Cycler Dice Real Time System II (Takara Bio). Quantification was done by Delta Delta Ct method, and *Rpl13a* was used as a reference gene.

### 2.5. Measurements of serine levels in serum and adipose tissue by GC/MS

Serum was mixed with equal volume of methanol, and then centrifuge at 13000×*g* for 10 min at 4 °C. Adipose tissues were grinded by Multi Beads Shocker (Yasui Kikai) with methanol and water in the proportion of 1:1 (by volume), and then centrifuge 13000×*g* for 10 min at 4 °C. Supernatant was mixed with equal volume of chloroform, and mixture was centrifuge at 13000×*g* for 10 min at 4 °C. The upper aqueous phase was taken into a tube and evaporated using Speedvac SPD 1010 (Thermo). Quantification of serine was performed by Agilent 5977 MSD single Quad mass spectrometer coupled to Agilent 7890 Gas Chromatography with selected ion monitoring (SIM) mode. Evaporated samples were derivatized by methoxiamine hydrochloride and N-methyl-N-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS, Pierce). Details of GC/MS setting were described in elsewhere [18]. Amounts of metabolite were calculated by integrated sum of area using Mass Hunter Quantitative software (Agilent), and the absolute concentration was calculated using serine standard curve.

### 2.6. Histological staining

After the excision, the adipose tissues were fixed with 4% paraformaldehyde (Wako), and embedded in paraffin. The paraffin sections of 3 μm thickness were subjected to hematoxylin and eosin staining. Sample slides were observed using BX61 microscope (Olympus, Japan).

### 2.7. Statistical analysis

Statistical analysis was performed using an unpaired or paired Student's t-test. Data are expressed as the mean ± SD, and significant differences are confirmed statistically when *p*-value is less than 0.05.

## 3. Results

### 3.1. PHGDH is abundantly expressed in mature adipocytes of white adipose tissue

Although mouse ENCODE transcriptome data shows that *Phgdh* mRNA is ubiquitously expressed in mouse tissues [11], the protein expression of PHGDH in mouse tissues has not yet been determined. Thus, we examined the tissue distribution of PHGDH protein using various mice tissue lysates. Western blotting analysis confirmed that the brain exhibited abundant expression of PHGDH protein. Interestingly, PHGDH was also abundantly expressed in visceral white adipose tissue (vWAT) and subcutaneous white adipose tissue (sWAT), whereas brown adipose tissue (BAT) contained little PHGHD (Fig. 1A). We also quantified mRNA levels of *Phgdh*, *Psat1*, and *Psph* in adipose tissues, and found that vWAT expressed relatively higher levels of these serine synthesis enzyme mRNAs (Fig. 1B). Although the protein expression of PHGDH in WAT was stronger than that in BAT, the protein levels of PSAT1 were almost comparable between vWAT, sWAT, and BAT (Fig. 1C). WAT is divided into the mature adipocyte fraction (MAF) and the stromal vascular fraction (SVF), which includes stromal cells, pericytes, immune cells, and preadipocytes [19]. Therefore, we also determined the localization of PHGDH in WAT using MAF and SVF samples from wild type mice. Most of PHGDH and PSAT1 were expressed in the MAF, suggesting that serine biosynthesis in WAT occurs in mature adipocytes (Fig. 1D). We further examined whether there are any changes in these serine biosynthesis enzymes during adipogenesis

Please cite this article in press as: K. Okabe, et al., Deletion of PHGDH in adipocytes improves glucose intolerance in diet-induced obese mice, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.08.180