Gly482Ser mutation impairs the effects of peroxisome proliferator–activated receptor γ coactivator-1α on decreasing fat deposition and stimulating phosphoenolpyruvate carboxykinase expression in hepatocytes

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Abstract

Peroxisome proliferator–activated receptor γ coactivator-1α (PGC-1α) is a transcriptional coactivator of nuclear receptor peroxisome proliferator–activated receptor γ that critically regulates glucose and fat metabolism. Although clinical evidence suggests that Gly482Ser polymorphism of PGC-1α is associated with an increased incidence of nonalcoholic fatty liver disease, a direct role for Gly482Ser mutation in altering PGC-1α actions on hepatocyte fat deposition remains to be explored. We hypothesized that Gly482Ser mutation impairs the abilities of PGC-1α in ameliorating overnutrition-induced hepatocyte fat deposition and in stimulating hepatocyte expression of cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C; encoded by a key PGC-1α target gene). In the present study, treatment of cultured hepatocytes with palmitate induced fat deposition, serving as a cell model of hepatic steatosis. Upon overexpression of wild-type PGC-1α, H4IIE cells exhibited a significant decrease in palmitate-induced hepatocyte fat deposition compared with control cells and/or cells upon overexpression of mutant PGC-1α (Gly482Ser). Overexpression of wild-type PGC-1α, but not mutant PGC-1α, also caused a significant increase in hepatocyte expression of carnitine palmitoyltransferase 1a, a rate-determining enzyme that transfers long-chain fatty acids into mitochondria for oxidation. In addition, overexpression of mutant PGC-1α did not stimulate PEPCK-C expression as overexpression of wild-type PGC-1α did, likely due to a decrease in the ability of mutant PGC-1αs in increasing PEPCK promoter transcription activity. Together, these results suggest that Gly482Ser mutation impairs the abilities of PGC-1α in decreasing fat deposition and in stimulating PEPCK-C expression in cultured hepatocytes.

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Abbreviations: NAFLD, nonalcoholic fatty liver disease; ACC1, acetyl-CoA carboxylase 1; FAS, fatty acid synthase; PPARγ, peroxisome proliferator–activated receptor γ; PGC-1α, peroxisome proliferator–activated receptor γ coactivator-1α; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; HNF-4α, hepatic nuclear factor-4α; CPT1α, carnitine palmitoyltransferase 1α; PGC-1β, peroxisome proliferator–activated receptor γ coactivator-1β.

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1. Introduction

Nutrient overload induces fat deposition in hepatocytes (hepatic steatosis), which is a characteristic of nonalcoholic fatty liver disease (NAFLD) [1]. When the liver develops overt inflammation and necrotic damage that are not associated with alcohol consumption, simple steatosis progresses to nonalcoholic steatohepatitis (NASH) [1, 2]. As the advanced form of NAFLD, NASH is considered one of the most common causes of liver cirrhosis and hepatocellular carcinoma [3–12]. In addition, hepatic steatosis is a major contributor of dyslipidemia, thereby increasing cardiovascular disease risk [13]. Given this, there is a critical need to increase understanding of the mechanisms underlying the development of hepatic steatosis.

In hepatocytes, excessive nutrients are converted into fats largely through lipogenesis, in which acetyl-CoA carboxylase 1 (ACCo1) and fatty acid synthase (FAS) are key rate-determining enzymes that increase the synthesis of free fatty acids as the required substrates for the generation of triglycerides [14, 15]. Of importance, nutritional and hormonal signals, that is, glucose and insulin, act through a number of transcription factors and transcriptional coactivators to tightly regulate the expression of enzymes for lipogenesis, as well as enzymes for fatty acid oxidation [14–17]. The latter is also a key metabolic pathway that controls hepatocyte fat deposition. Among transcription factors and transcriptional coactivators that are involved in nutrient metabolism, peroxisome proliferator–activated receptor γ (PPARγ) coactivator-1α (PGC-1α), a member of the transcriptional coactivator family of nuclear receptor PPARγ, exerts powerful effects on hepatic glucose and fat metabolism [18, 19]. Physiologically, PGC-1α is strongly induced in the liver by fasting [20]. This increases hepatic expression of genes for gluconeogenic enzymes including phosphoenolpyruvate carboxykinase (PCK) and glucose-6-phosphatase (G6Pase), through the mechanisms involving hepatic nuclear factor-4α (HNF-4α) and forkhead box protein O1 [20, 21]. As a result, PGC-1α protects mice from fasting-induced hypoglycemia. Moreover, PGC-1α critically stimulates rates of fatty acid oxidation. In support of this, reducing hepatic PGC-1α activity diminishes the hepatic gene program of fatty acid oxidation [19], which appears to account for fasting-induced hepatic steatosis in PGC-1α–deficient mice [22]. In contrast, PGC-1α overexpression in mouse primary hepatocytes increases the expression of carnitine palmitoyltransferase 1a (CPT1a) [19], activation of which increases the transfer of long-chain fatty acids from cytosol into mitochondria for oxidation. Because of this, PGC-1α is an essential regulator of hepatocyte fat deposition, thereby being a likely determinant in the development of hepatic steatosis.

In human subjects, PGC-1α is encoded by PPARGC1A, which, in regulating glucose and fat metabolism, is manifested by clinical aspects associated with Thr394Thr and Gly482Ser mutations of PGC-1α. Although it is known that Gly482Ser polymorphism of PGC-1α contributes to the development of insulin resistance and type 2 diabetes mellitus [23–25], Gly482Ser polymorphism of PGC-1α also appears to be associated with increased incidence of NAFLD [26]. However, a direct effect of Gly482Ser variation on altering PGC-1α actions on hepatocyte fat deposition remains to be determined. For the current study, we hypothesized that PGC-1α plays a direct role in regulating hepatocyte fat metabolism and gene expression and that this role is impaired by Gly482Ser mutation of PGC-1α. To test our hypothesis, we examined the effects of wild-type (Gly482) and mutant (Ser482) PGC-1α on hepatocyte fat deposition and expression of PEPCK-C, the product of a key downstream target gene of PGC-1α, in cultured hepatocytes. The rationale of the present study is that the results obtained provide cellular evidence to support the necessity of human subjects with Gly482Ser variation of PGC-1α in avoiding nutrient overload to decrease the incidence of NAFLD.

2. Methods and materials

2.1. Plasmid constructs

The plasmid containing the complementary DNA (cDNA) that encodes for wild-type PGC-1α with glycine (G) at the 482 site (pcDNA3.1-PGC-1α(G)) was a generous gift from Dr Youngmi Kim Pak (Kyung Hee University, Seoul, Korea). This plasmid served as the template to mutate glycine-482 to serine using site-directed mutagenesis according to the procedures provided with the kit (Invitrogen of Life Technologies, Grand Island, NY, USA). The resultant plasmid contained the cDNA that encodes for mutant PGC-1α with serine (S) at the 482 site and was referred to as pcDNA3.1-PGC-1α(S). The plasmid that contained the cDNA of HNF-4α (pcDNA3-HNF-4α) was a gift from Dr Jongsook Kim Kemper (University of Illinois, Chicago, IL, USA). An empty pcDNA3.1 plasmid was used as the control. Phosphoenolpyruvate carboxykinase promoter fragment (−1340 to +300) was amplified from human leukocyte genome DNA using polymerase chain reaction (PCR) with forward primers 5′-CGACCCGTTTCTAAAGTGGTTTGGTCG-GAGG-3′ and reverse primer 5′-GAAGATCTCTGCAGAGTGCCGG-3′. The PCR product was then cloned into the pGL3-basic vector to generate a luciferase reporter construct (pGL3-hPCK-luc).

2.2. Cell culture and treatment

H4IIE cells (rat hepatoma cells), HepG2 cells (human hepatoma cells), and LO2 cells (noncancerous human liver cells) were maintained in high-glucose Dulbecco modified Eagle medium that was supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μG/L streptomycin, as previously described [27, 28]. At 80% confluence, both H4IIE cells and HepG2 cells were transfected with pcDNA3.1-PGC-1α(G), pcDNA3.1-PGC-1α(S), and/or a control plasmid (Ctrl), as previously described [29], and using Lipofectamine 2000 (Invitrogen of Life Technologies, Grand Island, NY, USA) according to the procedures provided with the kit. Twenty-four hours after transfection, the transfected cells were subjected to the assays.

2.3. Examination of hepatocyte fat deposition

To examine hepatocyte fat deposition, the transfected cells were treated with palmitate (250 μM) or bovine serum albumin (BSA) in phosphate-buffered saline for an additional 24 hours
and were stained with Oil-Red-O for the last hour. A colorimetric assay was then used to quantify fat content [27,28], which was expressed in arbitrary unit after normalization by cell proteins.

2.4. Quantifications of hepatocyte gene expression

To analyze hepatocyte expression of genes for enzymes involved in fatty acid oxidation and lipogenesis, the total RNA of the transfected H4IIE cells was prepared and used for real-time reverse transcriptase (RT) PCR [27,28]. To analyze the effect of Gly482Ser mutation of PGC-1α on PEPCK-C expression, the messenger RNA (mRNA) and protein levels of PEPCK-C in the transfected HepG2 cells were quantified using real-time RT-PCR and Western blot, respectively.

2.5. Examination of hepatocyte insulin signaling

To determine changes in hepatocyte insulin signaling, the transfected H4IIE cells were treated with or without insulin (100 nM) for 30 minutes before harvest. Cell lysates were subjected to Western blots, as described below.

2.6. Analyses of transcription control of hepatocyte PEPCK expression

To address the effect of Gly482Ser mutation of PGC-1α on transcriptional control of PEPCK-C expression, HepG2 and LO2 cells were transfected with pcDNA3.1-PGC-1α (G), pcDNA3.1-PGC-1α (S), and/or a control plasmid, as described above. Meanwhile, both HepG2 and LO2 cells were cotransfected with pGL3-hPCK-luc and/or control plasmid pRL-SV40 in the presence or absence of pcDNA3-HNF-4α. After cotransfection for 48 hours, the cells were harvested, and cell lysates were subjected to the assay to measure luciferase activity using a commercial kit (Promega Corporation, Madison, WI, USA).

2.7. RNA isolation, reverse transcription, and real-time PCR

The total RNA was isolated from cultured cells. RNA isolation and real-time RT-PCR were conducted as previously described [27,29–31]. The mRNA levels were analyzed for human PGC-1α, PPARγ coactivator-1β (PGC-1β), CPT1a, ACC1, FAS, and/or PEPCK-C. A total of 0.1 μg RNA was used for the determination. Results were normalized to 18s ribosomal RNA.

Fig. 1 – Gly482Ser mutation impairs the ability of PGC-1α in decreasing hepatocyte fat deposition. H4IIE hepatocytes were transfected with pcDNA3.1-PGC-1α (G), pcDNA3.1-PGC-1α (S), and/or a control plasmid (Ctrl). After transfection for 24 hours, cells were treated with palmitate (Pal; 250 μM) or BSA (Ctrl) for an additional 24 hours. All experiments were performed at least in quadruplicates. A, Hepatocyte overexpression of wild-type or mutant PGC1α examined by Western blot analyses. B, Hepatocyte fat deposition. After the treatment regimen, cells were stained with Oil-Red-O for 1 hour. C, Quantification of fat content was performed using a colorimetric assay and expressed in arbitrary unit (AU) after normalization by cell proteins. Data are means ± SE, n = 4. **P < .01 palmitate vs negative control (BSA); ††P < .01 PGC-1α (G) vs control vector in the presence of palmitate; ‡P < .05 PGC-1α (S) vs PGC-1α (G) in the presence of palmitate.

Fig. 2 – Gly482Ser mutation impairs the ability of PGC-1α in increasing hepatocyte CPT1α expression. H4IIE hepatocytes were transfected with pcDNA3.1-PGC-1α (G), pcDNA3.1-PGC-1α (S), and/or a control plasmid (Ctrl). After transfection for 24 hours, the transfected cells were incubated in a growth medium for an additional 24 hours. Hepatocyte expression of genes related to fatty acid oxidation and lipogenesis was analyzed using real-time RT-PCR. Experiments were performed in quadruplicates. Data are means ± SE, n = 4. **P < .01 PGC-1α (G) vs Ctrl; ††P < .01 PGC-1α (S) vs PGC-1α (G).
2.8. Western blots

Lysates were prepared from cultured cells using the lysis buffer containing 50 mM HEPES (pH 7.4), 1% Triton X-100, 50 mM sodium pyrophosphate, 0.1 M sodium fluoride, 10 mM EDTA, 10 mM sodium orthovanadate, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 2 mM benzamidine, and 2 mM phenylmethylsulfonyl fluoride. The levels of PGC-1α, PEPCK, Akt1/2, and phospho-Akt (Ser473) were analyzed using rabbit antiserum as the primary antibody at a 1:1000 dilution. The blot was followed by a 1:10 000 dilution of goat antirabbit horseradish peroxidase-conjugated secondary antibody kit (Immobilon Western; EMD Millipore, Billerica, MA, USA).

2.9. Statistical analyses

Numeric data were presented as means ± SE. Statistical significance was assessed by unpaired, 2-tailed Student t tests. Differences were considered significant at the 2-tailed P < .05, as previously described [27–29,31]. All cell experiments were performed a minimum of 4 times and did not involve a power analysis.

3. Results

3.1. Gly482Ser mutation impairs the effect of PGC-1α overexpression on ameliorating hepatocyte fat deposition

To address a direct effect of Gly482Ser mutation of PGC-1α on altering hepatic fat metabolism, wild-type PGC-1α(G) or mutant PGC-1α(Ser482Ser, S) was overexpressed in H4IIE cells, which is a rat hepatocyte cell line that has been used to analyze overnutrition-related hepatocyte metabolic and

Fig. 3 – Gly482Ser mutation of PGC-1α does not alter hepatocyte insulin signaling. H4IIE hepatocytes were transfected with pcDNA3.1-PGC-1α(G), pcDNA3.1-PGC-1α(S), and control plasmid for 48 hours. The transfected cells were then treated with or without insulin (100 nM) for 30 minutes before harvest. Cell lysates were subjected to Western blot analysis. Akt1/2 and phospho-Akt (Ser473) were examined. Experiments were performed in quadruplicates.

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inflammatory responses [32]. Compared with the control (empty vector), transfection of the plasmid containing PGC-1α(G) or PGC-1α(S) caused a comparable increase in the amount of PGC-1α (Fig. 1A). Thus, the subsequent differences in metabolic responses between PGC-1α(G)- and PGC-1α(S)-overexpressing hepatocytes were attributable to Ser482Ser mutation of PGC-1α. As expected, palmitate treatment induced fat accumulation in empty vector-transfected hepatocytes (control; Fig. 1B, C). Upon overexpression of PGC-1α(G) but not overexpression of PGC-1α(S), hepatocyte fat accumulation was significantly decreased (Fig. 1B, C). Thus, Gly482Ser mutation appears to impair the ability of PGC-1α in ameliorating overnutrition-induced hepatocyte fat deposition.

3.2. Gly482Ser mutation blunts the effect of PGC-1α overexpression on stimulating hepatocyte CPT1α expression

To explore the insight of fat deposition, the expression of genes for key enzymes involved in fatty acid oxidation and lipogenesis was examined. Compared with controls, overexpression of PGC-1α(G) but not overexpression of PGC-1α(S) caused a significant increase in the mRNA levels of CPT1α (Fig. 2). However, overexpression of either PGC-1α(G) or PGC-1α(S) did not significantly alter the expression of PGC-1β, ACC1, or FAS expression. Thus, Gly482Ser mutation may blunt the effect of PGC-1α on increasing hepatocyte fatty acid oxidation, and PGC-1α likely has a limited role in altering hepatocyte lipogenesis.

3.3. Gly482Ser mutation of PGC-1α does not alter hepatocyte insulin signaling

In genetically modified mice, altering PGC-1α brings about controversial effects on liver insulin sensitivity [19,22]. In the present study, overexpression of PGC-1α(G) or PGC-1α(S) did not significantly alter hepatocyte Akt (Ser473) phosphorylation at either the basal or the insulin-stimulated condition compared with controls (Fig. 3). Thus, PGC-1α likely has a limited role in directly altering hepatocyte insulin signaling, and this action of PGC-1α remains unchanged in the presence of Gly482Ser mutation.

3.4. Gly482Ser mutation decreases the effect of PGC-1α overexpression on stimulating hepatocyte PEPCK-C expression

Stimulating PEPCK expression is a downstream event of PGC-1α actions [20,21]. We examined the extent to which Gly482Ser mutation alters PGC-1α action on stimulating hepatocyte PEPCK-C expression. In cultured HepG2 cells, overexpression of either PGC-1α(G) or PGC-1α(S) was evident (Fig. 4A). In addition, the expression of PGC-1α(S) at both protein and mRNA levels was comparable with that of PGC-1α(G) and was not altered by cooverexpression of HNF-4α. Under this condition, PEPCK-C expression was analyzed. Compared with the controls, overexpression of either PGC-1α(G) or PGC-1α(S) caused a slight but significant increase in the mRNA and/or protein levels of PEPCK-C in cells without overexpression of HNF-4α (Fig. 4B). In the presence of HNF-4α, the mRNA and protein levels of PEPCK-C were markedly increased in cells overexpressing PGC-1α(G) and/or PGC-1α(S). However, the efficacy of PGC-1α(S) overexpression at increasing PEPCK-C expression was much smaller than that of PGC-1α(G) overexpression. Thus, Ser482Ser mutation appears to impair the effect of PGC-1α on stimulating PEPCK-C expression when HNF-4α is at a high abundance.

3.5. Gly482Ser mutation decreases the effect of PGC-1α overexpression on stimulating hepatocyte PEPCK-C expression

To gain a molecular insight of how Gly482Ser mutation decreases the effect of PGC-1α on stimulating hepatocyte PEPCK-C expression, we performed the reporter gene analysis. In HepG2 cells, overexpression of PGC-1α(G) but not overexpression of PGC-1α(S) increased the PEPCK-C promoter activity in the presence of overexpressed HNF-4α (Fig. 5A). Similar results were also observed in LO2 cells (Fig. 5B). These results suggest that Ser482Ser mutation decreases the effect of PGC-1α on stimulating PEPCK-C expression through impairing the transcription activity of PGC-1α.

4. Discussion

Investigations of how white and brown adipose tissues developed led to the discovery of PGC-1α [33], and since...
then, mounting evidence has documented PGC-1α as an essential transcriptional coactivator that orchestrates nutrient metabolism in many tissues [34]. For example, hepatic PGC-1α is induced by fasting, which in turn works with HNF-4α and forkhead box protein O1 to stimulate the expression of genes for gluconeogenic enzymes and protects against fasting-induced hypoglycemia [20,21]. In addition, the activity of PGC-1α correlates well with the degree of liver fat deposition (hepatic steatosis), as this is supported by the data obtained from genetically modified mice [19,22]. Clinical studies demonstrate that Gly482Ser polymorphism of PGC-1α, which is believed to impair PGC-1α actions, is associated with increased incidence of NAFLD [26]. However, the cellular evidence validating the extent to which Gly482Ser variation impairs PGC-1α actions still remains to be explored.

In the present study, we demonstrated a direct effect of PGC-1α on decreased hepatocyte fat deposition. Notably, treatment of hepatocytes with palmitate induced fat deposition, and this effect of palmitate was partially blunted by overexpression of wild-type PGC-1α. These results served as the in vitro evidence to support a direct role for PGC-1α in protecting against overnutrition-induced hepatocyte fat deposition and are in agreement with the in vivo role of PGC-1α in protecting against hepatic steatosis [19,22]. Consistent with the powerful effect of PGC-1α on stimulating fatty acid oxidation [34], overexpression of wild-type PGC-1α caused an increase in CPT1α expression in cultured hepatocytes. Given the role of CPT1α in transferring long-chain fatty acid into mitochondria as a rate-determining step of fatty acid oxidation, PGC-1α stimulation of hepatocyte CPT1α expression contributed to the action of PGC-1α on ameliorating palmitate-induced fat deposition. Gly482Ser mutation nearly diminished the effects of PGC-1α on ameliorating palmitate-induced fat deposition and on increasing CPT1α expression, and Gly482Ser mutation appeared to reduce the ability of PGC-1α in stimulating hepatic fatty acid oxidation, thus contributing to the blunted effect on ameliorating palmitate-induced hepatocyte fat deposition. In addition to increasing CPT1α expression, PGC-1α stimulated PEPCK-C expression. This stimulatory effect of PGC-1α appeared to also contribute to the action of wild-type PGC-1α overexpression on ameliorating palmitate-induced hepatocyte fat deposition, as discussed below. Nonetheless, the present study provides cellular evidence that may underlie the increased incidence of hepatic steatosis in human subjects with Gly482Ser polymorphism of PGC-1α [26].

A stimulatory effect of PGC-1α on hepatic PEPCK-C expression has been repeatedly reported since the identification of PGC-1α as a transcriptional coactivator [20,21]. Physiologically, PGC-1α stimulation of hepatic PEPCK-C expression contributes to the effect of PGC-1α’s protection against fasting-induced hypoglycemia. Pathologically, however, roles for PGC-1α in stimulating PEPCK-C expression in relation to glucose and fat metabolic responses and insulin sensitivity are complicated and vary depending on the models studied. For instance, PGC-1α heterozygous disruption brings about a decrease in the effect of glucagon on inducing hepatic expression of PEPCK-C, which is accompanied by a decrease in hepatocyte insulin signaling [19]. In contrast, PGC-1α deficiency in mice protects against high-fat diet-induced insulin resistance [22]. In the latter study, however, PEPCK-C expression is not examined. Given this, the changes in hepatic PEPCK-C expression as a result of PGC-1α overexpression appear to have a limited role in regulating liver insulin sensitivity. Based on substantial evidence in the present study, both wild-type and mutant PGC-1α did not alter hepatocyte insulin signaling while Gly482Ser mutation impaired the ability of PGC-1α in stimulating hepatocyte PEPCK-C expression. If a similar case also exists in the in vivo condition, hepatic events in subjects with Gly482Ser polymorphism of PGC-1α likely have a limited role in the development of insulin resistance.

Cytoplasmic phosphoenolpyruvate carboxykinase is also considered an essential enzyme that generates glycerol-3-phosphate through glyceroneogenesis to provide a required substrate for triglyceride synthesis. Because of this, increased PEPCK-C contributes to fat deposition. This is particularly true in adipocytes/adipose tissue [35]. However, in the liver, PEPCK-C appears to act through a different mechanism to contribute to fat deposition [36]. When PEPCK-C is disrupted, the liver accumulates tricarboxylic acid cycle intermediates. The latter, in turn, inhibits hepatic fatty acid oxidation, thereby bringing about hepatic steatosis. Consistent with this mechanism in the present study, overexpression of mutant PGC-1α decreased hepatocyte PEPCK-C expression at both mRNA and protein levels when compared with wild-type PGC-1α. In addition, these effects of mutant PGC-1α were accompanied by increased hepatocyte fat deposition. Of interest, as discussed above, Gly482Ser mutation of PGC-1α decreased hepatocyte CPT1α expression, which is indicative of decreased fatty acid oxidation. Given this, it would be interesting to determine whether and how PEPCK-C is linked to CPT1α in hepatocytes in a future study.

Although confirming the role of PGC-1α in increasing transcription activity of PEPCK-C promoter, the present study also demonstrates Gly482 as a critical amino acid that is necessary for PGC-1α to fully increase transcription activity of PEPCK-C promoter in the presence of overexpressed HNF4α. However, it remains to be elucidated whether Gly482 is needed for PGC-1α to fully bind to PEPCK-C promoter, and whether Gly482 is needed for PGC-1α to fully interact with overexpressed HNF4α. These questions will be addressed by our future study. Nonetheless, it is confirmed that PGC-1α stimulates hepatocyte PEPCK-C expression, and this stimulatory effect of PGC-1α is impaired by Gly482Ser mutation. Our results are similar to those observed in the study by Choi et al [37], in which Gly482Ser mutation was shown to decrease the coactivator activity of PGC-1α on mitochondrial transcription factor A promoter. In contrast, a study by Nitz et al [38] indicated that Gly482Ser and Thr612Met variations do not impair the ability of PGC-1α in interacting with PPARγ. The reasons for the discrepancy are not clear but could be related to differences in promoters that were chosen for the studies.

In summary, the present study provides evidence supporting that Gly482Ser mutation impairs the abilities of PGC-1α in ameliorating overnutrition-induced fat deposition and in stimulating PEPCK-C expression. As such, we accept our hypothesis for the study. In addition, avoiding nutrient overload should be seriously considered for human subjects with Gly482Ser variation of PGC-1α to decrease the incidence of NAFLD. Given...
that human subjects with Gly482Ser variation of PGC-1α may only have physiological levels of PGC-1α, our study is limited in that we used PGC-1α overexpression to address the extent to which Gly482Ser mutation alters PGC-1α actions.

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