Regulatory Effects of Glycine on Hepatic Energy Metabolism and Related Gene Expression in Lipopolysaccharide-Challenged Piglets: Post-print

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Abstract

This study aimed to investigate the regulatory effects of glycine (Gly) on liver energy metabolism, key enzymes of energy metabolism, and mRNA expression of related regulatory factors in lipopolysaccharide (LPS)-challenged weaned piglets. Twenty-four Duroc × Landrace × Large White piglets were selected and divided into 4 groups with 6 replicates per group. The four groups were: 1) control group (basal diet); 2) LPS group (LPS + basal diet); 3) 1.0% Gly group (LPS + basal diet + 1.0% Gly); and 4) 2.0% Gly group (LPS + basal diet + 2.0% Gly). On day 28 of the experiment, piglets in the treatment groups received an intraperitoneal injection of 100 g/kg BW LPS, while the control group received an equal volume of saline. Experimental pigs were slaughtered 4 h after LPS or saline injection, and liver samples were collected for analysis. The results showed: 1) Compared with the LPS group, 1.0% Gly significantly increased the concentrations of adenosine triphosphate (ATP) and energy charge (EC) levels in the liver of piglets (P<0.05), significantly decreased the AMP/ATP ratio (P<0.05), and tended to decrease AMP concentration (P<0.10); 2.0% Gly tended to decrease the AMP/ATP ratio (P<0.10). 2) Compared with the LPS group, 1.0% Gly significantly decreased the mRNA expression levels of hexokinase 2 (Hexok2) and citrate synthase (CS) in the liver of piglets (P<0.05); 2.0% Gly significantly decreased the mRNA expression levels of Hexok2 and pyruvate kinase (PK) in the liver (P<0.05), and tended to decrease the mRNA expression level of CS in the liver (P<0.10). 3) Compared with the LPS group, 1.0% Gly significantly increased the mRNA expression level of AMP-activated protein kinase $ 1($AMPK$ $1) in the liver of piglets (P<0.05). In conclusion, dietary Gly supplementation can ameliorate liver energy metabolism disorder induced by LPS challenge and regulate the expression of related enzymes in metabolic

pathways such as glycolysis and the tricarboxylic acid cycle.

Full Text

Regulatory Role of Glycine on Energy Metabolism and Related Gene Expression in the Liver of Piglets Challenged with Lipopolysaccharide

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Abstract: This experiment was conducted to investigate the regulatory effects of glycine (Gly) on hepatic energy metabolism and the mRNA expression of key enzymes and regulatory factors involved in energy metabolism in lipopolysaccharide (LPS)-challenged weaned piglets. Twenty-four Duroc × Landrace × Yorkshire piglets were randomly assigned to four groups with six replicates per group and one pig per replicate. The four groups were: 1) control group (basal diet), 2) LPS group (LPS + basal diet), 3) 1.0% Gly group (LPS + basal diet + 1.0% Gly), and 4) 2.0% Gly group (LPS + basal diet + 2.0% Gly). On day 28 of the experiment, piglets in the treatment groups received an intraperitoneal injection of 100 g/kg BW LPS, while those in the control group received an equivalent volume of physiological saline. All pigs were slaughtered 4 h post-injection, and liver samples were collected for analysis.

The results showed that: 1) Compared with the LPS group, dietary supplementation with 1.0% Gly significantly increased hepatic adenosine triphosphate (ATP) concentration and energy charge (EC) level ($P < 0.05$), significantly decreased the AMP/ATP ratio ($P < 0.05$), and tended to reduce AMP concentration ($P < 0.10$). Supplementation with 2.0% Gly tended to decrease the hepatic AMP/ATP ratio ($P < 0.10$). 2) Dietary 1.0% Gly significantly downregulated the mRNA expression of hexokinase 2 (Hexok2) and citrate synthase (CS) in the liver compared with the LPS group ($P < 0.05$). Supplementation with 2.0% Gly significantly decreased the mRNA expression of hepatic Hexok2 and pyruvate kinase (PK) ($P < 0.05$) and tended to reduce CS mRNA expression ($P < 0.10$). 3) Compared with the LPS group, 1.0% Gly supplementation significantly increased the mRNA expression of hepatic AMP-activated protein kinase $ 1($AMPK$ $1) ($P < 0.05$).

In conclusion, dietary Gly supplementation can ameliorate LPS-induced hepatic energy metabolism disorders and modulate the expression of key enzymes in glycolysis and the tricarboxylic acid cycle in weaned piglets.

Keywords: piglets; lipopolysaccharide; glycine; liver; energy metabolism

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Introduction

The liver is a crucial immune organ in animals and a primary site for substance and energy metabolism. Lipopolysaccharide (LPS), a structural component of the outer membrane of Gram-negative bacteria, can induce immune stress in the body. During immune stress, immune cells become activated and release large quantities of inflammatory cytokines, leading to hepatic inflammatory responses, increased energy expenditure, and damage to liver structure and function.

Glycine (Gly) is the simplest amino acid structure and a non-essential amino acid that can be synthesized by the body, yet it plays vital physiological roles. As a functional amino acid, Gly can inhibit endotoxin-induced liver injury and protect hepatocytes from adenosine triphosphate (ATP) depletion. Additionally, as a glucogenic amino acid, Gly can be metabolized to produce alanine (Ala), which can enter the tricarboxylic acid cycle to provide energy. Gly is also a precursor for glutathione (GSH) synthesis, and reduced GSH participates in the tricarboxylic acid cycle and glucose metabolism, enabling high-energy production. Previous research has demonstrated that dietary supplementation with 0.5%, 1.0%, and 2.0% Gly enhanced the antioxidant capacity of suckling piglets. Supplementation with no more than 2% Gly in piglet diets is considered safe and beneficial for normal physiological function and feed intake, whereas excessive Gly may disrupt amino acid balance and exert toxic effects. Despite these known functions, few studies have investigated the effects of Gly on hepatic energy metabolism in piglets. Therefore, this study established an immune stress model by injecting LPS into weaned piglets and examined whether dietary Gly at 1% and 2% concentrations could alleviate hepatic energy metabolism disorders and determine the appropriate supplementation level.

1.1 Materials and Reagents

Glycine (purity > 99.5%) and alanine (purity > 99.5%) were purchased from Wuhan Amino Technology Co., Ltd. Lipopolysaccharide (E. coli serotype O55:B5) was obtained from Sigma-Aldrich and administered at a dose of 100 g/kg BW.

1.2 Experimental Animals and Design

Twenty-four weaned piglets [(21 ± 1) days old, Duroc × Landrace × Yorkshire, average body weight (7.17 ± 0.41) kg] with similar body condition were randomly allocated to four groups (six replicates per group, one pig per replicate) for a 28-day feeding trial. The groups were: 1) control (basal diet), 2) LPS (LPS + basal diet), 3) 1.0% Gly (LPS + basal diet + 1.0% Gly), and 4) 2.0% Gly (LPS + basal diet + 2.0% Gly). All diets were isonitrogenously balanced with alanine. On day 28, piglets in the treatment groups received an intraperitoneal injection of 100 g/kg BW LPS, while the control group received an equal volume of physiological saline. During the trial, ambient temperature was maintained at 25–28 °C. Each pen measured 1.20 m × 1.10 m. Pigs were fed powdered diets ad libitum with free access to water.

1.3 Experimental Diets

The basal diet was formulated according to NRC (1998) nutrient requirements for piglets. The composition and nutrient levels of the basal diet are presented in Table 1 . The premix provided per kilogram of diet: VA 12,000 IU, VB1 1.5 mg, VB6 3 mg, VB12 18 g, VD3 2,500 IU, VE 30 IU, VK3 3 mg, riboflavin 4 mg, nicotinic acid 40 mg, choline chloride 400 mg, folic acid 700 g, pantothenic acid 15 mg, biotin 100 g, Mn 20 mg, Se 0.36 mg, Zn 80 mg, Cu 25 mg, Fe 83 mg, I 0.48 mg. Digestible energy, lysine, methionine, tryptophan, and threonine were calculated values, while other nutrients were measured values.

1.4 Liver Sample Collection

Four hours after LPS or saline injection, piglets were anesthetized with intravenous sodium pentobarbital (80 mg/kg BW), slaughtered, and liver samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C until analysis.

1.5 Analytical Methods

1.5.1 Determination of Hepatic Energy Metabolite Concentrations Concentrations of ATP, ADP, and AMP in liver tissue were analyzed by high-performance liquid chromatography according to the method of Hou et al. Total adenine nucleotide (TAN) concentration and energy charge (EC) were calculated using the following formulas:

TAN = ATP + ADP + AMP
EC = (ATP + 0.5ADP) / (ATP + ADP + AMP)

1.5.2 mRNA Expression Analysis Target genes included: hexokinase 2 (Hexok2), phosphofructokinase (L-PFK), pyruvate dehydrogenase (PDH), pyruvate kinase (PK), isocitrate dehydrogenase (ICDH), citrate synthase (CS), acyl-coenzyme A oxidase (ACO), carnitine palmitoyltransferase 1 (L-CPT1), AMP-

activated protein kinase (AMPK), silent information regulator 1 (Sirt1), and proliferator-activated receptor-γ coactivator 1α (PGC1α). Total RNA extraction, cDNA synthesis, and real-time quantitative PCR were performed according to Liu et al. Real-time PCR primers (Table 2) were synthesized by Takara Bio (Dalian) Co., Ltd. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal reference gene, and relative mRNA expression was calculated using the $2-\Delta\Delta$CT method of Livak et al.

1.6 Statistical Analysis

Data were analyzed using SPSS 17.0 software for ANOVA and LSD multiple comparisons. Results are expressed as means $\pm$ SEM. Differences were considered significant at $P < 0.05$ and trends at $P < 0.10$.

Results

2.1 Hepatic Adenine Nucleotide Levels

As shown in Table 3 , LPS challenge significantly decreased hepatic ATP, ADP, and TAN concentrations compared with the control group ($P < 0.05$). Dietary supplementation with 1.0% Gly significantly increased hepatic ATP concentration and EC level ($P < 0.05$), significantly reduced the AMP/ATP ratio ($P < 0.05$), and tended to decrease AMP concentration ($P < 0.10$) compared with the LPS group. Supplementation with 2.0% Gly tended to reduce the hepatic AMP/ATP ratio ($P < 0.10$) but had no significant effects on other parameters ($P > 0.05$).

2.2 mRNA Expression of Genes Related to Glycolysis, Tricarboxylic Acid Cycle, and Fatty Acid β-Oxidation

Table 4 shows that LPS challenge significantly upregulated the mRNA expression of Hexok2 and PK in glycolysis and ICDHβ and CS in the tricarboxylic acid cycle ($P < 0.05$), while significantly downregulating L-PFK mRNA expression ($P < 0.05$) and tending to increase PDH mRNA expression ($P < 0.10$). Compared with the LPS group, 1.0% Gly supplementation significantly decreased hepatic Hexok2 and CS mRNA expression ($P < 0.05$). Supplementation with 2.0% Gly significantly reduced Hexok2 and PK mRNA expression ($P < 0.05$) and tended to decrease CS mRNA expression ($P < 0.10$).

2.3 mRNA Expression of Hepatic Energy Metabolism Regulatory Factors

As presented in Table 5 , LPS challenge significantly increased the mRNA expression of hepatic AMPK$ 2*and**Sirt*1($P < 0.05$).*Dietarysupplementationwith*1.0 $1 mRNA expression compared with the LPS group ($P < 0.05$).

Discussion

ATP is the direct energy source in the body, releasing substantial energy when hydrolyzed to ADP and inorganic phosphate or to AMP and pyrophosphate. Total adenine nucleotide (TAN) represents the sum of ATP, ADP, and AMP, reflecting mitochondrial capacity for high-energy phosphate production, oxidative respiratory activity, and cellular energy reserves. Energy charge (EC) level reflects the interconversion of high-energy phosphate bonds among ATP, ADP, and AMP, providing an effective assessment of energy reserve status. The AMP/ATP ratio is regulated by ATP; during stress responses, decreased ATP production or increased utilization elevates the intracellular AMP/ATP ratio, activating AMPK to trigger responses that restore cellular energy balance.

Our results demonstrate that 4 h post-LPS injection, hepatic ATP, ADP, and TAN concentrations were significantly reduced, indicating that LPS challenge suppressed hepatic mitochondrial energy metabolism and disrupted ATP homeostasis. Similar findings were reported by Kang et al. Dietary Gly supplementation significantly increased hepatic ATP concentration and EC level while decreasing AMP concentration and the AMP/ATP ratio. Consistent with our results, Zhou et al. observed that Gly treatment alleviated the decline in ATP concentration and improved cellular energy status in neonatal rat cardiomyocytes. These findings collectively suggest that Gly can mitigate LPS-induced energy metabolism disorders and promote energy production.

Glycolysis, the tricarboxylic acid cycle, and fatty acid β-oxidation are essential energy metabolic pathways. Hexok2, PFK, and PK catalyze key irreversible reactions in glycolysis, promoting ATP and pyruvate production. Pyruvate undergoes oxidative decarboxylation under the action of PDH to generate acetyl-CoA, which enters the tricarboxylic acid cycle for ATP production. CS is the rate-limiting enzyme in the first step of the tricarboxylic acid cycle, determining the rate of acetyl-CoA entry into the cycle. ICDH is another key enzyme in the tricarboxylic acid cycle, existing in three isoforms (ICDHα, ICDHβ, and ICDHγ), with ICDHβ and ICDHγ transporting metabolic intermediates from the cytoplasm to mitochondria for the tricarboxylic acid cycle. Fatty acid β-oxidation is also an important energy-producing pathway, with ACO and L-CPT1 serving as key enzymes in this process.

Our results revealed that 4 h after LPS injection, mRNA expression of Hexok2, PK, PDH, ICDHβ, and CS was significantly increased. Similarly, Sun et al. reported elevated Hexok and PK activities in the longissimus dorsi muscle of growing pigs injected with LPS. Dietary Gly supplementation significantly decreased the mRNA expression of Hexok2, PK, and CS. Moura et al. demonstrated that intracerebroventricular Gly administration in young rats significantly inhibited CS activity in the striatum. This may be attributed to Gly-induced elevation of ATP concentration, which subsequently suppressed the mRNA expression of rate-limiting enzymes in glycolysis and the tricarboxylic acid cycle. We found that Gly had no significant effects on the mRNA expression of key fatty acid β-

oxidation enzymes ACO and L-CPT1, possibly because Gly primarily regulates hepatic energy metabolism through glycolysis and the tricarboxylic acid cycle during the early stage of LPS challenge without significantly affecting fatty acid β-oxidation.

AMPK and Sirt1 are cellular energy sensors that can be activated under energy-restricted conditions and activate PGC1α through phosphorylation and deacetylation. When cellular energy stores decrease, the elevated AMP/ATP ratio activates AMPK, which restores homeostasis by limiting anabolic metabolism and promoting catabolic metabolism to increase ATP production. Sirt1 is closely associated with energy metabolism-related biological functions including gluconeogenesis and lipid accumulation, and can reduce the mRNA expression of genes related to fatty acid oxidation. PGC1α is a transcriptional coactivator closely linked to energy metabolism, playing important roles in glucose and fatty acid metabolism. Our results showed that LPS challenge significantly increased hepatic AMPK$ 2andSirt1$mRNAexpression$, $whiledietaryGlysupplementationincreasedAMPK$ 1$mRNAexpressio
$inducedeffectsonAMPK-relatedsignaling.ThisindicatesthatGlymayinfluencehepaticenergymetabolismth

Conclusions

1. Dietary Gly supplementation can ameliorate LPS-induced hepatic energy metabolism disorders in weaned piglets by modulating the expression of key enzymes including Hexok2, PK, and CS in glycolysis and the tricarboxylic acid cycle.
2. Dietary Gly supplementation has no significant effect on fatty acid β-oxidation.
3. Gly does not influence hepatic energy metabolism through AMPK, Sirt1, and PGC1α signaling pathways.
4. Considering the effects of different Gly levels on hepatic energy status and economic factors, 1% Gly supplementation is recommended.

References

[1] LIU Yulan, SHI Yin-xia, LU Jing, et al. Effect of lipopolysaccharide challenge on PPARγ expression in the hypothalamic-pituitary-adrenal axis of weaned piglets [J]. Chinese Journal of Animal Science, 2011, 47(1): 25-28.

[2] SCHMÖCKER C, WEYLANDT K H, KAHLKE L, et al. Omega-3 fatty acids alleviate chemically induced acute hepatitis suppression cytokines [J]. Hepatology, 2007, 45(4): 864-869.

[3] ZHONG Z, ENOMOTO N, CONNOR H D, et al. Glycine improves survival after hemorrhagic shock in the rat [J]. Shock, 1999, 12(1): 54-62.

[4] GU Jun-chao, MA Tao, WANG Yu. Discussion on the protective mechanism

of glycine and related diseases [J]. Beijing Medical Journal, 2005, 27(9): 560-562.

[5] ZHOU Shun-wu. Animal Biochemistry [M]. Beijing: Chemical Industry Press, 2008.

[6] YUAN Ping-ge, ZHANG Da-zhi. Mechanism and clinical application of reduced glutathione [J]. Drug Evaluation, 2006, 3(5): 385-390.

[7] WANG W, DAI Z, WU Z, et al. Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs [J]. Amino Acids, 2014, 46(8): 2037-2045.

[8] WU G. Amino acids: metabolism, functions, and nutrition [J]. Amino Acids, 2009, 37(1): 1-17.

[9] WU G, WU Z, DAI Z, et al. Dietary requirements of "nutritionally non-essential amino acids" by animals and humans [J]. Amino Acids, 2013, 44(4): 1107-1113.

[10] LIU Y L, HUANG J J, HOU Y Q, et al. Dietary arginine supplementation alleviates intestinal mucosal disruption induced by Escherichia coli lipopolysaccharide in weaned pigs [J]. British Journal of Nutrition, 2008, 100(3): 552-560.

[11] HOU Y Q, YAO K, WANG L, et al. Effects of α-ketoglutarate on energy status in the intestinal mucosa of weaned piglets chronically challenged with lipopolysaccharide [J]. British Journal of Nutrition, 2011, 106(3): 357-363.

[12] LIU Y L, CHEN F, ODLE J, et al. Fish oil enhances intestinal integrity and inhibits TLR4 and NOD2 signaling pathways in weaned pigs after LPS challenge [J]. The Journal of Nutrition, 2012, 142(11): 2017-2024.

[13] LIVAK K J, SCHMITTGEN T D. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method [J]. Methods, 2001, 25(4): 402-408.

[14] PI D A, LIU Y L, SHI H F, et al. Dietary supplementation of aspartate enhances intestinal integrity and energy status in weanling piglets after lipopolysaccharide challenge [J]. The Journal of Nutritional Biochemistry, 2014, 25(4): 456-462.

[15] KANG P, LIU Y L, ZHU H, et al. The effect of aspartate on the energy metabolism in the liver of weanling pigs challenged with lipopolysaccharide [J]. European Journal of Nutrition, 2015, 54(4): 581-588.

[16] ZHOU Jun-li, HUANG Yue-sheng, DANG Yong-ming, et al. Effect of glycine on energy metabolism in ischemic and hypoxic cardiomyocytes [J]. Progress in Modern Biomedicine, 2008, 8(6): 1093-1095.

[17] WOLF A, AGNIHOTRI S, MICALLEF J, et al. Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma

multiforme [J]. The Journal of Experimental Medicine, 2011, 208(2): 313–326.

[18] CURRIE P D, SULLIVAN D T. Structure and expression of the gene encoding phosphofructokinase (PFK) in Drosophila melanogaster [J]. The Journal of Biological Chemistry, 1994, 269(40): 24679–24687.

[19] ZANELLA A, BIANCHI P, BARONCIANI L, et al. Molecular characterization of PK-LR gene in pyruvate kinase-deficient Italian patients [J]. Blood, 1997, 89(10): 3847–3852.

[20] WIEGAND G, REMINGTON J. Citrate synthase: structure, control, and mechanism [J]. Annual Review of Biophysics and Biophysical Chemistry, 1986, 15(1): 97–117.

[21] MACDONALD M J, BROWN L J, LONGACRE M J, et al. Knockdown of both mitochondrial isocitrate dehydrogenase enzymes in pancreatic beta cells inhibits insulin secretion [J]. Biochimica et Biophysica Acta, 2013, 1830(11): 5104–5111.

[22] SUN H, HUANG Y, YIN C, et al. Lipopolysaccharide markedly changes glucose metabolism and mitochondrial function in the longissimus muscle of pigs [J]. Animal, 2016, 10(7): 1204–1210.

[23] MOURA A P, GRINGS M, PARMEGGIANI B D S, et al. Glycine intracerebroventricular administration disrupts mitochondrial energy homeostasis in cerebral cortex and striatum of young rats [J]. Neurotoxicity Research, 2013, 24(4): 502–511.

[24] LAVU S, BOSS O, ELLIOTT P J, et al. Sirtuins-novel therapeutic targets to treat age-associated diseases [J]. Nature Reviews Drug Discovery, 2008, 7(10): 841–853.

[25] LAGOUGE M, ARGMANN C, GERHART-HINES Z, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α [J]. Cell, 2006, 127(6): 1109–1122.

[26] LI Bo, TIAN Rui-xia, YANG Wei-jing, et al. Function of PGC-1α and metabolic diseases [J]. Chinese Journal of Biochemistry and Molecular Biology, 2011, 27(11): 1013–1018.

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