Effects of Glutamate on Intestinal Energy Metabolism in Lipopolysaccharide-Challenged Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of glutamate (Glu) on intestinal energy metabolism in weaned piglets challenged with lipopolysaccharide (LPS). Twenty-four weaned piglets were selected and allocated into 4 groups: control, LPS, LPS+1.0% Glu, and LPS+2.0% Glu groups, with 6 replicates per group and 1 pig per replicate. On day 28 of the experiment, pigs in the treatment groups were injected with 100 g/kg BW LPS, while the control group was injected with an equal volume of physiological saline, and slaughtered 4 h later to collect intestinal samples for analysis. The results showed that: 1) Compared with the control group, LPS challenge significantly decreased the contents of adenosine triphosphate (ATP), total adenine nucleotide pool (TAN) and energy charge (EC) ($P<0.05$), and significantly increased the adenosine monophosphate (AMP)/ATP ratio ($P<0.05$) in the jejunum of weaned piglets; compared with the LPS group, the LPS+2.0% Glu group significantly increased the contents of ATP, adenosine diphosphate (ADP) and TAN ($P<0.05$) in the jejunum. 2) Compared with the control group, LPS challenge highly significantly decreased the activities of citrate synthase and -ketoglutarate dehydrogenase complex in the ileum ($P<0.01$), and tended to decrease the activity of -ketoglutarate dehydrogenase complex in the jejunum ($P=0.092$); compared with the LPS group, except for the significantly decreased activity of citrate synthase in the ileum of the LPS+1.0% Glu group ($P<0.05$), Glu had no significant effect on the activities of key enzymes in the tricarboxylic acid cycle in the jejunum and ileum ($P>0.05$). 3) Compared with the control group, LPS challenge highly significantly decreased the mRNA expression levels of peroxisome proliferator-activated receptor coactivator 1 (PGC1) in the jejunum and silent information regulator 1 (Sirt1) and PGC1 in the ileum ($P<0.01$); compared with the LPS group, the LPS+2.0% Glu group tended to increase the mRNA expression levels of PGC1 in the jejunum ($P=0.067$) and Sirt1 in the ileum ($P=0.053$), and the LPS+1.0% Glu group tended to increase the mRNA expression level of Sirt1 in

the ileum (P=0.070). In conclusion, Glu can ameliorate the intestinal energy depletion state induced by LPS challenge.

Full Text

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Abstract

This study investigated the effects of glutamate (Glu) on intestinal energy metabolism in weaned piglets challenged with lipopolysaccharide (LPS). Twenty-four weaned piglets were assigned to four groups: control, LPS, LPS+1.0% Glu, and LPS+2.0% Glu, with six replicates per group and one pig per replicate. On day 28 of the experiment, pigs in the treatment groups were injected with 100 g/kg BW LPS, while control pigs received an equivalent volume of saline. Four hours post-injection, pigs were slaughtered and intestinal samples were collected for analysis. The results showed: 1) Compared with the control group, LPS challenge significantly decreased jejunal adenosine triphosphate (ATP) and total adenine nucleotide (TAN) contents and energy charge (EC) (P<0.05), while significantly increasing the AMP/ATP ratio (P<0.05). Dietary supplementation with 2.0% Glu significantly increased jejunal ATP, adenosine diphosphate (ADP), and TAN contents compared with the LPS group (P<0.05). 2) Relative to the control group, LPS challenge tended to decrease jejunal -oxoglutarate dehydrogenase complex activity (P=0.092) and extremely significantly decreased ileal citrate synthase and -oxoglutarate dehydrogenase complex activities (P<0.01). Compared with the LPS group, Glu supplementation had no significant effects on tricarboxylic acid (TCA) cycle key enzyme activities in jejunum and ileum (P>0.05), except that LPS+1.0% Glu significantly decreased ileal citrate synthase activity (P<0.05). 3) LPS challenge extremely significantly decreased mRNA expression of peroxisome proliferator-activated receptor coactivator-1 (PGC1) in jejunum and silent information regulator 1 (Sirt1) and PGC1 in ileum (P<0.01) compared with the control group. The LPS+2.0% Glu group showed a tendency to increase jejunal PGC1 (P=0.067) and ileal Sirt1 (P=0.053) mRNA expression, while the LPS+1.0% Glu group tended to increase ileal Sirt1 mRNA expression (P=0.070) compared with the LPS group. These findings indicate that dietary Glu supplementation can ameliorate LPS-induced intestinal energy depletion.

Keywords: glutamate; lipopolysaccharide; weaned piglets; intestine; energy metabolism

The intestine serves as the primary site for digestion and absorption while also functioning as a physiological barrier against pathogens and harmful substances [1]. Intestinal health and function require substantial energy expenditure [2]. During stress conditions, disrupted energy metabolism leads to decreased ATP content, cellular dysfunction or death, and consequent intestinal structural and functional damage [3]. Lipopolysaccharide (LPS), a component of Gram-negative bacterial membranes, can cause insufficient energy supply and intestinal injury [4]. Glutamate (Glu) is an acidic non-essential amino acid crucial for growth and development in young animals and intimately involved in intestinal mucosal growth and metabolism [5,6]. Stoll et al. [7] reported that approximately 90% of dietary Glu is metabolized in the porcine intestine, making it a primary energy source for the gut. The carbon skeleton of Glu can be converted to -oxoglutarate, which enters the tricarboxylic acid (TCA) cycle for oxidative energy production [8]. Additionally, Glu belongs to the arginine family of amino acids and can be converted to other family members such as aspartate, glutamine, and arginine, which further exert physiological functions [9]. However, research on Glu' s role in intestinal energy metabolism remains limited, and its molecular mechanisms are poorly understood. Therefore, this study established an immune stress model by injecting LPS into weaned piglets [10] to investigate Glu' s effects on intestinal energy metabolism, providing a theoretical basis for Glu to alleviate LPS-induced intestinal injury.

1.1 Experimental Materials

L-Glu (purity >99.1%) and L-alanine (purity >99.5%) were provided by Wuhan Amino Technology Co., Ltd. LPS (E. coli serotype O55:B5, Sigma) was dissolved in saline to a concentration of 500 g/mL for injection at 0.2 mL/kg BW (equivalent to 100 g/kg BW).

1.2 Experimental Animals and Design

Twenty-four healthy weaned piglets (Duroc × Landrace × Yorkshire) with similar body condition [average body weight (7.02±0.21) kg] were randomly allocated to four groups (n=6 per group, one pig per replicate) based on body weight. The experiment employed a single-factor design with the following treatments: 1) control (saline + basal diet), 2) LPS (LPS + basal diet), 3) LPS+1.0% Glu (LPS + basal diet + 1.0% Glu), and 4) LPS+2.0% Glu (LPS + basal diet + 2.0% Glu). The basal diet was formulated according to NRC (1998) nutrient requirements for piglets . All diets were isonitrogenously balanced with alanine. The experimental period lasted 28 days. On day 28, treatment pigs received LPS injection (100 g/kg BW) while control pigs received saline.

1.3 Animal Management

The experiment was conducted at the Hubei Key Laboratory of Animal Nutrition and Feed Science. Pigs were housed individually in pens (1.20 m × 1.10 m)

at 25–27 °C with ad libitum access to feed and water. Routine immunization and deworming were performed throughout the trial.

1.4 Intestinal Sample Collection and Processing

Four hours after LPS or saline injection on day 28, pigs were slaughtered and the small intestine was excised from the mesentery. Approximately 10 cm segments from the middle of jejunum and ileum were immediately placed on ice. Each segment was longitudinally opened along the mesentery, gently rinsed with 4 °C saline, blotted dry with filter paper, and the mucosa was scraped with a glass slide. Mucosal samples were aliquoted into 1.5 mL sterile cryovials, snap-frozen in liquid nitrogen, and stored at −80 °C.

1.5.1 Determination of Intestinal Mucosal Adenylate Contents

Adenylate contents were measured by reversed-phase high-performance liquid chromatography. Frozen mucosal tissue (0.1–0.2 g) was homogenized in 2 mL ice-cold 1.5 mol/L perchloric acid on ice and centrifuged at 3,000 r/min for 5 min at 4 °C. The supernatant (1 mL) was neutralized with 0.4 mL 2 mol/L potassium carbonate and centrifuged again under the same conditions. The final supernatant was stored at −80 °C until analysis.

Chromatographic conditions: Waters Breeze HPLC system with Waters XTerra MS C18 column (5 m × 4.6 mm × 150 mm); mobile phase: 50 mmol/L K HPO - KH PO buffer and chromatographic grade methanol (77:23, v/v) adjusted to pH 7.0 with phosphoric acid; flow rate: 1.0 mL/min; column temperature: 20 °C; UV detector at 260 nm; injection volume: 10 L.

External standard method was used to quantify ATP, ADP, and AMP contents. Standards were analyzed under identical conditions. Total adenine nucleotide (TAN) = ATP + ADP + AMP; Energy charge (EC) = (ATP + ½ ADP) / (ATP + ADP + AMP).

1.5.2 Determination of Citrate Synthase, -Oxoglutarate Dehydrogenase Complex, and Isocitrate Dehydrogenase Activities

Enzyme activities were determined by enzyme-linked immunosorbent assay (ELISA) according to Pi et al. [11] using commercial kits for porcine citrate synthase (#45126), -oxoglutarate dehydrogenase complex (#45157), and isocitrate dehydrogenase (#45234) from Shanghai Yuanye Bio-Technology Co., Ltd.

1.5.3 Determination of mRNA Expression of Energy Metabolism-Related Signaling Molecules

mRNA expression was quantified by real-time PCR according to Chen [12] using reagents from Takara Bio. Primers were designed using Primer Premier 6.0 based on published porcine gene sequences and synthesized by Takara Bio. All

genes showed amplification efficiencies near 100%. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal reference gene. Relative mRNA expression was calculated using the 2^(-ΔΔCT) method [13] and normalized to the control group.

1.6 Statistical Analysis

Data were analyzed by one-way ANOVA and LSD multiple comparisons using SPSS 22.0. Results are expressed as means ± SEM. Differences were considered extremely significant at P 0.01, significant at 0.01<P 0.05, and trends at 0.05<P 0.10.

2.1 Effects of Glu on Intestinal Mucosal Adenylate Contents in LPS-Challenged Piglets

As shown in , LPS challenge significantly decreased jejunal ATP and TAN contents and EC (P<0.05) while significantly increasing the AMP/ATP ratio (P<0.05) compared with the control group. Dietary supplementation with 2.0% Glu significantly increased jejunal ATP, ADP, and TAN contents (P<0.05) relative to the LPS group.

2.2 Effects of Glu on TCA Cycle Key Enzyme Activities in Intestinal Mucosa of LPS-Challenged Piglets

As shown in , LPS challenge tended to decrease jejunal -oxoglutarate dehydrogenase complex activity (P=0.092) and extremely significantly decreased ileal citrate synthase and -oxoglutarate dehydrogenase complex activities (P<0.01) compared with the control group. Except for significantly decreased ileal citrate synthase activity in the LPS+1.0% Glu group (P<0.05), Glu supplementation had no significant effects on TCA cycle key enzyme activities in jejunum and ileum (P>0.05).

2.3 Effects of Glu on mRNA Expression of Energy Metabolism-Related Signaling Molecules in Intestinal Mucosa of LPS-Challenged Piglets

As shown in , LPS challenge extremely significantly decreased mRNA expression of PGC1 in jejunum and Sirt1 and PGC1 in ileum (P<0.01) compared with the control group. The LPS+2.0% Glu group showed a tendency to increase jejunal PGC1 (P=0.067) and ileal Sirt1 (P=0.053) mRNA expression, while the LPS+1.0% Glu group tended to increase ileal Sirt1 mRNA expression (P=0.070) compared with the LPS group.

ATP is an unstable high-energy compound containing two high-energy phosphoanhydride bonds within its three phosphate groups. Hydrolysis of ATP to ADP releases substantial energy for various cellular activities. When ATP production fails to match utilization, intracellular ADP concentration increases, and two

ADP molecules are converted to one ATP and one AMP by adenylate kinase [8]. TAN serves as an energy mediator system whose magnitude reflects mitochondrial oxidative respiratory activity and capacity for generating high-energy phosphate compounds, as well as cellular energy reserves [14]. EC reflects the interconversion of high-energy phosphate bonds among ATP, ADP, and AMP, with normal maintenance depending on dynamic equilibrium between synthesis and degradation of high-energy compounds. Insufficient energy production or increased consumption affects EC levels [15]. Decreased ATP production or increased utilization elevates the intracellular AMP/ATP ratio, which activates AMP-activated protein kinase (AMPK) to restore cellular energy balance [16].

In this study, LPS challenge significantly decreased jejunal ATP, TAN, and EC while increasing the AMP/ATP ratio, consistent with Pi et al. [11]. Wang et al. [17] demonstrated that LPS affects the mitochondrial cytochrome oxidase system, impeding respiratory chain transfer and causing energy synthesis disorders with reduced ATP production. Dietary supplementation with 2.0% Glu significantly increased jejunal ATP, ADP, and TAN contents. Watford [18] reported that Glu metabolism provides abundant ATP for intestinal integrity and function. Blachier et al. [19] showed that Glu is oxidized by glutamate dehydrogenase in intestinal epithelial cells, undergoing transamination with oxaloacetate to produce -oxoglutarate and aspartate, with -oxoglutarate entering mitochondria for ATP production via the TCA cycle. These findings indicate that Glu alleviates LPS-induced energy metabolic disorders, promoting energy generation and enhancing metabolic and reserve capacity.

Citrate synthase, isocitrate dehydrogenase, and -oxoglutarate dehydrogenase complex are three rate-limiting enzymes of the TCA cycle that regulate critical biological processes including energy metabolism and biosynthesis [20]. Citrate synthase determines the rate of acetyl-CoA entry into the TCA cycle and serves as an important indicator of energy metabolic status [21]. Isocitrate dehydrogenase, present in mitochondria and cytoplasm, catalyzes isocitrate conversion to -oxoglutarate [22]. The -oxoglutarate dehydrogenase complex in the mitochondrial matrix catalyzes oxidative decarboxylation of -oxoglutarate to succinyl-CoA while generating reduced nicotinamide adenine dinucleotide (NADH) [23].

LPS challenge tended to decrease jejunal -oxoglutarate dehydrogenase complex activity and extremely significantly decreased ileal citrate synthase and -oxoglutarate dehydrogenase complex activities, consistent with Shi [24]. Glu supplementation had no significant effects on these TCA cycle key enzyme activities, possibly because elevated intestinal ATP content inhibited these three rate-limiting enzymes [20]. Alternatively, Glu may improve intestinal energy metabolism not by affecting enzyme activities but by converting to -oxoglutarate, a TCA cycle intermediate. Studies have shown that -oxoglutarate serves as an energy metabolic substrate for intestinal mucosa [25], and dietary -oxoglutarate supplementation improves energy metabolism in LPS-challenged intestinal mucosal cells [26].

AMPK is a highly conserved serine/threonine protein kinase ubiquitously ex-

pressed in eukaryotic cells that senses cellular energy status changes and maintains energy metabolic balance [27]. When decreased ATP production or increased utilization elevates the AMP/ATP ratio, AMPK activation promotes ATP regeneration and inhibits ATP consumption to maintain energy balance [28]. Additionally, AMPK activation can stimulate downstream Sirt1 activity [29]. Sirt1 is a nuclear protein that increases ATP content through enhanced hepatic gluconeogenesis, reduced lipid accumulation, and participation in pancreatic insulin secretion [30]. Sirt1 also mediates deacetylation of its downstream target PGC1 , influencing PGC1 activity and ultimately regulating mitochondrial and lipid metabolism genes [31]. PGC1 is a nuclear transcriptional coactivator involved in regulating mitochondrial biogenesis and glucose/fat metabolism [32].

LPS challenge extremely significantly decreased mRNA expression of jejunal PGC1 and ileal Sirt1 and PGC1 . Luo et al. [33] found that Sirt1 expression was inhibited during LPS-induced PC12 cell apoptosis. Glu alleviated LPS-induced decreases in jejunal PGC1 and ileal Sirt1 mRNA expression, suggesting that Glu modulates intestinal energy metabolism-related signaling pathways. As a member of the arginine family, Glu can be converted to other family amino acids including aspartate, glutamine, and arginine [9]. Kang et al. [34] demonstrated that dietary aspartate supplementation increased hepatic Sirt1 mRNA expression. Glu may increase intestinal Sirt1 mRNA expression through conversion to aspartate, subsequently activating downstream PGC1 to promote ATP production. The lack of significant effects on jejunal and ileal AMPK 1 and AMPK 2 mRNA expression in this study may be because the magnitude of energy change did not reach the threshold for AMPK activation. Research indicates that ATP levels must change beyond a certain threshold to activate AMPK [35].

In conclusion, Glu alleviates LPS-induced intestinal energy metabolic disorders in weaned piglets, possibly by modulating mRNA expression of energy metabolism-related regulatory factors Sirt1 and PGC1 , thereby promoting intestinal mucosal energy production.

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Note: Figure translations are in progress. See original paper for figures.