Epidermal growth factor activates EGFR/AMPK signalling to up-regulate the expression of SGLT1 and GLUT2 to promote intestinal glucose absorption in lipopolysaccharide challenged IPEC-J2 cells and piglets

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ABSTRACT

Epidermal growth factor (EGF) plays an important role in nutrients transport. The present study was to investigate the effects of EGF on glucose absorption in a cellular injury model (IPEC-J2 cells, in vitro study) and animal injury model (weaned piglets, in vivo study) established by lipopolysaccharide (LPS). In vitro study: porcine intestinal epithelial cells (IPEC-J2) were divided into four treatments: control group (Control); 100 ng/mL EGF treatment group (EGF); 1 μg/mL LPS treatment group (LPS) and 100 ng/mL EGF plus 1 μg/mL LPS treatment group (EGF + LPS). The results showed that EGF significantly increased the alkaline phosphatase (AKP) and sodium/potassium-transporting adenosine triphosphatase (Na⁺/K⁺-ATPase) activity, and significantly improved the mRNA and protein expression of SGLT1, GLUT2, EGF receptor (EGFR) and AMP-activated protein kinase α1 (AMPK-α1) in LPS-induced injured cells. In vivo experiment: twenty-four piglets weaned at 21 days were randomly assigned into: (1) control group (basal diets); (2) EGF group (basal diet + 2 mg/kg EGF); (3) LPS group (basal diet + injection with 100 μg/kg BW LPS) and (4) EGF + LPS group (basal diet + 2 mg/kg EGF + injection with 100 μg/kg BW LPS). Our results showed that EGF significantly increased the AKP and Na⁺/K⁺-ATPase activity, and significantly improved the mRNA expression of SGLT1, GLUT2, EGFR and AMPK-α1 in jejunal mucosa of piglets challenged with LPS. In conclusion, EGF can activate EGFR/AMPK signalling to up-regulate the expression of SGLT1 and GLUT2 as well as improve the AKP and Na⁺/K⁺-ATPase activity, thereby promoting intestinal glucose absorption in IPEC-J2 cells and piglets challenged by LPS.

HIGHLIGHTS

• EGF promotes SGLT1 and GLUT2 expression and AKP and Na⁺/K⁺-ATPase activity in LPS-induced injured porcine intestinal epithelial cells.
• EGF promotes SGLT1 and GLUT2 expression and AKP and Na⁺/K⁺-ATPase activity in jejunal mucosa of piglets challenged by LPS.
• Dietary supplementation with EGF activates EGFR/AMPK signalling to up-regulate the expression of SGLT1 and GLUT2 as well as improve the AKP and Na⁺/K⁺-ATPase activity to promote intestinal glucose absorption in lipopolysaccharide challenged piglets.

Introduction

Glucose is the main carbon and energy source of eukaryotic cells, and the transport of glucose to mammalian cells is a rate-limiting step in glucose utilisation (Chaudhry et al. 2012). It has been proved that there are at least two types of glucose transporters involved in glucose transport, one is sodium/glucose cotransporter 1 (SGLT1) with high affinity and low transport capacity and the other is glucose transporter 2 (GLUT2) with low affinity and high transport capacity (Bedford et al. 2015; Xu et al. 2015; Huerzeler et al. 2019). It is believed that intracellular glucose is transported to epithelial cells by SGLT1, which is located in the brush border membrane of the intestinal mucosa, and then through GLUT2, located in basal membrane of the intestinal tract, transporting to the portal vena cava (Chaudhry et al. 2012). However, when the glucose concentration in the intestinal lumen is too high, GLUT2 can cooperate with SGLT1 to accelerate
transport in the small intestine (Huang et al. 2007; Tang et al. 2016, 2018) and nutrients established as a trophic factor for the epithelial cell polypeptide comprising 53 amino acid residues, has absorption in animals. Epidermal growth factor (EGF), a small mitogenic polypeptide comprising 53 amino acid residues, has established as a trophic factor for the epithelial cell homeostasis (Tang et al. 2012). Thus, the study of the function of these glucose transporters not only provides potential drug targets for human related diseases, including obesity and diabetes, but also provides ideas for controlling nutrients absorption in animals.

Epidermal growth factor (EGF), a small mitogenic polypeptide comprising 53 amino acid residues, has established as a trophic factor for the epithelial cell homeostasis (Tang et al. 2016, 2018) and nutrients transport in the small intestine (Huang et al. 2007; Trapani et al. 2014; Wang L et al. 2019; Tang, Fang, et al. 2019; Wang L, Zhu, Li, et al. 2020). It has been established that EGF regulates the absorption of glucose in the intestinal tract via up-regulating the expression of SGLT1 (Cellini et al. 2005; Bedford et al. 2015; Xu et al. 2015; Wang CW et al. 2015; Wang L et al. 2019), but whether EGF regulates the absorption of glucose through GLUT2 is still controversial. For example, Xu et al. (2015) reported that EGF could up-regulate the expression of GLUT2 in jejunum and ileum of weaned piglets, indicating that GLUT2 participates in the regulation of glucose absorption, while, Bedford et al. (2015) reported that EGF had no effect on GLUT2 mRNA expression in weaned pigs. Our previous study showed that EGF has a good repair effect on oxidative damage of pig small intestinal epithelial cells stimulated by lipopolysaccharide (LPS) (Tang et al. 2018). In theory, in the process of repairing the damaged intestinal tract, more glucose is needed to meet the energy consumption of intestinal epithelial cells. Therefore, promoting intestinal glucose absorption is of great significance for the repair of damaged intestinal tract in humans and animals. Our previous study has confirmed that EGF can promote the absorption of glucose in LPS-induced injured porcine intestinal epithelial cells (IPE-J2) (Tang and Xiong 2021), which indirect indicated that the repair of damaged intestinal epithelial cells is related to promote the absorption of glucose. However, whether SGLT1-mediated glucose transport can meet the energy needs or not in the process of intestinal repair, and is it necessary to assist glucose absorption by mobilising the expression of GLUT2 in porcine intestine have rarely been reported. Thus, we hypothesised that EGF upregulates the expression of SGLT1 and GLUT2 to promote the intestinal glucose absorption in porcine intestine under damage conditions. To test this hypothesis we used LPS, a kind of endotoxin, to establish a cellular injury model (IPEC-J2 cells) and animal injury model (weaned piglets) to investigate the effects of EGF on the expression of glucose transporters SGLT1 and GLUT2 as well as to clarify the roles of SGLT1 and GLUT2 in glucose absorption during intestinal damage repair of piglets.

Materials and methods

Culture of intestinal epithelial cells

Porcine intestinal epithelial cells (IPEC-J2) were kindly provided by Institute of Subtropical Agriculture, Chinese Academy of Science (Changsha, China). IPEC-J2 cells were cultured as described previously (Tang et al. 2018). Briefly, cells were grown in uncoated plastic culture flasks (100 mm²) in DMEM-F12 medium (GE Healthcare life sciences, South Logan, Utah, USA) containing 10% foetal bovine serum (FBS, Gibco, Carlsbad, CA, USA), 1% antibiotics (Penicillin-Streptomycin) (Gibco, Carlsbad, CA, USA), and cultured in a humidified incubator at 37℃ with 5% CO₂ and 95% air. After 80% of fusion, cells digested with 0.25% trypsin-EDTA (Gibco, Carlsbad, CA, USA) and seeded in a 6-well cell culture plates (Corning, NY, USA) with approximately 1 × 10⁵ cells per well. After a 24 h of incubation, IPEC-J2 cells randomly divided into four groups (6 replicates/treatment, n = 6): (1) control group (0 ng/mL EGF + 0 μg/mL LPS), (2) EGF group (100 ng/mL EGF + 0 μg/mL LPS), (3) LPS (0 ng/mL EGF + 1.0 μg/mL LPS), and (4) EGF + LPS group (100 ng/mL EGF + 1.0 μg/mL LPS) respectively for 24 h. The EGF and LPS concentrations were adopted according to Tang et al. (2018). EGF was purchased from Peprotech (Rocky Hill, NJ, USA). LPS was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

Determination of AKP and Na⁺/K⁺-ATPase activity in IPEC-J2 cells

After a 24 h of EGF and/or LPS treatment, cells in 6-well culture plates were gently washed with PBS (Solarbio, Beijing, China) for twice, then RIPA Lysis Buffer R2220 (Solarbio, Beijing, China) was used to lyse IPEC-J2 cells according to the instructions of the manufacturer. Cellular protein concentration was determined using the bicinchoninic acid (BCA) protein assay reagent (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) at 562 nm according to the instructions of the manufacturer. Alkaline phosphatase (AKP) assay kit (A059-2-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was used to determine the AKP activity (n = 6) according to the instructions of the manufacturer. Briefly, different reagents were added into blank well (5 μL double distilled water, 50 μL buffer and 50 μL matrix fluid),
standard well (5 μL 0.1 mg/mL phenol standard fluid, 50 μL buffer and 50 μL matrix fluid), and measured well (5 μL sample, 50 μL buffer and 50 μL matrix fluid), fully mixed and water bath for 15 min at 37°C, then 150 μL chromogenic agent was added in each well and measured the OD at a wavelength of 520 nm used a enzyme-linked immune detector (Bio-Rad, USA). The AKP activity was calculated according to the following formulae:

\[
\text{AKP activity (U/g prot)} = \left\{ \frac{\left( \text{measured OD} - \text{blank OD} \right)}{\left( \text{standard OD} - \text{blank OD} \right)} \times \frac{\text{standard concentration (0.1 mg/mL)}}{\text{sample protein concentration (gprot/mL)}} \right\}
\]

Sodium/potassium-transporting adenosine triphosphatase (Na\(^+\)/K\(^+\)-ATPase) activity (n = 6) was measured as inorganic phosphate (Pi) released from cellular homogenates using a Na\(^+\)/K\(^+\)-ATPase assay kit (A0702-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. Briefly, different reagents were added into blank glass tube (0.16 mL double distilled water, 0.26 mL solution I, 0.08 mL solution II, 0.08 mL solution III) and measured glass tube (0.12 mL double distilled water, 0.1 mL sample, 0.04 mL solution X, 0.26 mL solution I, 0.08 mL solution II, 0.08 mL solution III) and measured glass tube (0.12 mL double distilled water, 0.1 mL sample, 0.04 mL solution X, 0.26 mL solution I, 0.08 mL solution II, 0.08 mL solution III) and incubated for 10 min at 37°C. Then 0.1 mL solution IV and 0.1 mL sample was add in blank glass tube, and 0.1 mL solution IV was added in measured glass tube, and centrifuged at 3500 rpm for 10 min after fully mixed. Supernatant was collected for Pi measurement. Different reagents were added into blank glass tube (0.3 mL double distilled water, 1.0 mL chromogenic agent), standard glass tube (0.3 mL phosphorus standard solution, 1.0 mL chromogenic agent), control glass tube (0.3 mL supernatant, 1.0 mL chromogenic agent) and Na\(^+\)/K\(^+\)-ATPase measured glass tube (0.3 mL supernatant, 1.0 mL chromogenic agent), and then reaction for 2 min, 1.0 mL solution VI was added in both glass tubes, fully mixed and reaction for 5 min at room temperature, then measured the OD value at a wavelength of 636 nm. The Na\(^+\)/K\(^+\)-ATPase activity was calculated according to the following formulae:

\[
\text{Na}\(^+\)/K\(^+\)–ATPaseactivity (U/mg prot) = \left\{ \frac{\left( \text{measured OD} - \text{control OD} \right)}{\left( \text{standard OD} - \text{standard OD} \right)} \times \frac{\text{standard concentration (0.02 μmol/mL)}}{\text{sample protein concentration (mgprot/mL)}} \times 6 \times 7.8 \right\}
\]

**Real-time PCR analysis of gene expression of SGLT1, GLUT2, EGFR and AMPK-α1 in IPEC-J2 cells**

After a 24 h of EGF and/or LPS treatment, total cell RNA was extracted and purified using TRIZol Reagent (Invitrogen, Carlsbad, CA, USA) following the protocol provided by the manufacturer. The mRNA expression of SGLT1, GLUT2, epidermal growth factor receptor (EGFR), AMP-activated protein kinase α1 (AMPK-α1)

were analysed by real-time quantitative RT-PCR as described previously (Tang, Su, et al. 2019). The RT-PCR was performed using the SYBR® Premix Ex Taq™ (Takara, Dalian, China) on an Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA, USA). The total volume of PCR reaction system was 25 μL (12.5 μL SYBR® Premix Ex TaqTM, 4 μLcDNA, 1 μL (10 mmol/L) forward/reverse primers and 8.5 μL dH2O). All PCRs were performed in triplicate on a 96-well RT-PCR plate under the following conditions: 95°C for 30 s followed by 39 cycles of 95°C for 5 s, 58°C for 30 s and 72°C for 60 s. The primers of genes (Sangon Biotech, Shanghai, China) were shown in Table 1. β-actin was used as a housekeeping gene to normalise target gene transcript levels. The formula 2\(^{-\left(\Delta\Delta Ct\right)}\), where \(\Delta\Delta Ct = (Ct_{Target} - Ct_{β-actin})\) treatment – (Ct_{Target} – Ct_{β-actin}) control was used to calculated the relative gene expression (Peng et al. 2020).

**Western blot analysis of SGLT1, GLUT2, EGFR and AMPK-α1 in IPEC-J2 cells**

After a 24 h of EGF and/or LPS treatment, cells in 6-well culture plates were gently washed with PBS (Solarbio, Beijing, China) for twice, then RIPA Lysis Buffer R2220 (Solarbio, Beijing, China) was used to lyse IPEC-J2 cells according to the instructions of the manufacturer. Cellular protein concentration was determined using the BCA protein assay reagent (Nanjing Jincheng Bioengineering Institute, Nanjing, China) at 562 nm according to the instructions of the manufacturer. Western blot was performed as described previously (Tang et al. 2018). Briefly, equal amount of protein samples of cell lysate was loaded for SDS-PAGE and subsequently transferred to PVDF membrane. The membrane was blocked with PBST buffer containing 5% skim-milk for 1 h at room
temperature followed by overnight hybridisation at 4 °C with the indicated primary anti-bodies: anti-SGLT1, anti-GLUT2, anti-EGFR, anti-AMPKα1 and anti-β-actin (Proteintech, Rosemont, IL, USA). After incubation with secondary antibody HRP goat anti-rabbit IgG (Proteintech, Rosemont, IL, USA) for 1 h, signals were detected using enhanced chemiluminescence kits (ECL-Plus, Thermo, Waltham, MA, USA), and then scanned for detection of fluorescence using the BioRad gel detection system.

**Animals and experimental design**

Twenty-four healthy piglets with similar birth order, weaned at 21 days with a mean body weight (BW) of 5.76 ± 0.38 kg were randomly assigned into four treatments (six piglets/treatment, three males and three females, n = 6). The first group fed a basal diet (Control group); the second group fed a basal diet plus 2 mg/kg EGF (ZYME FAST (Changsha) Biotechnology Co. LTD, Changsha, China) (EGF group); the third group fed a basal diet and injection with 100 μg/kg BW LPS (Sigma, Saint Louis, MO, USA) at day 8 and day 15 (LPS group); the fourth group fed a basal diet plus 2 mg/kg EGF and injection with 100 μg/kg BW LPS at day 8 and day 15 (EGF + LPS group). The composition and nutrient levels of basal diet were calculated values.

**Sample collection**

Six hours after the intraperitoneal injection of LPS, all pigs were anaesthetized with an injection of sodium pentobarbital (50 mg kg⁻¹ BW), bled by exsanguinations to death. Middle jejunum samples from all piglets (6 piglets per treatment) were collected, one piece of jejunum segment (about 5 cm) was fixed in 10% neutral buffered formalin for examination of intestinal morphology. For another piece of jejunum segment (about 5 cm), the mucosa was scraped gently after removal of surface chime, then immediately frozen in liquid nitrogen, and stored at −80 °C.

**Intestinal histomorphology**

Paraffin sections (approximately 5 mm) of jejunum (n = 6) were stained with haematoxylin and eosin, and villus height (VH) and crypt depth (CD) were measured using a light microscope with a computer-assisted morphometric system (BioScan Optimetric, BioScan Inc, Edmonds, WA, USA) according to previous study.

Table 1. Primers used for quantitative reverse transcription PCR.

| Gene     | Primers sequence | Product length | Accession no. or reference |
|----------|------------------|----------------|---------------------------|
| β-actin  | F: 5’-CATCTCGTGCTCAGCTGG-3’ R: 5’-TAATGCAGCAATTGCCCA-3’ | 116 bp | Tang et al. (2018) |
| SGLT1   | F:5’-ATATGGCCCTTATATCCCTT-3’ R:5’-AAATCGTGGTTGATAGGCCCA-3’ | 138 bp | NM_001164021.1 |
| GLUT2   | F:5’-CAGCTATTCTAGTAGCACTG-3’ R:5’-AAATCGTGGTTGATAGGCCCA-3’ | 151 bp | NM_001097417.1 |
| EGF     | F:5’-GCTATGATGTCGCCAAAGTCCGT-3’ R:5’-AGTGCATTCGCTCGTCTCC-3’ | 103 bp | NM_214007.1 |
| AMPK-α1 | F:5’-GGTGAATACTGCGCACCACA-3’ R:5’-TGGCACAATCCACTTTGCA-3’ | 72 bp | Tang and Xiong (2022) |

Table 2. Diet composition and nutrient levels (as fed basis, %).

| Ingredients          | Content | Item          | Nutrient levels |
|----------------------|---------|---------------|-----------------|
| Corn                 | 63.70   | DE/(MJ/Kg)    | 14.22           |
| Squeezed soybean meal| 16.00   | CP            | 19.59           |
| Expanded soybean     | 8.00    | Lys           | 1.33            |
| Fish meal            | 4.50    | Met + Cys     | 0.73            |
| Whey powder          | 2.00    | Ca            | 0.86            |
| Glucose              | 2.00    | Total phosphorus | 0.74      |
| Limestone            | 0.78    | Available phosphorus | 0.45 |
| CaHPO₄               | 1.30    |               |                 |
| L-Lysine-HCl         | 0.35    |               |                 |
| Met                  | 0.07    |               |                 |
| Thr                  | 0.06    |               |                 |
| NaCl                 | 0.24    |               |                 |
| Premix®              | 1.00    |               |                 |
| Total                | 100.00  |               |                 |

*The premix provided following per kilogram of diet*: VA 10,000 IU; VD₃ 1500 IU; VE 60 mg; VK₃ 1 mg; VB₁ 1.8 mg; VB₁₂ 0.024 mg; riboflavin 6 mg; folic acid 0.3 mg; biotin 4.5 mg; nicotinic acid 24 mg; D-pantothenic acid 15 mg; choline 1000 mg; Zn 125 mg; Fe 120 mg; Cu 150 mg; I 0.3 mg; Se 0.3 mg. 

**CP**, total phosphorus, Ca levels were measured values, and other nutrient levels were calculated values.

Met: methionine; Thr: threonine; Lys: lysine; DE: digestible energy; CP: crude protein; Cys: cysteine; Ca: calcium.
(Peng et al. 2020). Briefly, the fixed intestinal segment was embedded in paraffin, after which the tissue specimen was sliced to a thickness of 4 µm, mounted onto a glass slide, and then stained with haematoxylin/eosin. VH, CD of the jejunal were measured via a light microscope and the average of four measurements was taken as a replicate in each variable.

AKP and Na\(^{+/}\)/K\(^{+}\)-ATPase activity in jejunum mucosa

The jejunal mucosa tissues were homogenised in saline solution (1:4, weight: volume) and centrifuged at 3500 r/min for 15 min at 4 °C. The supernatants protein concentration was determined using the bicinchoninic acid (BCA) protein assay reagent (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) at 562 nm according to the instructions of the manufacturer. Then the supernatants were then diluted into the optimal content for detecting the activities of AKP and Na\(^{+/}\)/K\(^{+}\)-ATPase activity. The methods of determination of AKP and Na\(^{+/}\)/K\(^{+}\)-ATPase activity in jejunum mucosa (n = 6) were the same as the methods used in IPEC-J2 cells.

Real-time PCR analysis of gene expression of SGLT1, GLUT2, EGFR and AMPK-α1 in jejunum mucosa

Total RNA extraction from jejunal samples and the reverse transcription were conducted according to previous study (Peng et al. 2020). The methods of analysed mRNA expression of SGLT1, GLUT2, EGFR, AMPK-α1 (n = 6) in jejunum mucosa were the same as the methods used in IPEC-J2 cells.

Statistical analysis

All data are expressed as mean ± standard deviation (SD, n = 6). Data are statistically analysed with one-way ANOVA procedure by using the IBM SPSS statistics 21.0 software (SPSS, Inc. Chicago, IL, USA). Significant differences among the obtained means were determined by the Duncan’s multiple comparison test. Probability values < 0.05 were taken to indicate statistical significance.

Results

AKP and Na\(^{+/}\)/K\(^{+}\)-ATPase activity in IPEC-J2 cells

The effects of EGF on AKP and Na\(^{+/}\)/K\(^{+}\)-ATPase activity in IPEC-J2 cells challenged with LPS was presented in Figure 1. It showed that LPS induced severe damage to the AKP and Na\(^{+/}\)/K\(^{+}\)-ATPase, and their activity were significantly (p < 0.05) lower than that of other groups. While EGF can promote the activity of AKP and Na\(^{+/}\)/K\(^{+}\)-ATPase, and their activity were significantly (p < 0.05) higher than of other groups. EGF also promotes AKP (Figure 1(A)) and Na\(^{+/}\)/K\(^{+}\)-ATPase (Figure 1(B)) activity in LPS-induced injured cells (EGF ÷ LPS group), which is significantly higher (p < 0.05) than that of the LPS group and can reach a comparable level to that of the control group.

Gene expression of glucose transporters in IPEC-J2 cells

The effects of EGF on gene expression of glucose transporters in IPEC-J2 cells challenged with LPS were presented in Figure 2. Results showed that the LPS group had a lower expression of SGLT1 (Figure 2(B)), EGFR (Figure 2(C)) and AMPK-α1 (Figure 2(D)) compared with EGF and EGF + LPS groups (p < 0.05) and

Figure 1. Effects of EGF on AKP (A) and Na\(^{+/}\)/K\(^{+}\)-ATPase (B) activity of IPEC-J2 cells challenged by LPS (n = 6). EGF: epidermal growth factor; LPS: lipopolysaccharide; AKP: alkaline phosphatase; Na\(^{+/}\)/K\(^{+}\)-ATPase: sodium/potassium-transporting adenosine triphosphatase; *p < 0.05.
no difference compared with control group; and the EFG group had a higher expression of EGFR compared with control, LPS, and EGF + LPS groups (p < 0.05) and a higher expression of SGLT1 and AMPK-α1 compared with control, and LPS groups (p < 0.05); EGF + LPS group had a higher expression of GLUT2 (Figure 2(A)) compared with control, LPS and EGF groups (p < 0.05).

**Protein expression of glucose transporters in IPEC-J2 cells**

In accordance with above, the protein expression of GLUT2, SGLT1, EGFR and AMPK-α1 measured by western blot (Figure 3) showed that, LPS or EGF treated alone would not affect the expression of GLUT2 compared with control group (Figure 3(A)). Cells treated with EGF (EGF group) would promote the expression of SGLT1 (Figure 3(B)), EGFR (Figure 3(C)) and AMPK-α1 (Figure 3(D)) compared with control, and LPS groups. EGF plus LPS treated (EGF + LPS group) had a higher (p < 0.05) expression of GLUT2, SGLT1, EGFR and AMPK-α1 than those cells treated with LPS (LPS group).

**Intestinal histomorphology**

The results of intestinal morphology are presented in Figure 4. In comparison with the other groups, the LPS group exhibited a decrease (p < 0.05) of VH, villus height to crypt depth ratio (VCR), and an increase (p < 0.05) of CD except for EGF + LPS group; and the EGF group exhibited an increase (p < 0.05) of VH (Figure 4(A,B)), and VCR (Figure 4(A,D)) and a decrease (p < 0.05) of CD (Figure 4(A,C)) compared with other groups in the jejunum of piglets. The EGF + LPS group had a higher (p < 0.05) VH, and VCR compared with LPS group and had no difference compared to the control group.

**AKP and Na⁺/K⁺-ATPase activity in jejunum mucosa**

The effects of EGF on AKP and Na⁺/K⁺-ATPase activity in jejunum mucosa of piglets challenged with LPS were presented in Figure 5. It showed that the AKP (Figure 5(A)) and Na⁺/K⁺-ATPase (Figure 5(B)) activity in jejunum mucosa decreased significantly (p < 0.05) in LPS group compared with other groups, while, EGF group remarkably increased (p < 0.05) the AKP and Na⁺/K⁺-ATPase activity in jejunum mucosa compared with other groups. The EGF + LPS group had a higher AKP and Na⁺/K⁺-ATPase activity than the LPS group, and had no difference compared to the control group.
Figure 3. Effects of EGF on GLUT2, SGLT1, EGFR and AMPK-α1 protein expression in IPEC-J2 cells challenged by LPS (n = 6). (A) Western blot results of GLUT2 and SGLT1; (B) GLUT2 relative expression; (C) SGLT1 relative expression; (D) Western blot results of EGFR and AMPK-α1; (E) EGFR relative expression; (F) AMPK-α1 relative expression. EGF: Epidermal growth factor; LPS: lipopolysaccharide; SGLT1: sodium/glucose cotransporter 1; GLUT2: glucose transporter 2; EGFR: EGF receptor; AMPK-α1: AMP-activated protein kinase α1; *p < 0.05.

Figure 4. Effects of EGF on intestinal morphology of jejunum of weaned piglets challenged by LPS (n = 6). (A) Intestinal morphology of jejunum; (B) villus height of jejunum; (C) crypt depth of jejunum; (D) villus height to crypt depth of jejunum; EGF: epidermal growth factor; LPS: lipopolysaccharide; *p < 0.05.
Gene expression of glucose transporters in jejunum mucosa

The effects of EGF on gene expression of glucose transporters in jejunum mucosa of piglets challenged with LPS were presented in Figure 6. It showed that, as the same with the cell experiment, there was no difference in the expression of GLUT2 (Figure 6(A)) among the control group, EGF group and LPS group. Piglets fed with EGF-containing feed (EGF group) significantly (p < 0.05) promoted the expression of SGLT1 (Figure 6(B)), EGFR (Figure 6(C)) and AMPK-α1 (Figure 6(D)) compared with control, and LPS groups and significantly (p < 0.05) promoted the expression of EGFR and AMPK-α1 compared to EGF + LPS group. EGF + LPS group had a higher (p < 0.05) expression of GLUT2, SGLT1, EGFR and AMPK-α1 than that of the LPS group and the control group.

Figure 5. Effects of EGF on AKP (A) and Na⁺/K⁺-ATPase (B) activity of weaned piglets challenged by LPS (n = 6). EGF: Epidermal growth factor; LPS: lipopolysaccharide; AKP: alkaline phosphatase; Na⁺/K⁺-ATPase: sodium/potassium-transporting adenosine triphosphatase; *p < 0.05.

Figure 6. Effects of EGF on GLUT2 (A), SGLT1 (B), EGFR (C) and AMPK-α1 (D) mRNA expression in jejunum of weaned piglets challenged by LPS (n = 6). EGF: Epidermal growth factor; LPS: lipopolysaccharide; SGLT1: sodium/glucose cotransporter 1; GLUT2: glucose transporter 2; EGFR: EGF receptor; AMPK-α1: AMP-activated protein kinase α1; *p < 0.05.
Discussion

Intestinal tract is not only the main part of nutrients digestion and absorption, but also an important barrier for animals to prevent the toxins, allergens, and pathogens from the external environment into the circulation system (Arrieta et al. 2006; Tang et al. 2016; Tang, Liu, Zhong, et al. 2021). Thus, the normal integrity of epithelial cells is the structural basis for the intestinal absorption of nutrients. LPS is a major integral component of the outer membrane of gram-negative bacteria, which can induce cell injury and intestinal morphology destruction (Zhou et al. 2017; Tang et al. 2018). Intestinal morphology which often evaluated by VH, CD, and VCR can reflect the intestinal development and function, the increased VH and reduced CD, as well as the increased VCR in animals indicated a better intestinal function (Wang M, Huang, et al. 2020). The present study also found that the intestinal morphology of piglets subjected to LPS stimulation was severely damaged, manifested as a decreased VH and increased CD. Previous studies have demonstrated that EGF plays an important role in epithelial recovery and damaged intestinal repair (Tang et al. 2018; Wang L, Zhu, Yang, et al. 2020). The present study also confirmed that EGF treatment could restore intestinal morphology to a certain extent, manifested by increased VH and decreased CD, indicating that EGF could ameliorate the jejunal damage induced by LPS stress. In theory, during the process of the damaged intestinal repairing, more energy is needed to meet the energy consumption of intestinal epithelial cells.

Carbohydrates are the main source of energy for animals including piglets. Ingested carbohydrates are hydrolysed to monosaccharides by digestive enzymes in the gut, of which 80% monosaccharide is glucose. SGLT1 is the primary carrier protein responsible for the absorption of glucose from the lumen of the intestine across the brush border membrane of intestinal epithelial cells, which is dependent on Na+/K+-ATPase for energy supply (Drozdowski and Thomson 2006; Wang CW et al. 2015; Chen et al. 2016). Once inside the enterocytes, the glucose are either metabolised or diffuse out of the cell through the GLUT2, located on the basolateral membrane, into the blood circulation to maintain blood glucose balance (Wright et al. 1994; Chaudhry et al. 2012; Wang CW et al. 2015). Therefore, SGLT1, GLUT2 and Na+/K+-ATPase play important roles in glucose absorption in the small intestine. Although, studies have shown that EGF upregulated the intestinal glucose uptake by increasing the translocation of SGLT1 (Cellini et al. 2005; Bedford et al. 2015; Xu et al. 2015). Our previous study also confirmed that EGF could promote the glucose absorption in IPEC-J2 cells challenged by LPS (Tang and Xiong 2021), which can speculate that EGF repaired injured cells by increasing the absorption of glucose. However, the role of SGLT1 and GLUT2 in glucose absorption in the damaged intestine needs further confirmation.

In the present study, we used LPS to establish a cellular injury model (IPEC-J2) and animal injury model (piglets) to investigate the effects of EGF on the expression of glucose transporters SGLT1 and GLUT2 as well as to further clarify the roles of SGLT1 and GLUT2 in glucose absorption during intestinal damage repairing. The results showed that, in both IPEC-J2 cells and jejunum mucosa, LPS induced severe damage to the AKP and Na+/K+-ATPase activity, while EGF can promote AKP and Na+/K+-ATPase activity in LPS-induced injured cells and jejunum mucosa. AKP is a key enzyme in intestinal digestion and absorption, which can accelerate the uptake and transfer of nutrients, and provide energy for the body indirectly. At the same time, AKP is also an endogenous detoxification factor, which can remove LPS endotoxin secreted by pathogenic bacteria in the intestinal tract and protect intestinal health (Geddes and Philpott 2008). The increased AKP activity in EGF + LPS group suggested that EGF could promote the absorption capacity of glucose in damaged cells and intestine. Na+/K+-ATPase is an integral transmembrane protein in the basolateral membrane of intestinal epithelial cells, and the main role is to maintain the electrochemical gradient and osmotic pressure balance inside and outside the cell membrane and drive the co-transport of glucose molecules to SGLT1 with Na+ (Thorsen et al. 2014; Palanikumar et al. 2015; Chen et al. 2016).

SGLT1 and GLUT2 are two kinds of important glucose transporter. Previously, it was shown that EGF upregulated intestinal glucose uptake by increasing the translocation of SGLT1 (Chung et al. 2002; Cellini et al. 2005; Wang CW et al. 2015). However, recent studies indicated that GLUT2 can be trafficked to the apical membrane and contribute to glucose absorption in response to high glucose (Kellett et al. 2008; Chen et al. 2016). Results from the present study showed that LPS treatment had no effects on the expression of GLUT2 and SGLT1 in both IPEC-J2 cells and jejunum mucosa. EGF would promote the expression of SGLT in both IPEC-J2 cells and jejunum mucosa without affect the expression of GLUT2. Interestingly, EGF significantly increased the expression of GLUT2 and SGLT1 in LPS-induced damaged cells and jejunum...
mucosa. This indicates that in the process of intestinal repair, the glucose transported by SGLT1 is insufficient to meet the needs of the body, it is necessary to assist glucose absorption by mobilising the expression of GLUT2.

The biological function of EGF is related to its receptor, EGFR, a transmembrane glycoprotein abundantly located on the apical and basolateral aspect of villus enterocytes (Avissar et al. 2000; Tang et al. 2016). The binding of EGF with EGFR at the enterocytes surface activates a series of signalling pathways and plays a series of biological functions such as nutrients transport and intestinal repair (Wee et al. 2015; Tang et al. 2018; Wang L et al. 2019; Tang, Fang, et al. 2019; Tang, Liu, Liu 2021; Xue et al. 2021). AMPK is a key molecule in the regulation of biological energy metabolism and has been demonstrated to play important roles in the regulation of cellular glucose uptake (Carling 2004; Carling 2007; Dengler et al. 2017). It is widely accepted that the activation of AMPK leads to increased glucose transport in a variety of cells predominantly through the stimulation of the SGLT1 (Sopjani et al. 2010) and GLUT2 (Walker et al. 2005). The present study showed that EGF would promote the expression of EGFR and AMPK-α1 in normal or injury conditions. It indicated that under normal conditions, EGF up-regulates SGLT1 expression through the EGFR/AMPK signalling pathway to promote intestinal glucose absorption; while, in the process of intestinal repair, EGF up-regulates the expression of SGLT1 and GLUT2 through the EGFR/AMPK signalling pathway to jointly promote intestinal glucose absorption (Figure 7).

Figure 7. Schematic diagram of the mechanism of epidermal growth factor on glucose transport. (A) Under normal conditions, EGF up-regulates SGLT1 expression through the EGFR/AMPK signalling pathway to promote intestinal glucose absorption; (B) in the process of intestinal repair, EGF up-regulates the expression of SGLT1 and GLUT2 through the EGFR/AMPK signalling pathway to jointly promote intestinal glucose absorption. EGF: Epidermal growth factor; LPS: lipopolysaccharide; SGLT1: sodium/glucose cotransporter 1; GLUT2: glucose transporter 2; EGFR: EGF receptor; AMPK-α1: AMP-activated protein kinase α1.

Conclusions
In conclusion, EGF can activate EGFR/AMPK signalling to up-regulate the expression of SGLT1 and GLUT2 as well as improve the AKP and Na⁺/K⁺-ATPase activity, thereby promoting intestinal glucose absorption in IPEC-J2 cells and piglets challenged by LPS.

Ethical approval
The experimental procedures involving animals were approved by the animal welfare committee of the Guizhou Normal University (Guiyang, China) with an ethic approval number GZNU-2020-0042.
Disclosure statement
No potential conflict of interest was reported by the author(s).

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Data availability statement
The authors confirm that the data supporting the findings of this study are available within the article.

References
Arrieta MC, Bistritz L, Meddings JB. 2006. Alterations in intestinal permeability. Gut. 55(10):943–1520.
Avissar N, Wang H, Miller J, Iannoli P, Sax HC. 2000. Epidermal growth factor receptor is increased in rabbit intestinal brush border membrane after small bowel resection. Dig Dis Sci. 45(6):1145–1152.
Bedford A, Chen T, Huynh E, Zhu C, Medeiros S, Wey D, de Lange C, Li J. 2015. Epidermal growth factor containing culture supernatant enhances intestine development of early-weaned pigs in vivo: potential mechanisms. J Biotechnol. 196:197-19-19.
Carling D. 2004. The AMP-activated protein kinase cascade-a unifying system for energy control. Trends Biochem Sci. 29(1):18–24.
Carling D. 2007. The role of the AMP-activated protein kinase in the regulation of energy homeostasis. Novartis Found Symp. 286:72–81.
Cellini C, Xu J, Buchmiller-Crair T. 2005. Effect of epidermal growth factor on small intestinal sodium/glucose cotransporter-1 expression in a rabbit model of intrauterine growth retardation. J Pediatr Surg. 40(12):1892–1897.
Chaudhry RM, Scow JS, Madhavan S, Duenes JA, Sarr MG. 2012. Acute enterocyte adaptation to luminal glucose: a posttranslational mechanism for rapid apical recruitment of the transporter GLUT2. J Gastrointest Surg. 16(2):312–319.
Chen LH, Tuo BG, Dong H. 2016. Regulation of intestinal glucose absorption by ion channels and transporters. Nutrients. 8(1):43–53.
Chung BM, Wallace LE, Hardin JA, Gall DG. 2002. The effect of epidermal growth factor on the distribution of SGLT-1 in rabbit jejum. Can J Physiol Pharmacol. 80(9):872–878.
Dengler F, Rackwitz R, Pfannkuche H, Gabel G. 2017. Glucose transport across lagomorph jejum epithelium is modulated by AMP-activated protein kinase under hypoxia. J Appl Physiol. 123(6):1487–1500.
Drozdowski LA, Thomson ABR. 2006. Intestinal sugar transport. World J Gastroenterol. 12(11):1657–1670.
Ferenc K, Pietrzak P, Godlewski MM, Piwowarski J, Kiliaricz K, Guilloteau P, Zabielski R. 2014. Intrauterine growth retarded piglet as a model for humans-studies on the perinatal development of the gut structure and function. Reprod Biol. 14(1):51–60.
Geddes K, Philpott DJ. 2008. A new role for intestinal alkaline phosphatase in gut barrier maintenance. Gastroenterology. 135(1):8–12.
Hu L, Peng X, Chen H, Yan C, Liu Y, Xu Q, Fang Z, Lin Y, Xu S, Feng B, et al. 2017. Effects of intrauterine growth retardation and Bacillus subtilis PB6 supplementation on growth performance, intestinal development and immune function of piglets during the suckling period. Eur J Nutr. 56(4):1753–1765.
Huang Q, Li N, Zhu W, Li Q, Li J. 2007. Glutamine transporter ASCT2 was down-regulated in ischemic injured human intestinal epithelial cells and reversed by epidermal growth factor. J Repen Terent Nutr. 31(2):86–93.
Huerzeler N, Petkovic V, Sekulic-Jablanovic M, Kucharava K, Wright MB, Bodmer D. 2019. Insulin receptor and glucose transporters in the mammalian cochlea. Audiol Neurootol. 24(2):65–76.
Kellett GL, Brot-Laroche E, Mace OJ, Leturque A. 2008. Sugar absorption in the intestine: the role of GLUT2. Annu Rev Nutr. 28:35–54.
Li B, Li W, Hussain A, Zhang L, Wang C, Wang T. 2015. Effects of choline on meat quality and intramuscular fat in intrauterine growth retardation pigs. PLoS One. 10(6):e0129109.
Palanikumar M, Swapna G, Subha A, Balasubramanian P, Soudamani S, Gregory MD, Uma S. 2015. Chronic and selective inhibition of basolateral membrane Na-K-ATPase uniquely regulates brush border membrane Na absorption in intestinal epithelial cells. Am J Physiol Cell Phys. 308: C650–C656.
Peng P, Deng D, Chen S, Li C, Luo J, Romeo A, Li T, Tang X, Fang R. 2020. The effects of dietary porous zinc oxide supplementation on growth performance, inflammatory cytokines and tight junction’s gene expression in early-weaned piglets. J Nutr Sci Vitaminol. 66(4):311–318.
Sopjani M, Bhavsar SK, Fraser S, Kemp BE, Föller M, Lang F. 2010. Regulation of Na⁺-coupled glucose carrier SGLT1 by AMP-activated protein kinase. Mol Membr Biol. 27(2-3):137–144.
Tang X, Fang R, Pan G, Xiong K. 2019. Acute effect of epidermal growth factor on phosphate diffusion across intestinal mucosa of hens using the Ussing chamber system. Pakistan J Zool. 51:2209–2216.
Tang X, Liu X, Liu H. 2021. Mechanisms of epidermal growth factor effect on animal intestinal phosphate absorption: a review. Front Vet Sci. 8:670140.
Tang X, Liu B, Wang X, Yu Q, Fang R. 2018. Epidermal growth factor, through alleviating oxidative stress, protect
IPEC-J2 cells from lipopolysaccharides-induced apoptosis. IJMS. 19(3):848.

Tang X, Liu H, Yang S, Li Z, Zhong J, Fang R. 2016. Epidermal growth factor and intestinal barrier function. Mediators Inflamm. 2016:1927348.

Tang X, Liu X, Zhong J, Fang R. 2021. Potential application of Lonicera japonica extracts in animal production: from the perspective of intestinal health. Front Microbiol. 12:719877.

Tang X, Su W, Fang R. 2019. Effects of calcitonin on porcine intestinal epithelial cells proliferation, phosphorus absorption, and NaPi-Iib expression. Pakistan J Zool. 51:2167–2174.

Tang X, Xiong K. 2021. Effects of epidermal growth factor on glutamine and glucose absorption by IPEC-J2 cells challenged by lipopolysaccharide using the Ussing chamber system. Pakistan J Zool. 52:417–422.

Tang X, Xiong K. 2022. Intrauterine growth retardation affects intestinal health of suckling piglets via altering intestinal antioxidant capacity, glucose uptake, tight junction and immune responses. Oxid Med Cell Longev. 2022:2644205.

Thorsen K, Drengstig T, Ruoff P. 2014. Transepithelial glucose transport and Na⁺/K⁺ homeostasis in enterocytes: an integrative model. Am J Physiol Cell Physiol. 307(4):C320–C337.

Trapani V, Arduini D, Luongo F, Wolf FI. 2014. EGF stimulates Mg²⁺ influx in mammary epithelial cells. Biochem Biophys Res Commun. 454(4):572–575.

Walker J, Jijon HB, Diaz H, Salehi P, Churchill T, Madsen KL. 2005. S-Aminoimidazole-4-carboxamide riboside (AICAR) enhances GLUT2-dependent jejunal glucose transport: a possible role for AMPK. Biochem J. 385(Pt 2):485–491.

Wang CW, Chang WL, Huang IC, Chou FC, Chan FN, Su SC, Huang SF, Ko HH, Ko YL, Lin HC, et al. 2015. An essential role of cAMP response element-binding protein in epidermal growth factor-mediated induction of sodium/glucose cotransporter 1 gene expression and intestinal glucose uptake. Int J Biochem Cell Biol. 64:239–251.

Wang M, Huang H, Hu Y, Huang J, Yang H, Wang L, Chen S, Chen C, He S. 2020. Effects of dietary microencapsulated tannic acid supplementation on the growth performance, intestinal morphology, and intestinal microbiota in weaning piglets. J Anim Sci. 98:skaa112.

Wang L, Zhu F, Li J, Li YL, Ding XQ, Yin J, Xiong X, Yang HS. 2020. Epidermal growth factor promotes intestinal secretary cell differentiation in weaning piglets via Wnt/β-catenin signalling. Animal. 14(4):790–798.

Wang L, Zhu F, Yang H, Li J, Li Y, Ding X, Xiong X, Ji F, Zhou H, Yin Y. 2020. Epidermal growth factor improves intestinal morphology by stimulating proliferation and differentiation of enterocytes and mTOR signaling pathway in weaning piglets. Sci China Life Sci. 63(2):259–268.

Wang L, Zhu F, Yang H, Zhong J, Li Y, Ding X, Xiong X, Yin Y. 2019. Effects of dietary supplementation with epidermal growth factor on nutrient digestibility, intestinal development and expression of nutrient transporters in early-weaned piglets. J Anim Physiol Anim Nutr. 103(2):618–625.

Wang M, Huang H, Hu Y, Huang J, Yang H, Wang L, Chen S, Chen C, He S. 2020. Effects of dietary microencapsulated tannic acid supplementation on the growth performance, intestinal morphology, and intestinal microbiota in weaning piglets. J Anim Sci. 98:skaa112.

Wang L, Zhu F, Li J, Li YL, Ding XQ, Yin J, Xiong X, Yang HS. 2020. Epidermal growth factor promotes intestinal secretory cell differentiation in weaning piglets via Wnt/β-catenin signalling. Animal. 14(4):790–798.

Wang L, Zhu F, Yang H, Li J, Li Y, Ding X, Xiong X, Ji F, Zhou H, Yin Y. 2020. Epidermal growth factor improves intestinal morphology by stimulating proliferation and differentiation of enterocytes and mTOR signaling pathway in weaning piglets. Sci China Life Sci. 63(2):259–268.

Wang L, Zhu F, Yang H, Zhong J, Li Y, Ding X, Xiong X, Yin Y. 2019. Effects of dietary supplementation with epidermal growth factor on nutrient digestibility, intestinal development and expression of nutrient transporters in early-weaned piglets. J Anim Physiol Anim Nutr. 103(2):618–625.

Wang M, Huang H, Hu Y, Huang J, Yang H, Wang L, Chen S, Chen C, He S. 2020. Effects of dietary microencapsulated tannic acid supplementation on the growth performance, intestinal morphology, and intestinal microbiota in weaning piglets. J Anim Sci. 98:skaa112.