

Lipopolysaccharide-Induced Chorioamnionitis Induces Fetal Intestinal Injury and Affects Gut Colonization in Rats

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Abstract

Background: Chorioamnionitis is associated with an increased risk of multiple adverse outcomes in offspring, especially neonatal necrotizing enterocolitis (NEC), which is one of the serious gastrointestinal diseases in neonates. However, the underlying mechanism remains undefined. We hypothesize that lipopolysaccharide (LPS)-induced chorioamnionitis causes intestinal injury in offspring, thereby affecting the composition of the intestinal microbiome.

Methods: Pregnant Sprague Dawley rats were received intraperitoneal injections with 700 µg/kg Lipopolysaccharide (LPS) or saline at 15 days of gestation. Pups were allowed to deliver naturally, and euthanized at days 0,3 and 7 after birth. Intestinal tissue and faeces samples from offspring were collected to evaluate the effects of intrauterine infection on intestinal flora colonization and intestinal mucosal development.

Results: Significant intestinal injury of the offspring induced by prenatal LPS exposure was observed at day 0 and 3 after birth.. In addition, prenatal LPS exposure also induced significant changes in the intestinal microbiome of the offspring with a significant increase in *Proteobacteria (Escherichia-Shigella)* and a decrease in *Firmicutes* at 7 days after birth .

Conclusions: Thus, our findings suggest that LPS-induced chorioamnionitis induces intestinal injury in offspring and subsequently leads to NEC-like changes in the composition of the intestinal microbiome.

1. Background

Chorioamnionitis is used to refer to the intrauterine infection/inflammation, which includes amniotic fluid, placenta (decidua, chorion and amniotic membrane) and fetal infection caused by pathogenic microorganisms invading the amniotic cavity[1]. Intrauterine infection is linked with adverse neonatal outcomes including neonatal early-onset sepsis, neonatal necrotizing enterocolitis(NEC), patent ductus arteriosus, neonatal respiratory distress syndrome, bronchopulmonary dysplasia, cerebral palsy ,and hearing impairment [2–7]. Among them, intestinal injury, especially NEC is a serious gastrointestinal disease during the neonatal period, and the mortality rate is as high as 20%-30%[8]. Several studies have reported that intrauterine infection is associated with an increased risk of NEC[6, 9].However, the mechanisms linking intrauterine infections to neonatal intestinal injury remain unclear,and how such intestinal injury of the newborns develops into NEC has not been elucidated.

Several animal studies have demonstrated that intrauterine infections prevent fetal intestinal development during pregnancy[10],leading to the disruption of the tight junctional protein zonula occludens protein 1 (ZO-1) ,the increase of intestinal fatty acid-binding protein in serum [10–12] the loss of epithelial cell integrity, which further impaires epithelial differentiation. Research have shown that intrauterine infections cause direct injury to the placenta but indirect injury to the fetal intestine[13] based on the findings that lipopolysaccharide(LPS) cannot pass through the placenta. Furthermore, placental microbiome is unable to be detected in a model of LPS-induced intrauterine infection. It is widely

recognized that fetal gastrointestinal tract, amniotic fluid and placenta are sterile[14],and microorganisms quickly colonized in newborns only after delivery,even if there is still controversy[15, 16]. Therefore, whether the fetal intestinal injury caused by intrauterine infections have an influence on the initial colonization of gastrointestinal microbes in infants is of great significance. Studies have proved that immature or damage of the intestinal epithelial barrier caused by premature birth or antibiotic interference is associated with abnormal colonization of the intestinal flora[14, 17].Intestinal flora disorders are associated with the onset of NEC, presenting a bloom of *Proteobacteria*, specifically *Enterobacteriaceae* prior to NEC[18–20]. These results suggest that aberrant gut colonization and intestinal injury may have an important impact on the development of NEC.

We hypothesize that LPS-induced chorioamnionitis causes intestinal injury and subsequent affects the composition of the intestinal microbiome.To better understand the association between intrauterine infection and subsequent neonatal intestinal injury, and to explore the potential effects on the composition of the intestinal microbiome, we investigated the changes of the intestinal tissue structure, the intestinal epithelial barrier,and the composition of intestinal microbiome after establishing a LPS-induced rat model for intrauterine infection on.

2. Methods

2.1 Animals and sample collection

All animal studies were approved by the animal ethics committee of Guangxi Medical University(Guangxi,China) and in accordance with ARRIVE guidelines.The Sprague Dawley rats purchased from the Animal Experimental Center of Guangxi Medical University were used.All rats were housed with a 12-hour light cycle and free to get food and water during both housing and experimental time. At the gestation of day 15, pregnancy rats were randomly assigned to two groups, receiving a single 700 µg/kg intraperitoneal injection of LPS (*Escherichia coli* 055:B5;Sigma-Aldrich,St.Louis,MO) or the equivalent volume of saline for sham controls,respectively. All pregnancy rats were allowed to delivered naturally. Pups were allowed to stay together with their mothers after birth and allowed to breast-feed ad libitum. Pups were weighed and then euthanized by CO₂ inhalation at days 0,3 and 7 after birth. The terminal ileum was collected and fixed overnight in 10% formalin solution for immunohistochemical stainings and histology stainings. Feces were collected in a sterile container and stored at -80 °C until processing.

2.2 Antibodies and reagents

The rabbit antibody against human zonula occludens protein 1 (ZO-1) (catalog no.48588, 1:200; Signalway Antibody, Maryland, USA) and the biotin conjugated goat anti-rabbit (catalog no.SP-9000-6 ml) from Zsbio (Peking, China) were used as the primary and the secondary antibodies, respectively.

2.3 Hematoxylin and eosin stain

The formalin-fixed terminal ileum tissues were embedded in paraffin and cut into 4 µm thick sections. Intestinal ileum sections were stained with hematoxylin and eosin(H&E). Morphology changes in the intestinal ileum were assessed microscopically by a single investigator in a blinded manner [21]. The injury scores was as follows: Grade 0, normal mucosal villi; Grade 1, the subepithelial Gruenhagen's spaces developed with capillary congestion; Grade 2, extension of the subepithelial space with moderate lifting of epithelial layer from the lamina propria; Grade 3, massive epithelial lifting down the sides of villi;Grade 4, denuded villi with lamina propria and dilated capillaries exposed; and Grade 5, digestion and disintegration of lamina propria; hemorrhage and ulceration.

2.4 Immunohistochemistry

For staining of ZO-1 expressing cells, the ileum sections were boiled under high pressure in sodium-citrate buffer (pH 6.0) for 10 minutes for antigen retrieval. Subsequently, endogenous peroxidase activity was blocked by incubating with 3% H₂O₂ for 10 minutes. Nonspecific binding sites was blocked by normal goat serum for 30 minutes at room temperature. Thereafter, the slides were incubated with anti-ZO-1 antibodies for overnight at 4 °C. After washing, goat anti-rabbit biotin-conjugated secondary antibody were added to the sections and incubated for 10 minutes at room temperature. ZO-1 antibodies were recognized with streptavidin-biotin method (Zsbio ,Peking, China) and visualized with nickel-DAB. The nuclei were counterstained with hematoxylin. Slides were observed using a light microscope and scanned using the cellSens imaging system(Olympus,Tokyo,Japan). Stain-positive average optical density(AOD) were measured in 5 high power fields using Image J(1.52b software,National Institutes of Health, Bethesda, MD, USA). The average values of AOD in 5 high power fields at the end of the ileum of each animal were used for the analyses.

2.5 Fecal microbiome analysis:

Deoxyribonucleic acid(DNA)was isolated from feces samples using the E.Z.N.A.Stool DNA Kit (Omega, Inc, USA) according to the manufacturer's instructions. The extracted DNA samples was stored at -20°C until analysed. The V3-V4 hypervariable regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene were amplified using the following primers: 338F(5'-ACTCCTACGGGAGGCAGCAG-3') and 806R: (5'-GGACTACHVGGGTWTCTAAT-3').Polymerase chain reaction(PCR) was performed using the following 20 µl reactions: 10 ng of template DNA, 0.8 µl of forward primer(5 µM), 0.8 µl of reverse primer(5 µM), 2 µl of dNTPs (2.5 mM), 4 µl of 5 × FastPfu Buffer, 0.4 µl of FastPfu Polymerase, and deionized ultrapure water to adjust the volume. The PCR programme included an initial denaturation at 95 ° C for 3 minutes, then 27 cycles of 95 ° C for 30 seconds,55 ° C for 30 seconds, 72 ° C for 45seconds, and the final extension at 72°C for 10 minutes. There were 3 replicates per sample, and the PCR products of the same sample were mixed and detected by 2% agarose gel electrophoresis, followed by purification using AxyPrep DNA Gel Extraction Kit(Axygen,USA) according to the manufacturer's protocols. The purified PCR products were quantified using the Quant-iT PicoGreen dsDNA Assay Kit(Invitrogen,USA) on the QuantiFluor™-ST Blue Fluorescence System (Promega, USA), following the manufacturers' instructions. The amplicon libraries were established using the TruSeq™ DNA Sample Prep Kit (Illumina, USA) according to the manufacturer's instructions. The libraries were paired-end sequenced (2 × 300 bp) on the Illumina MiSeq

platform (Majorbio, Shanghai, China) with MiSeq Reagent Kit v2 (500 Cycles). The raw sequencing data were submitted to the Sequence Read Archive (SRA) of NCBI with the accession number PRJNA631599.

In order to obtain high-quality sequences, FLASH and Trimmomatic were used to quality filter and optimize the raw sequences according to the filtering criteria as previously described [22]. The high-quality sequences with 97% similarity were clustered to the same operational taxonomic units (OTUs) by Usearch (version 7.0 <http://drive5.com/uparse/>). The classification of the representative sequences of each OTU was analyzed by RDP Classifier (<http://rdp.cme.msu.edu/>) with the confidence threshold of 70% based on the 16S rRNA database of Silva (Release 128 <http://www.arb-silva.de>). OTU abundance information was normalized using a standard of sequence number corresponding to the sample with the least sequences. To compare the species richness and evenness between samples, we used Mothur 1.30.1 to calculate Alpha-diversity index consisted of Chao, Sobs and Shannon diversity indices.

2.6 Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). The data were shown as mean \pm SEM or median and interquartile range (IQR) based on the distribution. The Mann-Whitney U tests or unpaired student T tests were performed to compare the differences between the two groups. The *P* value less than 0.05 indicated statistically significant difference.

3. Results

3.1 The effect of LPS-induced intrauterine infection on survival of female and neonatal rats

In order to explore the appropriate dose of LPS for establishing an animal model of intrauterine infection, we observed maternal mortality, abnormal pregnancy rates, normal delivery rates and neonatal mortality by intraperitoneal injection of different doses of LPS in pregnant rats (Fig. 1A, *n* = 40 pregnant rats). We found that the increased doses of LPS did not cause premature delivery, but the fetus died in utero and were absorbed by the mothers or were directly responsible for the mother's death. All newborns were delivered at term. The normal delivery rates decreased with the increased LPS doses of 0, 0.3, 0.5, 0.7, 0.9 mg/kg, and the normal delivery rates were 100%, 50%, 33%, 25% and 0%. The abnormal pregnancy rates, which were the rates at which the fetus died in utero and were absorbed by the mothers, also varied with the dose of LPS. When LPS was administered at 0, 0.3, 0.5, 0.7 mg/kg, the abnormal pregnancy rates were 0%, 50%, 67%, 75%, and the maternal mortality were all at 0%. However, when the dose was raised to 0.9 mg/kg, the maternal mortality rate rose by 25% with an abnormal pregnancy rate of 75%. As a result, we chose a dose of 0.7 mg/kg LPS for subsequent experimental studies to maximize intestinal changes and adequate litters while minimizing the risk of maternal mortality. In order to ensure that pregnant rats have a similar response to chorioamnionitis by intraperitoneal injection of LPS at a dose of 0.7 mg/kg, we collected uterus specimens for pathological examination. Pregnant rats exposed to LPS had obvious inflammatory cell infiltration in the uterus (Fig. 1B).

During the experimental period of 7 days, we occasionally found the phenomenon that mothers ate their pups and apart from that, none of the pups die. The activities, feeding and hair color of all pups included in the LPS group and the sham group were normal after birth. There was no significant difference of birth weight and body weight gains between the LPS group and the sham group (Fig. 1C, n = 136 pups).

3.2 Histological analyses of intestinal ileum indicated that prenatal LPS exposure induced intestinal injury in neonatal rats

To determine whether prenatal LPS exposure would induce neonatal intestinal injury and has an effect on intestinal development, we collected distal ileum from different ages of pups for pathological histological analyses. We found that the overall structure of intestinal mucous from the sham groups were normal at any age, the intestinal villi were well-shaped and neatly arranged, the intestinal mucosal epithelial cells were not necrotic and exfoliated, and no inflammatory cells infiltration were observed in the intestinal mucosal layer (Fig. 2A). However, the intestinal structure of pups exposed to LPS at first days of life were disordered. Compared with the sham group, we could find shortened and irregular villi, edema of the submucosa, and the infiltration of a large number of inflammatory cells in the submucosa. At 3 days after birth, a large number of mucosal epithelial cells were denatured and swollen, submucosal edema was further aggravated and a large number of inflammatory cells were infiltrated. However, on the 7th day, the intestinal mucosa structure was better than before, villi structure were orderly arranged, no inflammatory cell infiltration was observed, but edema of some mucosal epithelial cells was still visible (Fig. 2A).

The results indicated that prenatal LPS exposure definitely induced fetal intestinal injury. Subsequently, we further assessed the injury using the intestinal injury score as previously described [21]. The injury scores of the pups exposed to LPS were significantly higher than that of the sham groups at d0 and d3 (Fig. 2B) ($P = 0.045$, n = 9 pups in the sham group and 7 pups in the LPS group at d0; $P = 0.038$, n = 7 pups in the sham group and 9 pups in the LPS group at d3). Although the difference did not reach significance, the injury scores of LPS group was still higher than the sham group at 7 days after birth ($P = 0.909$; LPS n = 7, Sham n = 6).

3.3 Immunohistochemical analysis suggested that prenatal LPS exposure caused the loss of intestinal wall integrity in neonatal rats

We further evaluated intestinal wall integrity by staining epithelial tight junction protein ZO-1, which plays an important role in maintaining intestinal wall barrier integrity by connecting intestinal epithelial cells [23, 24]. As shown in Fig. 2C, the intestinal epithelium ZO-1 protein in the sham groups showed a strong brown

staining and was evenly distributed on the top of the junction of ileal epithelial cells. However, the ZO-1 protein staining in the ileum tissue of the LPS group was weaker and unevenly distributed, showing discontinuous spots or short bands.

Compared to controls, intestinal ZO-1 expression in pups with prenatal LPS exposure decreased significantly at d0 and d3 (Fig. 2D) ($P=0.005$, $n=10$ pups in the sham group and 7 pups in the LPS group at d0; $P=0.003$, $n=8$ pups in the sham group and 10 pups in the LPS group at d3). At 7 days, we observed that the expression level of ZO-1 in the LPS group was slightly lower than that in the sham group, but there was no statistical difference ($P=0.327$; Sham $n=8$, LPS $n=7$).

3.4 LPS-induced intestinal injury changes the composition of the intestinal microbiome

We next investigated the fecal microbiota to determine if prenatal LPS exposure would affect gut colonization in rats. As shown in Fig. 3A, the Shannon diversity index in two groups gradually increased with the increase of age, and the diversity index of the sham group at d7 was significantly higher than that of pups at 3 days after birth ($P<0.05$), however, no significant difference was found in the diversity index between different ages in the LPS groups. Compared to sham controls, there were no significant differences in diversity index at any ages.

We further investigated the microbiota composition in different groups at different ages at the phyla and genus levels. At the phylum level, the composition of the microbiota in 20 fecal specimens of the two groups of pups revealed that the phyla of *Firmicutes*, *Proteobacteria* were the most abundant (Fig. 3B). The relative abundance of *Bacteroidetes* in the LPS group (0.045%) was significantly higher as compared to the sham group (0.006%) at 3 days of life ($P=0.045$) (Fig. 3C). Interestingly, we found that the relative abundance of the *Firmicutes* in the sham group increased significantly from 49.52–91.61% ($P=0.012$) and the relative abundance of the *proteobacteria* decreased significantly from 49.96–7.93% ($P=0.012$) with the increasing age, however, no significant differences were observed in the composition of the microbiota at the phylum level among different ages in the LPS groups. Furthermore, we found that the proportion of *Proteobacteria* and *Bacteroidetes* in the LPS group significantly increased ($P=0.012$ and 0.044) and the proportion of *Firmicutes* significantly decreased ($P=0.012$) when compared with that in the Sham group at 7 days after birth (Fig. 3D).

As shown in Fig. 4, at the genus level, the population of *Actinomyces* and *Enterococcus* in the LPS group at 3 days of life was lower than the Sham group ($P=0.036$ and 0.011), and the relative abundance of *Bacteroides* was higher in the LPS group compared to the Sham groups ($P=0.025$) (Figure 4B). At 7 days of life, fecal samples from the LPS group had higher levels of *Escherichia-Shigella* ($P=0.012$) and *Bacteroides* ($P=0.044$) compared to Sham group. Meanwhile, the proportion of *Lactobacillus*, *Rodentibacter* and *Veillonella* significant decreased in LPS group as compared to Sham group ($P=0.012, 0.036$ and 0.021) (Figure 4C).

4. Discussion

Chorioamnionitis is a serious problem as it is often clinically silent, and the diagnosis is based on pathological examination of the placenta or obvious clinical manifestations which was considered to reflect the more serious side of the continuum. This prenatal inflammation is associated with an increased risk of several complications in neonate including NEC[2–7]. However, the exact mechanism is not yet clear. Our study clearly shows that maternal prenatal LPS exposure indeed influences the normal development of intestine in utero and postnatal young rats. Intrauterine infection alone, not combined with prematurity, significantly reduced the expression of the tight junction protein ZO-1, which plays a key role in maintaining the integrity of paracellular intestinal barrier. Furthermore, our study also showed that intrauterine inflammation affected the colonization of intestinal microbiome in young rats with significantly increased the abundance of *Enterobacteriaceae*, *Escherichia-Shigella*, and significantly decreased the abundance of *Lactobacillus* and other beneficial bacteria, which is similar to the alteration of intestinal microbiome in human infants who develop into NEC[18–20].

The intestinal epithelium not only separates internal organs from the harmful environment of gut lumen, but also protects against incursion of toxins and foreign microorganisms[25, 26]. ZO-1 is one of the main transmembrane proteins that compose the tight junction of the intestinal epithelium, and plays an important role in maintaining the integrity of the intestinal mucosal barrier and intestinal permeability[27]. Disruption of the intestinal barrier is associated with downregulation of tight junction proteins and has been observed in many intestinal diseases such as NEC[28, 29]. It is well known that intrauterine infection is associated with an increased risk of NEC[6]. Animal studies have shown that intrauterine infections induced by prenatal LPS exposure induced fetal intestinal injury[10, 13, 30], however the detailed changes taking place at epithelial barrier during intrauterine infections is still not revealed. Our study shows that intrauterine infections induced by prenatal LPS exposure had a disrupted villous mucosal structure at birth, and this effect lasted for 3 days after birth and gradually recovered by 7 days, but the injury score still higher than sham group ($P > 0.05$) (Fig. 2B). Furthermore, the expression of tight junction protein ZO-1 at different ages between the two groups was consistent with the results of intestinal histological examinations (Fig. 2C–Figure 2D). These results support the findings in previous reports that prenatal inflammation exposure does disrupt the normal development of the intestinal mucosal barriers of offspring[10, 11, 31]. However, Fricke *et al.* [13] have reported that intestinal injury of the offspring induced by prenatal inflammation exposure persists into adulthood. The differences of animal models and the injury scores possibly contributes to the different results found in our model. In addition, the prior animal studies were focused on prematurity, whose intestinal mucosal barrier was immature[11–13]. Therefore, it was not clear whether inflammation or prematurity or even their combination induced intestinal injury. Our study demonstrated that intrauterine infection alone, not combined with prematurity, impaired the normal development of the intestinal mucosal barrier in offspring, and we speculate that such effect may initiate during gestation and last for one week or even longer in human infants.

Gut injury associated with abnormal colonization of the intestinal flora[17], since intestinal epithelium is not only the energy source of intestinal flora, but also the important habitat of intestinal flora[32]. However, the effects of gut injury induced by intrauterine infection on colonization of the intestinal flora of offspring are not known. Our data show that there were no significant differences in

diversity index at any ages of life when compared to sham controls, which is consistent with the results of previous study on human infants [33]. While *Firmicutes* and *Proteobacteria* dominated in all the samples, there is an increase of the *Firmicutes* with corresponding a decrease of *Proteobacteria* in sham groups, which is similar to the change of intestinal microbiome in full term infants [34]. However, no obvious change was found in the intestinal microbiome in pups whose mothers exposure to LPS during the period of our observation. Interestingly, when compared to the sham group, we found that pups whose mothers exposure to LPS had lower *Firmicutes* (*Lactobacillus*) and higher *Proteobacteria* (*Escherichia-Shigella*) in their intestinal microbiome at 7 days of samples. Using a mice models, Elgin et al reported [35] that pups exposure to maternal inflammation did not induce alterations in the composition of the microbiome. However, Puri et al reported [33] that the relative abundance of family *Mycoplasmataceae* (phylum *Tenericutes*), genus *Prevotella* (phylum *Bacteroidetes*) and genus *Sneathia* (phylum *Fusobacteria*) was higher in preterm infants with chorioamnionitis, they found that aberrant intestinal colonization induced by chorioamnionitis was associated with later sepsis. It is possible that the different results of microbial composition with maternal inflammation between our study and those of others are different research species, different experimental designs and different environments.

Several studies have reported [18–20], that dysbiosis in early colonizing organisms often prior to the onset of NEC in human infants, which is characterized by an increase in *Proteobacteria*, especially *Enterobacteriaceae*. It is well known that intrauterine infection is associated with an increased risk of NEC [6]. We found that intrauterine infections induced both intestinal injury and aberrant intestinal colonization in their offspring. Historically, the gastrointestinal tract of infants is generally considered to be sterile before birth [14], and the intestinal microbiome begins to colonize gradually after birth, even if there is still controversy [15, 16]. Moreover, recent animal study have found that the placenta of pregnant mice injected with LPS had no detectable microbiome [13]. Our study revealed that intestinal injury induced by intrauterine infections was present at birth, suggesting that intestinal injury may begin in utero. However, pups whose mothers exposure to LPS did not show abnormal changes in their intestinal microbiome until 7 days after birth. Based on these findings, we speculate that intrauterine infection may hinder the normal intestinal development of the fetus and cause intestinal injury to the fetus. Intestinal injury induced by intrauterine infection may impair the the ability of the intestine to resist the invasion of bacteria and infection of normal sterile tissue. This results in NEC-like changes in intestinal colonization, characterized by an explosion in *Proteobacteria* (*Escherichia-Shigella*) and a decrease in *Firmicutes*. Bacteria pass through injured intestinal epithelial barrier, leading to tissue destruction, which develops into NEC [36]. Our data provide possible explanation for the increased risk of NEC in infants exposed to intrauterine infection.

There are some shortcomings in our study. First, the sample size in this study is limited, and the results can be confused by differences between models and shams factors such as gestational age or experimental operation. Second, our current studies have not observed either the long-term effects of intrauterine infection on the intestinal microbiota of the offspring or the effects of intrauterine infection on the development of intrauterine intestinal mucosa in the fetus. Therefore, studies with much larger scale with much longer follow-up are necessary to address these issues.

5. Conclusion

Our findings demonstrate that intrauterine infection does induce intestinal injury to the offspring, and the effect may initiate in utero. The intrauterine infection induces significant alterations of the composition of intestinal microbiota after intestinal injury, which is similar to the microbiota in human infants who develop into NEC. Our results provide support from animal evidence for understanding the mechanism why infants exposed to intrauterine infections before birth have a higher incidence of NEC.

Abbreviations

NEC: necrotizing enterocolitis; LPS: lipopolysaccharide; ZO-1: zonula occludens protein 1; AOD: average optical density; DNA: Deoxyribonucleic acid; rRNA: ribosomal ribonucleic acid; PCR: Polymerase chain reaction; OTUs: operational taxonomic units

Declarations

Ethics approval and consent to participate

Our animal study protocol was approved by the animal ethics committee of Guangxi Medical University (Guangxi, China). We performed the animal experiments following the *Guiding Opinions on the Treatment of Laboratory Animals* issued by the Ministry of Science and Technology of the People's Republic of China and the *Laboratory Animal-Guideline for Ethical Review of Animal Welfare* issued by the National Standard GB/T35892-2018 of the People's Republic of China.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials presented in this study are available from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

QH designed the study, performed experiments, collected and analyzed data, wrote the first draft of the manuscript. SL performed experiments. YZ performed experiments and collected data. BW conceptualized the study, edited and revised the manuscript. FB performed experiments and collected data. YC designed the study, interpreted data, and finalized the manuscript. All authors have approved the version of the submitted manuscript.

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Figures



Figure 1

Different doses of intraperitoneal lipopolysaccharide (LPS) result in different maternal and fetal outcomes. (1A) Increased the dose of LPS leads to an higher rates of mater death or abnormal pregnancy that the fetus were absorbed by their mothers. (1B) Pregnant rats exposed to LPS had obvious inflammatory cell infiltration in the uterus(Magnification: 200×, Scale bar: 100µm). (1C) Body weight of pups rats of different ages in Sham groups and LPS groups.



Figure 2

Pathological changes in ileum tissues of two groups at different ages (2A)(Magnification: 200×, Scale bar: 100µm). Injury score of samples in different ages from two groups(2B). Immunolocalisation of ZO-1 in the ileum(2C, 2D). The staining positive protein of ZO-1 was evenly distributed at the top of the junction of small intestinal mucosal epithelial cells in the sham groups, and the positive expression of protein was dark brown, while ZO-1 was unevenly distributed and the staining became shallow in the LPS groups(2C) (Magnification: 400×, Scale bar: 50µm). Dynamic comparison of the average optical density values of ZO-1 in the ileum between LPS groups and sham groups at different ages(2D). Asterisk indicates significantly differences(* P <0.05).

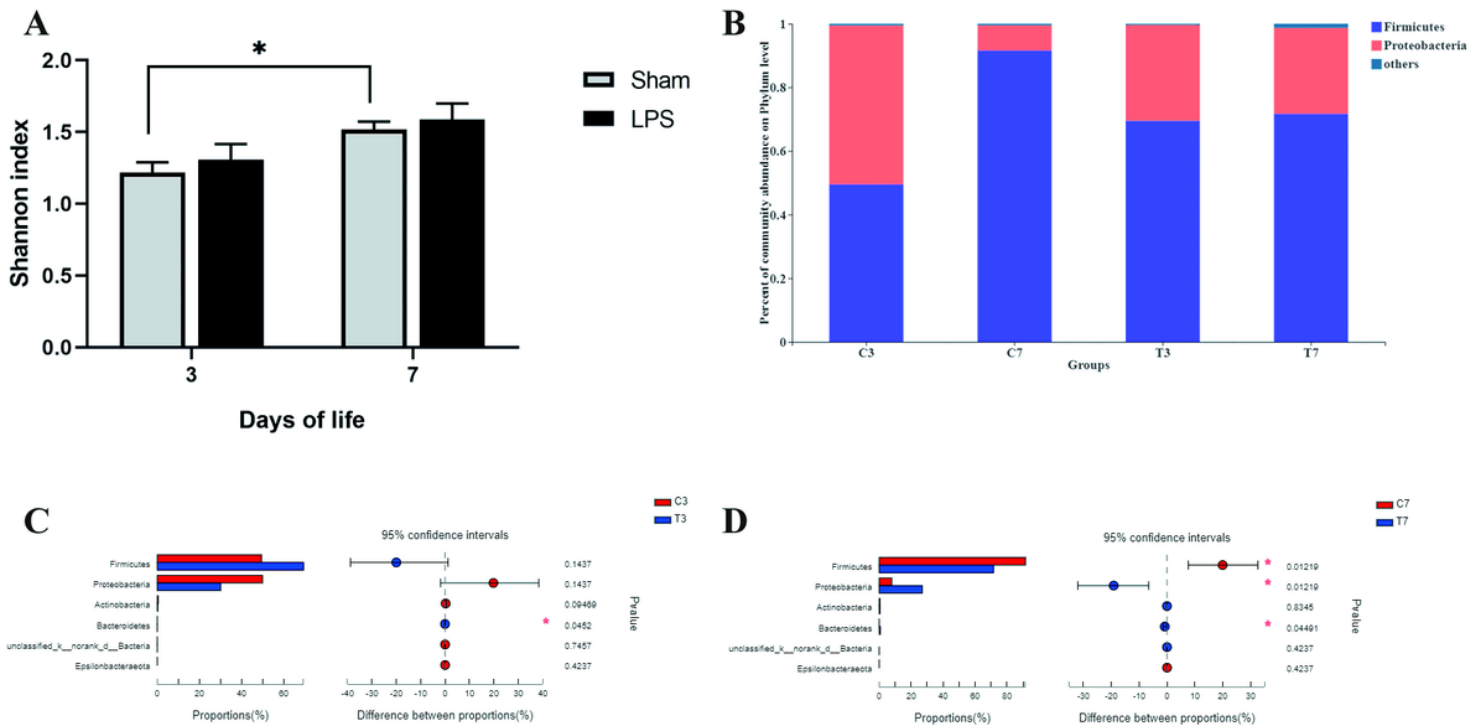


Figure 3

Shannon diversity index of samples in different ages from two groups (3A). Microbiota composition at phylum level in fecal samples from LPS groups and sham groups at different ages(3B). Comparison of the relative abundance of microbial communities between the LPS groups and sham groups on d3, d7. C, Sham group; T, LPS group (3C, 4D). Asterisk indicates significantly differences(* P <0.05).

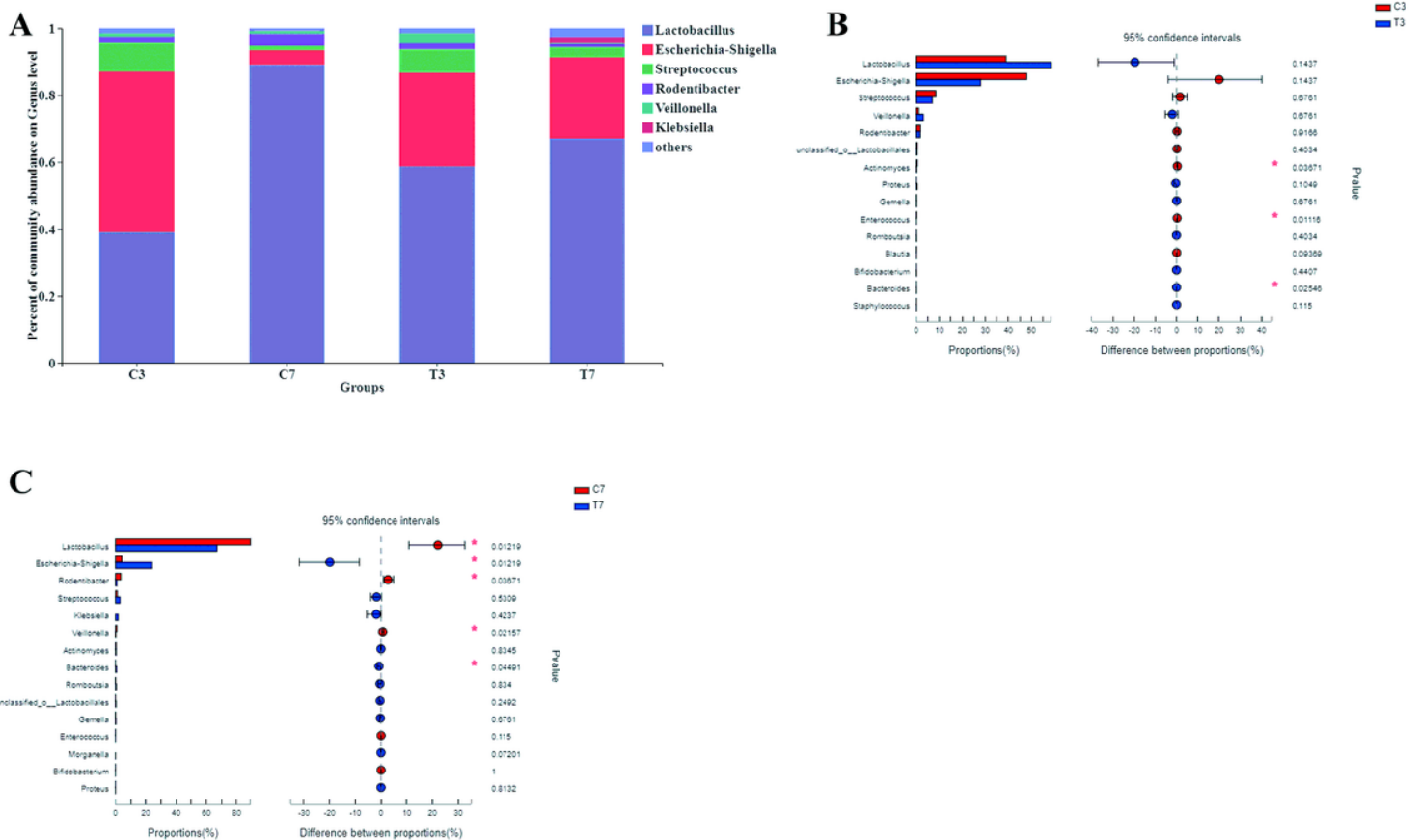


Figure 4

Microbiota composition at genus level in fecal samples from LPS groups and sham groups at different ages(4A). Comparison of the relative abundance of microbial communities between the LPS groups and sham groups on d3, d7. C, Sham group; T, LPS group(4B, 4C). Asterisk indicates significantly differences(* P <0.05).

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