

Calcium-Sensitive Receptors Alters Intestinal Microbiota Metabolites Especially SCFAs and Ameliorates Intestinal Barrier Damage in Neonatal Rat Endotoxemia

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Purpose: The calcium-sensing receptor (CaSR) acts as a major modulator of tissue responses related to calcium homeostasis and expresses highly in the mammalian intestine. Endotoxemia tends to impair intestinal barrier function and poses significant obstacles in clinical treatment. This work is designed to decipher whether CaSR can protect lipopolysaccharide (LPS)-induced intestinal barrier dysfunction in neonatal rats by targeting intestinal metabolites.

Patient and Methods: In this study, we utilized gas chromatography (GC) combined with liquid chromatography-mass spectrometry (LC-MS) to quantitatively analyze SCFAs and metabolites in fecal samples of 24 neonatal rats with LPS induced endotoxemia.

Results: Our results showed that CaSR alleviated endotoxin damage to the intestinal tight junction structure and upregulated the levels of butyric acid, propionic acid, valeric acid, and isovaleric acid in short-chain fatty acids (SCFAs). Non-targeted metabolomics analysis indicated that CaSR improved intestinal metabolic disorders by regulating glycerophospholipid metabolism, α -linolenic acid metabolism, as well as sphingolipids metabolism.

Conclusion: CaSR can alter intestinal microbiota metabolites, especially SCFAs, and improve intestinal barrier damage in neonatal rat endotoxemia.

Keywords: calcium-sensing receptor, lipopolysaccharide, endotoxemia, short chain fatty acids, metabolomics

Introduction

Infection remains a chief reason for neonatal mortality around the world. From 2000 to 2010, 64% of deaths of under 5-year-old children were due to infections, of which 40.3% died in the neonatal period. Despite the decreased percentage of infectious deaths (51.8%) by 2013, the percentage of infectious deaths in the neonatal period increased to 44%.^{1,2} Most infections are driven by Gram-negative bacteria. Particularly, lipopolysaccharide (LPS) is a major ingredient of the Gram-negative bacteria's outer membrane, which enters the bloodstream to activate nuclear transcription factors, induce the release of inflammatory factors, activate inflammation-related cell signaling pathways, and trigger cascade reactions, eventually leading to a series of pathological and physiological changes in the body, namely endotoxemia.³ Endotoxemia can manifest as neonatal sepsis in neonates, especially in premature infants, and is the most severe condition of neonatal infection that accounts for 11–30% of all neonatal deaths, often accompanied by shock, necrotizing enterocolitis, and neurological infections.^{4,5} Recent research has found that increased intestinal permeability and barrier dysfunction are implicated in endotoxemia development.⁶

The calcium-sensing receptor (CaSR), belongs to the G protein-coupled receptor superfamily, is widely distributed in the gastrointestinal tract, nervous system, cardiovascular system, breast, and bone tissues, and its expression is regulated by calcium ions, inflammatory factors, and various bacterial metabolites.⁷ CaSR is expressed in the apical and basolateral membranes of villus cells of the small intestine, as well as in surface and crypt epithelial cells of the colon in rodents and humans.^{8,9} CaSR plays an important role in the gut. In mice with intestinal epithelial specific CaSR knockout, intestinal barrier integrity was reduced, intestinal microbiome composition was altered, intestinal pattern recognition receptor expression was altered, and local and systemic innate responses were skewed from regulatory to irritant, thus aggravating colitis induced by DSS.^{10,11} CaSR is considered a potential immunotherapeutic target for intestinal homeostasis in a manner. CaSR agonist (R-568) can reverse the secretion of the intestinal fluid triggered by cholera toxin and heat-stable *Escherichia coli* enterotoxin by stimulating phosphodiesterases to degrade cyclic nucleotides. The allosteric activator of CaSR may provide a unique therapy for secretory diarrheas.¹² However, the function of CaSR in neonatal sepsis has not been investigated, and the purpose of this study is to explore the role of CaSR in neonatal rats.

The intestinal microbiota is pivotal in the production and absorption of nutrients, metabolism, and immune system maintenance in the host.¹³ Accumulating studies have indicated intestinal dysbacteriosis as one of the etiological factors in a variety of diseases, including irritable bowel syndrome, diverticulosis, celiac disease, and type 2 diabetes.^{14–17} The critical role of intestinal microbiota in many gastrointestinal and extra-gastrointestinal diseases has also been confirmed.¹⁸ Gastrointestinal microbiota includes over 1500 species and 50 phyla, with over 99% of intestinal microbiota belonging to *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. Of these, *Bacteroidetes* and *Firmicutes* are the major components of healthy intestinal microbiota.^{19,20} The intestinal microbiota status was evaluated by the ratio of *Firmicutes* to *Bacteroidetes*.²¹

Short-chain fatty acids (SCFAs), consisting of acetic, propionic, and butyric acid, are common metabolites in the intestinal microbiota. These SCFAs are the cardinal energy sources of intestinal epithelial cells, which contribute to maintaining the intestinal mucosal mechanical barrier.²² SCFAs promote the production of antimicrobial peptides including lysozyme, defensin, and mucin genes, increase the antimicrobial peptide secretion, and intensify the intestinal immune function.²³ Also, SCFAs are closely related to host physiological functions.²⁴ Emerging studies^{25,26} report that SCFAs, as ligands of GPCRs, are pivotal modulators of host metabolism and intestinal microorganisms. Moreover, SCFAs exert anti-inflammatory functions in the colon epithelium and advance the intestinal barrier via promoting the assembly of the tight junction.^{27,28} Consequently, SCFAs are accepted as pivotal parameters for evaluating intestinal homeostasis, in particular intestinal barrier integrity.

This work quantitatively analyzed metabolites, especially SCFAs, in fecal samples using gas chromatography (GC) coupled with liquid chromatography-mass spectrometry (LC-MS) to further explore the protective mechanism of CaSR on endotoxemia-induced intestinal injury.

Materials and Methods

Animals and Design of Experiments

Twenty-four Sprague-Dawley (SD) neonatal rats (5-day old, 12.5–15 g) supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd. [SCXK-2021-0011] were raised in standard conditions (21°C–23°C, relative humidity 40%–70%, a cycle of 12 h light and 12 h darkness), with standard laboratory diet and drinking water at liberty. The operations were ratified by the Ethics Committee of First Affiliated Hospital of Harbin Medical University [IACUC No. 2022114] and followed the *Guide for the Care and Use of Laboratory Animals*. Animals need to be executed for tissue sampling and analysis. According to the regulations of animal welfare and animal protection, we performed cervical dislocation on animals under deep anesthesia to minimize the pain of experimental animals. The successful establishment of the animal model was confirmed by observing the drowsiness, activity, and trembling of animals. Twenty-four neonatal rats were randomized into the control, LPS, and CaSR agonist (R-568) groups. As reported in previous studies, neonatal rats experienced intraperitoneal injections of LPS (10 mg/kg) for establishing endotoxemia models.^{29–31} Rats in the control group were subjected to injection with an equal amount of physiological saline. In the CaSR agonist group, rats were given R-568 solution (10 mg/kg) by intraperitoneal injections 1 h before LPS injection. After 24 h of LPS intervention, the rats were intraperitoneally injected

with 10% chloral hydrate solution and euthanized by cervical dislocation. The intestinal tissue was removed in a sterile environment and fixed with 2.5% glutaraldehyde or stored at -80°C . All tissue samples were retained at -80°C for reserve.

Ultrastructure of Intestinal Epithelial Intercellular Tight Junctions

The ileum tissue was subjected to 2-h fixation in 2.5% glutaraldehyde at 4°C , embedding in an Epon mixture with a 1:1 ratio of Epon 812 and acetone before cutting into 60–80 nm sections and dying with uranyl acetate together with lead citrate, followed by observation with a transmission electronic microscope (TEM) (HT7700, available from Hitachi, Tokyo, Japan).

Non-Targeted Metabolomics Analysis

Fecal samples were collected from 8 rats in each group of control group, LPS group and CaSR agonist group. The fecal samples (25 mg) and the solution of methanol: water (4:1, v/v) were blended at 50 Hz for 6 min in a Wonbio-96c high-throughput tissue crusher (supplied by Wanbo Biotechnology Co., Ltd., Shanghai, China), which was placed for 30 min at -20°C to precipitate proteins. Next, the mixture was subjected to 15-min centrifugation (13,000 g, 4°C) to collect the supernatant for LC-MS/MS.

UHPLC-Q Exactive system (Thermo Fisher Scientific) was utilized as an instrument platform for LC-MS assay. In detail, the HSS T3 column (100 mm \times 2.1 mm i.d., 1.8 μm) was employed for separating 2 μL of samples, which was then entered into mass spectrometry measurement. The mobile phases consisted of 0.1% formic acid each in acetonitrile: isopropanol (1:1, v/v). Subsequently, the harvest of the mass spectrometric data was achieved with Thermo UHPLC-Q Exactive Mass Spectrometer that was equipped with an electrospray ionization (ESI) source under the positive or negative ion mode. The raw data of metabolites were analyzed by uploading to the Majorbio cloud platform. For accurate quality, the MS fragment spectra were appraised in the Human Metabolome Database (HMDB), followed by quantification of the isotope ratio of differential metabolites. The control group, LPS group and CaSR agonist group were compared pairwise. The mapping of the differential metabolites into their biochemical pathways was achieved via the KEGG (Fisher's exact test, P adjust < 0.05) for metabolic enrichment pathway analysis. Afterward, multivariate statistical analyses were executed with the ropls R package, including partial least-squares discriminant analysis (PLS-DA) and orthogonal PLS-DA (OPLS-DA). The significantly differential metabolites were determined with the screening criteria of variable importance in projection (VIP) ≥ 1 and $P < 0.05$.

Detection of SCFAs

The fecal samples of rats were collected, weighed (25 mg), and transferred into a 2 mL test tube. The samples were added with 500 μL water comprising 0.5% phosphoric acid and then ground at 50 Hz for 3 min, repeated twice. After 15-min centrifugation at 4°C and 13,000 g, the supernatant was transferred to a centrifuge tube (1.5 mL) and added with N-butanol solvent (0.2 mL) comprising internal standard 2-ethyl butyric acid (10 mg/mL). Then, the sample was subjected to 10-s vortex and 5-min centrifugation at 4°C and 13,000 g. Subsequently, the sample was passed via a filter (0.22 mm) and analyzed by Agilent 8890 B gas chromatography (supplied by Agilent Technologies, CA, USA) coupled with a mass selective detector (MSD, Agilent 5977B). The analyte compounds were isolated with an HP-FFAP capillary column ((30 m long \times 0.25 mm diameter \times 0.25 μm film thickness). The GC parameters included a constant flow rate (1 mL/min), injection volume (1 μL), inlet temperature (260°C), splitting mode (10:1), as well as a solvent delay (2.5 min). The temperature of the GC column was initially maintained at 80°C and increased to 120°C ($40^{\circ}\text{C}/\text{min}$) and then 200°C ($10^{\circ}\text{C}/\text{min}$). Besides, the temperatures of the ion source and transmission line were kept at 230°C . The scanning mode was selected ion monitor (SIM). In the sample, the SCFA compounds' absolute content was quantified with the application of Masshunter software (v10.0.707.0, Agilent, USA).

Statistic Methods

IBM SPSS Statistics 26.0 was implemented for statistical analysis based on Student's *t*-test (two-group comparison) or one-way analysis of variance (ANOVA) coupled with Tukey's multiple comparison tests (multi-group comparison). Data were indicated as mean \pm standard deviation (SD). A notable difference was noted in a *p*-value below 0.05.

Results

CaSR Agonist Protects the Integrity of Intestinal Epithelial Tight Junctions in LPS-Induced Neonatal Rat Endotoxemia

Twenty-four neonatal rats were randomized into the control, LPS, and CaSR agonist (R-568) groups. Neonatal rats experienced intraperitoneal injections of LPS (10 mg/kg) for establishing endotoxemia models. In the CaSR agonist group, rats were given R-568 solution (10 mg/kg) by intraperitoneal injections 1 h before LPS injection. The rats were euthanized 24 h after LPS intervention. The intestinal epithelial tight junctions' integrity prevents microbial toxins along with other pernicious intracellular contents from crossing the intestinal epithelium.³² Tight junctions are crucial parameters of the intestinal barrier's integrity and impermeability. To assess the effect of CaSR agonists on the integrity of tight junction of intestinal epithelium, We compared the tight junction structure and other organelles between the control group, LPS group and CaSR agonist-treated under transmission electron microscopy (TEM). In the control group, tight connective structures and organelles were observed in the intestinal epithelial cells, which were normal with many large lipid droplets. In LPS group, the microvilli of intestinal epithelial cells were sparse, the compact connective structure was cracked, the desmosomes were reduced, the intracellular lipid droplets were significantly reduced, and the mitochondria were slightly swollen. In the agonist group, the tight junction structure returned to normal, the intracellular lipid droplets were smaller than those in the control group, the number of lipid droplets was significantly increased than that in the LPS group, the nucleus was not swollen, and some mitochondria were slightly swollen (Figure 1). These partial results suggest that administration of CaSR agonists mitigated LPS-induced intestinal epithelial tight junction breakdown and lipid droplet reduction.

CaSR Agonists Alters the Fecal Metabolites in LPS-Induced Neonatal Rat Endotoxemia

The intestinal microbiota's potential mechanism participating in host metabolism has relevance to the metabolites produced by intestinal microbiota. To investigate the impact of changes in intestinal metabolic pathways, we used LC-MS non-targeted metabolomics to analyze fecal metabolites. After normalization, missing value encoding, and filtering

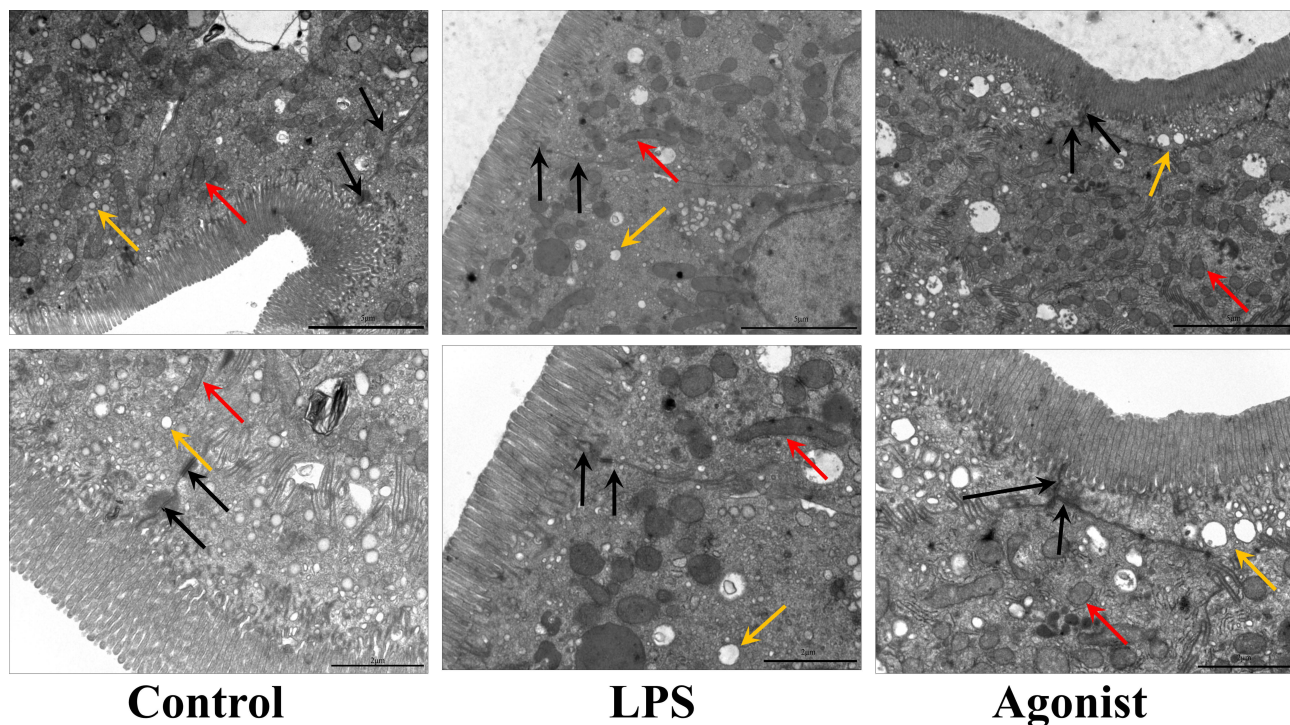


Figure 1 Transmission electron microscopy (TEM) of intestinal epithelial cells. Tight junction (black arrow); mitochondria (red arrow); lipid-droplet (yellow arrow). Scale above, 5 μ m. The scale below, 2 μ m.

low-quality peaks, 10,644 peaks were detected in the positive ion mode while 9059 peaks, were in the negative ion mode. We performed PCA analysis of the control group, LPS group and CaSR agonist group in two groups, as shown in Figure 2A, and found that LPS significantly changed intestinal metabolite profiles compared with the control group. At the same time, as shown in Figure 2B, the resolved metabolite profile of the CaSR agonist group was also significantly changed compared with that of the LPS group. The same results were also shown using OPLS-DA score analysis (Figures 2C and D).

According to the screening criteria of $VIP > 1.0$, $fold > 1.0$ or < 1.0 , and $P < 0.05$, 1801 definite metabolites (1101: positive; 700: negative) were noted in three groups. As shown in the volcanic plot (Figure 3A), the Agonist group presented significant changes in 695 metabolites compared to the LPS group, of which 357 were upregulated and 338 were downregulated. The heat map analysis showed notable alternations in the top 50 metabolites of the LPS and Agonist groups, which contributed to distinguishing the metabolic differences (Figure 3B). Lipids, benzene, lipid molecules, carboxylic acids, as well as organic oxygen compounds, were the chief differential metabolites. We subsequently made an analysis of potential metabolic pathways following the matched differential metabolites. Given KEGG classification, the differential metabolites between the LPS and Agonist groups included 7 types of metabolic pathways, namely metabolism, cellular processes, drug development, human diseases, biological systems, and environmental and genetic information processing (Figure 3C). Intriguingly, enriched lipid metabolism was noted in the metabolic category, and pathogenic *E. coli* infection was markedly enriched in the human disease category (Figure 3C).

The topology method was utilized for pathway enrichment analysis following the differential metabolites between LPS and Agonist groups (Figure 4). KEGG topology analysis disclosed 10 enriched metabolic pathways. Of these, the degradation of furfural, biosynthesis of cutin, cork base, and wax, α -linolenic acid metabolism, glycerol phospholipid metabolism, sphingolipid metabolism, biosynthesis of tyrosine, phenylalanine, and tryptophan, metabolism of serine, glycine, and threonine, phenazine biosynthesis, arginine biosynthesis, sesquiterpene biosynthesis, and triterpene biosynthesis were the main metabolic pathways of CaSR intervention, which may be related to alleviating intestinal mucosal barrier damage. Glycerol phospholipid, sphingolipid, and α -linolenic acid metabolism were filtered out to be the major metabolic pathways in the LPS and Agonist groups ($P < 0.05$).

CaSR Agonists Affects the Levels of SCFAs in LPS-Induced Neonatal Rat Endotoxemia

The preliminary results observed an elevation in the SCFAs-related bacteria abundance in the intestinal microbiota after CaSR intervention. Considering the non-targeted metabolomics conducted by LC/MS, we adopted GC/MS to estimate

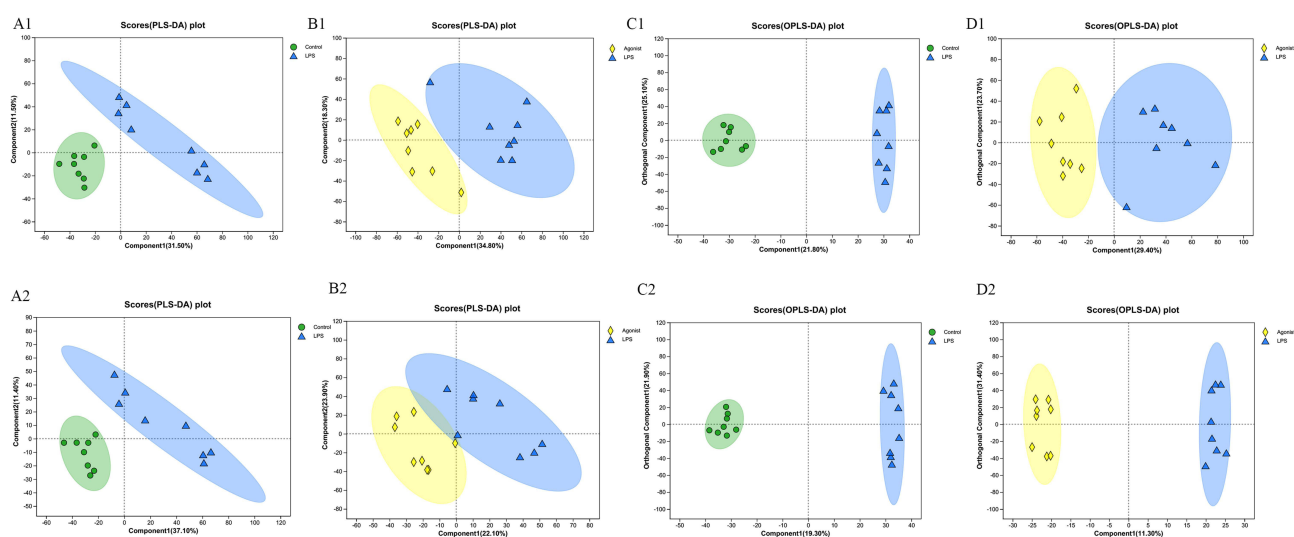


Figure 2 CaSR altered the fecal metabolites. (A) Scores plots of PLS-DA between the control and LPS groups ($n = 8$ mice per group). (B) Scores plots of PLS-DA between the LPS and Agonist groups ($n = 8$ mice per group). (C) Scores plots of OPLS-DA between the control and LPS groups. (D) Scores plots of OPLS-DA between the LPS and Agonist groups. 1 and 2 are in the positive and negative modes, respectively.

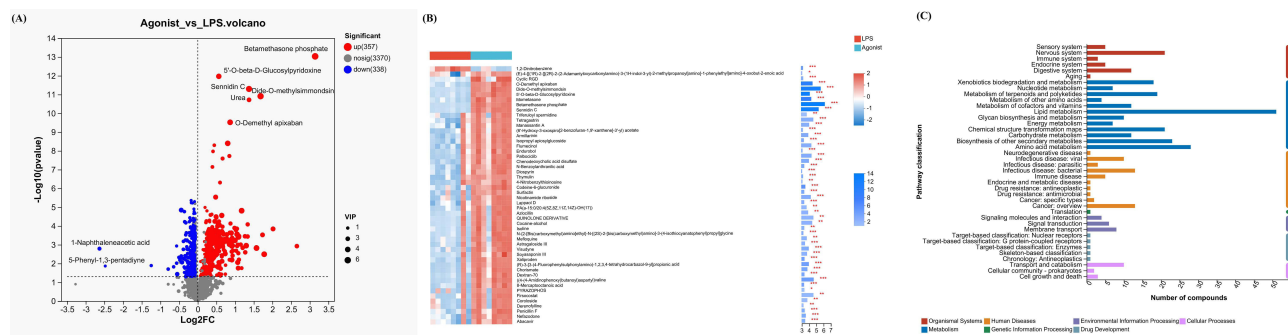


Figure 3 Metabolites in the LPS and Agonist groups. **(A)** A volcano plot of metabolites. The horizontal coordinate is the multiple change value of the difference in metabolite expression between the two groups; the vertical coordinate is the statistical test value of the difference in metabolite expression. Both the horizontal and vertical values were logized. Each dot in the diagram represents a specific metabolite, and the size of the dot represents the Vip value. The more the point on the left and right and the point on the upper side, the more significant the expression difference is. **(B)** The top 50 significantly altered metabolites in the LPS and Agonist groups. Each column represents a sample, with the sample name below; The color represents the relative expression of the metabolite in the group of samples. The corresponding relationship between the color gradient and the numerical size is shown in the gradient color block. The right side is the metabolite VIP bar chart. The bar length indicates the contribution value of the metabolite to the difference between the two groups. The larger the value, the greater the difference between the two groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Control vs LPS. **(C)** KEGG pathways enriched by differential genes between Agonist group and LPS group.

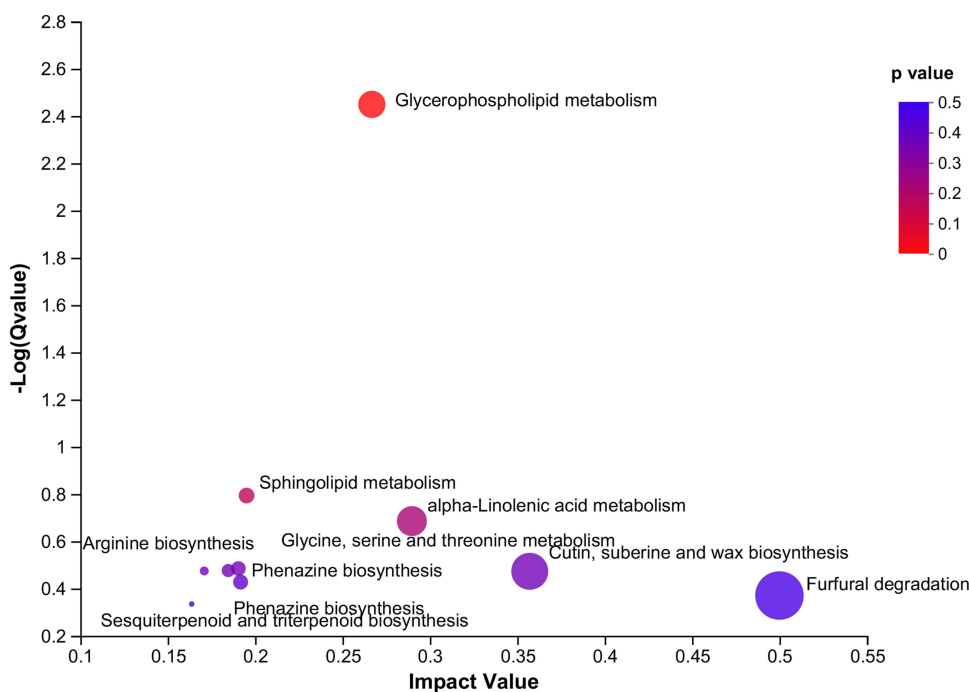


Figure 4 Pathway impact in topology analysis. The size and color of each circle were based on the pathway impact value and P-value, respectively.

the concentration of SCFAs because of the strong volatility of SCFAs. The concentration and total content of 7 types of SCFAs were shown in Figure 5. Elevated total content of SCFAs was observed in the LPS group in contrast to the control group ($P < 0.001$), with distinct decreases in acetic acid and isobutyric acid (respectively $P < 0.001$ and $P < 0.05$). Relative to the LPS group, the CaSR group exhibited an enhancement in 4 SCFAs, namely propionic, butyric, isovaleric, and valeric acid (Figure 5).

Discussion

Neonatal sepsis is a refractory and fatal disease among neonates with substantial morbidity and mortality, which poses great challenges for pediatric management worldwide.³³ Further improving sepsis diagnosis and therapy has far-reaching

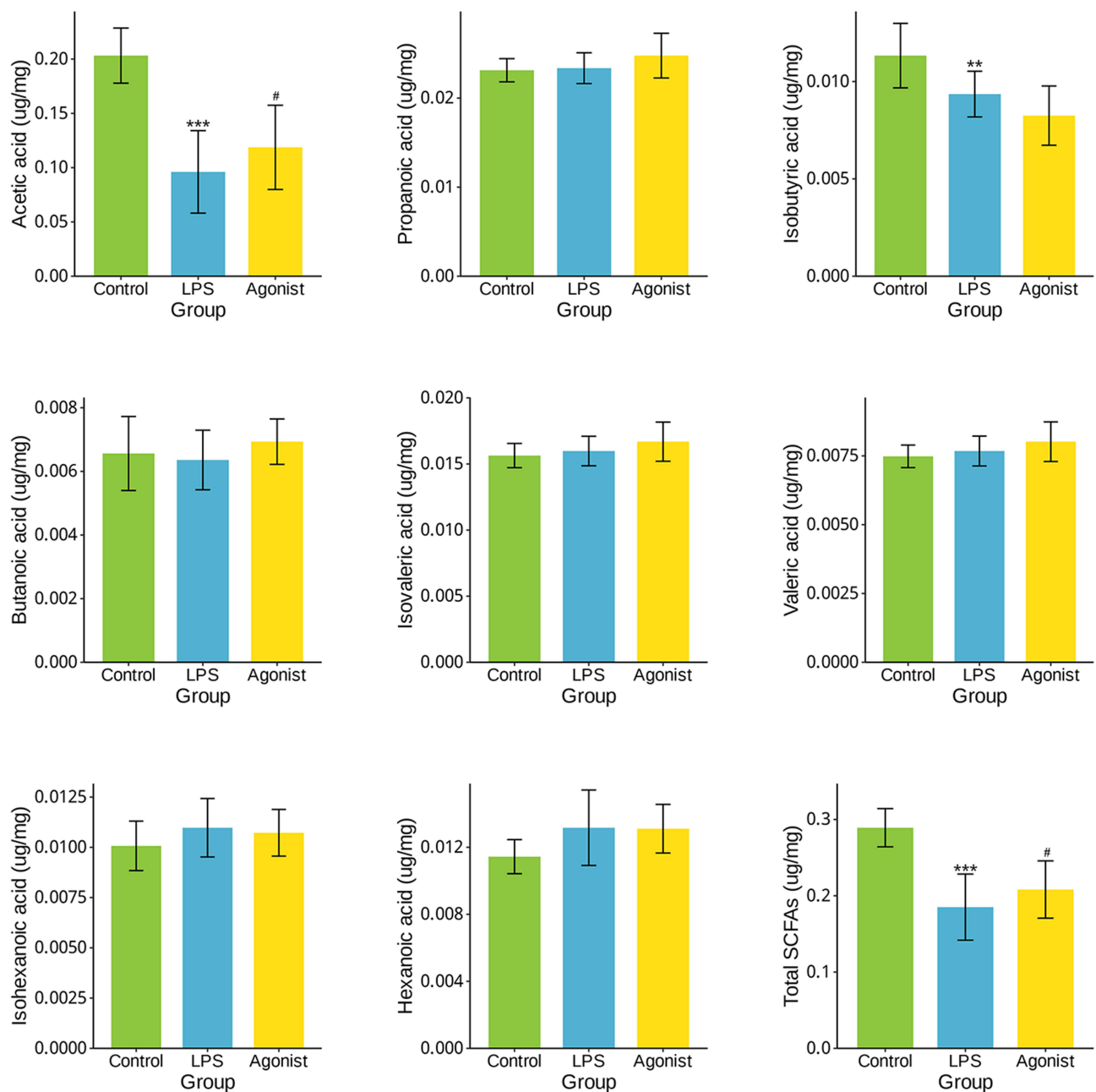


Figure 5 SCFAs contents in different groups. **P < 0.01, ***P < 0.001, Control vs LPS; #P < 0.05, Agonist vs LPS.

significance for global health.³⁴ The intestine is widely deemed as the organ dysfunction “engine”.³⁵ As important components of the intestine, intestinal microflora mediates diverse physiological functions including metabolism and biosynthesis. Nevertheless, critical diseases result in various alterations in microbial diversity, such as a decrease in diversity and excessive growth of pathogenic bacteria.^{36,37} Prescott et al³⁸ found a significant dose-effect relationship between intestinal ecological disorders and sepsis progression through clinical observation of 43,095 hospitalized patients. Damage to the intestinal barrier and changes in intestinal microflora can be considered both as a result of sepsis and a cause of its progression. The mechanism and therapeutic potential of intestinal microflora in the occurrence and development of sepsis are increasingly highlighted, which may effectively contribute to improving the prognosis of sepsis.³⁹

SCFAs possess notable anti-inflammatory activities and maintain glycolipid metabolism homeostasis.⁴⁰ Emerging evidence has suggested the critical role of SCFAs in intestinal homeostasis, barrier function, inflammatory responses, and

epithelial cell integrity.⁴¹ As a research hotspot in recent years, the role of SCFAs in sepsis has also been gradually explored. Consistently, our results confirmed that the total level of SCFAs in the LPS-induced sepsis model was significantly reduced. Nastasi et al found that SCFAs, especially propionic acid, and butyric acid, can inhibit the expressions of cytokines IL-6 and IL-12 induced by LPS in human mature dendritic cells.⁴² SCFAs trigger the generation of prostaglandin E2 and the IL-10 levels by stimulating GPCRs, and can also activate inflammasomes, promoting the production and secretion of IL-18.⁴³ In clinical studies using SCFAs to treat inflammatory diseases, the improvement of clinical and histological indicators in inflammatory bowel diseases supports the direct anti-inflammatory effect of butyric acid at the inflammatory site.^{44,45} In type 2 diabetes, a metabolic disorder with low-grade inflammation, the reduction of butyric acid-producing organisms can be observed.⁴⁶ Multiple studies^{47,48} have shown that SCFAs suppress histone acetylation, thereby inhibiting gene transcription, while OKUMURA and FU et al^{49,50} indicated that intravenous injection of sodium butyrate (NaB) inhibits NF- κ B activation and restores the production of tight junction proteins ZO-1 and claudin-1 to block the vicious cycle of cytokine storms, improve intestinal barrier function, and increase the survival rate. A negative correlation was noted between systemic butyric acid levels and sepsis, and an increase in butyric acid levels may improve the condition of sepsis. This study found that Relative to the LPS group, the Agonist group exhibited an enhancement in propionic, butyric, isovaleric, and valeric acid, suggesting that the reduction of intestinal inflammation may have relevance with elevated butyric acid levels. Accumulating evidence supports the critical role of SCFAs in shaping host metabolism through inflammatory pathways in the local and peripheral immune systems. Increasing attention also concerns the potential of SCFAs as critical molecular signals between host and microbiota, or as metabolic substrates for controlling host cell metabolism.

Various junction proteins in intestinal epithelial cells, such as claudin, occludin, ZO-1, ZO-2, etc., form a mechanical barrier for epithelial cells. The integrity of tight junctions is crucial for maintaining intestinal balance.⁵¹ SCFAs can upregulate tight junction proteins in septic intestinal tissues, thereby improving intestinal function. The dietary approach based on SCFAs has gradually become a focus of researchers. SCFAs, especially butyrates, are prominent substrates for retaining colonic epithelium. Butyrate is a favorable fuel for colon cells, and intestinal epithelial cells are the main sites of butyrate isolation in vivo.^{52–54} SCFAs also act in the epithelial barrier integrity by coordinating the tight junction protein modulation. Between the lumen and the hepatic portal vein system, tight junction proteins also modulate the intracellular molecular pathways. The increased permeability is related to the bacteria translocation and/or the bacterial cell wall components, thus triggering related inflammatory cascade reactions.⁵⁵ Butyric acid seems to be the most important regulatory factor for tight junction proteins and enhances intestinal barrier function via enhancing the claudin-1 and ZO-1 levels and the occludin redistribution.⁵⁶ As reported, butyrate reverses the abnormal level of ZO-1, reduces LPS translocation, and inhibits neutrophil infiltration, pro-inflammatory cytokine secretion, as well as macrophage activation thereby relieving liver injury in rats.⁵⁷ Meanwhile, butyric acid can activate the function of PPAR- γ , elevate the expressions of in vivo and in vitro transporters such as MCT-1 and MCT-4, and promote the absorption of SCFAs by intestinal epithelial cells to provide energy for intestinal epithelial cells.⁵⁸ SCFAs upregulate the expressions of ZO-1, occludin, claudin-3, and claudin-4 by activating AMPK, inhibiting the MLCK/MLC2 pathway, and phosphorylating PKC β 2.⁵⁹ Mitochondria are known to maintain the energy homeostasis of intestinal epithelial cells.⁶⁰ SCFAs can significantly promote the genes encoding mitochondrial adenosine triphosphate (ATP) synthase subunits and mitochondrial uncoupling proteins to maintain mitochondrial function.⁶¹ Moreover, energy balance disorder is a typical characteristic of inflammatory intestinal tissue. Observation under TEM found that the tight junction structure of the intestinal epithelium in neonatal rats with endotoxemia was damaged, and mitochondria were slightly swollen. CaSR intervention alleviated the damage to the tight junction structure of the intestinal epithelium. These results may be attributed to alternations in the intestinal metabolite composition, contributing to reduced production of SCFAs and damage to tight junction structures. CaSR may regulate this process and participate in the protective effect of the intestinal barrier. This paper adopted LC/MS for revealing the intestinal metabolic profile in neonatal rats with endotoxemia. The metabolic spectrum results showed significant differences in the metabolites between each group, with the differential metabolites mainly related to lipid metabolism including carboxylic acids, phospholipids, and lipid molecules. Through the KEGG topology analysis of the LPS and Agonist groups, we found that the significantly differential metabolites were mainly implicated in sphingolipids metabolism, α -linolenic acid metabolism, and glycerol phospholipid metabolism. CaSR notably augmented the α -linolenic acid abundance. As a polyunsaturated fatty

acid with an anti-inflammatory effect, α -linolenic acid plays an anti-inflammatory and antioxidant role in a variety of ways, including acting as a substrate for cyclooxygenase/oxygenase to regulate the production of lipid mediators such as eicosanoid.^{62,63} CaSR may alleviate inflammatory responses by regulating a variety of unsaturated fatty acid metabolic pathways.

To summarize, Targeted and non-targeted metabolomics analysis indicates that CaSR may be involved in regulating the production of SCFAs and a wide range of metabolites. Specific alternations in the microbiota-metabolism axis may contribute to the protective influence of CaSR on the intestinal barrier in endotoxemia.

Conclusion

In conclusion, our results demonstrate that CaSR agonists alter intestinal metabolites and ameliorate intestinal damage caused by endotoxemia. However, it is unclear how CaSR acts on endotoxemia through metabolite changes, possibly by affecting the synthesis of SCFAs and fecal metabolites to reduce intestinal damage, and further evidence is needed.

Data Sharing Statement

Data supporting the outcomes of this work can be acquired from the corresponding author following reasonable requirements.

Ethics Approval and Informed Consent

The authors are responsible for the work to ensure that questions relating to the accuracy or completeness of the work are properly examined and resolved. The operations were ratified by the Ethics Committee of First Affiliated Hospital of Harbin Medical University [IACUC No. 2022114] and followed the Guide for the Care and Use of Laboratory Animals.

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Author Contributions

C.J. finished study design, Y.S., X.L., M.J. finished experiments on animals, J.S., F.M. finished data analysis, Y.S., J. S. finished manuscript editing. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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