

Prophylactic Short-Chain Fatty Acid Supplementation Modulates Circulating Immunometabolic Biomarkers and Reduces Organ-Injury Markers in CLP-Induced Sepsis

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Submitted: 14 May 2026 Revised: 9 June 2026 Accepted: 29 June 2026 Published: 23 October 2026

Background: Sepsis involves profound immune and metabolic disturbances. This study investigated whether prophylactic short-chain fatty acid (SCFA) supplementation alters circulating immunometabolic biomarkers and biochemical markers of hepatic and renal injury in experimental sepsis.

Methods: Male Wistar rats were assigned to four groups (n = 12/group) with independent 24-h and 48-h subgroups. Sepsis was induced by cecal ligation and puncture (CLP). A mixture of sodium acetate, propionate, and butyrate (60:20:20 molar ratio; total dose 500 mg/kg) was administered intraperitoneally for 7 days before CLP and discontinued after sepsis induction. Circulating immunometabolic and organ-injury-related markers were measured.

Results: SCFA supplementation increased circulating G protein-coupled receptor 41 (GPR41)- and G protein-coupled receptor 43 (GPR43)-related marker concentrations and produced an overall increase in glucagon-like peptide-1 (GLP-1). Angiopoietin-like protein 4 (ANGPTL4) increased significantly in septic animals at both time points, whereas C1q/tumor necrosis factor-related protein 3 (CTRP3) was not significantly altered by SCFA supplementation within septic animals. Circulating NOD-like receptor family pyrin domain-containing protein 3 (NLRP3)-related marker concentrations were reduced at 24 h and 48 h, while NLRP6-related marker concentrations were reduced at 48 h. SCFA supplementation also reduced alanine aminotransferase (ALT), urea, and creatinine at both time points and aspartate aminotransferase (AST) at 48 h. Other adipokine and metabolic marker responses were marker- and time-dependent.

Conclusion: Prophylactic SCFA supplementation was associated with changes in circulating immunometabolic biomarkers and reductions in biochemical markers of hepatic and renal injury in CLP-induced sepsis. These findings require further tissue-based and therapeutic investigation.

Keywords: short-chain fatty acids; sepsis; immunometabolism; circulating biomarkers; organ injury

Introduction

Sepsis is a complex and life-threatening syndrome characterized by a dysregulated host response to infection, leading to systemic inflammation, immune dysfunction, and multi-organ failure. Despite advances in antimicrobial therapy and supportive care, sepsis remains a major cause of mortality worldwide, largely due to the inability to effectively modulate the host response [1,2]. Increasing evidence indicates that sepsis should not be viewed solely as an uncontrolled inflammatory condition, but rather as a dynamic and multifactorial disorder involving simultaneous immune activation, immune suppression, and profound metabolic reprogramming.

In recent years, the concept of immunometabolism has provided a new framework for understanding the pathophysiology of sepsis. Immune cell function is closely linked to metabolic pathways, and shifts in cellular metabolism

directly influence inflammatory responses, cytokine production, and tissue repair processes [3,4]. During sepsis, metabolic reprogramming is not restricted to immune cells but extends to multiple organ systems, including adipose tissue, liver, and the gastrointestinal tract. This systemic metabolic dysregulation is reflected by alterations in adipokines, lipid-binding proteins, and enteroendocrine signaling molecules, which collectively contribute to disease progression and organ dysfunction [5].

Among the emerging regulators of immunometabolic homeostasis, the gut microbiota has gained increasing attention. The intestinal microbiota interacts bidirectionally with the host immune system and plays a crucial role in maintaining immune tolerance and metabolic balance [6]. In sepsis, this homeostatic relationship is profoundly disrupted. Dysbiosis, reduced microbial diversity, and depletion of beneficial commensal bacteria impair the produc-

tion of microbiota-derived metabolites and increase intestinal permeability, thereby amplifying systemic inflammation [7,8]. This has led to the recognition of the gut as a central organ in the pathogenesis of sepsis and the concept of a “microbiota–immune axis” in systemic inflammatory diseases.

Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are among the most important metabolites produced by microbial fermentation of dietary fibers in the gut. SCFAs exert pleiotropic effects on both immune and metabolic pathways and are increasingly recognized as key mediators linking the microbiota to host physiology [9]. These molecules influence immune responses through multiple mechanisms, including activation of G protein-coupled receptors (GPR41 and GPR43), inhibition of histone deacetylases (HDACs), and modulation of nuclear receptors, such as peroxisome proliferator-activated receptor γ (PPAR- γ). Through these pathways, SCFAs regulate immune cell differentiation, cytokine production, epithelial barrier integrity, and metabolic signaling [9,10].

Beyond their local effects in the intestine, SCFAs have systemic immunometabolic functions. They modulate adipose tissue inflammation by regulating adipokine secretion, influence lipid metabolism through fatty acid-binding proteins (FABPs), and affect enteroendocrine signaling by stimulating the release of incretin hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) [11,12]. Together, these effects position SCFAs as systemic mediators influencing interconnected immune and metabolic processes relevant to the pathophysiology of sepsis.

An experimental study has shown that SCFA supplementation can attenuate inflammation, improve gut barrier integrity, and reduce organ injury in sepsis models [13]. However, a previous study has focused on individual SCFAs (particularly butyrate) or evaluated limited sets of inflammatory markers [14]. Recent evidence indicates that SCFAs regulate interconnected immune and metabolic processes through G protein-coupled receptor (GPCR) signaling, HDAC inhibition, and effects on epithelial barrier function and systemic immune homeostasis [15]. However, their coordinated effects on adipokines, lipid-binding proteins, and enteroendocrine mediators have not been comprehensively characterized in experimental sepsis [15].

Importantly, sepsis is increasingly recognized as a condition involving coordinated alterations across multiple biological systems rather than isolated signaling pathways. Therefore, evaluating SCFA effects within a broader immunometabolic framework may provide a more accurate understanding of their therapeutic potential. In this context, the simultaneous assessment of signaling pathways involving the microbiota, metabolic regulators, and immune mediators represents a novel integrative approach.

Based on this rationale, the present study investigated the effects of combined SCFA supplementation on immunometabolic alterations in a polymicrobial sepsis model induced by cecal ligation and puncture (CLP). We hypothesized that prophylactic SCFA supplementation would modulate circulating immunometabolic biomarkers associated with sepsis and attenuate sepsis-associated elevations in biochemical markers of hepatic and renal injury. By evaluating multiple circulating biomarkers within a single experimental framework, this study aimed to characterize the systemic effects of exogenously administered SCFAs and to identify potential targets for further investigation.

Materials and Methods

Animal Welfare and Regulatory Compliance

All experimental procedures involving animals were conducted in accordance with internationally accepted ethical standards for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Bursa Uludag University (Approval No. 2024-14/08). All experimental procedures complied with the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines 2.0 and were performed in accordance with the European Directive 2010/63/EU for the protection of animals used for scientific purposes, as well as the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [16,17]. Throughout the study, all efforts were made to minimize animal suffering, and humane endpoints were strictly applied.

Animals and Experimental Design

A total of 48 male Wistar rats (8–10 weeks old, 200–250 g) were used in this study. Animals were obtained from the Experimental Animal Research Center of Bursa Uludag University and housed under controlled environmental conditions (22 ± 2 °C, 50–60% humidity, 12-hour light/dark cycle) with ad libitum access to standard laboratory chow and water. Animals were randomly allocated into four experimental groups ($n = 12$ per group): (1) Sham + PBS(control), (2) Sham + SCFA, (3) CLP + PBS(sepsis), and (4) CLP + SCFA. To evaluate time-dependent effects, each group was further subdivided into two independent subgroups ($n = 6$ per subgroup) corresponding to the 24 h and 48 h endpoints. Due to the large number of biomarkers analyzed and the potential physiological impact of repeated blood sampling, serial measurements from the same animal were avoided. Instead, separate subgroups were designated for each time point, and each time point was treated as an independent dataset in the statistical analysis. Randomization was performed using a simple randomization method. Male animals were specifically selected to reduce variability associated with hormonal fluctuations of the estrous cycle, which may influence immune and metabolic responses. The po-

tential influence of sex on the study outcomes is acknowledged as a limitation.

SCFA Preparation and Administration

A SCFA mixture consisting of sodium acetate, sodium propionate, and sodium butyrate was prepared in sterile phosphate-buffered saline (PBS) at a molar ratio of 60:20:20 [18,19]. The SCFA mixture was administered intraperitoneally once daily for 7 consecutive days before CLP, for a total combined dose of 500 mg/kg [20,21]. Thus, the reported dose of 500 mg/kg refers to the total combined dose of the three SCFAs rather than to the dose of each component. Animals in the Sham + PBS and CLP + PBS groups received an equivalent volume of sterile PBS according to the same administration schedule. SCFA supplementation was prophylactic only and was discontinued at the time of sepsis induction; no further SCFA was administered after CLP.

Induction of Sepsis

Polymicrobial sepsis was induced using the CLP model. Before surgery, anesthesia was induced in an induction chamber using 4% sevoflurane in oxygen delivered through a calibrated vaporizer. After loss of the righting reflex, anesthesia was maintained with 2–3% sevoflurane through a fitted nose cone during the surgical procedure. The animals breathed spontaneously throughout anesthesia, and endotracheal intubation or mechanical ventilation was not required. Anesthetic depth was assessed by the absence of the pedal withdrawal reflex, and respiratory rate, breathing pattern, mucosal color, and body temperature were monitored throughout the procedure. Body temperature was maintained using a thermostatically controlled warming pad. No perioperative or postoperative analgesic agents were administered because their potential pharmacological effects on the inflammatory and immunometabolic endpoints evaluated in this study were considered a possible confounding factor in the experimental design. Following adequate anesthesia, the abdominal area was shaved and aseptically prepared. A midline laparotomy was performed along the linea alba, and the cecum was exteriorized. After assessment of its size and contents, the cecum was ligated below the ileocecal valve using a 3-0 silk suture without causing intestinal obstruction. The ligated cecum was then punctured three times through-and-through on the anti-mesenteric surface using an 18-G needle, and a small amount of fecal material was gently extruded to ensure patency. The cecum was returned to the abdominal cavity, and the incision was closed in layers. To compensate for fluid loss, animals received 600 μ L sterile PBS intraperitoneally. Two hours after surgery, all animals were administered ceftriaxone (80 mg/kg, subcutaneously) diluted in saline (25 mL/kg) to mimic clinical sepsis management following source control [22,23]. In sham-operated animals, laparotomy was performed, and the cecum was gently ma-

nipulated, but neither ligation nor puncture was performed; the abdomen was subsequently closed in the same manner as in CLP-operated animals. Successful induction of sepsis was supported by the standardized CLP procedure and by the development of characteristic sepsis-associated biochemical and inflammatory alterations in the untreated CLP group, including significant elevations in circulating NLRP3- and NLRP6-related marker concentrations and in ALT, AST, urea, and creatinine levels.

Sample Collection and Processing

At 24 or 48 h following CLP induction, animals were placed under deep terminal anesthesia using 7% sevoflurane delivered in 100% oxygen through a fitted nose cone. Adequate anesthetic depth was confirmed by the absence of palpebral and pedal withdrawal reflexes. Once deep anesthesia was established, approximately 8 mL of blood per animal was collected by cardiac puncture for biochemical analysis. Following sample collection, additional blood was withdrawn under continued deep anesthesia to complete euthanasia by terminal exsanguination. Death was confirmed by the cessation of cardiac activity and spontaneous respiration. Blood samples were transferred into EDTA-containing tubes and centrifuged at 3000 rpm for 10 minutes at 4 °C to obtain plasma. The resulting plasma samples were aliquoted into sterile tubes and stored at –80 °C until further analysis. Care was taken to avoid repeated freeze–thaw cycles to preserve sample integrity.

Biochemical and Immunometabolic Measurements

Plasma levels of GPR41 (Cat. No: SRB-T-7), GPR43 (Cat. No: SRB-T-6), GLP-1 (Cat. No: SRB-T-84809), GLP-1R (Cat. No: SRB-T-5), PYY (Cat. No: SRB-T-85535), adiponectin (Cat. No: 201-11-0759), omentin (Cat. No: SRB-T-81157), resistin (Cat. No: SRB-T-81078), leptin (Cat. No: 201-11-0562), ANGPTL4 (Cat. No: SRB-T-3), CTRP1 (Cat. No: SRB-T-88941), CTRP3 (Cat. No: SRB-T-2), PPAR- γ (Cat. No: 201-11-0285), FABP4 (Cat. No: SRB-T-0942), FABP5 (Cat. No: SRB-T-87341), NLRP3 (Cat. No: SRB-T-81850), and NLRP6 (Cat. No: SRB-T-8) were measured using commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kits (SunRedBio, Shanghai, China) according to the manufacturer's instructions. All samples and standards were analyzed in duplicate, and concentrations were calculated from standard curves generated for each assay. Biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine, were determined using an automated biochemical analyzer (Roche Cobas c501, Roche Diagnostics, Mannheim, Germany) with commercially available diagnostic reagent kits according to the manufacturer's protocols. All measurements were performed in duplicate, and quality control procedures were applied throughout the analytical process.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA, USA). Data are presented as mean $\pm$ standard deviation (SD). The normality of data distribution was assessed using the Shapiro–Wilk test. Because independent groups of animals were used for the 24 h and 48 h endpoints, the two time points were analyzed separately, and no repeated-measures analysis was applied. For each biomarker at each time point, data were analyzed using an ordinary two-way analysis of variance (ANOVA), with sepsis status (Sham vs. CLP) and SCFA treatment (vehicle vs. SCFA) included as between-subject factors. The analysis assessed the main effects of sepsis status and SCFA treatment, as well as their interaction. Where appropriate, planned simple-effects comparisons were performed using Šidák's multiple-comparisons test. The effect of SCFA treatment was evaluated by comparing vehicle and SCFA-treated animals separately within the Sham and CLP groups. In contrast, the effect of sepsis was evaluated by comparing Sham and CLP animals separately within the vehicle- and SCFA-treated conditions. Adjusted p -values are reported for these multiple comparisons. A p -value of less than 0.05 was considered statistically significant. An exploratory post hoc power analysis was performed using G*Power (version 3.1.9.7; Heinrich Heine University, Düsseldorf, Germany) [24], based on observed effect sizes for selected outcome variables. The resulting power estimates ($1-\beta$) exceeded 0.80 across the evaluated outcomes. Given the post hoc nature of these calculations, the estimates were interpreted descriptively and were not used to establish the adequacy of the sample size.

Results

Changes in Circulating GPR41- and GPR43-Related Marker Levels

SCFA supplementation was associated with increased circulating concentrations of GPR41- and GPR43-related immunoreactive proteins, whereas sepsis status had no significant effect on either marker. For GPR41, no significant sepsis status $\times$ SCFA treatment interaction was observed at either 24 h or 48 h ($p > 0.05$), and sepsis status had no significant main effect at either time point ($p > 0.05$). In contrast, SCFA treatment showed a significant main effect at both 24 h ($p < 0.01$) and 48 h ($p < 0.001$), indicating an overall increase in circulating concentrations of GPR41-related markers. Although Šidák-adjusted within-group comparisons did not reach statistical significance at 24 h in either sham or septic animals ($p > 0.05$), circulating GPR41-related marker concentrations were significantly higher following SCFA supplementation at 48 h in both sham ($p < 0.01$) and septic animals ($p < 0.05$).

A similar pattern was observed for GPR43. No significant interaction between sepsis status and SCFA treatment

was detected at 24 h ($p > 0.05$) or 48 h ($p > 0.05$), and sepsis status had no significant main effect at either time point ($p > 0.05$). SCFA treatment, however, produced a significant main effect on circulating GPR43-related marker concentrations at both 24 h ($p < 0.001$) and 48 h ($p < 0.001$). Šidák-adjusted comparisons confirmed significantly higher concentrations following SCFA supplementation in sham animals at both 24 h ($p < 0.05$) and 48 h ($p < 0.05$), and in septic animals at 24 h ($p < 0.01$) and 48 h ($p < 0.05$).

Collectively, these findings demonstrate that prophylactic SCFA supplementation was associated with increased concentrations of circulating GPR41- and GPR43-related immunoreactive proteins, largely independent of sepsis status. These circulating measurements should not be interpreted as direct evidence of altered receptor expression or activation of GPR41/GPR43 signaling pathways (Fig. 1).

To facilitate interpretation of the findings, an author-generated summary table was constructed from the experimental results of the present study. This table does not present additional statistical analyses; rather, it provides a conceptual overview of the biological domains influenced by SCFA supplementation (Table 1).

Changes in Circulating Enteroendocrine Markers

Enteroendocrine responses showed distinct patterns across the measured components of the PYY–GLP-1 axis. PYY concentrations were not significantly affected by sepsis status or SCFA supplementation at 24 h, with no significant interaction between these factors ($p > 0.05$). At 48 h, however, a significant sepsis status $\times$ SCFA treatment interaction emerged ($p < 0.05$), together with a significant effect of sepsis status ($p < 0.05$). Šidák-adjusted comparisons showed that PYY concentrations were significantly lower in SCFA-treated septic animals than in SCFA-treated sham animals at 48 h ($p < 0.01$). In contrast, SCFA supplementation did not significantly alter PYY concentrations within either sham or septic animals at either time point ($p > 0.05$). Circulating GLP-1 concentrations exhibited a consistent treatment-associated response, with significant main effects of SCFA supplementation at both 24 h ($p < 0.05$) and 48 h ($p < 0.05$), indicating an overall elevation in GLP-1 concentrations across sepsis-status groups. No significant sepsis status $\times$ SCFA treatment interaction or main effect of sepsis was detected for GLP-1 at either time point ($p > 0.05$), and the SCFA-associated increases did not reach significance in the individual vehicle-versus-SCFA comparisons within the sham and septic cohorts ($p > 0.05$). Despite this treatment-related increase in circulating GLP-1, GLP-1R levels remained comparatively stable, with no significant effects of sepsis status, SCFA supplementation, or their interaction at either 24 or 48 h ($p > 0.05$), and no significant differences were detected in any of the Šidák-adjusted pairwise comparisons ($p > 0.05$). Collectively, these findings indicate that SCFA supplementation was associated with an overall increase in circulating GLP-1 concentrations with-

Table 1. Summary of the observed effects of sepsis and prophylactic SCFA supplementation on circulating biomarkers.

Biological domain	Representative markers	Sepsis-associated findings	SCFA-associated findings
Biochemical markers of hepatic and renal injury	ALT, AST, urea, creatinine	Vehicle-treated septic animals showed significantly higher ALT, AST, urea, and creatinine concentrations than vehicle-treated sham animals at both 24 h and 48 h.	SCFA treatment significantly reduced ALT, urea, and creatinine concentrations in septic animals at both 24 h and 48 h. AST concentrations were significantly reduced at 48 h but not at 24 h. These findings indicate attenuation of circulating biochemical markers of hepatic and renal injury and should not be interpreted as direct evidence of improved organ function.
Circulating inflammasome-related markers	NLRP3, NLRP6	Sepsis was associated with significantly higher concentrations of circulating NLRP3- and NLRP6-related markers at both time points.	SCFA treatment significantly reduced circulating NLRP3-related marker concentrations in septic animals at both 24 h and 48 h. NLRP6-related marker concentrations were significantly reduced in septic animals at 48 h but not at 24 h. These circulating measurements do not directly demonstrate altered inflammasome activation.
Circulating SCFA receptor-related markers	GPR41, GPR43	Sepsis status had no significant main effect on circulating GPR41- or GPR43-related marker concentrations at either 24 h or 48 h	SCFA treatment produced significant overall increases in GPR41- and GPR43-related marker concentrations at both time points. For GPR41, within-group increases reached significance in sham and septic animals at 48 h, whereas GPR43 concentrations were significantly increased in both sham and septic animals at 24 h and 48 h.
Enteroendocrine markers	GLP-1, GLP-1R, PYY	GLP-1 and GLP-1R showed no significant main effect of sepsis at either time point. PYY showed a significant sepsis status $\times$ SCFA treatment interaction and a significant sepsis effect at 48 h, but not at 24 h.	SCFA treatment produced a significant overall increase in GLP-1 concentrations at both 24 h and 48 h, although within-group comparisons were not significant. SCFA did not significantly alter PYY concentrations within sham or septic animals at either time point, and no significant effect on GLP-1R was observed.
Circulating adipokines	Adiponectin, leptin, resistin, omentin	Sepsis was associated with lower adiponectin and leptin concentrations and higher omentin and resistin concentrations, indicating a heterogeneous adipokine response.	SCFA treatment had significant overall effects on adiponectin at both time points, although the increases in septic animals were not statistically significant. Leptin was unaffected by SCFA treatment. Omentin showed an SCFA treatment effect at 24 h, driven by an increase in sham animals. Resistin concentrations were significantly reduced by SCFA treatment in both sham and septic animals at 48 h.
Lipid-binding proteins	FABP4, FABP5	FABP4 and FABP5 concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at both 24 h and 48 h.	For FABP4, an overall SCFA treatment effect was detected at 24 h, but no significant within-group treatment differences were observed. FABP5 concentrations were significantly reduced by SCFA treatment in septic animals at 48 h but not at 24 h.
Circulating metabolic regulatory markers	ANGPTL4, PPAR- γ , CTRP1, CTRP3	ANGPTL4 concentrations were lower in vehicle-treated septic animals, whereas CTRP1 concentrations were higher and CTRP3 concentrations were lower than in vehicle-treated sham animals at both time points. PPAR- γ showed no significant main effect of sepsis.	SCFA treatment significantly increased ANGPTL4 concentrations in septic and sham animals at both 24 h and 48 h. SCFA did not significantly alter CTRP1 concentrations within septic animals. CTRP3 concentrations were numerically higher following SCFA treatment in septic animals, but did not reach statistical significance; a significant increase was observed only in sham animals at 24 h. PPAR- γ showed a modest overall effect of SCFA treatment at 24 h, with no significant within-group differences.

Abbreviations: SCFA, short-chain fatty acid; GPR41, G-protein-coupled receptor 41; GPR43, G-protein-coupled receptor 43; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; PYY, peptide YY; ANGPTL4, angiopoietin-like protein 4; CTRP, C1q/tumor necrosis factor-related protein; PPAR- γ , peroxisome proliferator-activated receptor gamma; FABP, fatty acid-binding protein; NLRP, NOD-like receptor family pyrin domain-containing protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

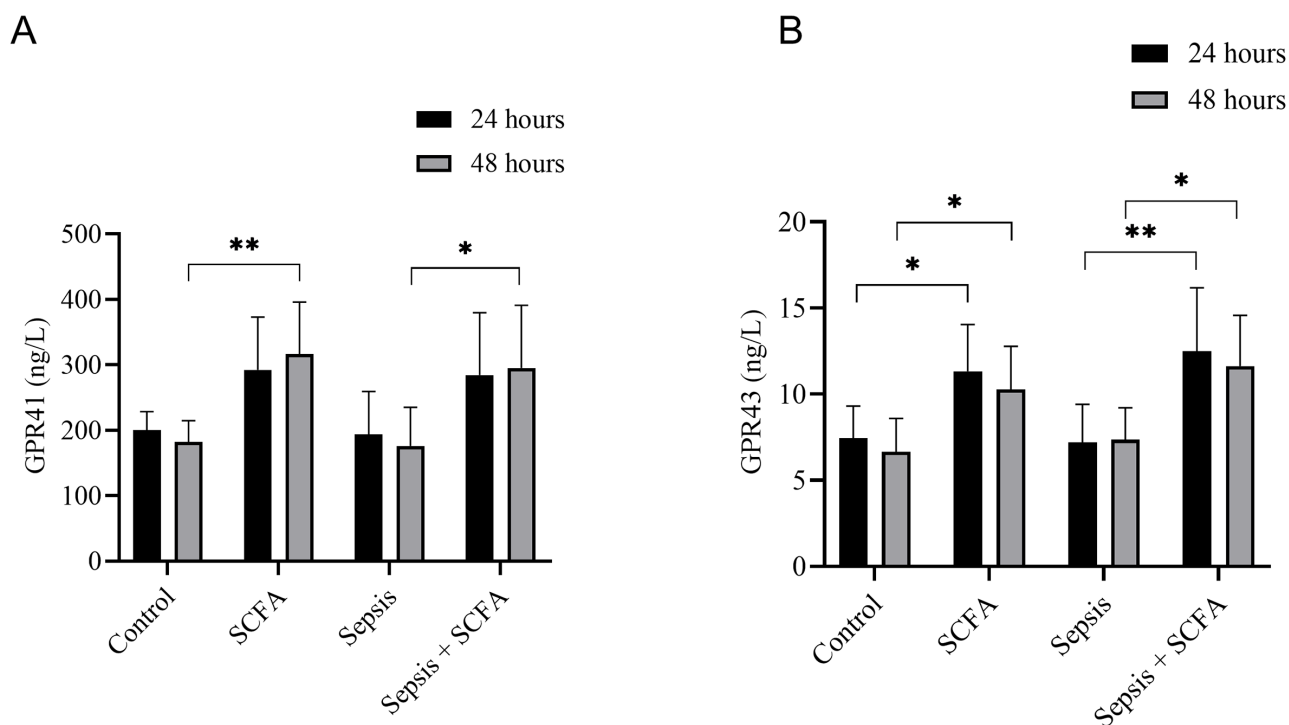


Fig. 1. Effects of SCFA supplementation on circulating GPR41- and GPR43-related marker concentrations in experimental sepsis. (A) circulating GPR41-related marker concentrations; (B) circulating GPR43-related marker concentrations. * $p < 0.05$, ** $p < 0.01$. SCFA, short-chain fatty acid; GPR41, G-protein-coupled receptor 41; GPR43, G-protein-coupled receptor 43.

out a corresponding alteration in GLP-1R levels, while its effects on PYY were more dependent on the septic state and sampling time (Fig. 2; Table 1).

Alterations in Circulating Adipokine Profiles

Circulating adipokine profiles were markedly altered under septic conditions. However, no significant sepsis status $\times$ SCFA treatment interaction was detected for adiponectin, leptin, omentin, or resistin at either 24 or 48 h (all $p > 0.05$). Adiponectin concentrations showed significant main effects of both sepsis status and SCFA treatment at 24 h ($p < 0.01$ and $p < 0.05$, respectively) and 48 h ($p < 0.01$ for both). Šídák-adjusted comparisons showed that adiponectin concentrations were significantly lower in vehicle-treated septic animals than in vehicle-treated sham animals at both 24 h and 48 h ($p < 0.05$ for both), and remained lower in SCFA-treated septic animals than in their sham counterparts at 48 h ($p < 0.05$). Although SCFA supplementation was associated with higher adiponectin concentrations in septic animals at both time points, these differences did not reach statistical significance ($p > 0.05$). Leptin showed a pronounced sepsis-associated reduction at both time points, with a significant main effect of sepsis at 24 and 48 h ($p < 0.001$ for both). Šídák-adjusted comparisons confirmed significantly lower leptin concentrations in septic animals under vehicle-treated conditions at 24 h ($p < 0.01$) and 48 h ($p < 0.001$), and under SCFA-treated con-

ditions at both time points ($p < 0.01$). SCFA treatment itself had no significant effect on leptin concentrations at either time point ($p > 0.05$). In contrast, omentin concentrations were increased under septic conditions, with significant main effects of sepsis status at both 24 h and 48 h ($p < 0.01$ for both). Vehicle-treated septic animals exhibited significantly higher omentin concentrations than vehicle-treated sham animals at both time points ($p < 0.05$). A significant overall effect of SCFA treatment was detected for omentin at 24 h ($p < 0.05$), with a significant increase in sham animals ($p < 0.05$). In contrast, no significant effect of SCFA was observed in septic animals or at 48 h ($p > 0.05$). Resistin concentrations were likewise elevated under septic conditions, with significant main effects of sepsis status at both 24 h and 48 h ($p < 0.001$ for both). Šídák-adjusted comparisons showed significantly higher resistin concentrations in septic animals than in the corresponding sham animals under vehicle-treated conditions at 24 h ($p < 0.05$) and 48 h ($p < 0.01$), and under SCFA-treated conditions at both time points ($p < 0.01$). SCFA treatment did not significantly affect resistin concentrations at 24 h ($p > 0.05$); however, at 48 h, a significant overall SCFA treatment effect was observed ($p < 0.001$), accompanied by significant reductions in resistin concentrations in both sham and septic animals ($p < 0.05$ for both) (Fig. 3; Table 1).

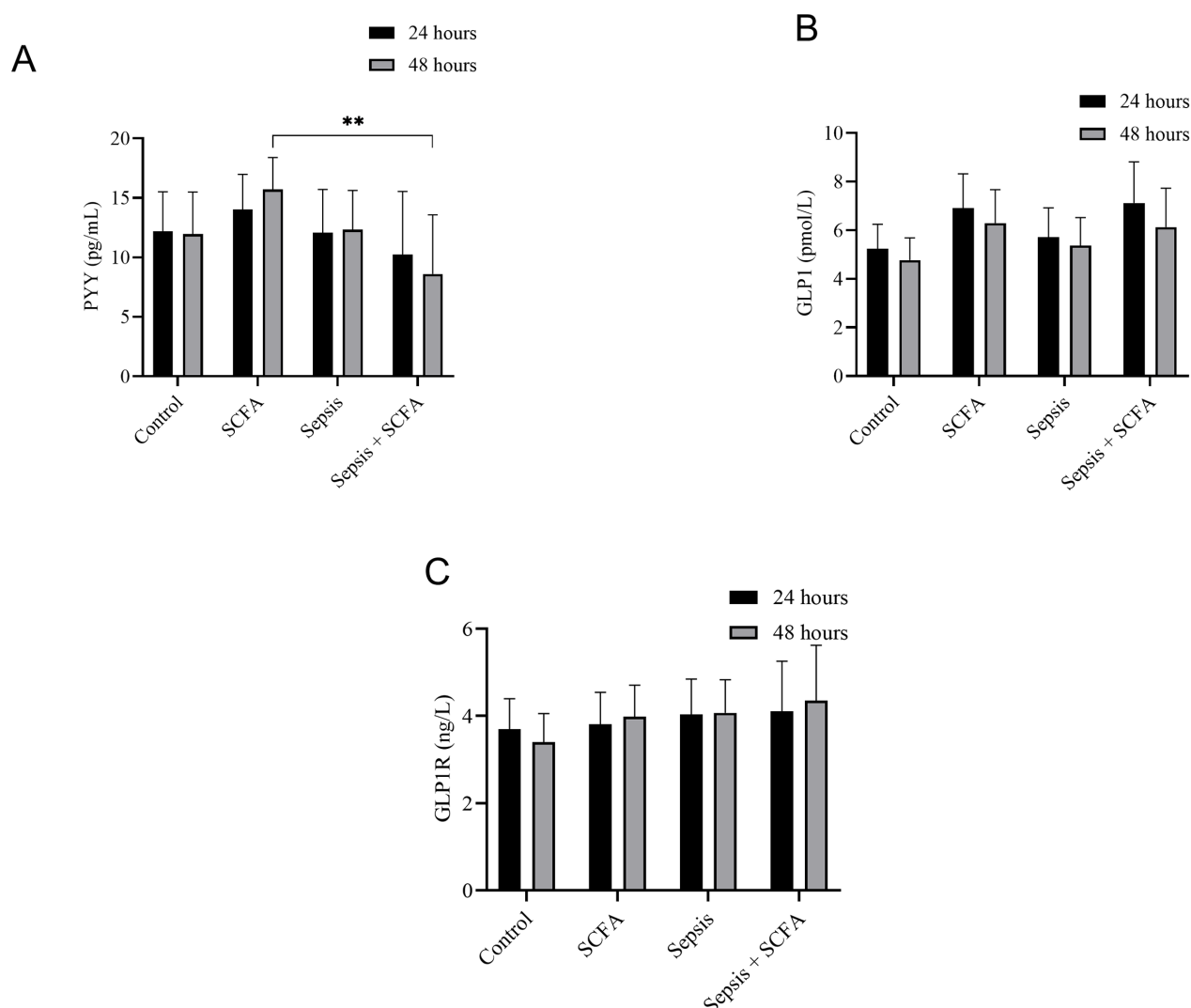


Fig. 2. Effects of SCFA supplementation on enteroendocrine markers (PYY, GLP-1, and GLP-1R) in experimental sepsis. (A–C) represent the following enteroendocrine markers: (A) PYY, (B) GLP-1, and (C) GLP-1R. Black bars represent 24 h and gray bars represent 48 h. Data are presented as mean $\pm$ SD ($n = 6$ /group/time point). Two-way ANOVA followed by Šídák-adjusted multiple comparisons was performed separately at each time point. ** $p < 0.01$. ANOVA, analysis of variance; SD, standard deviation.

Modulation of Immunometabolic Regulators

ANGPTL4 showed a marked sepsis- and SCFA-dependent response. Significant interactions between sepsis status and SCFA treatment were observed at both 24 h ($p < 0.05$) and 48 h ($p < 0.05$). Šídák-adjusted comparisons showed that ANGPTL4 concentrations were significantly lower in vehicle-treated septic animals than in vehicle-treated sham animals at both 24 h ($p < 0.001$) and 48 h ($p < 0.0001$). SCFA treatment significantly increased ANGPTL4 concentrations in septic animals at both time points ($p < 0.0001$ for both), as well as in sham animals at 24h ($p < 0.01$) and 48h ($p < 0.001$). Following SCFA treatment, ANGPTL4 concentrations no longer differed significantly between sham and septic animals at either time point ($p > 0.05$). In contrast, CTRP1 showed a sepsis-associated increase. No significant interaction was detected at either

24 h or 48 h ($p > 0.05$), while a significant main effect of sepsis status was observed at both time points. CTRP1 concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at 24 h ($p < 0.05$) and 48 h ($p < 0.05$). SCFA treatment did not significantly alter CTRP1 concentrations in septic animals at 24 h ($p > 0.05$). At 48 h, concentrations were numerically lower following SCFA treatment, although the difference did not reach statistical significance ($p > 0.05$). CTRP3 exhibited the opposite sepsis-associated pattern, with significant main effects of sepsis status at both 24 h and 48 h ($p < 0.001$ for both), and no significant interactions ($p > 0.05$). CTRP3 concentrations were significantly lower in septic than in sham animals under vehicle-treated conditions at both 24 h and 48 h ($p < 0.05$ for both). SCFA treatment significantly increased CTRP3 concentrations in

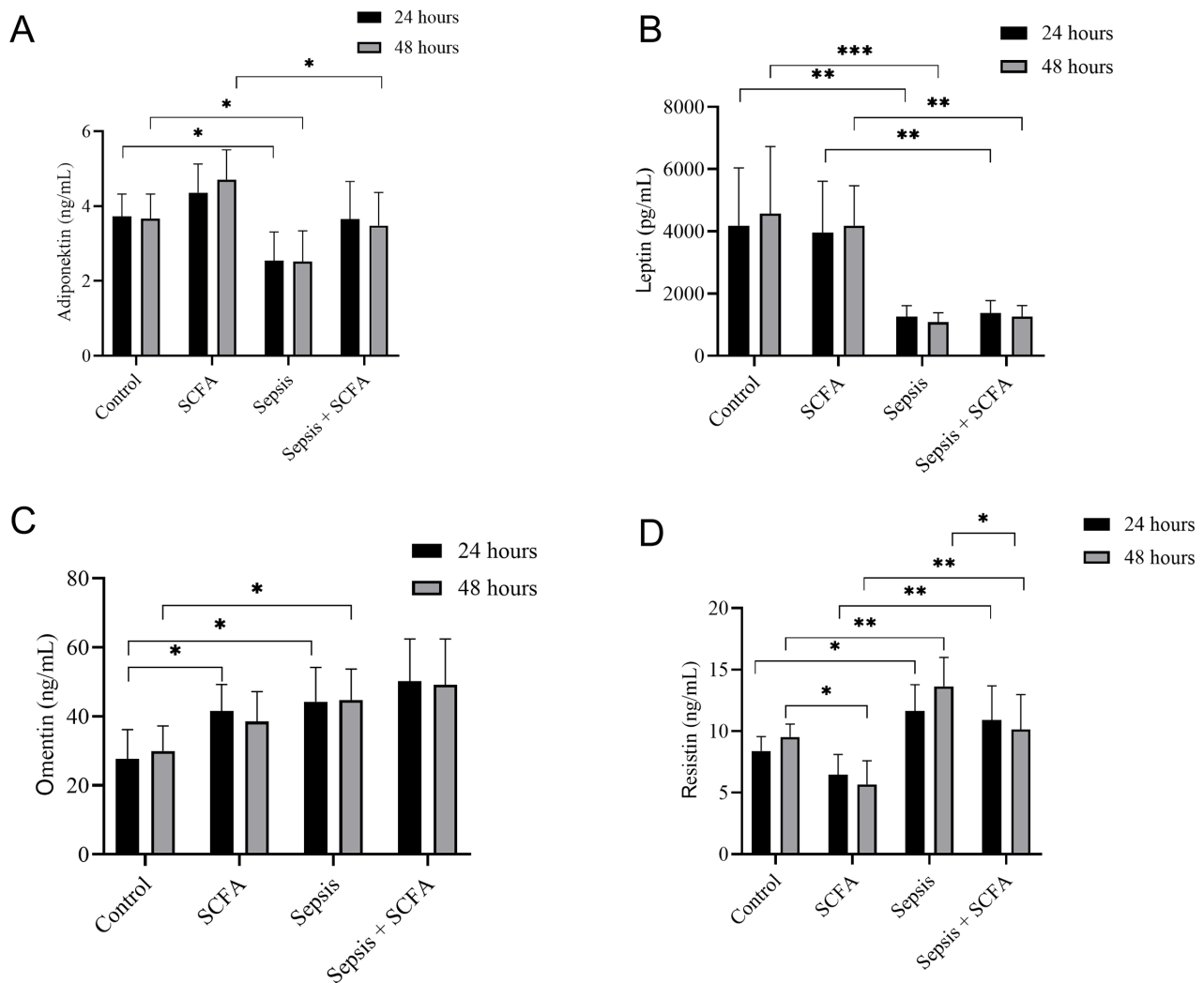


Fig. 3. Effects of SCFA supplementation on circulating adipokine concentrations (adiponectin, leptin, omentin, and resistin) in experimental sepsis. (A–D) represent the following circulating adipokines: (A) adiponectin, (B) leptin, (C) omentin, and (D) resistin. Black bars represent 24 h and gray bars represent 48 h. Data are presented as mean $\pm$ SD ($n = 6$ /group/time point). Two-way ANOVA followed by Šidák-adjusted multiple comparisons was performed separately at each time point. Only selected biologically relevant pairwise comparisons are shown for clarity. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

sham animals at 24 h ($p < 0.01$), but not at 48 h ($p > 0.05$). In septic animals, CTRP3 concentrations were numerically higher following SCFA treatment at both time points, although these differences were not statistically significant ($p > 0.05$). For PPAR- γ , no significant interaction between sepsis status and SCFA treatment was detected at either 24 h or 48 h ($p > 0.05$), and sepsis status had no significant main effect at either time point ($p > 0.05$). A modest overall main effect of SCFA treatment was observed at 24 h ($p < 0.05$), but Šidák-adjusted comparisons revealed no significant treatment effects within either sham or septic animals at either time point ($p > 0.05$) (Fig. 4; Table 1).

Changes in Lipid Handling Markers

FABP4 and FABP5 concentrations were significantly affected by sepsis status, although their responses to SCFA treatment differed. For FABP4, no significant sepsis status $\times$ SCFA treatment interaction was observed at either 24 h or 48 h ($p > 0.05$). FABP4 concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at both 24 h and 48 h ($p < 0.05$ for both). Although a significant overall main effect of SCFA treatment was detected at 24 h ($p < 0.05$), Šidák-adjusted comparisons showed no significant effect of SCFA treatment within either sham or septic animals at either time point ($p > 0.05$). Similarly, no significant interaction between sepsis status and SCFA treatment was observed for FABP5 at either 24 h or 48 h ($p > 0.05$). FABP5 concen-

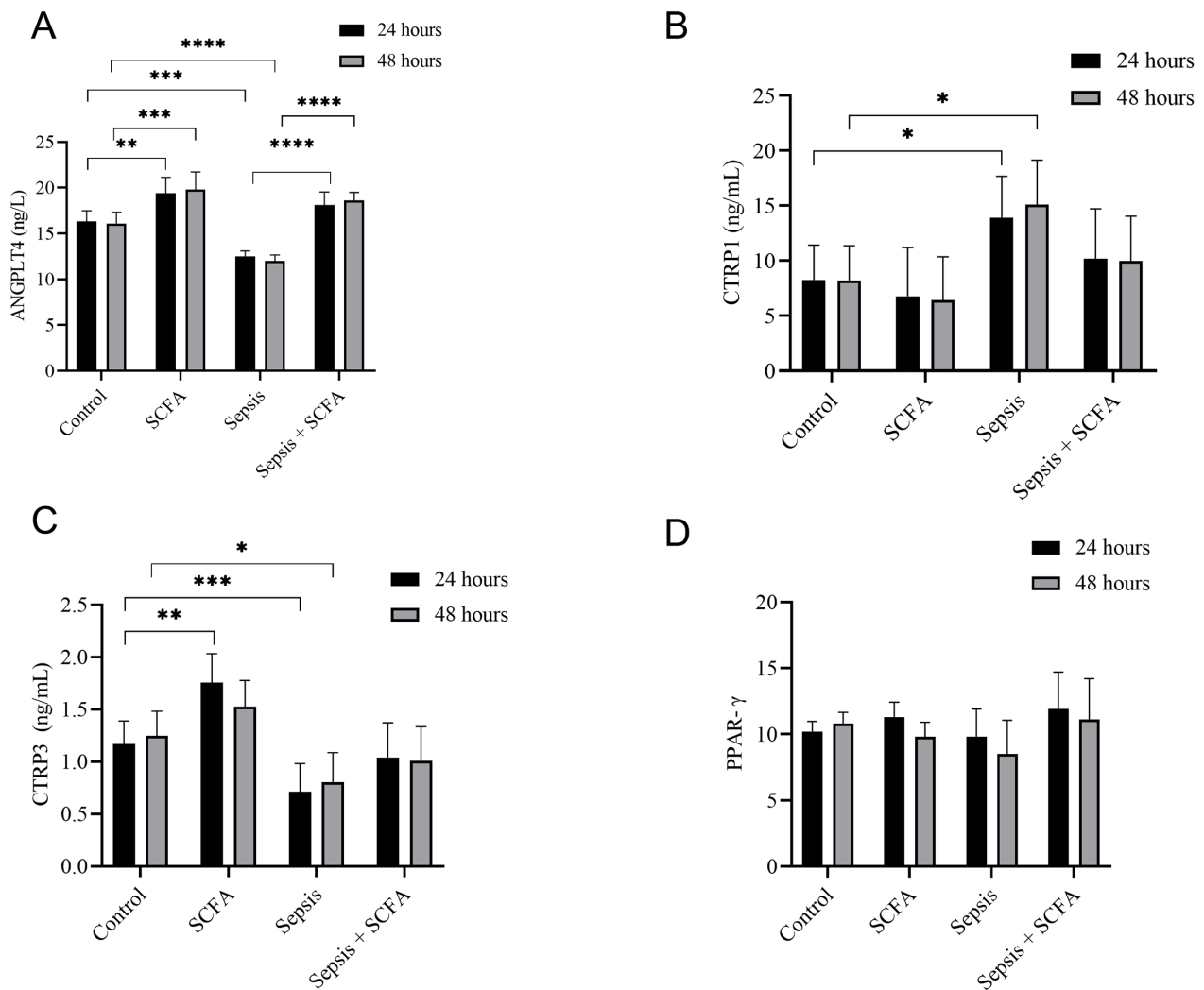


Fig. 4. Effects of SCFA supplementation on immunometabolic regulatory markers (ANGPTL4, CTRP1, CTRP3, and PPAR-γ). (A–D) represent the following immunometabolic regulatory markers: (A) ANGPTL4, (B) CTRP1, (C) CTRP3, and (D) PPAR-γ. Black bars represent 24 h and gray bars represent 48 h. Data are presented as mean ± SD (n = 6/group/time point). Two-way ANOVA followed by Šidák-adjusted multiple comparisons was performed separately at each time point. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

trations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at 24 h ($p < 0.05$) and 48 h ($p < 0.001$). SCFA treatment did not significantly reduce FABP5 concentrations in septic animals at 24 h ($p > 0.05$); however, a significant reduction was observed at 48 h ($p < 0.01$), while no significant effect was detected in sham animals at either time point ($p > 0.05$) (Fig. 5; Table 1).

Changes in Circulating NLRP3- and NLRP6-Related Marker Levels

Circulating NLRP3- and NLRP6-related marker concentrations were significantly altered under septic conditions. For NLRP3, no significant interaction between sepsis status and SCFA treatment was detected at either 24

h or 48 h ($p > 0.05$). A significant main effect of sepsis status was observed at both time points ($p < 0.0001$ for both). Šidák-adjusted comparisons showed that circulating NLRP3-related marker concentrations were significantly higher in septic than in sham animals under both vehicle-treated conditions ($p < 0.0001$ at both time points) and under SCFA-treated conditions at 24 h ($p < 0.001$) and 48 h ($p < 0.0001$). SCFA treatment significantly reduced circulating NLRP3-related marker concentrations in septic animals at both 24 h and 48 h ($p < 0.05$ for both), whereas no significant effect was observed in sham animals ($p > 0.05$ at both time points).

For NLRP6, no significant sepsis status × SCFA treatment interaction was observed at either 24 h or 48 h ($p > 0.05$). A significant main effect of sepsis status was de-

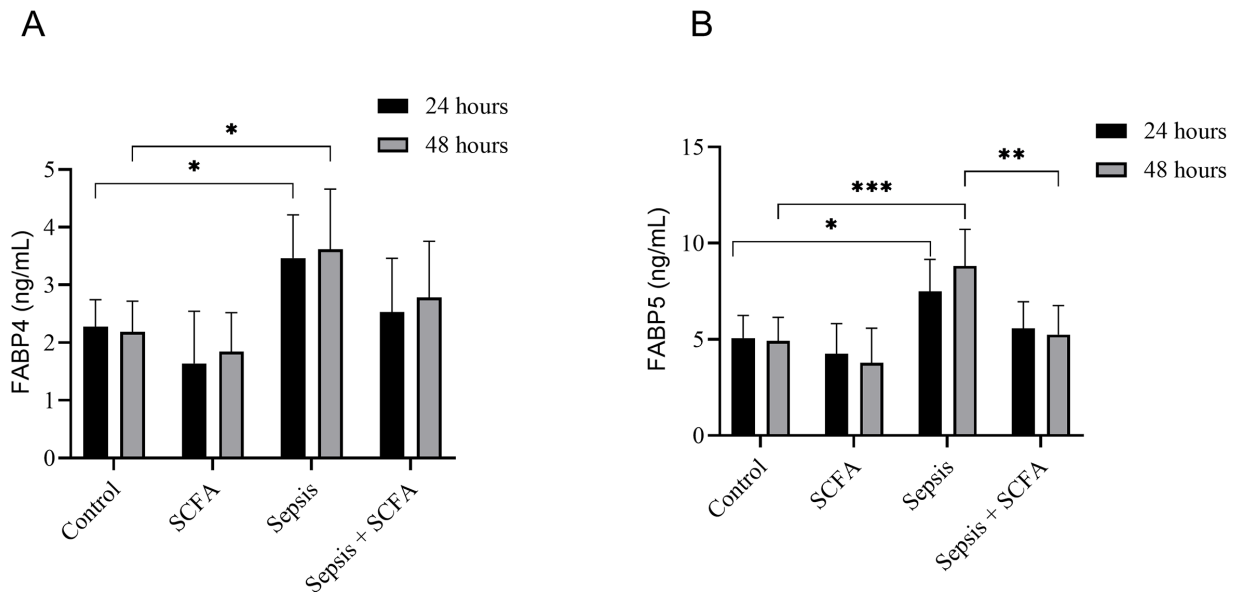


Fig. 5. Effects of short-chain fatty acid (SCFA) supplementation on lipid-binding proteins (FABP4 and FABP5) in experimental sepsis. (A,B) represent the following lipid-binding proteins: (A) FABP4 and (B) FABP5. Black bars represent 24 h and gray bars represent 48 h. Data are presented as mean $\pm$ SD ($n = 6$ /group/time point). Two-way ANOVA followed by Šídák-adjusted multiple comparisons was performed separately at each time point. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

tected at both time points (24 h: $p < 0.01$; 48 h: $p < 0.001$), whereas a significant main effect of SCFA treatment was observed only at 48 h ($p < 0.05$). Šídák-adjusted comparisons showed that circulating NLRP6-related marker concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at both 24 h and 48 h ($p < 0.01$ for both). Under septic conditions, SCFA treatment did not significantly alter NLRP6-related marker concentrations at 24 h ($p > 0.05$), whereas a significant reduction was observed at 48 h ($p < 0.05$). No significant effect of SCFA treatment was observed in sham animals at either time point ($p > 0.05$).

Collectively, these findings demonstrate that sepsis was associated with increased circulating NLRP3- and NLRP6-related marker concentrations. Prophylactic SCFA supplementation was associated with lower circulating NLRP3-related marker concentrations in septic animals at both time points and with lower NLRP6-related marker concentrations at 48 h. Because these measurements were obtained from plasma and no tissue-level inflammasome expression, assembly, or downstream functional activity was assessed, these findings should not be interpreted as direct evidence of altered inflammasome activation (Fig. 6).

Effects of SCFA Supplementation on Biochemical Markers of Hepatic and Renal Injury

Circulating biochemical markers associated with hepatic and renal injury were significantly altered under septic conditions, although the magnitude of the response to SCFA supplementation varied across markers.

For ALT, a significant interaction between sepsis status and SCFA treatment was observed at both 24 h and 48 h ($p < 0.05$ for both). Šídák-adjusted comparisons showed that ALT concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at both 24 h and 48 h ($p < 0.001$ for both). SCFA treatment significantly reduced ALT concentrations in septic animals at both time points (24 h: $p < 0.05$; 48 h: $p < 0.01$), whereas no significant effect of SCFA treatment was observed in sham animals ($p > 0.05$ at both time points). ALT concentrations did not differ significantly between SCFA-treated sham and septic animals at either 24 h or 48 h ($p > 0.05$).

For AST, no significant sepsis status $\times$ SCFA treatment interaction was detected at either 24 h or 48 h ($p > 0.05$). AST concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at both 24 h and 48 h ($p < 0.01$ for both). SCFA supplementation did not significantly affect AST concentrations in sham animals at either sampling point ($p > 0.05$). In septic animals, the reduction in AST concentration following SCFA treatment did not reach statistical significance at 24 h ($p > 0.05$), whereas a significant reduction was observed at 48 h ($p < 0.01$).

Urea concentrations showed a pronounced sepsis-associated increase. A significant sepsis status $\times$ SCFA treatment interaction was observed at both 24 h ($p < 0.01$) and 48 h ($p < 0.001$). Urea concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at both time points ($p <$

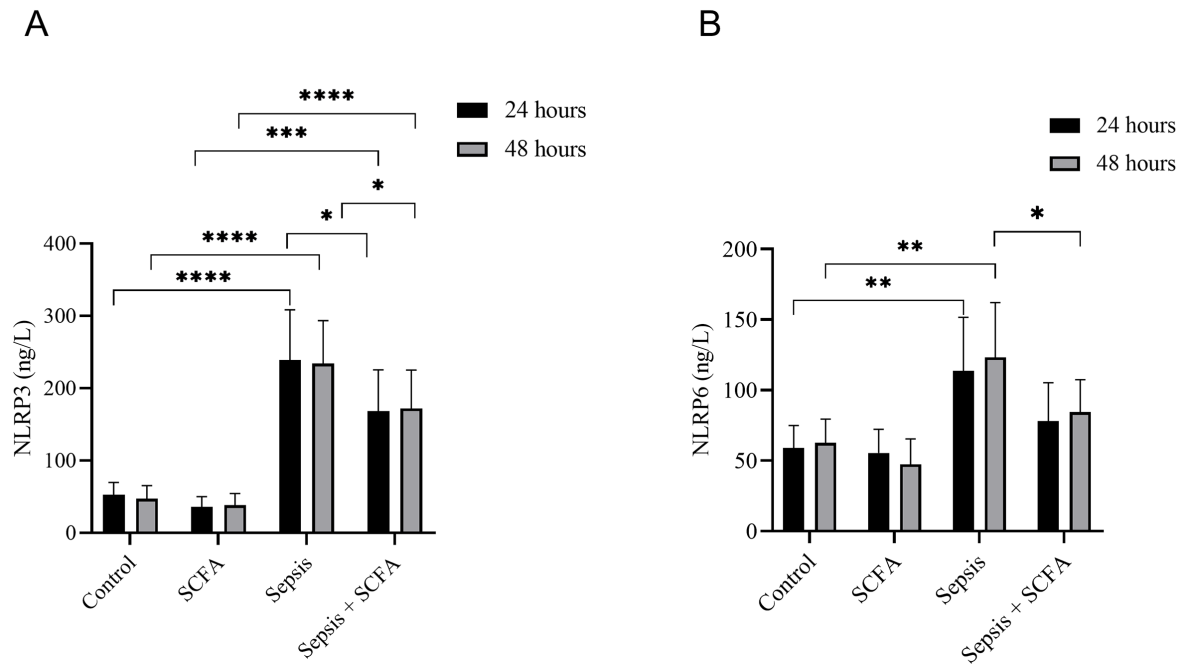


Fig. 6. Effects of SCFA supplementation on circulating NLRP3- and NLRP6-related marker concentrations in experimental sepsis. (A,B) represent the following inflammasome-related markers: (A) NLRP3 and (B) NLRP6. Black bars represent 24 h and gray bars represent 48 h. Data are presented as mean $\pm$ SD ($n = 6$ /group/time point). Two-way ANOVA followed by Šídák-adjusted multiple comparisons was performed separately at each time point. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

0.0001 for both). SCFA treatment significantly reduced urea concentrations in septic animals at 24 h ($p < 0.01$) and 48 h ($p < 0.001$), whereas no significant effect was observed in sham animals ($p > 0.05$ at both time points).

For creatinine, the interaction between sepsis status and SCFA treatment was significant at 24 h ($p < 0.05$), but not at 48 h ($p > 0.05$). Creatinine concentrations were significantly higher in septic than in sham animals under both vehicle- and SCFA-treated conditions at both sampling points ($p < 0.0001$ for all comparisons). SCFA treatment significantly reduced creatinine concentrations in septic animals at both 24 h and 48 h ($p < 0.001$ for both), whereas its effect in sham animals was not statistically significant at either time point ($p > 0.05$).

Collectively, these findings demonstrate that prophylactic SCFA supplementation was associated with reductions in several circulating biochemical markers of sepsis-associated hepatic and renal injury. The most consistent reductions were observed for ALT, urea, and creatinine at both time points, whereas AST was significantly reduced only at 48 h. Because direct functional assessments and histopathological evaluation of the liver and kidney were not performed, these changes should not be interpreted as direct evidence of improved organ function (Fig. 7).

To provide an integrated overview of the measured outcomes, Table 1 summarizes the observed sepsis- and SCFA-associated changes across the evaluated domains of circulating biomarkers. This summary is descriptive and

should not be interpreted as evidence of pathway activation or specific mechanistic relationships.

Discussion

Sepsis is a multidimensional syndrome involving profound disturbances in immune and metabolic homeostasis, intestinal function, endothelial integrity, and multiple organ systems [25,26,27]. Microbiota-derived metabolites such as SCFAs have attracted increasing attention because of their capacity to influence several immune, endocrine, and metabolic processes. In the present study, prophylactic administration of a combined SCFA formulation was associated with changes across multiple circulating biomarker domains in CLP-induced sepsis, including GPR41- and GPR43-related markers, enteroendocrine mediators, adipokines, metabolic regulatory proteins, lipid-binding proteins, NLRP3- and NLRP6-related markers, and biochemical markers of hepatic and renal injury. These findings demonstrate a broad systemic association between prophylactic SCFA supplementation and circulating immunometabolic biomarkers; however, they do not establish the activation or inhibition of specific molecular pathways.

One notable finding was an increase in circulating concentrations of GPR41- and GPR43-related immunoreactive proteins following SCFA supplementation. SCFA treatment exerted significant overall effects on GPR41 at both time points, with significant within-group increases

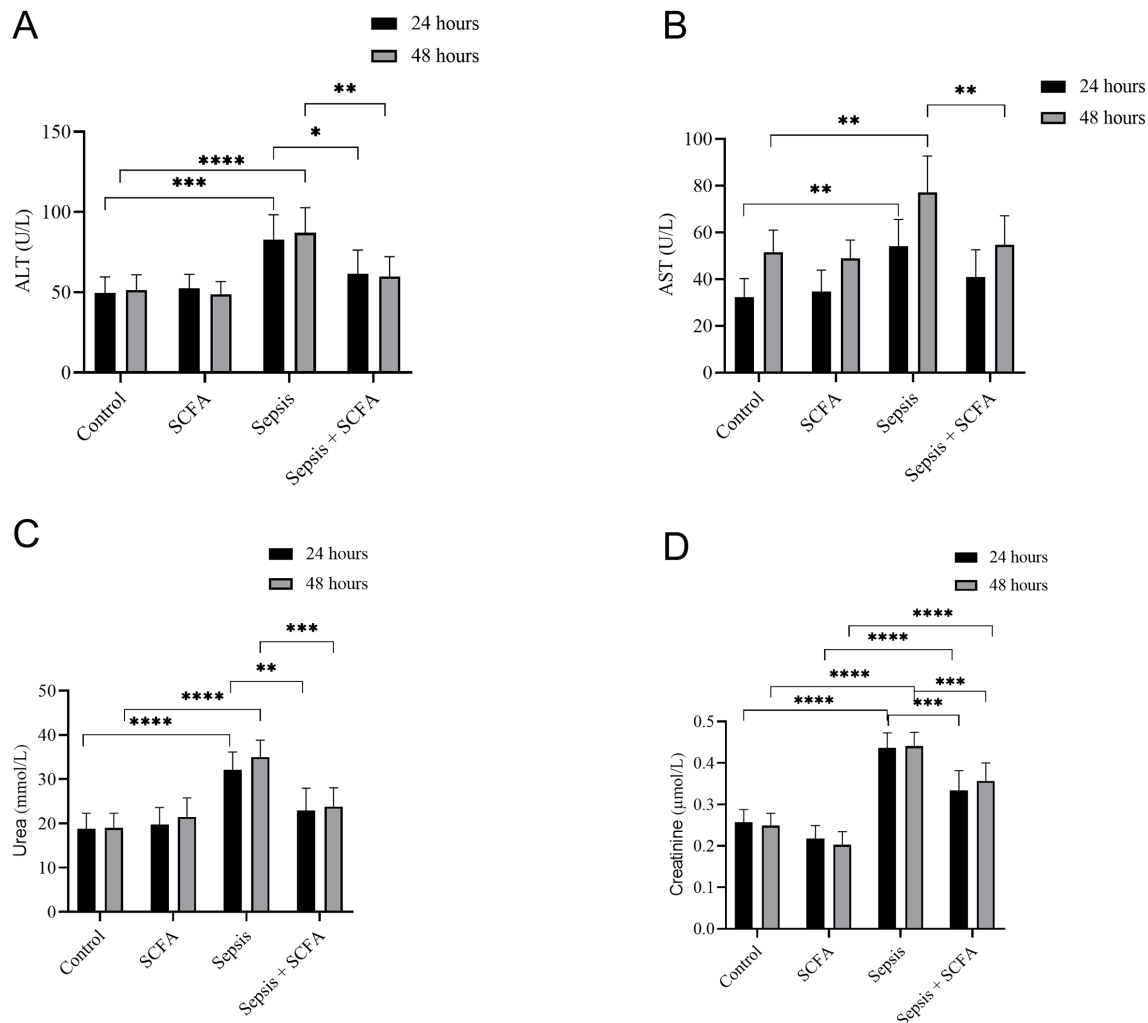


Fig. 7. Effects of short-chain fatty acid (SCFA) supplementation on circulating biochemical markers of hepatic and renal injury (ALT, AST, urea, and creatinine) in experimental sepsis. (A–D) represent the following biochemical markers: (A) ALT, (B) AST, (C) urea, and (D) creatinine. Black bars represent 24 h and gray bars represent 48 h. Data are presented as mean $\pm$ SD ($n = 6$ /group/time point). Two-way ANOVA followed by Šidák-adjusted multiple comparisons was performed separately at each time point. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

in sham and septic animals at 48 h. GPR43-related marker concentrations were significantly increased following SCFA treatment in both sham and septic animals at 24 h and 48 h. GPR41 (FFAR3) and GPR43 (FFAR2) are established receptors for acetate, propionate, and butyrate, and their activation has been associated with immune and metabolic effects in previous experimental studies [28,29,30]. However, the present study measured circulating immunoreactive protein concentrations rather than receptor expression, localization, or downstream signaling in relevant tissues. Therefore, the observed increases should be interpreted as SCFA-associated changes in circulating concentrations of GPR41- and GPR43-related markers, rather than as direct evidence of enhanced receptor expression or pathway activation.

The enteroendocrine findings demonstrated marker- and time-dependent responses to SCFA supplementation. PYY concentrations were not significantly affected by sepsis status or SCFA treatment at 24 h. At 48 h, a significant sepsis status $\times$ SCFA treatment interaction was observed, with lower PYY concentrations in SCFA-treated septic animals than in SCFA-treated sham animals; however, SCFA supplementation did not significantly alter PYY concentrations within either sham or septic animals. In contrast, SCFA treatment produced a significant overall effect on circulating GLP-1 concentrations at both 24 h and 48 h, although individual within-group comparisons did not reach statistical significance. GLP-1R-related circulating concentrations remained stable across experimental groups. Previous studies have demonstrated relationships between SCFAs and enteroendocrine responses through

FFAR2/FFAR3-associated mechanisms [31,32,33]; however, the present circulating measurements do not provide direct evidence of such receptor-mediated signaling. Overall, the findings indicate selective and context-dependent changes in circulating enteroendocrine markers following prophylactic SCFA supplementation.

Circulating adipokine concentrations exhibited heterogeneous responses to sepsis and SCFA supplementation. Septic animals exhibited lower adiponectin and leptin concentrations together with higher omentin and resistin concentrations, indicating that acute sepsis was associated with a complex adipokine profile rather than a uniform pro-inflammatory shift. Adiponectin, an adipokine with well-established metabolic and anti-inflammatory functions [34,35,36], was reduced under septic conditions. Although SCFA treatment produced a significant overall effect on adiponectin concentrations at both time points, the increases observed within septic animals did not reach statistical significance. Leptin concentrations were markedly lower in septic animals and were not significantly affected by SCFA treatment. Resistin concentrations were elevated during sepsis and were significantly reduced by SCFA treatment in both sham and septic animals at 48 h, but not at 24 h. Omentin concentrations increased under septic conditions, whereas SCFA treatment produced a significant effect at 24 h, primarily evident in sham animals. Given the reported vasculoprotective and anti-inflammatory properties of omentin [37], its increase during sepsis may reflect a component of a complex systemic response; however, the present data do not establish the underlying mechanism. Collectively, these findings indicate marker- and time-dependent changes in circulating adipokine concentrations following SCFA supplementation.

ANGPTL4, CTRP1, CTRP3, and PPAR- γ showed distinct patterns across experimental conditions. ANGPTL4 concentrations were significantly lower in vehicle-treated septic animals than in vehicle-treated sham animals. They were significantly increased by SCFA treatment in both sham and septic animals at 24 h and 48 h. Following SCFA supplementation, ANGPTL4 concentrations no longer differed significantly between sham and septic animals. ANGPTL4 has established roles in lipid metabolism, vascular permeability, and endothelial biology [38,39,40]; however, the current circulating measurements do not permit conclusions about changes in lipid handling or endothelial function. CTRP1 concentrations were higher and CTRP3 concentrations lower in vehicle-treated septic animals than in their sham counterparts. SCFA treatment did not significantly alter CTRP1 concentrations within septic animals, while the numerical increases observed in CTRP3 concentrations in septic animals also failed to reach statistical significance [41,42,43]. Therefore, these findings should not be interpreted as restoration of CTRP1 or CTRP3 during sepsis. PPAR- γ concentrations were not significantly affected by sepsis status, and although

a modest overall SCFA treatment effect was detected at 24 h, no significant within-group treatment differences were observed. Because PPAR- γ activity is predominantly regulated at the cellular, transcriptional, and tissue levels [44,45], circulating PPAR- γ -related immunoreactivity should not be considered a direct measure of PPAR- γ pathway activity.

Circulating FABP4 and FABP5 concentrations were significantly higher in vehicle-treated septic animals than in vehicle-treated sham animals at both time points. SCFA treatment produced marker- and time-dependent effects. For FABP4, a significant overall treatment effect was detected at 24 h, although no significant within-group effect of SCFA supplementation was observed in either sham or septic animals. For FABP5, SCFA treatment significantly reduced concentrations in septic animals at 48 h but not at 24 h. FABP4 and FABP5 are involved in lipid trafficking and have been associated with metabolic and inflammatory processes [46,47]. Nevertheless, the present findings reflect only changes in circulating concentrations and do not directly demonstrate improved lipid utilization or metabolic adaptation during sepsis.

The findings regarding circulating NLRP3- and NLRP6-related markers also deserve consideration. Both markers were markedly increased following CLP-induced sepsis. SCFA supplementation was associated with significantly lower concentrations of circulating NLRP3-related markers in septic animals at 24 h and 48 h. In contrast, a significant reduction in circulating concentrations of NLRP6-related markers was observed at 48 h but not at 24 h. NLRP3 and NLRP6 are components of inflammasome-related pathways that have important roles in inflammatory responses, cellular injury, and host-microbiota interactions [48,49,50,51]. However, because the present study measured circulating immunoreactive protein concentrations rather than tissue expression, inflammasome assembly, caspase activation, or downstream functional responses, these findings cannot be interpreted as direct evidence of altered inflammasome activation. Elevated circulating NLRP3- and NLRP6-related marker levels may reflect multiple processes, including cellular injury, protein release, or other sepsis-associated systemic changes. Accordingly, the observed reductions following SCFA supplementation should be interpreted conservatively as changes in circulating concentrations of inflammasome-related markers. Further studies incorporating tissue-level expression and functional assessment are required to determine the biological mechanisms underlying these observations.

Changes in circulating biochemical markers of hepatic and renal injury accompanied the alterations observed in immunometabolic markers. Septic animals exhibited significantly elevated ALT, AST, urea, and creatinine concentrations compared with vehicle-treated sham animals. Prophylactic SCFA administration significantly reduced ALT, urea, and creatinine concentrations in septic animals at both

24 h and 48 h, whereas AST was significantly reduced only at 48 h. Previous experimental studies have reported that butyrate and other SCFAs may influence inflammatory, pyroptotic, endothelial, and mitochondrial processes relevant to organ injury [52,53,54]. However, the present study assessed circulating biochemical markers only and did not include histopathological evaluation or direct functional assessment of hepatic or renal tissue. Therefore, the observed reductions should be interpreted as attenuation of circulating biochemical markers associated with hepatic and renal injury rather than as evidence of restored or improved organ function. Further studies incorporating tissue histology and direct functional assessments are required to determine whether these biochemical changes correspond to structural or functional organ protection.

Collectively, the present results demonstrate that prophylactic SCFA supplementation was associated with changes across several circulating biomarker domains during experimental sepsis. These included receptor-related markers, enteroendocrine mediators, adipokines, metabolic regulatory proteins, lipid-binding proteins, inflammasome-related markers, and biochemical markers of hepatic and renal injury. The direction and statistical significance of these effects varied by individual marker and sampling time. Because the study did not assess tissue-level signaling, microbiota composition, histopathology, or direct organ function, these findings should be interpreted as associations with systemic biomarkers rather than evidence of coordinated pathway activation or a defined mechanism of action.

Several limitations should be acknowledged. First, only male animals were studied; therefore, potential sex-dependent responses remain unclear. Second, the study evaluated circulating biomarkers without parallel tissue-level assessment of gene or protein expression, receptor localization, or downstream functional signaling. Third, survival analysis and histopathological injury scoring were not performed. Furthermore, SCFA administration was exclusively prophylactic and discontinued at the time of sepsis induction; therefore, the findings cannot be interpreted as evidence of therapeutic efficacy after sepsis onset. Gut microbiota composition, intestinal barrier integrity, and endogenous SCFA concentrations were also not directly assessed. Consequently, the present study cannot determine whether the observed changes in circulating biomarkers were attributable to host–microbiota interactions or to the direct systemic effects of exogenously administered SCFAs. Future studies should incorporate post-sepsis therapeutic dosing, microbiota profiling, tissue-level mechanistic analyses, histopathological assessment, and outcome-based endpoints such as survival. Despite these limitations, the present findings demonstrate that prophylactic SCFA supplementation is associated with significant marker- and time-dependent changes in circulating immunometabolic biomarkers and biochemical markers of hepatic and renal

injury in experimental sepsis, providing a rationale for further mechanistic and therapeutic investigation.

Conclusion

In conclusion, prophylactic SCFA supplementation modulated sepsis-associated immunometabolic alterations and attenuated several markers of organ injury in a CLP-induced experimental sepsis model. These effects were accompanied by changes in SCFA-responsive receptor-associated markers, adipokine profiles, inflammasome-related mediators, and metabolic regulators, suggesting a broad influence on interconnected immune and metabolic networks. Although the present findings do not directly demonstrate alterations in gut microbiota composition and were obtained in a preventive rather than therapeutic setting, they support the concept that microbiota-derived metabolites may contribute to the regulation of host responses during sepsis. Further studies incorporating microbiota profiling, tissue-level validation, survival analyses, and post-sepsis therapeutic intervention protocols are required to clarify the translational relevance of these findings.

Availability of Data and Materials

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

PE contributed to the conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing of the original draft. YBY contributed to the experimental procedures, sample collection, investigation, data interpretation, and critical revision of the manuscript. MSY contributed to the laboratory analyses, data interpretation, validation, and critical revision of the manuscript. SÇ contributed to the conceptualization, methodology, supervision, project administration, and critical revision of the manuscript. All authors read and approved the final version of the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Bursa Uludag University (Approval No. 2024-14/08). All procedures were conducted in accordance with the ARRIVE guidelines 2.0 and were performed in accordance with the European Directive 2010/63/EU for

the protection of animals used for scientific purposes, as well as the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Acknowledgment

Not applicable.

Funding

This study was supported by the Bursa Uludag University Scientific Research Projects Coordination Unit (Grant No. TGA-2023-1455).

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in Manuscript Preparation

During the preparation of this manuscript, the authors used OpenAI's ChatGPT (GPT-4) solely for language editing, grammar correction, and improvement of readability. The AI tool was not used for data generation, data analysis, result interpretation, or for drawing scientific conclusions. After its use, the authors thoroughly reviewed, verified, and revised all content to ensure accuracy and originality.

References

- [1] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016; 315: 801–810. <https://doi.org/10.1001/jama.2016.0287>
- [2] Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *Lancet* (London, England). 2020; 395: 200–211. [https://doi.org/10.1016/S0140-6736\(19\)32989-7](https://doi.org/10.1016/S0140-6736(19)32989-7)
- [3] O'Neill LAJ, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nature Reviews. Immunology*. 2016; 16: 553–565. <https://doi.org/10.1038/nri.2016.70>
- [4] Willmann K, Moita LF. Physiologic disruption and metabolic reprogramming in infection and sepsis. *Cell Metabolism*. 2024; 36: 927–946. <https://doi.org/10.1016/j.cmet.2024.02.013>
- [5] Blaak EE, Canfora EE, Theis S, Frost G, Groen AK, Mithieux G, et al. Short chain fatty acids in human gut and metabolic health. *Beneficial Microbes*. 2020; 11: 411–455. <https://doi.org/10.3920/BM2020.0057>
- [6] Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Research*. 2020; 30: 492–506. <https://doi.org/10.1038/s41422-020-0332-7>
- [7] Kullberg RFJ, Wiersinga WJ, Haak BW. Gut microbiota and sepsis: from pathogenesis to novel treatments. *Current Opinion in Gastroenterology*. 2021; 37: 578–585. <https://doi.org/10.1097/MOG.0000000000000781>
- [8] Sung J, Rajendraprasad SS, Philbrick KL, Bauer BA, Gajic O, Shah A, et al. The human gut microbiome in critical illness: disruptions, consequences, and therapeutic frontiers. *Journal of Critical Care*. 2024; 79: 154436. <https://doi.org/10.1016/j.jcrc.2023.154436>
- [9] Fusco W, Lorenzo MB, Cintoni M, Porcari S, Rinninella E, Kait-sas F, et al. Short-Chain Fatty-Acid-Producing Bacteria: Key Components of the Human Gut Microbiota. *Nutrients*. 2023; 15: 2211. <https://doi.org/10.3390/nu15092211>
- [10] Liu XF, Shao JH, Liao YT, Wang LN, Jia Y, Dong PJ, et al. Regulation of short-chain fatty acids in the immune system. *Frontiers in Immunology*. 2023; 14: 1186892. <https://doi.org/10.3389/fimmu.2023.1186892>
- [11] Du Y, He C, An Y, Huang Y, Zhang H, Fu W, et al. The Role of Short Chain Fatty Acids in Inflammation and Body Health. *International Journal of Molecular Sciences*. 2024; 25: 7379. <https://doi.org/10.3390/ijms25137379>
- [12] Nogal A, Valdes AM, Menni C. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes*. 2021; 13: 1–24. <https://doi.org/10.1080/19490976.2021.1897212>
- [13] Liu J, Jin Y, Ye Y, Tang Y, Dai S, Li M, et al. The Neuro-protective Effect of Short Chain Fatty Acids Against Sepsis-Associated Encephalopathy in Mice. *Frontiers in Immunology*. 2021; 12: 626894. <https://doi.org/10.3389/fimmu.2021.626894>
- [14] Wei Y, Li T, Zhao D, Sun T, Ma C, Zhang L, et al. Sodium butyrate ameliorates sepsis-associated lung injury by enhancing gut and lung barrier function in combination with modulation of CD4⁺Foxp3⁺ regulatory T cells. *European Journal of Pharmacology*. 2024; 963: 176219. <https://doi.org/10.1016/j.ejphar.2023.176219>
- [15] Mann ER, Lam YK, Uhlig HH. Short-chain fatty acids: linking diet, the microbiome and immunity. *Nature Reviews. Immunology*. 2024; 24: 577–595. <https://doi.org/10.1038/s41577-024-01014-8>
- [16] Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*. 2020; 18: e3000410. <https://doi.org/10.1371/journal.pbio.3000410>
- [17] National Research Council. Guide for the Care and Use of Laboratory Animals. 8th edn. National Academies Press: Washington DC, USA. 2011. <https://doi.org/10.17226/12910>
- [18] van der Hee B, Wells JM. Microbial Regulation of Host Physiology by Short-chain Fatty Acids. *Trends in Microbiology*. 2021; 29: 700–712. <https://doi.org/10.1016/j.tim.2021.02.001>
- [19] Golpour F, Abbasi-Alaei M, Babaei F, Mirzababaei M, Parvardeh S, Mohammadi G, et al. Short chain fatty acids, a possible treatment option for autoimmune diseases. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2023; 163: 114763. <https://doi.org/10.1016/j.biopha.2023.114763>
- [20] Shah S, Fillier T, Pham TH, Thomas R, Cheema SK. Intraperitoneal Administration of Short-Chain Fatty Acids Improves Lipid Metabolism of Long-Evans Rats in a Sex-Specific Manner. *Nutrients*. 2021; 13: 892. <https://doi.org/10.3390/nu13030892>
- [21] Liao H, Li H, Bao H, Jiang L, Du J, Guo Y, et al. Short Chain Fatty Acids Protect the Cognitive Function of Sepsis Associated Encephalopathy Mice via GPR43. *Frontiers in Neurology*. 2022; 13: 909436. <https://doi.org/10.3389/fneur.2022.909436>
- [22] Drechsler S, Osuchowski M. Cecal Ligation and Puncture. *Methods in Molecular Biology* (Clifton, N.J.). 2021; 2321: 1–8. https://doi.org/10.1007/978-1-0716-1488-4_1
- [23] Wilson CN, Vance CO, Lechner MG, Matuschak GM, Lechner AJ. Adenosine A1 receptor antagonist, L-97-1, improves survival and protects the kidney in a rat model of cecal ligation and puncture induced sepsis. *European Journal of Pharmacology*. 2014; 740: 346–352. <https://doi.org/10.1016/j.ejphar.2014.07.012>
- [24] Lakens D. Sample Size Justification. *Collabra: Psychology*. 2022; 8: 33267. <https://doi.org/10.1525/collabra.33267>
- [25] Wiersinga WJ, van der Poll T. Immunopathophysiology of human sepsis. *EBioMedicine*. 2022; 86: 104363. <https://doi.org/10.1016/j.ebiom.2022.104363>
- [26] Witmer NH, Linzer CR, Boudreau RL. Fndc5 is translated

- from an upstream ATG start codon and cleaved to produce irisin myokine precursor protein in humans and mice. *Cell Metabolism*. 2024; 36: 879–881. <https://doi.org/10.1016/j.cmet.2024.02.008>
- [27] Yu J, Liu X. Gut microbiota and sepsis: mechanisms, clinical correlations, and therapeutic prospects. *Frontiers in Medicine*. 2026; 13: 1793041. <https://doi.org/10.3389/fmed.2026.1793041>
- [28] Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *The Journal of Biological Chemistry*. 2003; 278: 11312–11319. <https://doi.org/10.1074/jbc.M211609200>
- [29] Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M. Free Fatty Acid Receptors in Health and Disease. *Physiological Reviews*. 2020; 100: 171–210. <https://doi.org/10.1152/physrev.00041.2018>
- [30] Schlatterer K, Peschel A, Kretschmer D. Short-Chain Fatty Acid and FFAR2 Activation - A New Option for Treating Infections? *Frontiers in Cellular and Infection Microbiology*. 2021; 11: 785833. <https://doi.org/10.3389/fcimb.2021.785833>
- [31] Kim CH. Short-, medium-, versus long-chain fatty acids: mechanisms of immunomodulation and disease pathogenesis. *Cellular & Molecular Immunology*. 2026; 23: 594–606. <https://doi.org/10.1038/s41423-026-01412-z>
- [32] Liu M, Lu Y, Xue G, Han L, Jia H, Wang Z, et al. Role of short-chain fatty acids in host physiology. *Animal Models and Experimental Medicine*. 2024; 7: 641–652. <https://doi.org/10.1002/am.e2.12464>
- [33] Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*. 2009; 461: 1282–1286. <https://doi.org/10.1038/nature08530>
- [34] Zhao S, Kusminski CM, Scherer PE. Adiponectin, Leptin and Cardiovascular Disorders. *Circulation Research*. 2021; 128: 136–149. <https://doi.org/10.1161/CIRCRESAHA.120.314458>
- [35] Baldelli S, Aiello G, Mansilla Di Martino E, Campaci D, Muthanna FMS, Lombardo M. The Role of Adipose Tissue and Nutrition in the Regulation of Adiponectin. *Nutrients*. 2024; 16: 2436. <https://doi.org/10.3390/nu16152436>
- [36] Gianoli S, Tang J, Odegard KC, Yuki K, Koutsogiannaki S. Harnessing adiponectin for sepsis: current knowledge, clinical insights and future therapies. *Critical Care (London, England)*. 2025; 29: 300. <https://doi.org/10.1186/s13054-025-05516-2>
- [37] Hussein AA, Ahmed NA, Sakr HI, Atia T, Ahmed OM. Omentin roles in physiology and pathophysiology: an up-to-date comprehensive review. *Archives of Physiology and Biochemistry*. 2024; 130: 800–813. <https://doi.org/10.1080/13813455.2023.2283685>
- [38] Kersten S. Role and mechanism of the action of angiopoietin-like protein ANGPTL4 in plasma lipid metabolism. *Journal of Lipid Research*. 2021; 62: 100150. <https://doi.org/10.1016/j.jlr.2021.100150>
- [39] Cho DI, Ahn JH, Kang BG, Hwang I, Cho HH, Jun JH, et al. ANGPTL4 Prevents Atherosclerosis by Preserving KLF2 to Suppress EndMT and Mitigates Endothelial Dysfunction. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2025; 45: 1742–1761. <https://doi.org/10.1161/ATVBAHA.125.322700>
- [40] Ziveri J, Le Guennec L, Dos Santos Souza I, Barnier JP, Walter SM, Diallo Y, et al. Angiopoietin-like 4 protects against endothelial dysfunction during bacterial sepsis. *Nature Microbiology*. 2024; 9: 2434–2447. <https://doi.org/10.1038/s41564-024-01760-4>
- [41] Zhang H, Zhang-Sun ZY, Xue CX, Li XY, Ren J, Jiang YT, et al. CTRP family in diseases associated with inflammation and metabolism: molecular mechanisms and clinical implication. *Acta Pharmacologica Sinica*. 2023; 44: 710–725. <https://doi.org/10.1038/s41401-022-00991-7>
- [42] Schmid A, Vlacil AK, Schuett J, Karrasch T, Schieffer B, Schäffler A, et al. Anti-Inflammatory Effects of C1q/Tumor Necrosis Factor-Related Protein 3 (CTRP3) in Endothelial Cells. *Cells*. 2021; 10: 2146. <https://doi.org/10.3390/cells10082146>
- [43] Namazi G, Mehranian M, Mafi A, Salami R, Raygan F. Serum levels of C1q/TNF-related protein-1 (CTRP1) and CTRP3 in patients with coronary artery disease. *ARYA Atherosclerosis*. 2025; 21: 34–41. <https://doi.org/10.48305/arya.2025.43204.3006>
- [44] Małodobra-Mazur M, Oldakowska M, Dobosz T. Exploring PPAR Gamma and PPAR Alpha's Regulation Role in Metabolism via Epigenetics Mechanism. *Biomolecules*. 2024; 14: 1445. <https://doi.org/10.3390/biom14111445>
- [45] Alex S, Lange K, Amolo T, Grinstead JS, Haakonsson AK, Szalowska E, et al. Short-chain fatty acids stimulate angiopoietin-like 4 synthesis in human colon adenocarcinoma cells by activating peroxisome proliferator-activated receptor γ . *Molecular and Cellular Biology*. 2013; 33: 1303–1316. <https://doi.org/10.1128/MCB.00858-12>
- [46] Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nature Reviews. Drug Discovery*. 2008; 7: 489–503. <https://doi.org/10.1038/nrd2589>
- [47] Makowski L, Brittingham KC, Reynolds JM, Suttles J, Hotamisligil GS. The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and I κ B kinase activities. *The Journal of Biological Chemistry*. 2005; 280: 12888–12895. <https://doi.org/10.1074/jbc.M413788200>
- [48] Paik S, Kim JK, Shin HJ, Park EJ, Kim IS, Jo EK. Updated insights into the molecular networks for NLRP3 inflammasome activation. *Cellular & Molecular Immunology*. 2025; 22: 563–596. <https://doi.org/10.1038/s41423-025-01284-9>
- [49] Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *International Journal of Molecular Sciences*. 2019; 20: 3328. <https://doi.org/10.3390/ijms20133328>
- [50] Wlodarska M, Thaïss CA, Nowarski R, Henao-Mejia J, Zhang JP, Brown EM, et al. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell*. 2014; 156: 1045–1059. <https://doi.org/10.1016/j.cell.2014.01.026>
- [51] Shen C, Lu A, Xie WJ, Ruan J, Negro R, Egelman EH, et al. Molecular mechanism for NLRP6 inflammasome assembly and activation. *Proceedings of the National Academy of Sciences of the United States of America*. 2019; 116: 2052–2057. <https://doi.org/10.1073/pnas.1817221116>
- [52] Tian X, Yuan L, Zeng Y. Butyrate attenuates SA-AKI by inhibiting pyroptosis via the STING-GSDMD axis. *Biochemical and Biophysical Research Communications*. 2025; 743: 151143. <https://doi.org/10.1016/j.bbrc.2024.151143>
- [53] Andrade-Oliveira V, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJF, de Almeida DC, et al. Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. *Journal of the American Society of Nephrology: JASN*. 2015; 26: 1877–1888. <https://doi.org/10.1681/ASN.2014030288>
- [54] Nicese MN, Bijkerk R, Van Zonneveld AJ, Van den Berg BM, Rotmans JJ. Sodium Butyrate as Key Regulator of Mitochondrial Function and Barrier Integrity of Human Glomerular Endothelial Cells. *International Journal of Molecular Sciences*. 2023; 24: 13090. <https://doi.org/10.3390/ijms241713090>