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Modulatory effects of exogenous estradiol during endotoxemia

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ABSTRACT

This study explores the effects of exogenous estradiol on inflammatory, metabolic, and organ-function responses to lipopolysaccharide (LPS)-induced endotoxemia using a murine model. Male, female, and estradiol-supplemented female mice were administered equal dose of LPS injections and observed over a 6-hour period for systemic physiological changes, including blood pressure, glomerular filtration rate (GFR), blood gases, and temperature. Post-mortem analyses evaluated inflammatory pathway markers, biomarkers of organ injury, and targeted metabolomics from plasma samples. Estradiol supplementation was associated with attenuated early markers of hepatic and cardiac injury, reduced cardiac nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) expression, and changes from baseline post-LPS on metabolite profiles compared with control female and male mice. These effects were organ- and endpoint-dependent, with renal functional differences emerging primarily at later time points. Metabolite changes involving L-carnitine, methionine sulfoxide, and octanoic acid were consistent with altered metabolic and redox responses, but do not directly demonstrate changes in mitochondrial or antioxidant function. Together, these findings indicate that elevated estradiol levels are associated with modulation of early inflammatory and metabolic responses during endotoxemia, rather than uniform protection across organs or sexes. This work highlights biological sex and hormonal status as important variables in endotoxemia models and provides hypothesis-generating evidence for future studies examining hormone-dependent regulation of immune-metabolic pathways during systemic inflammation.

1. Introduction

Sepsis and endotoxemia are life-threatening conditions arising from a dysregulated immune response to infection, leading to widespread inflammation, multi-organ failure, and high mortality. Progression to septic shock is characterized by persistent hypotension, tissue hypoperfusion, and metabolic dysfunction, further increases the risk of death (Mahapatra and Heffner, 2025). In the United States, sepsis contributes to over 250,000 deaths annually, with global mortality exceeding 11 million per year (Rudd et al., 2020). Despite improvements in supportive care, sepsis remains one of the most complex and lethal conditions in critical care medicine.

A growing body of evidence indicates that biological sex is a key determinant of outcomes and physiological responses during sepsis (Min et al., 2024). Epidemiological studies report that females experience lower mortality and reduced organ failure compared to males (Wanrooij et al., 2023), and that sepsis is more prevalent in men, who exhibit a 1.3-fold higher annual relative risk than women (Min et al., 2024). Among patients with septic shock admitted to Intensive Care Units

(ICUs), males are overrepresented and show worse clinical trajectories, including longer ICU and hospital stays, higher ICU mortality, greater likelihood of 90-day and 1-year readmission, and increased 1-year mortality (Campanelli et al., 2018; Thompson et al., 2022).

Although the mechanisms driving these sex differences remain unclear, several factors have been proposed, including the beneficial effects of genes expressed from the second X chromosome in females and the immunomodulatory actions of sex hormones such as estrogen, particularly 17 β -estradiol (E2). Beyond its role in reproductive physiology, E2 broadly regulates immune responses by modulating cytokine production, enhancing endothelial barrier integrity, and reducing oxidative stress, all of which are critical during the inflammatory and metabolic dysregulation of sepsis (Xiang et al., 2021; Zhong et al., 2023; Chakrabarti et al., 2008). Its role in sepsis-associated immune responses has been supported by previous studies (Dias et al., 2022; Grimaldi et al., 2005). Estrogen and 17 β -estradiol have been shown to enhance endothelial function, suppress excessive cytokine release, and improve mitochondrial metabolism—factors that may collectively improve survival during septic insult (Trenti et al., 2018). However, the precise

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molecular and physiological mechanisms by which estrogen confers protection in sepsis remain incompletely understood.

A central pathway implicated in the immune response to sepsis is the Toll-like receptor 4 (TLR4) signaling cascade (Kim et al., 2023). Upon recognition of lipopolysaccharide (LPS), a bacterial endotoxin, TLR4 activation triggers downstream signaling through adapter proteins such as cluster of differentiation 14 (CD14) and TIR domain containing adapter molecule 1 (TICAM1), ultimately leading to the translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and the expression of pro-inflammatory cytokines (Ciesielska et al., 2020). Dysregulation of this pathway contributes to the cytokine storm and multi-organ dysfunction observed in inflammation and septic shock (Kumar et al., 2020). Given that NF- κ B is a redox-sensitive transcription factor, estrogen's known antioxidant effects may intersect with this pathway to suppress excessive inflammation.

To characterize early and sustained physiological responses to LPS-induced endotoxemia, we employed two complementary timepoints. A 6-hour model captured acute inflammatory, metabolic, and organ-specific changes across all groups, including estradiol-supplemented females. A separate 24-hour model, limited to naturally cycling females stratified by estrous phase, was used to assess later-stage renal function via GFR and determine whether endogenous estradiol influences delayed kidney responses to LPS.

We tested the hypothesis that chronic exogenous estradiol (E2, 17 β -estradiol, 1.7 mg, 60-day release) mitigates inflammatory and metabolic consequences of sepsis by modulating TLR4 signaling and preserving organ function. Using a standard LPS-induced endotoxemia model, we compared male, female, and estradiol-supplemented female mice across systemic hemodynamics, inflammatory gene expression, organ injury biomarkers, in-vivo glomerular filtration rate (GFR), and targeted plasma metabolomics. Here, "improved clinical outcomes" refer to changes in early organ injury biomarkers, inflammatory signaling (particularly NF- κ B), and metabolite profiles consistent with altered metabolic and redox responses, rather than direct measures of survival, organ recovery, or clinical efficacy. By integrating these findings across sex and hormone status, we aim to define estrogen's mechanistic role in inflammation and septic progression, as well as inform future sex-specific studies and therapeutic strategies.

2. Methods

2.1. Animal preparation

Studies were performed in male & female C57BL/6 J mice weighing 15–25 g (Jackson Laboratories, Bar Harbor, ME). Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals, and all protocols were approved by the University of California San Diego Institutional Animal Care and Use Committee (IACUC). All methods were carried out in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments).

This study was designed as a mechanistic, hypothesis-generating investigation to examine early inflammatory, metabolic, and organ-specific responses to endotoxemia rather than to assess survival or long-term outcomes. Sample sizes ($n = 5$ – 10 per group, depending on endpoint) were selected based on prior experience with this endotoxemia model and comparable studies demonstrating reliable detection of biologically meaningful differences in inflammatory signaling, organ injury biomarkers, and metabolomic profiles (Hamesch et al., 2015). Animals were randomly assigned to experimental groups prior to saline or LPS administration.

2.2. Verification of estrus phase

To verify the hormone stage of both groups of female mice, vaginal lavage with normal saline was applied to both treated and untreated female mice at baseline to obtain cell samples. Estrous cycle staging was

performed to stratify female mice by endogenous estrogen status at the time of experimentation and to examine hormone-associated differences in inflammatory and physiological responses, rather than to control or normalize outcomes across groups. The vaginal smear samples were obtained using the framework and methodology of previous studies (McLean et al., 2012).

Three primary cell types were detected in vaginal smear samples: (1) nucleated epithelial cells, (2) cornified squamous epithelial cells, and (3) leukocytes (McLean et al., 2012). The relative ratio of cell types observed in smears was used to identify the stage of the estrous cycle of the mouse on the day of sample collection in accordance with previous studies (McLean et al., 2012). These phases are summarized below and shown graphically in Fig. 1.

1. Proestrus (Fig. 1D): Cells are almost exclusively clusters of round, well-formed nucleated epithelial cells.
2. Estrus (Fig. 1E): Cells are predominantly cornified squamous epithelial cells, present in densely packed clusters
3. Metestrus (Fig. 1F): Small darkly stained leukocytes predominate. Cornified squamous epithelial cells may also be observed, often in fragments.
4. Diestrus (Fig. 1G): Rare cornified squamous epithelial cells may still be present, however leukocytes still predominate.

2.3. Preparation of reagents and solutions

The prepared LPS solution was composed of 10 mg lipopolysaccharides from *Escherichia coli* O128:B12 (L2755, Sigma Aldrich, St. Louis, MO) diluted with 5 mL of ultrapure water to reach a concentration of 2 mg/mL. The selected LPS dose (10 mg/kg, intraperitoneal) was chosen to reliably and consistently induce systemic inflammation and early organ injury markers within a controlled, non-lethal time window, as established in prior murine endotoxemia studies (Hamesch et al., 2015). This model reflects acute endotoxemia rather than polymicrobial sepsis or septic shock.

2.4. Animal preparation

To exacerbate the sex difference in the immune response to endotoxemia, a 17 β -Estradiol (E2) pellet was implanted into female mice, thus increasing the total concentration of E2 within the blood of the animal. Estradiol group, or Exogenous Estradiol Treatment (EET), mice were anesthetized and then had a 1.7 mg pellet of 17 β -Estradiol (Innovative Research of America, Sarasota, FL) implanted beneath the skin in the interscapular region. The mice were then allowed to recover for 3 weeks before being used in experimentation in the 6-hour endotoxemia model. Mice used in the 24-hour endotoxemia model were not dosed with 17 β -Estradiol. This estradiol dosing regimen has been widely used to achieve sustained high-physiological to supraphysiological circulating estradiol levels in murine studies examining inflammatory and cardiovascular modulation (Cavasin et al., 2003). Circulating estradiol concentrations were not directly measured in the present study. The control group was comprised of healthy animals that were treated with an intraperitoneal (IP) injection of saline with a fixed volume of 100 μ L. To induce endotoxemia, the endotoxemic group was comprised of animals given an IP injection of LPS solution with a fixed injection volume of 100 μ L (10 mg/kg LPS). All animals were instrumented with a carotid catheter for measuring the systemic and blood gas parameters. All protocols were approved by our local IACUC.

2.5. Systemic parameters

Arterial pressure and heart rate (HR) were recorded continuously from the aorta (MP150, Biopac, Santa Barbara, CA) at a 2 kHz sampling rate. Blood pressure recordings were used to calculate online MAP, systolic blood pressure (SBP), and diastolic blood pressure (DBP) using

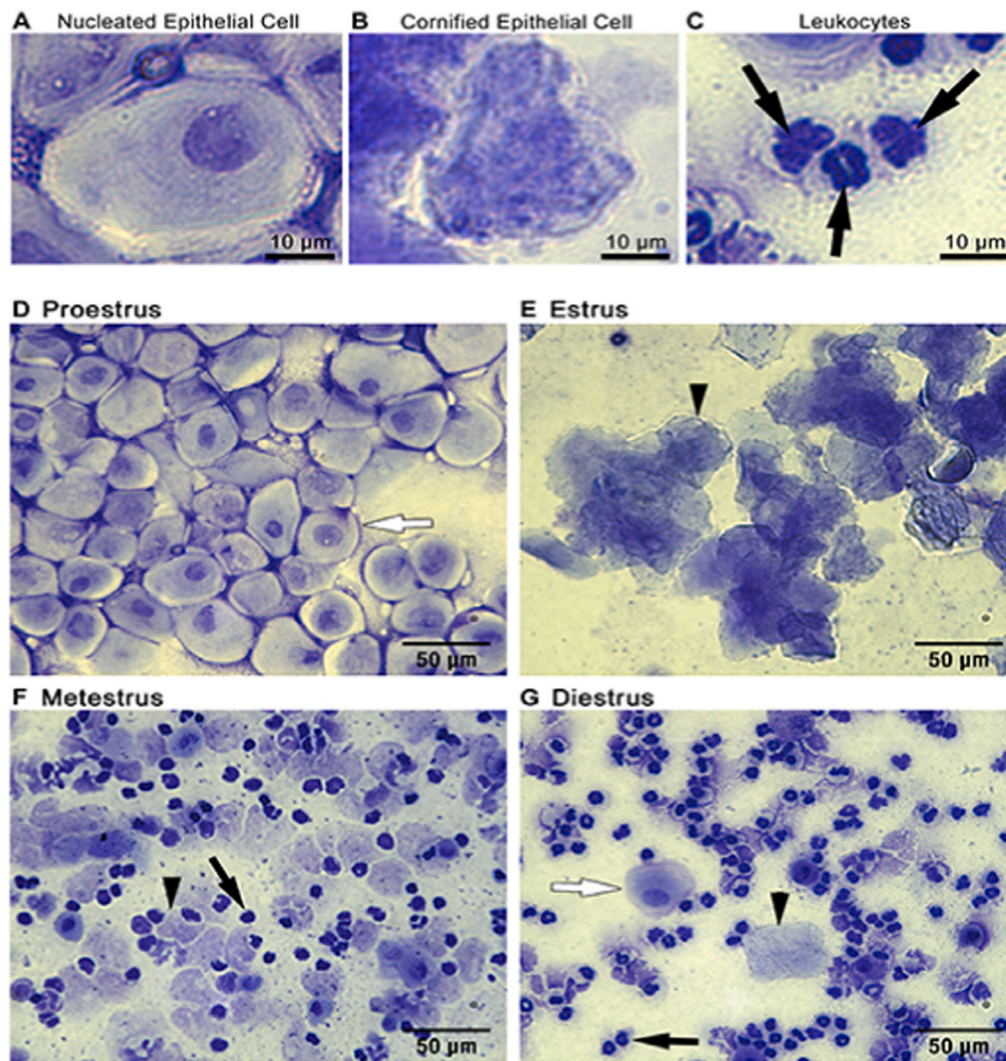


Fig. 1. Microscope slides from a vaginal lavage, reproduced from [McLean et al. \(2012\)](#). Three main cell types are detected in vaginal smear samples: (A) nucleated epithelial cells, (B) cornified squamous epithelial cells, and (C) leukocytes. The ratio of these cell types present in the smear can be used to identify mice in (D) proestrus, (E) estrus, (F) metestrus, or (G) diestrus as described in *representative results*. Black arrowheads in E, F and G point to representative cornified squamous epithelial cells. Black arrows in C, F and G point to representative leukocytes. White arrows in D and G highlight representative nucleated epithelial cells.

AcqKnowledge software (Biopac). Hematocrit was measured from centrifuged arterial blood samples taken in heparinized tubes (65 µL). Additionally, arterial blood was collected in heparinized glass capillary tubes (65 µL) and immediately analyzed for oxygen partial pressure (PO₂), carbon dioxide partial pressure (PCO₂), pH, hemoglobin O₂ saturation (SO₂), glucose, and lactate (ABL90; Radiometer America, Brea, CA). Glomerular filtration rate (GFR) was recorded with a Transdermal GFR Measurement System (TGFR, MediBeacon, St. Louis, MO). Acquisition of systemic physiological data was performed using automated instrumentation and analysis pipelines.

2.6. Transdermal glomerular filtration rate (GFR)

Glomerular filtration rate (GFR) was assessed noninvasively using the MediBeacon TGFR System (St. Louis, MO), which enables continuous monitoring of renal function in conscious, freely moving mice ([Scarfe et al., 2018](#)). The system uses a fluorescent tracer, MB-102 (FITC-sinistrin), administered via intraarterial injection. The FITC-sinistrin is freely filtered by the glomerulus and neither reabsorbed nor secreted, its plasma half-life directly reflects GFR ([Ellery et al., 2015](#)). After injection, the tracer is excited at 480 nm and its emitted light (520 nm) is detected transdermally using a photodetector placed

on the shaved anterior surface in males and dorsal surface in females (to avoid interference from reproductive tissues) ([Ellery et al., 2015](#)). The system generates an elimination kinetics curve from the fluorescence decay, and the GFR is calculated based on the tracer's plasma clearance. GFR was selected as a delayed functional endpoint, as changes in renal filtration often evolve later than early inflammatory and biochemical markers during endotoxemia.

2.7. 6-hour endotoxemia model

Briefly, baseline measurement blood sample was obtained from all mice at the beginning of experimentation. The control group was given the saline injection while the LPS group was given the prepared LPS solution as described above. Mice were anesthetized briefly with 2 % vol. inhaled isoflurane (Drägerwerk AG, Lübeck, Germany) and placed on a heated pad while obtaining blood samples of 100 µL from the aortic catheter at baseline, 1 hr, 2 hr, 4 hr, and 6 hr post-injection. Samples were subsequently centrifuged in a 0.5 mL microcentrifuge tube in a benchtop centrifuge (Sorval ST 8 R, Thermo Scientific, Waltham, MA) following which RBC and plasma samples were obtained for metabolomics analysis. Blood samples were spun at 4500 RPM for 7 min in a chamber cooled to 4 ° Celsius. The 6-hour time point was selected to

capture early inflammatory, metabolic, and organ-injury responses to LPS prior to secondary hemodynamic changes. No animals died during the 6-hour endotoxemia experiments.

2.8. 24-hour endotoxemia model

A separate group of animals was used for the 24-hour model, which was centered on measuring systemic and renal functional changes using GFR over 24 h after endotoxemia onset. Mice were given an IP injection with either saline or the LPS solution as prescribed before according to their group. The following day, animals were briefly anesthetized using isoflurane 5 % in compressed room air for induction (Drägerwerk AG, Lübeck, Germany) and then maintained at 1.5 %, except during surgical procedures when it was increased to 2.5 %. Animals were placed on a heating pad to preserve core body temperature at 37 °C and allowed to freely breathe from a nosecone delivering anesthesia. Animals were instrumented with an aortic catheter for hemodynamic assessment, blood sampling and intravenous infusion, respectively. Animals were allowed to stabilize for 10 min and baseline measurements were collected. Both control and LPS female mice had a vaginal lavage applied to obtain cell samples to determine the phase of the hormonal cycle stage of the mice. No EET mice were used in this experiment, instead delineating the female mice by the naturally higher or lower estradiol of their observed estrus phase. This model was designed to assess delayed renal functional responses associated with endogenous estrogen status rather than chronic estradiol supplementation, and therefore estradiol pellets were not used in this experiment. The GFR tracer was then applied and measured over 1 h to collect GFR levels.

2.9. Harvesting tissues

Animals were euthanized via cervical dislocation immediately following collection of the final blood sample or 1 h after GFR determination. Following euthanasia, tissue samples were obtained for the brain, heart, lung, liver, kidney and plasma. Plasma was obtained through centrifugation of the final blood sample in a 1.5 mL microcentrifuge tube in a benchtop centrifuge (Sorval ST 8 R, Thermo Scientific, Waltham, MA). Blood samples were spun at 4500 RPM for 7 min in a chamber cooled to 4 °C Celsius. Analyses of plasma and tissue samples were performed by the UC San Diego Histology and Immunoassay Core using standardized, validated protocols. Biomarkers of organ injury and inflammation were quantified using commercially available mouse-specific enzyme-linked immunosorbent assay (ELISA) kits, and inflammatory markers were assessed using flow cytometry and quantitative polymerase chain reaction (qPCR) based approaches. Investigators conducting downstream analyses were blinded to experimental group allocation during data processing. Detailed information regarding assay kits, detection limits, and sensitivity is provided in [supplemental material 1](#).

2.10. Metabolomic analyses

Metabolomic analyses were completed from plasma samples. Briefly, metabolites were extracted in cold methanol:acetonitrile:water (5:3:2, v/v/v). After vortexing at 4 °C for 10 min, extracts were separated from the protein pellet by centrifugation for 10 min at 10,000 g at 4 °C. Analysis by Ultra-High-Pressure Liquid Chromatography-Mass Spectrometry was performed using a UHPLC coupled online to an Exactive mass spectrometer (ThermoFisher, Bremen, Germany) using a five min C18 gradient in positive and negative ion modes. Metabolite peaks were integrated and annotated using EI-MAVEN with the KEGG database.

2.11. Statistical analysis

All values are expressed as mean $\pm$ SD. Data was analyzed using Two-Way Analysis of Variance (ANOVA). When appropriate, post hoc

analyses were performed with the Tukey's honestly significant difference test (Tukey's HSD). All statistics were calculated using R (version 4.4.1, R Foundation for Statistical Computing, Vienna, Austria). Results were considered significant if $p < 0.05$. Metabolomic analyses focused on a small, literature-informed subset of pre-specified metabolites selected a priori based on relevance to inflammatory, metabolic, and redox pathways. As this was a targeted, hypothesis-driven analysis rather than an untargeted screen, global multiple-comparison correction was not applied. Statistical interpretation emphasizes consistency across related endpoints rather than reliance on isolated comparisons.

2.12. Code availability

All software components of this work are publicly available at <https://github.com/Tajanko/turbo-enigma>. Software components are open-source and may be distributed and reused according to the MIT License.

3. Results

Most outcomes, including inflammatory signaling, organ damage markers, and metabolomics, were measured using a 6-hour model to capture early-phase responses. For GFR, we instead employed a 24-hour model, as GFR changes manifest later in the inflammatory progression and its consequences. In this model, mice were stratified by estrous phase to investigate the effect of natural estrogen levels on renal function. These complementary timepoints allowed us to distinguish immediately from delayed responses to systemic inflammation and estradiol exposure. Across organ biomarkers, differences between LPS-treated groups were not uniform across all endpoints or comparators, consistent with organ- and marker-specific sensitivity to estradiol exposure.

3.1. Organ function biomarkers

3.1.1. Liver

To assess liver inflammation and injury, the levels of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined in each experimental group. The plasma concentration of ALT and the plasma concentration of AST were quantified to establish the relative level of hepatic function between the experimental groups. [Fig. 2](#) shows lower plasma concentrations of ALT and AST when estradiol levels were greater compared with the other LPS experimental groups.

3.1.2. Kidney

Kidney injury was assessed by measuring measured levels of blood urea nitrogen (BUN) and creatinine in each experimental group. The plasma concentration of BUN and creatinine were quantified to establish the relative level of renal function between the experimental groups. [Fig. 3](#) shows LPS-associated changes in BUN and creatinine across groups; differences between estradiol-treated, male, and untreated female mice were endpoint-dependent.

Kidney function was further assessed through the measurement of transdermal GFR. The results were not absolute and instead served to demonstrate the relative differences between groups. Because GFR changes can emerge later, GFR was evaluated at 24 h in a separate cohort stratified by estrous phase. Estrus-phase females showed higher GFR than diestrus-phase females after LPS ([Fig. 4](#)), consistent with hormone-associated differences in renal filtration during endotoxemia.

3.1.3. Heart

Cardiac C-reactive protein (CRP) and cardiac troponin were measured as biomarkers of cardiac inflammation and injury across all groups. Troponins serve as highly specific indicators of cardiomyocyte damage in models of myocardial infarction, ischemia-reperfusion, and

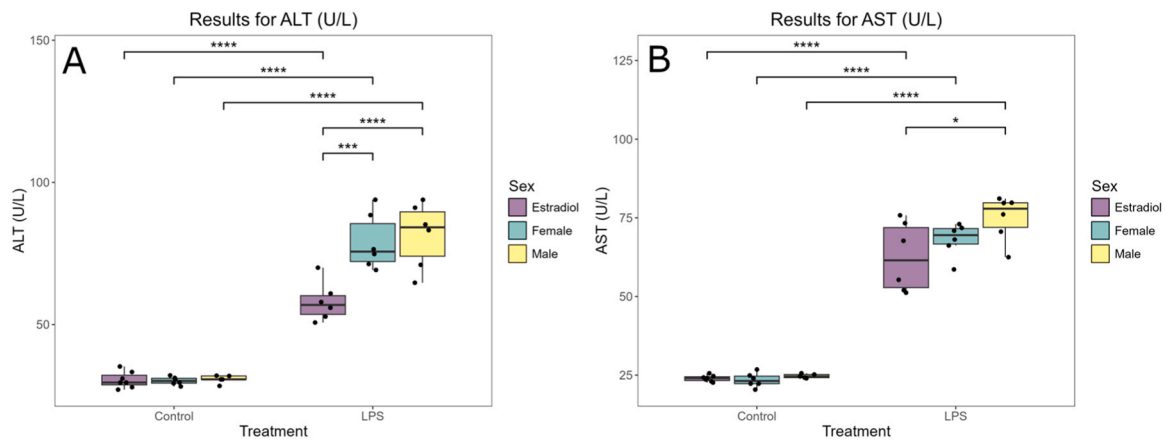


Fig. 2. ALT and AST levels. The plasma concentration of ALT (A) and AST (B) from animals 6 hrs after LPS shown as mean $\pm$ SD. ALT and AST increased after LPS; ALT values were lower in the Estradiol group relative to other LPS groups and AST values were lower in the Estradiol and Female group relative to the Male group. Control groups ($n = 5$), LPS-challenged groups: Estradiol ($n = 6$), Female LPS ($n = 6$), and Male LPS ($n = 6$). Significance is indicated as follows: $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, and $****P < 0.0001$.

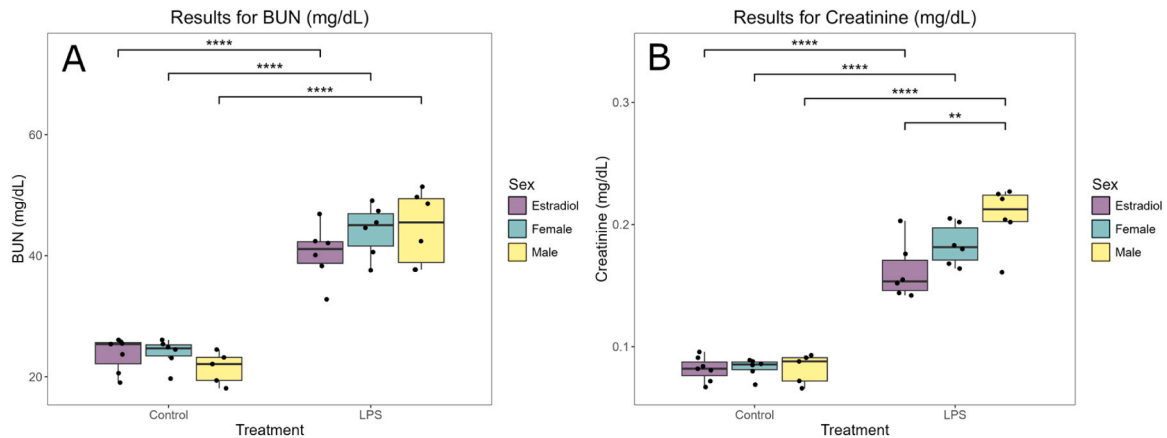


Fig. 3. BUN and creatinine levels. The plasma concentration of BUN (A) and creatinine (B) from animals 6 hrs after LPS shown as mean $\pm$ SD. BUN and creatinine increased after LPS; creatinine values were lower in the Estradiol and Female group relative to the Male group. Control groups ($n = 5$), LPS-challenged groups: Estradiol ($n = 6$), Female LPS ($n = 6$), and Male LPS ($n = 6$). Significance is indicated as follows: $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, and $****P < 0.0001$.

cardiotoxicity. Together, CRP and troponin provide a robust assessment of cardiovascular injury. LPS increased cardiac injury markers, and the Estradiol group showed lower troponin relative to comparator LPS groups (Fig. 5), consistent with attenuation of this injury marker in the early phase.

3.2. Inflammatory pathway markers

To investigate where estradiol may exert its effects within the TLR4 pathway, we selected three upstream markers, CD14, TLR4, and TICAM1, as checkpoints, along with NF- κ B as our primary endpoint. CD14 and TLR4 mediate LPS recognition and receptor activation, while TICAM1 propagates the signal to downstream effectors (Lu et al., 2008). NF- κ B was emphasized due to its central role in inflammatory gene regulation and its well-established dose-dependent activation by LPS (Sharif et al., 2007). The influence of estradiol on NF- κ B signaling during endotoxemia remains poorly defined, representing a key gap addressed in this study. The pathway is illustrated in Fig. 6. Fig. 7

Among the inflammatory pathway markers measured in the heart, NF- κ B showed the clearest divergence between LPS-treated groups. Although all LPS-challenged mice exhibited expected increases relative to controls, NF- κ B expression was significantly lower in the Estradiol group than in both Male and Female LPS groups, indicating that elevated estradiol is consistent with lower downstream signaling. Consistent with

this, TICAM1, an adapter linking TLR4 activation to NF- κ B, was reduced in estradiol-treated mice relative to the Female LPS group. In contrast, CD14 and TLR4 were similarly elevated across all LPS-treated groups, consistent with estradiol-associated differences downstream of receptor engagement. Together, these cardiac results are consistent with a model in which estradiol selectively attenuates NF- κ B-driven cardiac inflammation during endotoxemia.

3.3. Metabolomics

3.3.1. Filtering for important metabolites

To identify potential mechanisms by which estradiol modulates inflammation during endotoxemia, we performed a targeted, hypothesis-driven analysis of 117 measured metabolites, focusing a priori on L-carnitine, methionine sulfoxide, and octanoic acid based on existing literature linking these metabolites to inflammation, oxidative stress, and estradiol signaling. L-carnitine supports mitochondrial β -oxidation and has anti-inflammatory effects through NF- κ B inhibition, but its modulation by estradiol remains unexplored. Methionine sulfoxide, a marker of oxidative stress, activates the antioxidant enzyme MsrA, which in turn suppresses NF- κ B and TNF- α , yet no studies have examined EET's interaction with this pathway. Octanoic acid, a medium-chain fatty acid, reduces NF- κ B expression while activating JAK2/STAT3 signaling in LPS-stimulated macrophages, suggesting a

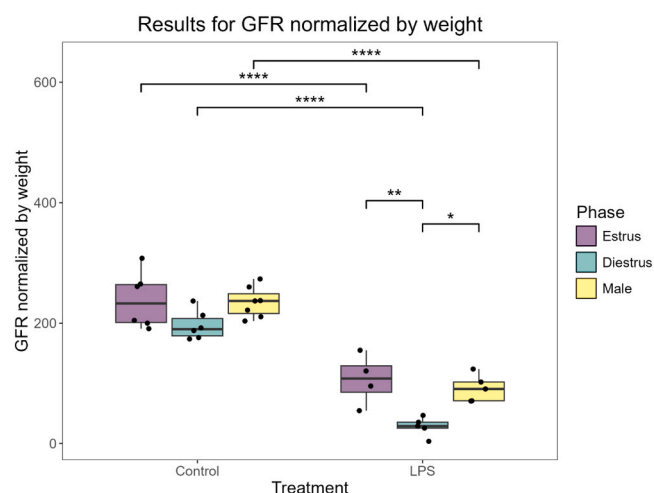


Fig. 4. Glomerular filtration rate. Results are shown as mean $\pm$ SD. GFR was lower after LPS; estrus-phase females and males exhibited higher GFR than diestrus-phase females, consistent with estrous-phase-associated differences in renal filtration. Control groups ($n = 6$), LPS-challenged groups: Estrus LPS ($n = 4$), Diestrus LPS ($n = 6$), and Male LPS ($n = 6$). Significance is indicated as follows: $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, and $****P < 0.0001$.

complex regulatory role that has not been evaluated in conjunction with estradiol. These gaps in literature motivated our focus on these three metabolites.

3.3.2. L-Carnitine

L-carnitine, a key metabolite in fatty acid β -oxidation, was measured to assess estradiol's influence on immunometabolic responses to LPS (Fig. 8). At baseline, L-carnitine levels were significantly higher in estradiol-treated females than in male or female LPS groups, consistent with baseline differences in substrate availability and/or response kinetics associated with estradiol exposure in responses to endotoxemia. Six hours after LPS injection, L-carnitine levels no longer differed between groups, indicating a convergent systemic response. Normalized data further showed increases in the Male and Female LPS groups relative to estradiol-treated mice. Normalized values reflect fold-change from baseline and should be interpreted alongside baseline differences between groups (Fig. 9, 10).

3.3.3. L-methionine sulfoxide

L-methionine sulfoxide, an oxidative stress biomarker generated by

ROS-mediated methionine oxidation, was examined to assess estradiol's influence on redox balance during endotoxemia. At baseline, levels were significantly higher in estradiol-treated females than in Male or Female LPS groups, suggesting estradiol may prime an early oxidative stress response, potentially via the methionine sulfoxide reductase A pathway. Six hours after LPS, both Male and Female LPS groups showed significant increases relative to controls, indicating a delayed oxidative response. Normalized data further supported this "catch-up" pattern, with higher fold-changes in both LPS groups compared to estradiol-treated mice; the Male LPS group also increased significantly relative to Male controls. These findings suggest that high systemic estradiol may accelerate early oxidative stress responses, modulating the surge in L-methionine sulfoxide following LPS exposure.

3.3.4. Octanoic acid

Octanoic acid (caprylate), a medium-chain fatty acid metabolized through mitochondrial β -oxidation, was evaluated to assess estradiol's impact on energy metabolism and inflammatory modulation during sepsis. At baseline, octanoic acid levels were significantly higher in estradiol-treated females than in Male or Female LPS groups, suggesting estradiol may prime early mitochondrial metabolic responses to endotoxemia. Six hours after LPS, octanoic acid levels in both LPS groups increased to match estradiol-treated mice, eliminating baseline differences; the Male LPS group also increased significantly relative to Male controls. Normalized data showed a pronounced rise in the Male LPS group compared to both the Estradiol and Male Control, consistent with a delayed "catch-up" response. These findings are consistent with differences in metabolic response timing and β -oxidation-related substrate dynamics.

4. Discussion

The main findings of this study are that estradiol (specifically 17 β -estradiol, E2) modulates inflammation, preserves organ function, and stabilizes metabolic homeostasis more effectively than either male or untreated female counterparts in a murine model of endotoxemia. Estradiol-treated females showed improved cardiac, hepatic, and renal markers, reduced expression of key inflammatory mediators, and distinct metabolomic profiles at baseline and after LPS challenge. Using a well-established LPS-induced endotoxemia model, we examined the systemic and molecular effects of chronic estradiol treatment and found that estradiol both primes physiological systems prior to LPS exposure and attenuates the inflammatory and metabolic dysregulation typical of inflammation and septic progression. Because our primary outcomes were measured in a 6-hour LPS endotoxemia model, our conclusions

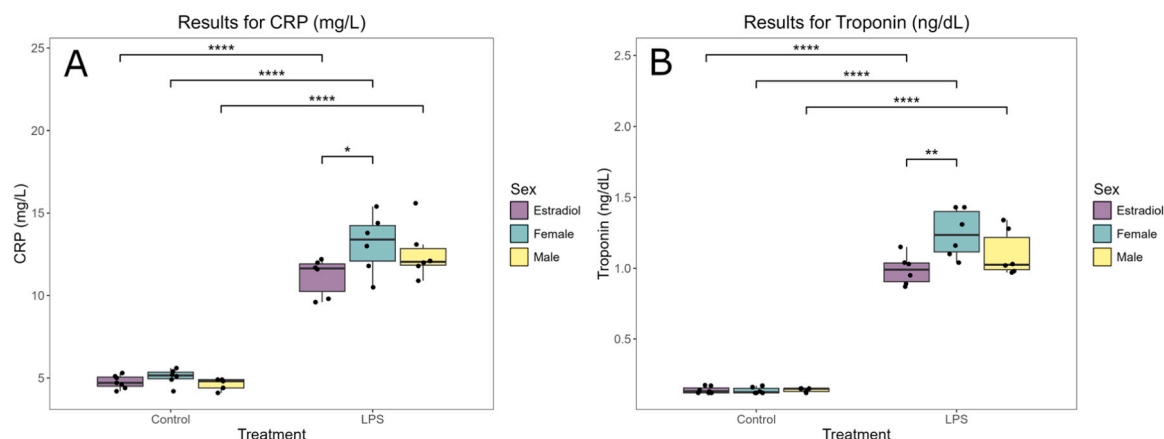


Fig. 5. CRP and Troponin levels. The plasma concentration of CRP (A) and troponin (B) from animals 6 hrs after LPS shown as mean $\pm$ SD. CRP and troponin increased after LPS; values were lower in the Estradiol and Male group relative to Female group. Control groups ($n = 6$), LPS-challenged groups: Estrus LPS ($n = 4$), Diestrus LPS ($n = 6$), and Male LPS ($n = 6$). Significance is indicated as follows: $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, and $****P < 0.0001$.

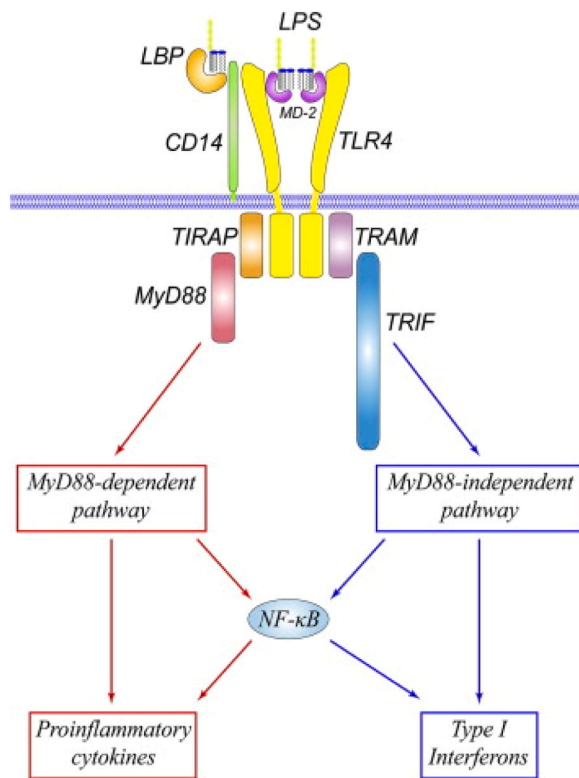


Fig. 6. The LPS/TLR4 signal transduction pathway, reproduced from Lu et al. (2008). LPS recognition is facilitated by lipopolysaccharide binding protein (LBP) and CD14 and is mediated by TLR4/Myeloid Differentiation factor 2 (MD-2) receptor complex. TICAM1 (formerly TRIF) mediates the MyD88-independent pathway. Both pathways facilitate the expression of NF-κB and thus the activation of proinflammatory cytokine and Type I interferon genes. For our pathway analysis CD14, TLR4, TICAM1, and NF-κB were selected as critical nodes.

most directly reflect early-phase inflammatory and metabolic responses rather than later-stage inflammation or polymicrobial sepsis.

4.1. Organ biomarkers

Analysis of organ injury biomarkers showed that estradiol supplementation provides consistent protection across hepatic, renal, and cardiac tissues during LPS challenge. Estradiol-treated mice exhibited significantly lower ALT and AST levels than untreated LPS groups, indicating reduced hepatocellular injury. Renal function was also preserved, with lower BUN and creatinine levels in the Estradiol group, and improved GFR in females in estrus further supporting estrogen's role in maintaining renal perfusion and filtration during systemic inflammation. Notably, GFR was assessed in a complementary 24-hour cohort stratified by estrous phase rather than exogenous pellets; thus, the renal protection associated with higher endogenous estradiol is consistent with, but not identical to, the 6-hour exogenous estradiol paradigm. In the heart, troponin and CRP levels were markedly reduced in estradiol-treated mice, indicating decreased myocardial stress and inflammation. Together, these findings suggest that estradiol confers broad protection against organ injury during endotoxemia, likely through coordinated modulation of vascular, metabolic, and immune responses. Importantly, the organ-protective phenotype aligns with the signaling pattern observed in cardiac tissue, upstream TLR4 activation appears preserved, while downstream NF-κB is reduced, consistent with a model in which estradiol limits inflammatory amplification rather than inflammation detection or sensing.

4.2. TLR4 inflammatory pathway markers

Analysis of inflammatory pathway markers provides mechanistic insight into how estradiol modulates the host response to septic insult. While upstream TLR4 components (CD14 and TLR4) were similarly elevated across all LPS-challenged groups, downstream signaling diverged with estradiol treatment. NF-κB expression was significantly reduced in the Estradiol group compared to Male and Female LPS groups, indicating suppression of the pro-inflammatory transcriptional response. TICAM1 showed a downward trend with estradiol, which is directionally consistent with dampened myeloid differentiation primary response 88 (MyD88) independent signaling; however, this result did not reach significance here and should be interpreted cautiously. This downstream inhibition aligns with reduced organ injury markers and suggests that estradiol does not block immune recognition, but limits amplification of inflammatory signaling. Overall, these findings highlight a selective anti-inflammatory action of estradiol, particularly through attenuation of NF-κB expression in cardiac tissue.

4.3. L-Carnitine

L-carnitine, synthesized primarily in the liver and kidneys, is essential for mitochondrial fatty acid β-oxidation by transporting long-chain fatty acids into the mitochondrial matrix (Longo et al., 2016). During sepsis, when energy demands rise and glucose metabolism becomes impaired, this pathway is critical for sustaining ATP production. Prior studies also indicate that L-carnitine supplementation reduces inflammatory cytokines and improves survival in sepsis models (Keshani et al., 2024). The data shows that estradiol-treated LPS mice begin the challenge with higher circulating L-carnitine. One interpretation is that estradiol supports earlier substrate availability for β-oxidation and immunometabolic stability; however, targeted assays of mitochondrial flux and cytokine output will be needed to directly connect L-carnitine shifts to NF-κB-dependent inflammation.

4.4. L-methionine sulfoxide and methionine sulfoxide reductase A

L-methionine sulfoxide, a marker of oxidative stress generated through ROS-mediated oxidation of methionine residues, was significantly elevated in estradiol-treated LPS mice. Its reduction is catalyzed by methionine sulfoxide reductase A (MsrA), a key antioxidant enzyme that protects against LPS-induced septic shock and suppresses proinflammatory signaling through inhibition of the ROS-MAPK-NF-κB pathway (Singh et al., 2017). MsrA deficiency leads to heightened IL-6 and TNF-α production, increased ROS, enhanced NF-κB activation, and elevated iNOS expression, underscoring the enzyme's central role in regulating oxidative and inflammatory responses. Because ROS strongly activates NF-κB during sepsis, early increases in methionine sulfoxide in estradiol-treated mice suggest that estrogen may modulate oxidative stress by enhancing MsrA-dependent defenses that limit NF-κB-driven inflammation. This relationship supports a model in which estradiol amplifies antioxidant capacity and thereby attenuates downstream inflammatory signaling during endotoxemia. We did not measure MsrA activity or ROS directly, so this proposed linkage remains speculative and should be tested in future work.

4.5. Octanoic acid and fatty acid metabolism

Octanoic acid (caprylic acid), a medium-chain fatty acid used in mitochondrial β-oxidation, was elevated at baseline in estradiol-treated mice. Because fatty acid oxidation is often impaired in sepsis (Longo et al., 2016), early increases in octanoic acid may indicate preserved mitochondrial function. Prior work shows that octanoic acid modulates inflammation: TNF-α and MCP-1 increase while IL-10 and IL-1β decrease with treatment, and anti-inflammatory effects occur through upregulation of ABCA1 and p-JAK2/p-STAT3 signaling (Zhang et al., 2023).

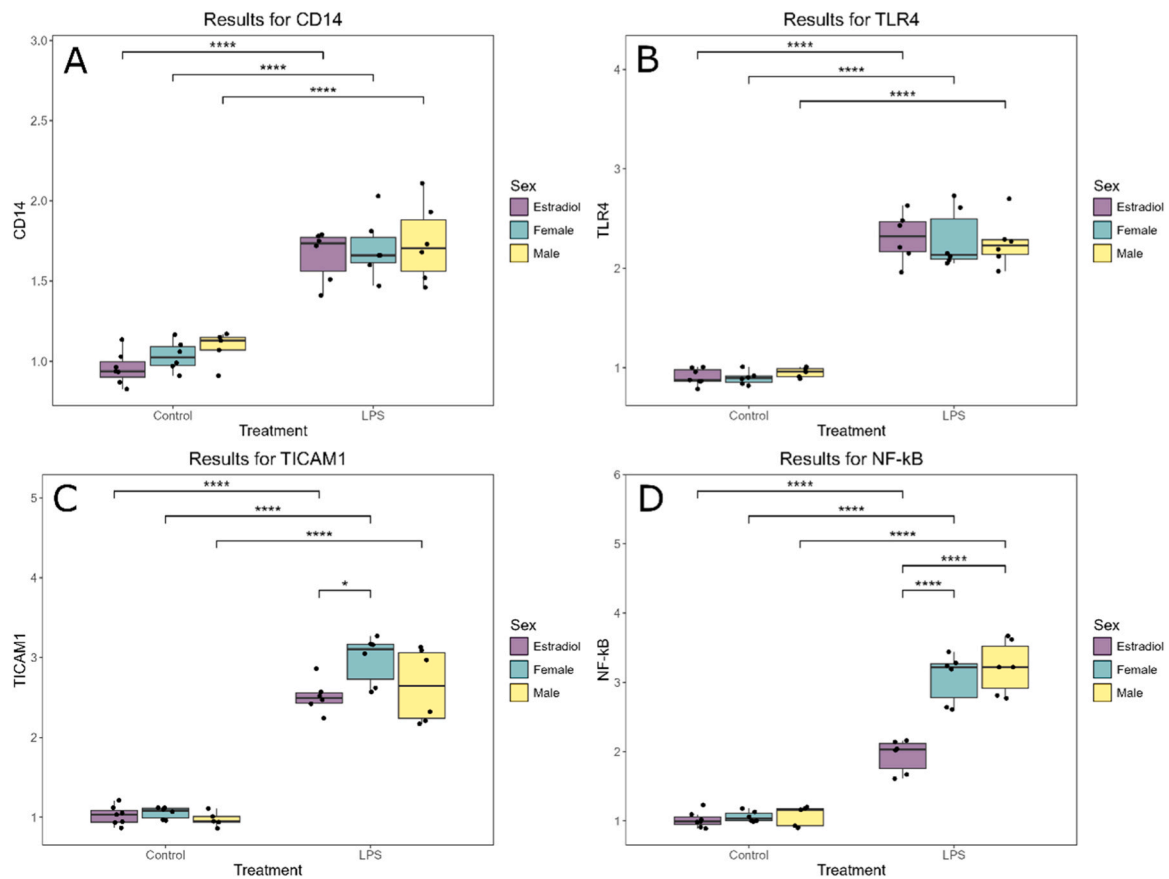


Fig. 7. Inflammatory pathway marker levels. Expression levels of CD14 (A), TLR4 (B), TICAM1 (C), and NF-κB (D) were measured in cardiac tissue via qPCR and normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), then presented relative to control group values as mean $\pm$ SD. While CD14 and TLR4 levels increased similarly across all LPS groups, the Estradiol group exhibited a significant reduction in NF-κB expression compared to both Female and Male LPS groups. TICAM1 showed a trend toward reduction in the Estradiol group compared to the other LPS groups. Control groups ($n = 5$), LPS-challenged groups: Estradiol ($n = 6$), Female LPS ($n = 6$), and Male LPS ($n = 6$). Significance is indicated as follows: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, and **** $P < 0.0001$.

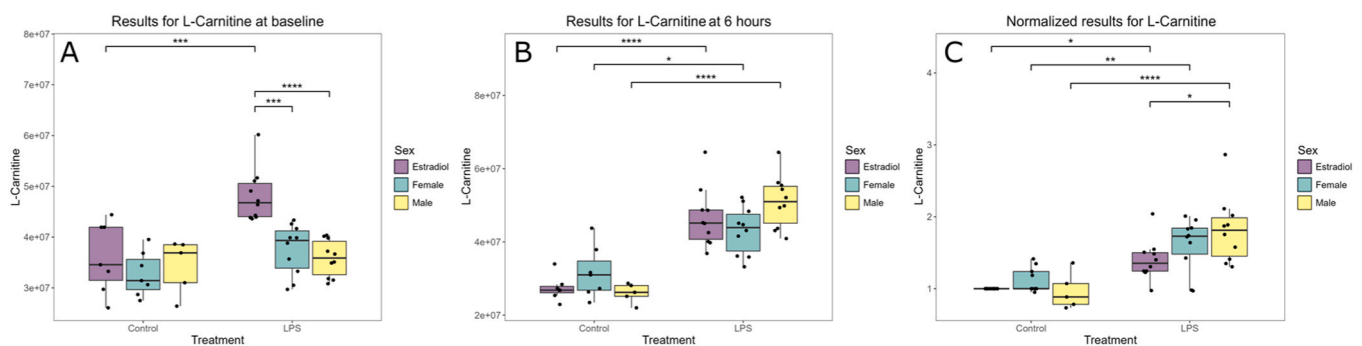


Fig. 8. L-carnitine levels. Plasma L-carnitine concentrations at baseline (A), 6 h post-injection (B), and normalized to baseline levels (C). Results are shown as mean $\pm$ SD. Elevated baseline L-carnitine in the Estradiol group is consistent with estradiol-associated differences in metabolic state prior to LPS. Control groups ($n = 5$), LPS-challenged groups: Estradiol ($n = 10$), Female LPS ($n = 10$), and Male LPS ($n = 10$). Significance is indicated as follows: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, and **** $P < 0.0001$.

Octanoic acid has also been shown to suppress cytokine production and downregulate TLR4, MyD88, NF-κB, TNF-α, IKKα, and IKKβ expression (Zhang et al., 2019). The higher baseline levels in estradiol-treated females are consistent with differences in mitochondrial substrate availability and/or earlier metabolic engagement; whether this directly mediates reduced inflammatory signaling was not tested.

4.6. Summary and conclusions

This study highlights the powerful hormonal effects, specifically of estradiol, as modulator agent in the context of systemic inflammation induced by endotoxemia and potentially septic shock, providing multifaceted protection across inflammatory, metabolic, and organ function domains. By integrating data from organ injury biomarkers, inflammatory pathway markers, and targeted metabolomics, we demonstrate that estradiol-treated mice exhibit a distinctive protective phenotype that

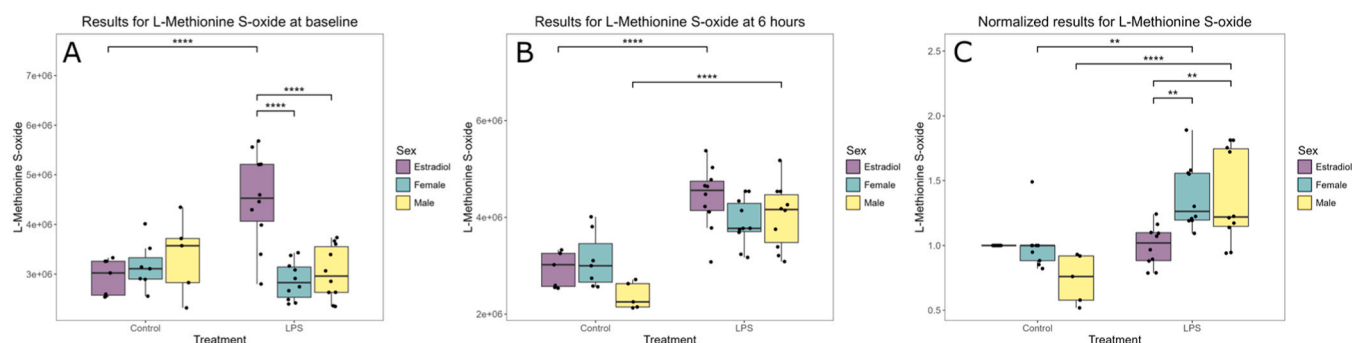


Fig. 9. L-methionine sulfoxide levels. Plasma concentrations of L-methionine sulfoxide were measured at baseline (A), 6 h post-LPS injection (B), and normalized to baseline values (C). Elevated baseline levels in the Estradiol group are consistent with differences in redox status or oxidative stress response kinetics; Methionine Sulfoxide Reductase A (MsrA) activity was not directly measured. In contrast, the Male and Female LPS groups exhibited a delayed reactive increase in L-methionine sulfoxide, reflected in significantly higher normalized values. Control groups ($n = 5$), LPS-challenged groups: Estradiol ($n = 10$), Female LPS ($n = 10$), and Male LPS ($n = 10$). Significance is indicated as follows: $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, and $****P < 0.0001$.

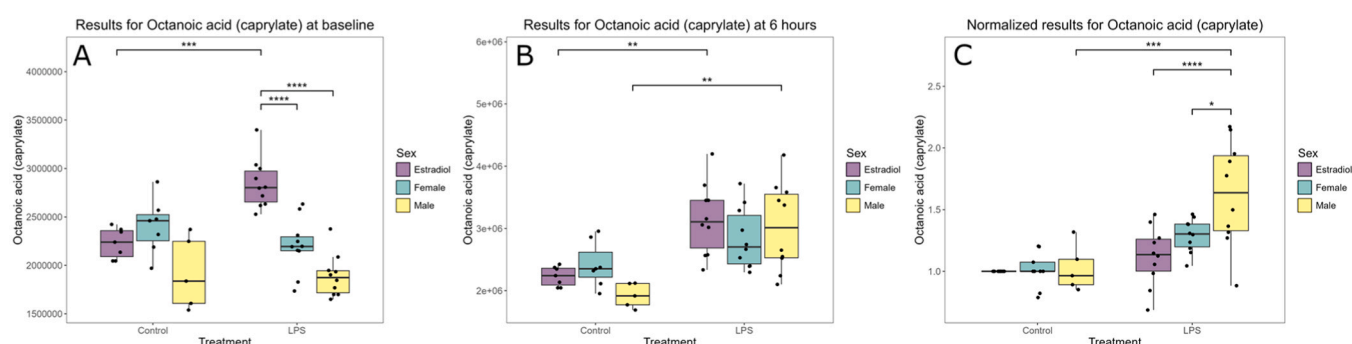


Fig. 10. Octanoic acid levels. Plasma concentrations of octanoic acid were measured at baseline (A), 6 h post-LPS injection (B), and normalized to baseline values (C). Elevated baseline octanoic acid in the Estradiol group is consistent with estradiol-associated differences in lipid metabolism at baseline. The Male LPS group demonstrated a delayed elevation at 6 h and an exaggerated normalized response compared to controls and the Estradiol group, consistent with a reactive metabolic shift in the absence of estradiol. Control groups ($n = 5$), LPS-challenged groups: Estradiol ($n = 10$), Female LPS ($n = 10$), and Male LPS ($n = 10$). Significance is indicated as follows: $*P < 0.05$, $**P < 0.005$, $***P < 0.001$, and $****P < 0.0001$.

limits the physiological impact of LPS-induced endotoxemia. The suppression of NF- κ B signaling, preservation of mitochondrial substrates, and mitigation of oxidative stress collectively point to a systemic reprogramming of the host response under the influence of estradiol. These findings suggest that estradiol not only buffers against acute immune activation, but also catalyzes the metabolic response during endotoxemia and septic insult. Several alternative explanations should be considered. Chronic estradiol pellets may induce baseline metabolic differences independent of LPS responsiveness, meaning some group differences could reflect starting-state divergence rather than purely differential response. In addition, because metabolite selection was literature-informed and focused on three candidates, broader pathway-level shifts may be underrepresented. Future studies matching baseline metabolic state across groups or incorporating untargeted metabolomics would help isolate estradiol's causal role. Key limitations include the use of an acute endotoxemia model, limited timepoints for most endpoints, and reliance on transcript-level pathway markers in a single tissue. These constraints motivate follow-up work in LPS induce inflammation and polymicrobial sepsis models, as well as protein-level/in situ signaling readouts.

CRediT authorship contribution statement

Tajanko Felix Nicholas: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cynthia R. Muller:** Supervision, Project administration, Methodology, Investigation. **Munoz Carlos J.:** Writing – review & editing,

Supervision, Investigation. **Cabrales Pedro J.:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

All the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.biocel.2026.106919](https://doi.org/10.1016/j.biocel.2026.106919).

Data availability

Data will be made available on request.

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