Female mice exhibit less renal mitochondrial injury but greater mortality using a comorbid model of experimental sepsis

Authors

Lee Ann MacMillan-Crow
Philip R Mayeux

Affiliations

Lee Ann MacMillan-Crow, Ph.D.
Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Philip R. Mayeux, Ph.D.
Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Corresponding Author:
Lee Ann MacMillan-Crow, Ph.D.
Contact information:
Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, 4301 West Markham Street, Slot 638, Little Rock, AR 72205
E-mail lmcrow@uams.edu

Abstract

Given the inherent heterogeneity of the septic patient population and possible comorbid conditions, it is not surprising that the influence of gender on incidence and outcomes are still unclear. The goal of this study was to use a clinically relevant murine model of sepsis, cecal ligation and puncture (CLP) in CD1 mice, with and without uniphrectomy as a comorbid condition to investigate possible gender differences in renal mitochondrial function and dynamics. High resolution respirometry on fresh kidney biopsies was used to measure renal respiratory complex activities. At 18h post-CLP with nephrectomy male mice showed significant reductions in complex I, II, and III activities, while females were less effected; only complex I was significantly reduced from sham mice. Taken together, our studies revealed, for the first time, gender differences in mitochondrial respiratory activity even in the absence of sepsis. We also examined expression of key mitochondrial fission and fusion proteins. In both genders and in both CLP models, protein expression of the primary fission protein, DRP1 was significantly decreased. No changes were observed in female mice in either CLP model; whereas, male mice demonstrated a slight reduction in MFN1 and the short form of OPA1 after CLP, and modest increase in MFN2 with CLP plus nephrectomy. In both genders CLP with nephrectomy produced a greater increase in serum blood urea nitrogen, a biomarker of renal injury, than without nephrectomy. However, CLP with nephrectomy produced significantly lower 96-hour survival in females. Our results suggest that the CLP nephrectomy comorbid model of sepsis may be an appropriate model to study gender differences a select group of predisposed individuals.

1. Introduction

Susceptibility, incidence and outcomes in human sepsis are influenced by multiple factors such as age, genetics, and comorbid medical conditions. Another potentially important factor may be gender [1]. There are examples in the human literature where mortality in females with sepsis was less than [2], higher [3,4] or no different [5] than in males. Given the inherent heterogeneity of the septic patient population, it is not surprising that the influence of gender on incidence and outcomes are still unclear [2,6,7].

There is growing interest in the role of mitochondrial dysfunction in the development of multiple organ failure associated with human sepsis [8-10]. Acute kidney injury (AKI) is a frequent complication of sepsis that can greatly increase mortality [11]. Several animal studies [10,12-14], including our own [15-17], have revealed that sepsis induces renal mitochondrial injury. Interestingly, energy production and mitochondrial function can be influenced by sex hormones, especially in times of stress [18,19].

There are relatively few studies on the pathophysiology of sepsis in the presence of comorbid conditions, such as chronic kidney disease [20,21]. To our knowledge no one has evaluated whether gender alters the effects of sepsis on mitochondrial function in any organ or if outcomes are different when there are comorbidities.

Mitochondria are dynamic organelles, which continually undergo morphological changes, including fission and fusion, and mitophagy, ultimately leading to biosynthesis of new functional mitochondria (biogenesis) [22-26]. It is clear that an imbalance of mitochondrial fission and fusion is observed in many disease states, and disruption of fusion limits the response to acute toxic stress by preventing efficient respiration and compensatory mixing of functional mitochondria with damaged ones. Mitochondrial fission and fusion are regulated by a group of GTPase proteins. DRP1 (dynamin-related protein 1) promotes fission by interacting with receptor proteins in the mitochondrial outer membrane. Proteins called mitofusins (MFN1/2), and optic atrophy protein type 1 (OPA1) are GTPase's that participate in the fusion process of the mitochondrial outer and inner membranes.

The goal of this study was to evaluate if mitochondrial function and dynamics are altered differently in male and female mice made septic by cecal ligation and puncture (CLP), a clinically relevant

model of severe sepsis. The second goal was to examine the effects of gender on mitochondrial function and dynamics during sepsis with the comorbid condition of uniphrectomy.

2. Materials and Methods

2.1. Ethical statement and animals

All animals were housed and handled in accordance to *National Institute of Health Guide for the Care of Laboratory Animals* with approval by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. Male and female CD1 mice at 8 weeks of age were purchased from Charles River (Wilmington, MA USA). Mice were housed for 7 days with free access to food and water prior to use. Following surgery all mice received buprenorphine (0.05 mg/kg, subcutaneous) for pain.

2.2. Cecal ligation and puncture (CLP) model of sepsis and uniphrectomy

Following a midline laparotomy under isoflurane anesthesia, the cecum was ligated 1.5 cm from the tip with a 4-0 silk suture and punctured twice with a 21 gauge needle. An approximately 1 mm column of fecal material was expressed from each puncture. The cecum was isolated but neither ligated nor punctured in control sham-operated mice (Sham). In those mice undergoing uniphrectomy, the right kidney artery and vein were ligated with 4-0 silk and the right kidney was removed immediately prior to ligation and puncture of the cecum. The incision was closed in two layers using 4-0 silk. All mice received 1 ml of pre-warmed saline at the end of surgery and were placed on a heating pad and monitored for 6 hours. Mice were given imipenem/cilastatin (14 mg/kg in 40 ml/kg saline) subcutaneous at 6 hours and for studies extending beyond 18 hours, animals received additional doses of imipenem/cilastatin (7 mg/kg) at 12-hour intervals beginning at 18 hours.

2.3. Survival study

Core body temperature was used as an indicator of pending death [27]. It was measured every 6 hours using a rectal probe. Mice were considered nonsurvivors if they died or had to be euthanized when two consecutive readings of core body temperature were less than 28.0^{o}C.

2.4. Blood chemistry

Blood chemistry (BUN) was determined in heparinized blood (arterial) using a hand-held clinical chemistry

analyzer, iSTAT^{TM}, and cartridges (CHEM8^{+}) as described by the manufacturer (Vetscan®, Abaxis, USA).

2.5. High resolution respirometry (HRR)

Mitochondrial respiratory complex activity was measured in saponin permeabilized renal biopsies (representing cortex and medulla) by high resolution respirometry (HRR) (Oroboros instruments - Oxygraph-2k, Innsbruck, Austria), according to substrate-inhibitor-titration (SIT) protocol as described earlier [15,28-30]. Briefly, representative renal biopsies (cortex and medulla) of the kidneys were incubated with 100 µg/ml Saponin prepared in MiRO5 buffer, followed by 3x washes with MiRO5 buffer [60 mM K-lactobionate, 0.5 mM EGTA, 3 mM $MgCl_2$, 20 mM Taurine, 10 mM KH2PO4, 20 mM HEPES, 110 mM BSA, and 1 g/L sucrose, pH 7.0]. Mitochondrial respiration was initiated by adding 2 mM malate and 10 mM glutamate and maximum active respiration was achieved by adding 2.5 mM ADP. Rotenone (0.1 mM) was then added to completely inhibit complex I respiration. To measure complex II and III respiration 10 mM succinate was added followed by 2mM malonate to inhibit complex II respiration (complex II activity), and 10 µM antimycin A to inhibit complex III respiration (complex III activity). Inhibitor concentrations were selected based on experimental determination of doses needed to maximally reduce substrate-induced respiration. Finally, data analysis was done using DATLAB 4.2 software (Oroboros), and tissue respiration was shown as oxygen flux (pmol/mg/s).

2.6. Renal extract preparation for Western blot analysis

Renal extracts from whole kidney homogenates were prepared by using radioimmuno-precipitation assay (RIPA) buffer (Pierce, USA) with 1 mM PMSF (Sigma, USA), 1.2 mM Na_3VO_4 (Sigma, USA), 2.5 mM NaF (Sigma, USA), 1mM DTT (Sigma, USA) and protease inhibitor cocktail (Pierce, USA). Protein concentrations were determined by Coomassie Plus Protein Assay Reagent (Pierce, USA). Renal extracts (20-35 µg) were resolved onto SDS-PAGE gel and then transferred to PVDF membrane. Western blot analysis was performed using antibodies against proteins: DRP1 (1:1000, BD Bioscience, #611112); MFN1 (1:1000, Abcam, #ab57602); MFN2 (1:1000, Abcam, # ab124773); OPA1 (1:1000, Abcam, #ab42364); and β-actin (1:1000, Sigma, #

A5441). Actin expression was used as a loading control for all western blot experiments. Probed membranes were washed three times and immune-reactive proteins were detected using horseradish peroxidase conjugated secondary antibodies (Seracare KPL, USA) and enhanced chemiluminescence (Thermoscientific, USA). Densitometry evaluation on scanned membrane was performed using AlphaEase FC software.

2.7. Statistical analysis

Results are presented as mean ± standard error of the mean (SEM) using Graph Pad Prism software. An unpaired Student's *t*-test was used when comparing differences between the means of two groups at a 95% level of confidence. Survival curves were analyzed using Mantel-Cox log rank test. Differences with a P value < 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Gender differences in mitochondrial dysfunction using both CLP models

Aged male mice of the inbred C57BL/6 strain are particularly susceptible to sepsis-induced renal injury. [31] We previously reported in 40-week old male C57BL/6 mice that CLP produces a rapid decline in renal mitochondrial complex activities.[15] Researchers are now considering that outbred strains of mice, such as the CD1 strain, may better model the genetically heterogeneous human population [32] and do develop sepsis-induced renal injury at a younger age. [20] To examine the effects of sepsis on renal mitochondrial respiratory complex activities, we measured complex activities in renal biopsies from male and female CD1 mice at 18 h following sham or CLP surgery with both kidneys intact (non-nephrectomy) or with uniphrectomy (the more severe model). The activities of renal mitochondrial respiratory complexes I, II, III, and IV were assessed by high resolution respirometry (HRR), which has the advantage of using an intact tissue biopsy, rather than relying on mitochondrial isolation. Interestingly, respiratory complex II and IV activities were significantly higher in females than in males following sham surgery (**Table 1**).

Table 1. Respiratory complex activities in male and female mice				
	Complex I	Complex II	Complex III	Complex IV
Male	23.8 ± 3.0	30.2 ± 6.5	17.3 ± 2.7	10.7 ± 0.3
Female	23.3 ± 2.0	50.0 ± 3.5*	16.7 ± 1.6	27.7 ± 2.9*
*P < 0.05 compared to male; n = 4 per group.				

Using the non-nephrectomy CLP model male complex I and III activities were significantly reduced (**Figure 1A**), whereas female mice showed no respiratory defect (**Figure 1B**). Next, mitochondrial function was evaluated using the nephrectomy CLP model. Male mice showed significant reductions in complex I, II, and III activities (**Figure 1C**), while females were less effected; only complex I was significantly reduced from sham mice (**Figure 1D**). Taken together, our studies revealed, for the

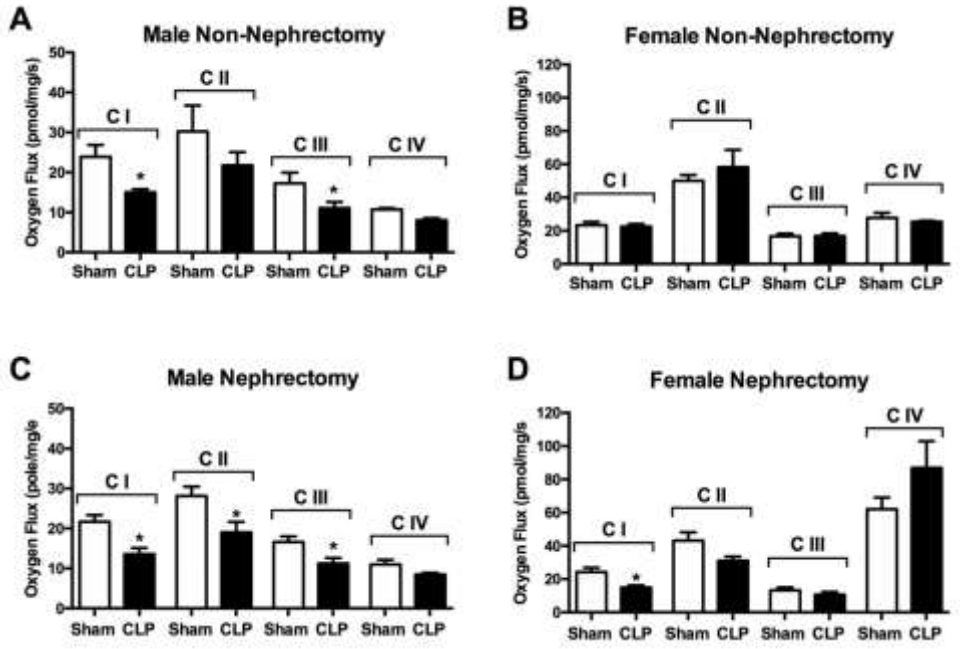


Figure 1. Male mitochondrial respiratory function more impaired than female in both CLP models. Graph showing respiratory complex I, II, III, and IV activity of the electron transport chain using HRR in kidney biopsies from Sham and CLP male (A, C) and female (B, D) mice using the standard CLP model (non-nephrectomy; A, B) and the more severe CLP model (nephrectomy; C, D). Values were expressed as mean ± SEM (n = 5). *P < 0.05 compared to the corresponding Sham group.

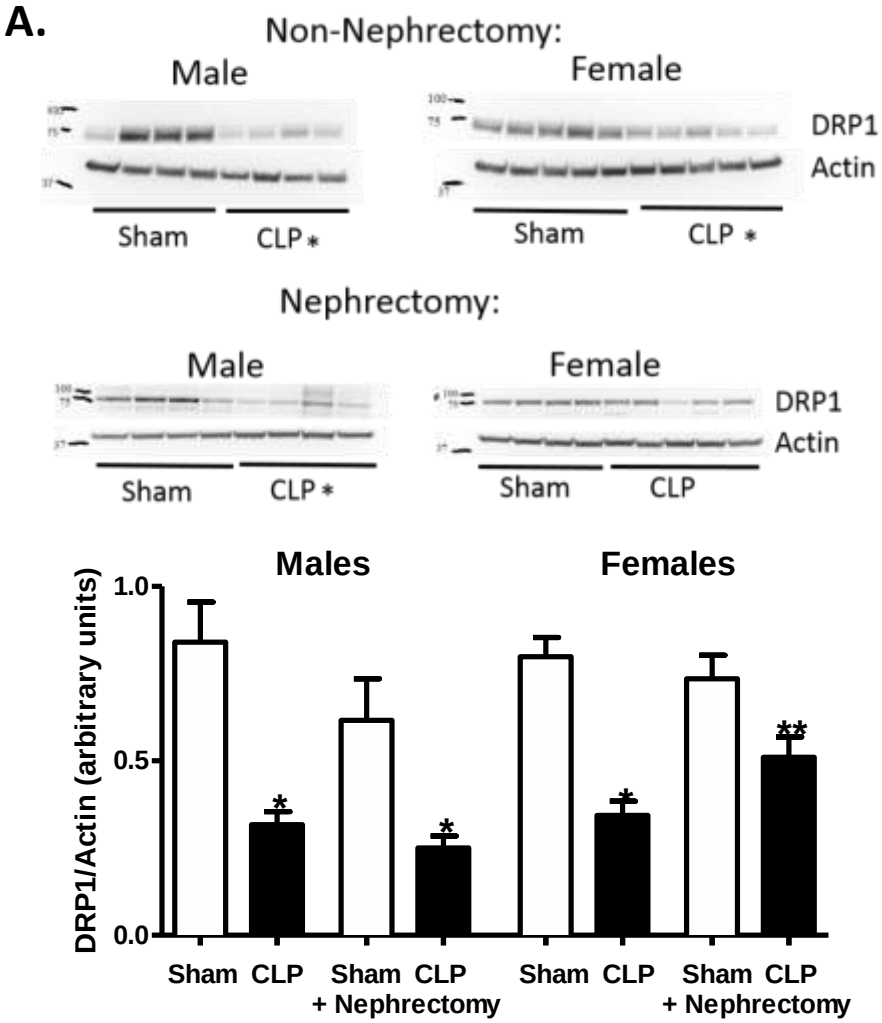
first time, gender differences in mitochondrial respiratory activity even in the absence of sepsis. Moreover, mitochondrial respiratory activity in the kidney from female mice are better preserved than in male mice following sepsis even with the comorbid condition of uniphrectomy. These findings suggest that, female mice are more protected from sepsis induced mitochondrial dysfunction compared to male mice.

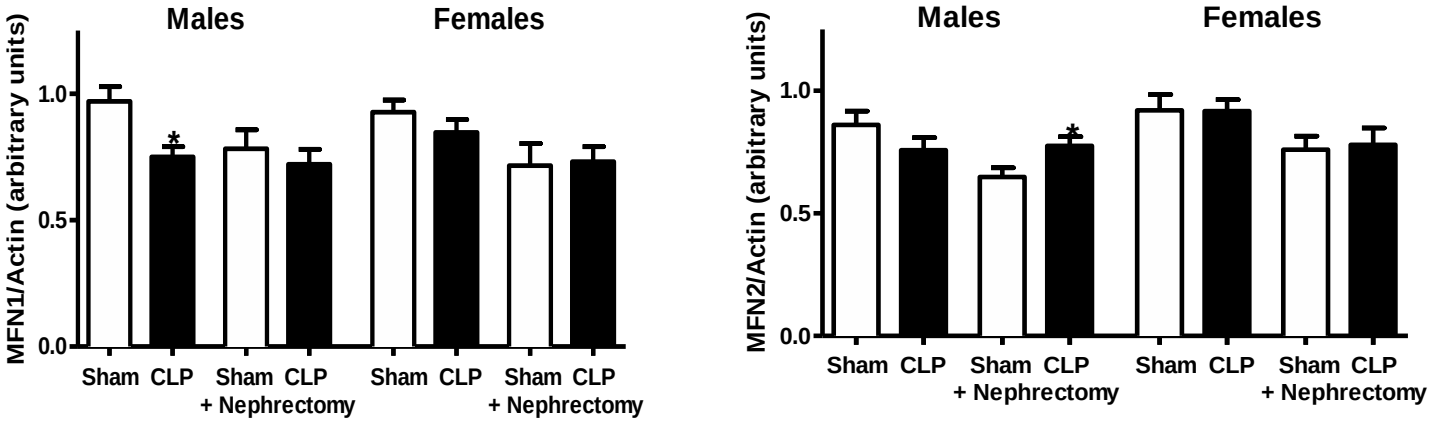
3.2. Effects of CLP on renal mitochondrial fusion machinery in both genders.

Given the gender differences on mitochondrial respiration and prior reports showing that sepsis alters mitochondrial dynamics [12,13] we examined protein expression of key mitochondrial fission and fusion machinery in renal tissue 18 h following sham or CLP surgery. Interestingly, in both genders and in both CLP models, protein expression of the primary fission protein, DRP1 was significantly decreased (**Figure 2A**). Interestingly, female mice exposed to CLP + nephrectomy had higher levels of DRP1 compared to CLP alone. Next, we evaluated key proteins involved with mitochondrial fusion including mitofusins (MFN1/2) and optic atrophy protein type 1 (OPA1), which are GTPase's that regulate the fusion process of the outer and inner membranes, respectively. No changes were observed in female mice in either CLP model, whereas male mice demonstrated a slight reduction in MFN1 and the short form of OPA1 after CLP, and modest increase in MFN2 with CLP plus nephrectomy (**Figure 2 B & C**). The significance of these findings remain unclear, but we predict that male mice are attempting to compensate for the profound loss of DRP1, while female mice lack this compensation.

Female mice exhibit less renal mitochondrial injury but greater mortality using a comorbid model of experimental sepsis

October 2018





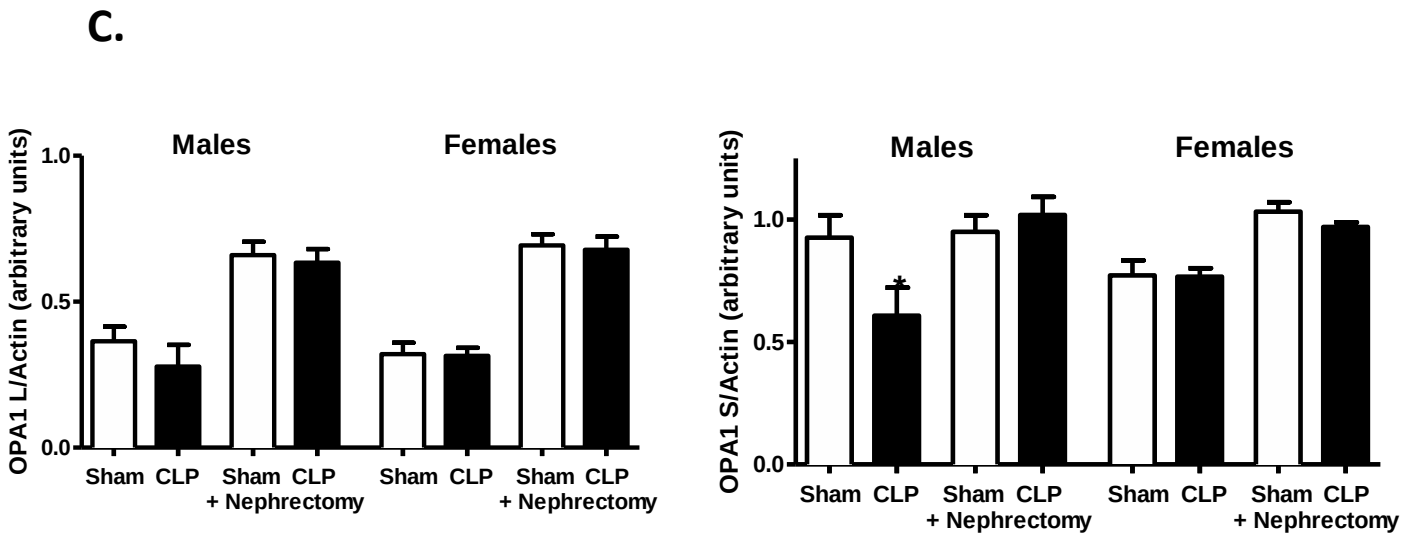


Figure 2. **Sepsis reduces DRP1 expression in both genders**. Renal extracts (20-35 ug) were resolved on SDS-PAGE gels and immunoblotted. (**A**) Representative DRP1 western blots showing distinct protein bands of DRP1 (~75 kDa) in Sham and CLP kidneys. Densitometry evaluation of each blot (normalized to actin) are shown below. (**B**) Densitometry evaluation of MFN1 and MFN2 in both genders and both CLP models. (**C**) Densitometry evaluation of OPA1 L (long form) and OPA1 S (short form) in both genders and both CLP models. Actin was used as a loading control for all western blots. Values were expressed as Mean ± SEM (n =4). **P* < 0.05 compared to the corresponding Sham group; **P < 0.05 compared to the corresponding CLP non-nephrectomy group.

To our knowledge DRP1 has never been evaluated in renal tissue after CLP-induced sepsis. The profound decline in renal DRP1 levels differ from other studies evaluating brain and liver tissue, which showed increased DRP1 expression after sepsis.[12,33] Both of these studies showed that the putative DRP1 inhibitor, mdivi, provided protection from sepsis-mediated brain and liver damage. However, the specificity of mdivi as a DRP1 inhibitor has been challenged since there are reports that mdivi also inhibits complex I respiration.[34] Many reports suggest that mitochondrial fission is a pre-requisite for mitophagy, thus it is possible that the decline in DRP1 observed in our CLP models reduces the onset of mitophagy, which could lead to accumulation of damaged mitochondria. The consequence of reduced mitophagy is unclear. Some studies suggest that reduced mitophagy might provide protection, while others suggest this exaggerates injury. [35,36] Clearly, further studies are needed to clarify

the possible role of altered mitophagy in the kidney during sepsis. However, since DRP1 was profoundly reduced in both genders and in both CLP models it is unlikely that this contributed to the differences observed in mitochondrial function between genders.

3.3. Morbidity and survival

Hypothermia and elevated blood urea nitrogen (BUN) are hallmarks of sepsis-induced systemic inflammation and renal injury, respectively in mice. Both males and females exhibited similar hypothermia at 18 h following CLP without or with nephrectomy (**Figure 3A**). BUN levels (**Figure 3B**) in male and female mice at 18 h following non-nephrectomy CLP were only modestly elevated with levels in females reaching statistical significance compared to sham. The more severe model CLP with nephrectomy resulted in a greater increase in BUN levels in both males and females compared to sham and compared to non-nephrectomy. The presence of comorbid conditions, including renal insufficiency can greatly increase mortality in the septic patient.[37-39] To examine potential gender differences in mortality in mice subjected to CLP with nephrectomy, we performed survival studies. CLP-induced sepsis with accompanying nephrectomy resulted in a significantly lower survival rate in females compared to males (**Figure 3C**). Female mice were more likely to die from CLP-induced sepsis with nephrectomy than were male mice with a hazard ratio of 6.8.

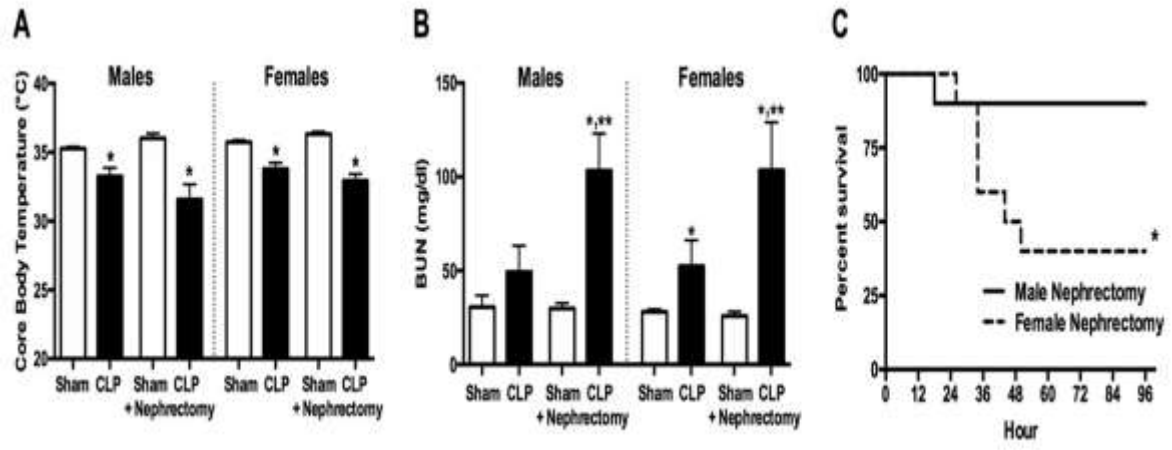


Figure 3. Gender differences in morbidity and mortality. (**A**) Core body temperature at 18 h was decreased in both septic males and females with or with nephrectomy. (B) Serum BUN levels at 18 h were increased in both males and females following CLP with nephrectomy. Values were expressed as Mean ± SEM (n = 4-5). **P* < 0.05 compared to the corresponding Sham group; **P < 0.05 compared to the corresponding CLP non-nephrectomy group. Survival curves in male and female mice subject to CLP with nephrectomy are presented in (**C**). **P* < 0.05 compared to the male survival curve as analyzed by the log-rank test (n = 10 per group).

4. Conclusion

As mentioned in the introduction, prior published reports are mixed on the impact that gender has on outcome after sepsis in the human population as well as in animal models. This isn't too surprising given the complexity of sepsis and the diverse selection of animal models used to study sepsis (multiple strains of mice and rats, various ages, and organs of interest). For example, our prior study utilized aged male mice (40-week-old C57 strain) which demonstrated reductions in complexes I-III after CLP and a 50% survival rate.[15] Our new results using a younger mice from an outbred strain show similar results regarding mitochondrial function but no mortality, suggesting that age was a primary factor for the increased mortality of our prior study. Another co-morbidity observed in septic patients is underlying renal injury, which led us to assess whether mice with only one

kidney would sustain more mitochondrial injury after CLP compared with mice with both kidneys. Mice subjected to CLP plus nephrectomy showed increased mitochondrial damage (males worse than females); however only female mice displayed increased mortality in the nephrectomy plus CLP model. No mortality was observed in either gender in the CLP model alone by 24 h (data not shown). In the inbred mouse strain C3H/HeN, female mice subjected to CLP were reported to have a lower mortality rate than males [40] even with prior hemorrhage as the comorbid condition.[41] However, there are significant differences between our study and those studies. We used an outbred strain, all mice received broad spectrum antibiotics, and nephrectomy was the comorbid condition. However, our results do correlate with human studies showing that the females with severe sepsis (as in our CLP + nephrectomy model) have a higher risk of death compared to males. [3,4]

In summary, our data indicate that female mice demonstrated less sepsis induced renal mitochondrial respiratory injury compared to males; however, overall mortality was greater. How or why this occurs remains unclear. CLP-induced sepsis is a model of multiple organ failure. One possibility is that renal mitochondrial function simply does not correlate with survival after CLP with nephrectomy in this strain of mice due to the stress of nephrectomy on other organs. Another possibility is that reduced mitochondrial respiration at complexes I-III leads to a form of 'hibernation' [9] allowing male mice to recover from CLP, whereas female mice might not undergo this adaptation. Alternatively, other mitochondrial pathways (e.g. mitophagy or biogenesis) could participate in the gender dependent effects after CLP in the kidney or other organs. In any regard, our results suggest that the CLP nephrectomy model may be an appropriate model to study gender differences a select group of predisposed individuals susceptible to the lethal effects of sepsis.

5. Acknowledgments

This work was supported by a grant from the NIH (R01 GM106419). We would also like to acknowledge the excellent technical assistance by Alex Harb and Hong Wu.

6. References

1. Angele MK, Pratschke S, Hubbard WJ, Chaudry IH. Gender differences in sepsis: cardiovascular and immunological aspects. 2014; 5: 12-9.

2. Schroder J, Kahlke V, Staubach KH, Zabel P, Stuber F. Gender differences in human sepsis. 1998; 133: 1200-5.

3. Sakr Y, Elia C, Mascia L, Barberis B, Cardellino S, Livigni S, et al. The influence of gender on the epidemiology of and outcome from severe sepsis. 2013; 17: R50.

4. Pietropaoli AP, Glance LG, Oakes D, Fisher SG. Gender differences in mortality in patients with severe sepsis or septic shock. 2010; 7: 422-37.

5. Mahmood K, Eldeirawi K, Wahidi MM. Association of gender with outcomes in critically ill patients. 2012; 16: R92.

6. Failla KR, Connelly CD. Systematic Review of Gender Differences in Sepsis Management and Outcomes. 2017; 49: 312-24.

7. Papathanassoglou E, Middleton N, Benbenishty J, Williams G, Christofi MD, Hegadoren K. Systematic review of gender-dependent outcomes in sepsis. 2017; 22: 284-92.

8. Pool R, Gomez H, Kellum JA. Mechanisms of Organ Dysfunction in Sepsis. 2018; 34: 63-80.

9. Arulkumaran N, Deutschman CS, Pinsky MR, Zuckerbraun B, Schumacker PT, Gomez H, et al. Mitochondrial Function in Sepsis. 2016; 45: 271-81.

10. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. 2002; 2002/07/23: 219-23.

11. Murugan R, Kellum JA. Acute kidney injury: what's the prognosis? 2011; 2011/02/24: 209-17.

12. Gonzalez AS, Elguero ME, Finocchietto P, Holod S, Romorini L, Miriuka SG, et al. Abnormal mitochondrial fusion-fission balance contributes to the progression of experimental sepsis. *Free radical research* 2014.

13. Tran M, Tam D, Bardia A, Bhasin M, Rowe GC, Kher A, et al. PGC-1alpha promotes recovery after acute kidney injury during systemic inflammation in mice. 2011; 121: 4003-14.

14. Zolezzi JM, Silva-Alvarez C, Ordenes D, Godoy JA, Carvajal FJ, Santos MJ, et al. Peroxisome proliferator-activated receptor (PPAR) gamma and PPARalpha agonists modulate mitochondrial fusion-fission dynamics: relevance to reactive oxygen species (ROS)-related neurodegenerative disorders? *PLoS One* 2013; 8: e64019.

15. Patil NK, Parajuli N, Macmillan-Crow LA, Mayeux PR. Inactivation of renal mitochondrial respiratory complexes and manganese superoxide dismutase during sepsis: mitochondria targeted antioxidant mitigates injury. *American journal of physiology Renal physiology* 2014; 2014/02/07: F734-F743.

16. Singh P, Parajuli N, Mayeux PR, Macmillan-Crow LA. Renal mitochondrial lipid peroxidation during sepsis. 2016; 2: 116.

17. Sims CR, Macmillan-Crow LA, Mayeux PR. Targeting mitochondrial oxidants may facilitate recovery of renal function during

infant sepsis. *Clinical pharmacology and therapeutics* 2014; 96: 662-4.

18. Demarest TG, McCarthy MM. Sex differences in mitochondrial (dys)function: Implications for neuroprotection. 2015; 47: 173-88.

19. Ventura-Clapier R, Moulin M, Piquereau J, Lemaire C, Mericskay M, Veksler V, et al. Mitochondria: a central target for sex differences in pathologies. 2017; 131: 803-22.

20. Doi K, Leelahavanichkul A, Hu X, Sidransky KL, Zhou H, Qin Y, et al. Pre-existing renal disease promotes sepsis-induced acute kidney injury and worsens outcome. 2008; 74: 1017-25.

21. Leelahavanichkul A, Huang Y, Hu X, Zhou H, Tsuji T, Chen R, et al. Chronic kidney disease worsens sepsis and sepsis-induced acute kidney injury by releasing High Mobility Group Box Protein-1. *Kidney Int* 2011; 2011/08/13: 1198-211.

22. Zolezzi JM, Silva-Alvarez C, Ordenes D, Godoy JA, Carvajal FJ, Santos MJ, et al. Peroxisome proliferator-activated receptor (PPAR) gamma and PPARalpha agonists modulate mitochondrial fusion-fission dynamics: relevance to reactive oxygen species (ROS)-related neurodegenerative disorders? *PLoS One* 2013; 8: e64019.

23. Jendrach M, Mai S, Pohl S, Voth M, Bereiter-Hahn J. Short- and long-term alterations of mitochondrial morphology, dynamics and mtDNA after transient oxidative stress. *Mitochondrion* 2008; 8: 293-304.

24. Fan X, Hussien R, Brooks GA. H2O2-induced mitochondrial fragmentation in C2C12 myocytes. *Free Radic Biol Med* 2010; 49: 1646-54.

25. Watanabe T, Saotome M, Nobuhara M, Sakamoto A, Urushida T, Katoh H, et al. Roles of mitochondrial fragmentation and reactive oxygen species in mitochondrial dysfunction and myocardial insulin resistance. *Exp Cell Res* 2014; 323: 314-25.

26. Leonard AP, Cameron RB, Speiser JL, Wolf BJ, Peterson YK, Schnellmann RG, et al. Quantitative analysis of mitochondrial morphology and membrane potential in living cells using high-content imaging, machine learning, and morphological binning. *Biochim Biophys Acta* 2015; 1853: 348-60.

27. Warn PA, Brampton MW, Sharp A, Morrissey G, Steel N, Denning DW, et al. Infrared body temperature measurement of mice as an early predictor of death in experimental fungal infections. 2003; 2003/04/12: 126-31.

28. Parajuli N, Campbell LH, Marine A, Brockbank KG, Macmillan-Crow LA. MitoQ blunts mitochondrial and renal damage during cold preservation of porcine kidneys. *PLoS One* 2012; 2012/11/10: e48590.

29. Parajuli N, Shrum S, Tobacyk J, Harb A, Arthur JM, Macmillan-Crow LA. Renal cold storage followed by transplantation impairs expression of key mitochondrial fission and fusion proteins. *PLoS One* 2017; 12: e0185542.

30. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods Mol Biol* 2012; 810: 25-58.

31. Miyaji T, Hu X, Yuen PS, Muramatsu Y, Iyer S, Hewitt SM, et al. Ethyl pyruvate decreases sepsis-induced acute renal failure and multiple organ damage in aged mice. 2003; 64: 1620-31.

32. Doi K, Leelahavanichkul A, Yuen PS, Star RA. Animal models of sepsis and sepsis-induced kidney injury. 2009; 2009/10/07: 2868-78.

33. Deng S, Ai Y, Gong H, Feng Q, Li X, Chen C, et al. Mitochondrial dynamics and protective effects of a mitochondrial division inhibitor, Mdivi-1, in lipopolysaccharide-induced brain damage. 2018; 496: 865-71.

34. Bordt EA, Clerc P, Roelofs BA, Saladino AJ, Tretter L, Adam-Vizi V, et al. The Putative Drp1 Inhibitor mdivi-1 Is a Reversible Mitochondrial Complex I Inhibitor that Modulates Reactive Oxygen Species. 2017; 40: 583-94.

35. Tang C, He L, Liu J, Dong Z. Mitophagy: Basic Mechanism and Potential Role in Kidney Diseases. 2015; 1: 71-9.

36. Zhao C, Chen Z, Qi J, Duan S, Huang Z, Zhang C, et al. Drp1-dependent mitophagy protects against cisplatin-induced apoptosis of renal tubular epithelial cells by improving mitochondrial function. 2017; 8: 20988-1000.

37. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, Kurosawa S, Stepien D, Valentine C, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. 2013; 93: 1247-88.

38. Sims CR, Nguyen TC, Mayeux PR. Could Biomarkers Direct Therapy for the Septic Patient? 2016; 357: 228-39.

39. Romanovsky A, Morgan C, Bagshaw SM. Pathophysiology and management of septic acute kidney injury. *Pediatr Nephrol* 2013; 2013/02/13: 1-12.

40. Zellweger R, Wichmann MW, Ayala A, Stein S, DeMaso CM, Chaudry IH. Females in proestrus state maintain splenic immune functions and tolerate sepsis better than males. 1997; 25: 106-10.

41. Diodato MD, Knoferl MW, Schwacha MG, Bland KI, Chaudry IH. Gender differences in the inflammatory response and survival following haemorrhage and subsequent sepsis. 2001; 14: 162-9.