Dichloroacetate improves hepatic and systemic energy metabolism during sepsis

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Abstract

Dramatic metabolic reprogramming between an anabolic resistance and catabolic tolerance occurs within the immune system in response to systemic infection with the sepsis syndrome. While metabolic tissues such as the liver are subject to end-organ damage during sepsis and are the primary cause of sepsis death, how their metabolic and energy reprogramming during sepsis state ensures survival is unclear. Employing comprehensive metabolomic screening, targeted lipidomic screening, and transcriptional profiling in a mouse model of septic shock, we show that hepatocyte lipid metabolism, mitochondrial TCA energetics, and redox balance are significantly reprogrammed after cecal ligation and puncture (CLP). We identify increases in TCA cycle metabolites citrate, cis-aconitate, and itaconate with reduced fumarate, elevated triglyceride synthesis, and lipid droplet accumulation in the septic hepatocytes. Transcription analysis of liver tissue supports and extends the hepatocyte findings. Plasma metabolomics show systemic hypoglycemia and increased concentrations of free fatty acids, ketones and corticosterone in parallel with liver reprogramming. Strikingly, the administration of the pyruvate dehydrogenase kinase (PDK) inhibitor dichloroacetate (DCA) reverses dysregulated hepatocyte and systemic metabolism and mitochondrial dysfunction. DCA administered during sepsis arrests anorexia and weight loss, restores VO2 levels as an index of increased carbohydrate oxidation and promotes physical activity. We suggest that sepsis inflicts an energy demand and supply crisis with distinct shifts in hepatocyte and systemic mitochondrial function. Targeting the mitochondrial PDC/PDK energy homeostat rebalances life-threatening energy deregulation caused by bacterial sepsis.
Introduction

Sepsis represents a life-threatening condition that results from dysregulated inflammatory responses to disseminated and uncontrolled infection. If not diagnosed and treated early after its onset, sepsis may limit survival by inducing early shock and subsequent immunometabolic paralysis. Sepsis accounts for one in three hospital deaths in the U.S. and millions of deaths each year globally, highlighting its hazard to public health.\(^1,2\) The high mortality rate associated with sepsis reflects the lack of a clinically viable molecular-based therapeutic target. Therefore, understanding the pathogenesis of sepsis at both the molecular and organismal levels is a major gap in public health and of the utmost importance.

Sepsis is a biphasic phenomenon, with clearly defined acute and chronic phases.\(^3,4\) The acute phase of sepsis is driven by high energy consumption and hyper-inflammation characterized by oxidative stress, followed by cellular reprogramming to a low energy, anti-inflammatory state of immunometabolic paralysis, with accompanying organ failure.\(^2,5\) In addition to inflammatory responses, dysregulation of the host’s metabolism during sepsis plays a vital role in its progression and severity.\(^6-8\) Compared to healthy individuals, energy homeostasis is significantly altered in septic patients.\(^7,8\) The early phase of sepsis is a hypermetabolic condition marked by increased catabolism of fats, proteins and carbohydrates, associated with high rates of oxygen consumption and ATP synthesis.\(^9-11\) Following the acute phase is a hypometabolic state where ATP production and mitochondrial respiration decrease.\(^9\) Immune cells and some organ cells enter an energy
conserving “hibernation-like” state as a protective mechanism to lower the metabolic demands of the cell and help with its recovery \(^6,12,13\). However, staying in this hypometabolic state for a prolonged period can lead to organ dysfunction and failure \(^12,13\).

Of particular interest is that, during the hyper-inflammatory anabolic phase of sepsis, an increase in the expression and activity of pyruvate dehydrogenase kinase 1 (PDK1) consistently occurs \(^4\). This enzyme is one of four PDK isoforms that reversibly phosphorylates serine residues on pyruvate dehydrogenase complex (PDC) E1\(\alpha\) subunit, inhibiting the conversion of pyruvate to acetyl coenzyme A (acetyl CoA) \(^14\). Inhibition of this important enzymatic activity that connects glycolysis to tricarboxylic cycle (TCA), oxidative phosphorylation (OXPHOS) and the lipogenic pathway is thought to be one of the main mechanisms that is driving dysfunction of mitochondrial respiration and cell bioenergetics observed during sepsis \(^15,16\), suggesting that PDK may be a novel, druggable target for treatment of sepsis.

There a few reports that indicate changes in hepatic metabolism during sepsis but these studies are not conclusive \(^17\). Therefore, there is limited knowledge regarding the metabolic changes and infection related liver dysfunction in response to sepsis. Therefore, we set out to characterize the global hepatic manifestations of sepsis, with the overall goal of identifying master regulators of the hepatic metabolic response to polymicrobial infection.
Results

Sepsis Impairs Hepatic Mitochondrial Metabolism

To test the long-term hepatic transcriptional changes elicited by sepsis we performed RNA-seq in whole livers thirty-hours post cecal ligation puncture (CLP). At this time point, septic mice exhibit tolerance, in which immunometabolic paralysis and end organ dysfunctions decrease survival. Ingenuity pathway analysis (IPA) of the top physiological pathways subject to alteration in response to sepsis revealed a significant increase in the acute phase response pathway, highlighting a persistent inflammatory state in the liver into the chronic phase of sepsis (Fig. 1a). Of particular interest were the findings that oxidative phosphorylation and mitochondrial dysfunction were the top two enriched pathways in the liver of septic mice (Fig. 1a). To gain further insight into the effects of sepsis on the transcriptional regulation of mitochondrial function we performed gene set enrichment analysis (GSEA) of the oxidative phosphorylation pathway (GO:0006119). Septic mice at 30 h decreased the transcriptional output of OXPHOS components, as evidenced by a negative GSEA enrichment score (Fig. 1b). Given the connection between oxidative phosphorylation and the tricarboxylic acid (TCA) cycle, we next asked if polymicrobial infection would elicit similar alterations in this pathway. In accordance with the changes observed in the oxidative phosphorylation pathway, we found a negative enrichment score for TCA cycle enzymes (GO:0006099) in the liver of CLP mice (Fig. 1c). Thus, our transcriptome data indicate that sepsis impairs mitochondrial metabolism in the liver.

Next we wanted to assess if the transcriptional changes elicited by sepsis would manifest in altered hepatic TCA cycle metabolism. Therefore, we performed global
unbiased metabolomic screening in isolated hepatocytes from control and septic mice by Ultrahigh Performance Liquid Chromotography-Tandem Mass Spectroscopy (UPLC-MS/MS). In line with altered transcriptional regulation of the TCA cycle, we found that sepsis altered the relative abundance of multiple metabolites involved in the TCA cycle (Fig. 1d). In particular, significant elevation of citrate and cis-aconitate was observed in septic hepatocytes at 30h (Fig. 1e&f). Unlike macrophages, which shift their ratio of succinate and α-ketoglutarate to favor succinate accumulation over α-ketoglutarate 18-20, we found no changes in the levels of these metabolites in hepatocytes (Fig. 1G&H). Furthermore, unlike macrophages 21, levels of fumarate decreased, but malate levels were unchanged in hepatocytes from septic animals (Fig. 1I&J). Monocytes, macrophages and dendritic cells reprogram the TCA cycle during acute inflammation to a catabolic tolerance phenotype by shunting cis-aconitate to itaconate through the enzymatic action of aconitase decarboxylase (ACOD1; also known as immune responsive gene 1 [IRG1]) 21-24. Accordingly, we also found that sepsis significantly induces ACOD1 and elevates itaconate in isolate hepatocyte preparations (Fig. 1K&L).

**Sepsis Impairs Hepatic Redox Balance and Promotes Oxidative Stress**

Perturbations in oxidative phosphorylation have been shown to promote the production of reactive oxygen species (ROS) 25. Furthermore, itaconate has been shown to induce genes involved in oxidative stress regulation through activation of the master NRF2 KEAP1 antioxidative pathway in macrophages 23. Therefore, we examined if sepsis invokes a gene expression profile involved in regulation of ROS. GSEA of the ROS metabolic process pathway (GO:0072593) revealed a positive enrichment, highlighting
the transcriptional induction of genes involved in the regulation of oxidative stress in livers of septic mice (Fig. 2a). Given the induction of ROS metabolic genes we wanted to determine if sepsis alters key metabolites involved in redox balance, specifically the cysteine-glutathione transsulfuration redox regulatory cycle. Hepatocytes isolated during sepsis-induced tolerance did not adjust intracellular homocysteine levels (Fig. 2b), however, cystathionine significantly decreased (Fig. 2c). Furthermore, we found a trend for decreased cysteine levels in the liver \((p=0.0534)\) (Fig. 2d) and significantly decreased glycine (Fig. 2e). Most dramatically, septic mice depleted hepatocyte glutathione (Fig. 2f). During oxidative stress, the pool of glutathione shifts from reduced glutathione (GSH) to oxidized glutathione (GSSG). Additional evidence of increased hepatic oxidative stress was a significant shift in the cellular glutathione pools, favoring GSSG over GSH in CLP mice compared to sham controls (Fig. 2g).

**Sepsis promotes hepatic steatosis**

Thus far, our data revealed that sepsis induces significant impairments in hepatocyte OXPHOS, alterations in the TCA cycle, and induction of oxidative stress, findings that are also metabolic hallmarks of fatty liver disease \(^{26}\). Because perturbations in global lipid profiles occur in septic patients \(^{11}\), we evaluated the effects of CLP on the hepatic lipid metabolism pathway. In support of our hypothesis that sepsis alters hepatic lipid metabolism, transcriptome data indicate significant repression of lipid metabolism in livers of septic mice (Fig. 3a). Sepsis also decreased transcription output that supports fatty acid metabolic process components in the liver, as evidenced by a negative GSEA enrichment score (Fig. 3b).
Since hepatic lipid metabolism significantly changes during sepsis, we further investigated the effect of sepsis on both the fatty acid oxidation and biosynthetic processes pathways. Slight changes occurred in the expression profiles of genes involved in fatty acid oxidation in response to CLP after 30 h. In contrast, we found a striking increase in genes involved in fatty acid biosynthesis (Fig. 3c). We then asked whether the transcriptional alterations elicited by sepsis would be manifested in hepatic lipid metabolites. Consistent with our transcriptional profiling, our unbiased metabolomic screening in hepatocytes identified lipid metabolism as the top metabolic pathway altered in sepsis (Fig. 3d). Our metabolomic screen identified increases in virtually all fatty acids and acylcarnitine derivatives assayed (Fig. 3e). Given the consistency between our transcriptome data and metabolomics panel, we performed lipidomic screening in the liver of sham vs CLP mice to capture the global consequences of sepsis on hepatic lipid metabolism. Strikingly, we find over 500 lipid species increased in septic hepatocytes (Fig. 3f). As a consequence of alterations in lipid metabolism, we observe an increase in lipid droplet formation in liver tissues of CLP mice (Fig. 3g), confirming the presence of hepatic steatosis.

**Sepsis-induced anorexia increases plasma free fatty acids**

The onset of anorexia and a switch from glucose metabolism to alternative fuel sources during sepsis has been well established. Consistent with this principle, septic mice housed in metabolic chambers exhibited onset of anorexia, as evidenced by a marked decrease in total food intake (Fig. 4a). While anorexia induced by systemic inflammation is highly conserved, the extent to which sepsis-associated anorexia is associated with altered plasma metabolites is unclear. To address this knowledge gap, we
performed global metabolomic screen in plasma from sham and CLP mice 30 hours post-surgery. We find plasma metabolites known to be altered in the fasting state are indeed altered in response to polymicrobial infection. Specifically, sepsis induces a significant decrease in the relative abundance of plasma glucose compared to sham mice (Fig. 4b). Similar to previous reports, increased circulating levels of corticosterone, a primary mediator of the fasting response also occurred (Fig. 4c). During nutrient deprivation, ketogenesis increases circulating ketones. Likewise, we found elevated levels of circulating ketone bodies 3-hydroxybutyrate, 2-hydroxybutyrate, and 2-aminobutyric in septic mice (Fig. 4d-f). Fasting also liberates free fatty acids and increases their levels in plasma. Consistent with a fasting response, sepsis increased 75 lipid species in the circulation (Fig. 4g). Collectively, these data indicate sepsis induces a systemic starvation state, concomitant with repressed mitochondrial energetics and nutrient changes in hepatocytes.

**PDK inhibition attenuates sepsis-induced transcriptional and metabolic changes in the liver**

Given the establishment of hepatocyte mitochondrial dysfunction and steatosis during sepsis, we asked whether pharmacological targeting of the hepatic PDK/PDC axis would mitigate the disruption of key metabolic and bioenergetic processes induced by sepsis. We previously reported that PDK inhibition by the pyruvate analog and pan-PDK inhibitor dichloroacetate (DCA) promotes PDC-dependent immunometabolic adaptations to sepsis and increases survival. Therefore, we hypothesized that PDK inhibition
would also ameliorate sepsis-induced metabolic dysfunction in the liver. To test this postulate, we administered DCA to septic mice 24 h after CLP onset and assessed the global transcription profile of liver 6 h later via RNA-seq. DCA treatment of septic mice reversed the majority of sepsis-regulated gene networks (Fig. 5a). DCA also restored to nearly sham levels the top physiological pathways altered in response to sepsis, such as inflammation mediators, lipid triglycerides synthesis, redox control and mitochondrial TCA function (Fig. 5b).

Given our reversal of the sepsis-regulated hepatic transcriptome by DCA, we next determined whether it would have functional effects on the hepatic metabolome. We repeated our unbiased metabolomic profiling in isolated hepatocytes from septic mice and found that TCA metabolites that accumulate in hepatocytes during sepsis—citrate, cis-aconitate, and itaconate—returned almost to sham levels after DCA treatment (Fig. 5c). Of particular note, we also observed that DCA treatment did not restore fumarate levels, but rather decreased this TCA intermediate decreased even further than measured in septic livers not exposed to the drug (Fig. 5c).

Next, we focused on metabolites proximal to the glutathione synthesis redox pathway, given the transcriptional reversal of the conjugation to glutathione pathway in DCA treated septic mice and distal to methionine (Fig. 5b). We found that DCA not only caused a reversal of cystathionine and taurine low levels in septic mice, but also increased accumulation of these metabolites in the liver after DCA (Fig. 5d). No significant differences occurred in glycine. The increased levels of transsulfuration redox metabolites in DCA-treated mice resulted in a reduced oxidative burden in hepatocytes during sepsis, as evidenced by an increased GSSG/GSH ratio (Fig. 5e).
Finally, given the pronounced steatotic phenotype present during sepsis, and the restoration of mitochondrial function and redox balance in response to DCA treatment, we next determined whether PDK inhibition would also reverse the fatty liver phenotype of sepsis. Metabolomics revealed broad reductions in hepatic mono-, di- and triglycerides in septic mice treated with DCA (Fig. 5f), indicating that PDK activation can control the development of hepatic steatosis during sepsis (Fig. 5g).

**DCA rescues systemic energy crisis during polymicrobial infection**

The above findings strongly suggest that PDK inhibition reverses many sepsis-induced metabolic and transcriptional changes in the liver. We next investigated the systemic effects of DCA treatment on energy metabolism in CLP mice. Plasma metabolite screening in CLP mice revealed alterations in over 250 metabolites in response to CLP, with the majority of these metabolites decreasing after DCA treatment (Fig. 6a&b). When we further examined the 198 sepsis-dysregulated metabolites normalized by DCA treatment, we found that the majority involve pathways of glycine and methionine metabolism that fuel glutathione (Fig. 6c) and promote the tolerant and antioxidant catabolic phase of sepsis. Consistent with our findings in livers from septic mice, these data indicate that DCA restores circulating metabolite levels comparable to concentrations measured in sham controls and identify a reversal in sepsis-induced systemic energy depletion following PDK inhibition.
Given that DCA restores levels of sepsis-dysregulated circulating metabolites, we hypothesized that PDK inhibition might also reverse the sepsis-induced anorexia response and starvation. To test this hypothesis, we housed septic mice treated with or without DCA in metabolic cages for after sepsis onset. Consistent with previous reports, CLP induced a dramatic decrease in body weight within 24 h of surgery (Fig. 6d). Strikingly, DCA administration completely normalized body weight in septic mice (Fig. 6d) and increased physical activity (data not shown). DCA-induced body weight correction may result from decreasing anorexia, as food intake concomitantly increased in DCA-treated septic mice (Fig. 6e). While modestly increases in respiratory exchange ratio (RER) occurred, DCA restored VO2 to sham values. We interpret the VO2 increase as indicating that PDK inhibition readjusts carbohydrate oxidation during sepsis starvation, consistent with PDC activation (Fig. 6f&g). Together, our findings identify PDK as a significant driver of systemic energy imbalance during sepsis and a therapeutic target for its reversal by increasing PDC oxidation of glucose carbons. Contributing to this effect are reversing anorexia, increasing food intake and restoring mitochondrial function (Fig. 6h).
Considerable attention has been focused recently on the immunometabolic consequences of inflammation in immune cells. However, significant gaps exist in our understanding of the metabolic adaptations induced by systemic inflammation from sepsis in vital organs. Addressing this limitation is critical because sepsis survival depends on restoring both organ and immune cell homeostasis following dysregulated and disseminated inflammation. The present study reveals major disruption of several key aspects of hepatic mitochondrial metabolic and bioenergy functions in septic mice, including TCA cycle activity, OXPHOS, redox balance and lipid metabolism. In addition, at the organismal level, anorexia increase as an early response to sepsis followed by an altered circulating lipid profile and increased levels of corticosterone, a pattern consistent with a state of fasting or starvation. Importantly, PDK inhibition by DCA highlights the PDK/PDC as a critical gatekeeper of energy metabolism during life-threatening systemic infection. Within one hour of its administration, DCA reversed the starvation phenotype and, within six hours, restored overall mitochondrial capacity to meet the demands used to regenerate immune and organ physiologic competence. Overall, these findings support the notion that dysregulated mitochondrial reprogramming in hepatocytes contributes to a sepsis-induced systemic energy supply and demand crisis and the vulnerability of PDC to significant post-transcriptional inhibition during sepsis.

Upregulation of PDKs inactivates PDC to skew carbohydrate metabolism away from mitochondrial oxidation and promotes cytoplasmic reduction of glycolytically-derived pyruvate to lactate, as the central feature of the Warburg response. Upregulating oxidative phosphorylation also shifts immune cells to Warburg glycolysis during cell activation.
and alters levels of TCA cycle citrate, cis-aconitate, succinate, and fumarate metabolite signaling properties. For example, succinate primes inflammation through succinate dehydrogenase (SDH)-mediated ROS generation and ATP synthesis. SDH-derived ROS enhances IL-1β production by activating HIF-1α and activating the NLRP3 inflammasome. Inhibition of SDH activity increases IL-10 production and skews inflamed immune cells towards an anti-inflammatory response. Fumarate, on the other hand, possesses anti-inflammatory properties as it inhibits pro-inflammatory cytokine production either by activating the NRF2 pathway or via inhibiting pathways like NF-κB and MAPK that lie downstream of TLR signaling in immune cells. Decreased fumarate did not align with succinate in hepatocytes at the time point assayed.

Cis-aconitate decarboxylation to itaconate represses glycolysis and dampens inflammation in monocytes and macrophages. In response to LPS stimulation, macrophages significantly upregulate IRG1 and accumulate itaconate. Direct inhibition of SDH by itaconate limits IL-1β levels. Transcriptional regulation of the NRF2/Keap1 and IκBζ-ATF3 pathways by itaconate also mute IL-1β expression. In line with what occurs in macrophages, we observed an increase in IRG1, citrate, cis-aconitate, and itaconate in septic hepatocytes at 30 h. While we did not fully characterize the hepatic function of itaconate, a recent paper demonstrates its anti-inflammatory effects during Ischemia-reperfusion (IR) injury in the liver. The deletion of IRG1 heightens inflammation and liver damage and renders hepatocytes susceptible to oxidative injury after I/R injury. Further, itaconate administration reduces liver damage and inflammation associated with I/R in IRG1 KO mice, also emphasizing its anti-inflammatory and hepatoprotective effects. Given the immunomodulatory effects of itaconate and its
accumulation in the liver in our study, we hypothesize that itaconate directs hepatocyte shifts in both TCA cycle metabolism and mitochondrial energetics. DCA reduces itaconate levels, which we also find in a human monocyte model of sepsis \(^{16}\).

ROS also may direct hepatic responses to sepsis. Similar to immune cells, liver cells increase ROS production during sepsis \(^{46,47}\). We found that the thiol antioxidant glutathione decreased, concomitant with an increase in oxidized glutathione, in hepatocytes during sepsis. Overall, our findings support the notion that sepsis disrupts hepatic redox balance, as reflected in alterations in glycine, cystathionine and taurine levels in the liver.

Sepsis also profoundly alters lipid metabolism, resulting in significant fatty acid and glycerol metabolic shifts in plasma, changes reported to predict prognosis in septic patients \(^{48}\). Sirt1 and Sirt6 NAD-dependent deacetylases play an essential role in regulating the balance between glycolysis and fatty acid oxidation \(^{49}\). Specifically, Sirt1 upregulates PGC-1-dependent fatty acid oxidation during the shift from the acute to the late phase of sepsis. However, we do not observe a fatty acid oxidation transcription profile in septic livers. Yet another vital lipid metabolic pathway in immune cells is de novo lipogenesis \(^{50}\). Macrophages generate malonyl-CoA and subsequent malonylation of glycolytic enzymes \(^{51}\). While we did not measure malonyl-CoA and acetyl-CoA levels, our metabolomics screening did show an increase in hepatic citrate levels. Furthermore, our transcriptional studies identify a cohort of genes in livers from septic animals that are involved in fatty acid biosynthesis. Our data are consistent with human data where patients who died from sepsis had evidence of hepatic steatosis affecting 5%-80% of liver
parenchyma. These data emphasize the robust steatotic phenotype conserved between mice and humans.

The combination of anorexia and weight loss is an important clinical manifestation of sepsis in humans. Representative animal models demonstrated significant reductions in food intake and body weight in response to infection. Consistent with a fasting state, we find a circulating metabolomic signature enriched in ketone bodies and glucocorticoids with decreased glucose levels. During the fasting period, free fatty acids released from adipose tissues are taken up at a nonsaturable rate by the liver, where they are packaged into triglycerides and transported out and into circulation by very low-density lipoproteins (VLDL), which make them available as an energy source during periods of energy crisis. In addition to the fasting metabolomics signature observed in septic mice, we found that sepsis significantly elevates levels of 75 lipid species in the plasma. We propose nutrient depletion as another mechanism contributing to the development of sepsis-induced steatosis.

PDC is a master metabolic regulator controlling the conversion of pyruvate to acetyl-CoA in the mitochondria. A part of the metabolic reprogramming that occurs in immune cells in response to inflammatory signaling is inactivation of PDC. PDK is a negative regulator of the PDC, as it phosphorylates PDC and inhibits the conversion of pyruvate to acetyl-CoA. During sepsis, the expression and activity of PDK1 in immune cells is heightened, contributing to the dysfunction of mitochondria metabolism. One mechanism driving this observed increase is through pro-inflammatory mediators such as LPS and interferon gamma (IFN-γ). Another possible mechanism contributing to PDK activation in the context of sepsis is through glucocorticoid signaling. In fact, starvation...
is well established to activate the PDK pathway. Based on our findings of elevated glucocorticoid levels in both the circulation and the liver, we hypothesize the stress hormone pathway as a contributor to PDK activation during sepsis.

We reported in a sepsis monocyte model that DCA reduced TCA cycle catabolic effects, concurrent with increasing amino acid anaplerotic catabolism of branched-chain amino acids, leading to increased TCA-driven anabolic energetics. In the present study, we find restoration of TCA metabolites, decreased triglyceride accumulation, lessening of lipid synthesis and oxidative stress rebalances in septic mice after DCA administration. Not only does DCA reverse sepsis-induced gene expressions and metabolic profiles to baseline levels, it also leads to an increase in antioxidants, body weight and food intake, suggesting systemic improvements in response to DCA and reversal of anorexia. Overall we demonstrate that hepatic dysfunction and a systemic energy crisis during sepsis improves after targeting the PDC/PDK axis with DCA. Furthermore, we show sepsis not only affects immunity, but it dysregulates hepatic and organism-wide lipid metabolism and redox homeostasis in the liver. The net results is an energy crisis between low nutrient supply and high energy demand for immunity and organ function.

Further work elucidating the mechanistic pathways involved in the dysfunction of lipid metabolism and the impact of TCA metabolite alterations is warranted. In particular, further investigation specifically into the PDK pathway to determine which isoform underlies the hepatic manifestations of sepsis. This is warranted given multiple isoforms of PDK expressed in the liver and DCA inhibits multiple isoforms of PDK. The results of this study fill a gap in understanding how sepsis at the molecular level compromises
liver function. It also further informs the novel therapeutic targeting potential of the PDC/PDK homeostat.
Material and Methods

Animal Experiments

Male C57BL/6J mice aged 8-10 weeks were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were subject to a 12:12 hour dark/light cycle with *ad libitum* access to standard rodent chow and water. Cecal ligation puncture model was used to induce sepsis as previously described. Briefly, cecum was ligated and punctured two times with a 22-gauge needle. Contents were then returned and incision was closed in two layers (peritoneum and skin). Sham operation where abdominal incision was made but cecum not ligated or punctured was used as a control. Subcutaneous fluids (1ml normal saline) were given to each animal. Mice were euthanized 30 hours post-surgery for tissue collection. Dichloroacetate (DCA) (Sigma; MO, USA) was administered (25mg/kg) intraperitoneally at various time points post-surgery: for metabolomic screening and RNA-seq DCA was administered 24 hours post-surgery and tissues collected 6 hours post DCA administration (30 hours post-surgery), for metabolic cages DCA was administered immediately post-surgery.

Hepatocyte Isolation

Hepatocytes were isolated via portal vein perfusion and collagenase digestion as previously described. Following perfusion, liver cells were liberated by gentle dissociation in DMEM (ThermoFisher; CA, USA). Cells were then filtered through nylon mesh to remove cellular debris and connective tissue and resulting cells pelleted by centrifugation at 50g for 1 minute. After three washes with DMEM cells were counted and viability assessed via Trypan Blue exclusion.
RNA-sequencing

RNA was isolated from whole liver tissues using Trizol and the RNeasy RNA isolation kit (Qiagen; MD, USA) according to manufacturer’s protocol. One microgram of high quality RNA (RIN>8) was used as a template for library generation using the Illumina TruSeq RNA Sample Prep Kit v2 (Illumina; CA, USA) according to the manufacturer’s protocol. Generated libraries were then poly(A) enriched for mRNA prior to sequencing. Indexed samples were sequenced at 100bp-paired-end protocol with the NovaSeq 6000 (Illumina), generating approximately 20-30 million reads per sample. Sequenced reads were aligned to the University of California Santa Cruz (UCSC) mm10 reference genome using STAR v2.5 as previously described. The mapped read counts were quantified by Subread feature Counts v1.5.0-p1. Differentially expressed genes (DEGs) were determined by DESeq2 v1.14.1 using a false discovery rate of 0.05. Ingenuity Pathway Analysis (Qiagen) and Gene Set Enrichment Analysis v4.0.3 (GSEA) were further used as previously described.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy

Hepatocytes were isolated described above for metabolomic screening via Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS) (Metabolon; NC, USA). Briefly, 150-200 microliter cell pellets per animal were used as starting material. Samples were prepared using the automated MicroLab STAR system (Hamilton; NV, USA). Proteins were precipitated using methanol under vigorous shaking for 2 minutes followed by centrifugation. Prior to analysis organic solvents were removed.
with TurboVap (Zymark; MA, USA) and overnight storage under nitrogen. Dried samples were reconstituted with solvents compatible with the three following analytical methods:

1) reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), 2) RP/UPLC-MS/MS with negative ion mode ESI and 3) HILIC/UPLC-MS/MS with negative ion mode ESI. Resulting samples were analyzed with the ACQUITY UPLC (Waters; MA, USA) and a Q-Exactive high resolution/accurate mass spectrometer (ThermoScientific; MA, USA) interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. An acidic positive ion condition was used on an aliquot optimized to detect more hydrophilic compounds. Another acidic positive ion condition was ran but chromatographically optimized for hydrophobic compounds. Basic negative ion optimized conditions was also used on a separate C18 column. Resulting raw data was extracted and peaks identified using Metabolon’s hardware and software. Compounds were identified by comparing to known library entries of purified standards or recurrent unknown entities. A library of authenticated standards containing retention time/index (RI), mass to charge ratio (m/z) and chromatographic data (MS/MS spectral data) on all library compounds. Three criteria are used to identify chemicals: 1) RI within a narrow window of proposed identification, 2) accurate mass match to the library ± 10 ppm and 3) MS/MS forward and reverse scores between the experimental data and authentic standards. Peaks were quantified using area under the curve (AUC). For complex lipid panel lipids were extracted with methanol:dichloromethane in the presence of internal standards. Extracts were concentrated under nitrogen and reconstituted in 250ml of 10mM ammonium acetate dichloromethane:methanol (50:50). Mass spec analysis was performed in a Shimazdu LC
with nano PEEK tubing and the Sciex Slexlon-5500 QTRAP (Sciex; MA, USA). Both negative and positive mode electrospray was used. Individual lipid species were quantified by taking the peak area ratios of target compounds and their assigned internal standards then multiplying by the concentration of added internal standards. Lipid class concentrations were calculated from the sum of all molecular species within a class, and fatty acid compositions were determined by calculating the proportion of each class comprised by individual fatty acids.

**Oil Red O Staining**

Livers were frozen in optimal cutting temperature (OCT) media (FisherScientific; NH, USA) and sectioned with a cryostat (Leica; Wetzler, Germany) at 4mm. Liver sections were fixed at room temperature for 15 minutes with 4% paraformaldehyde followed by washing three times with phosphate buffered saline (PBS). Liver sections were then incubated with Oil Red O as previously described \(^{74,75}\). Liver sections were then washed three times with PBS and cover slipped with Fluoroshield Mounting Media (Sigma). Stained slides were visualized with a brightfield microscope (Nikon; NY, USA).

**Indirect Calorimetry**

To measure whole body energy expenditure in live animals, mice were housed individually in metabolic chambers of PhenoMaster (Indirect calorimetry system; TSE systems, Germany) and acclimatized for 3 days with free access to food and water. The Energy expenditure (Oxygen consumption (VO2), Respiratory exchange ratio (RER) and
food intake were obtained continuously during a 12 h light and 12 h dark cycle for four
days and measured for last 24 h after adaptation.

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Figure Legends

Figure 1: Sepsis Impairs hepatic mitochondrial metabolism. (A) Top 5 canonical pathways subject to transcriptional alterations in the liver identified by Ingenuity Pathway Analysis of RNA-seq of sham versus CLP mice (n = 4 mice per group). Blue represents a negative z-score and orange represents a positive z-score. Shading indicates intensity of pathway activation/inhibition. (B) GSEA of the oxidative phosphorylation pathway (GO:0006119) indicating a negative normalized enrichment score (NES = -0.853). (C) GSEA of the TCA cycle pathway (GO:0006099) indicating a negative enrichment score (NES = -1.1956). (D) Schematic representation of hepatic TCA cycle metabolites altered during chronic sepsis. Red denotes a metabolite increased in response to sepsis; green indicates a metabolite decreased in response to sepsis; black indicates a metabolite unchanged in response to sepsis; grey indicates a metabolite not measured in our metabolomic screening. (E-J) Relative metabolite levels measured by Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS) from livers of sham and CLP mice 30 hours post-surgery (n = 5 sham; 8 CLP). (K) rlog transformed counts from RNA-seq of sham and CLP mice 30 hours post-surgery. (L) Relative itaconate levels in livers of sham and CLP mice measured by UPLC-MS/MS (n = 5 sham; 8 CLP). * denotes p<0.05.

Figure 2: Impaired hepatic redox balance in septic mice. (A) GSEA of the ROS metabolic pathway (GO:0072593) from RNA-seq from livers comparing sham to CLP showing a positive enrichment score (NES = 1.117) (n = 4 mice per group). (B-F) Relative metabolite levels of metabolites involved in redox balance measured by UPLC-MS/MS (n = 5
sham; 8 CLP). (G) % of glutathione pool that is either oxidized or reduced in livers from sham and CLP mice 30 hours post-surgery. * denotes p<0.05, **** p<0.0001.

Figure 3: Sepsis promotes hepatic steatosis. (A) Top 5 induced and repressed physiological pathways in the liver of sham versus CLP mice identified by IPA of RNA-seq. (B) GSEA of the fatty acid metabolic pathway (GO:0019395) from RNA-seq from livers comparing sham to CLP showing a negative enrichment score (NES = -1.018) (n = 4 mice per group). (C) Heatmap representation of average log2 fold change of genes involved in fatty acid oxidation (GO:0019395) and fatty acid biosynthetic process (GO:0006633) in CLP versus sham operated mice (n = 5 sham; n = 8 CLP). (D) IPA of top 5 metabolic pathways significantly altered in the liver in response to sepsis identified by global metabolomic screening. (E) Heatmap representation of log2 fold change of different lipid species in sham and CLP mice measured by UPLC-MS/MS. (F) Targeted lipidomic screening by UPLC-MS/MS of livers from sham and CLP mice 30 hours post-surgery (n = 5 sham; n = 8 CLP). (G) Oil red O staining of frozen liver sections from sham and CLP mice 30 hours post-surgery. Scale bar is 50µm. Image is representative of 3 independent mice per group.

Figure 4: Sepsis-induced anorexia increases plasma free fatty acid levels. (A) Cumulative food intake over 24-hour period following sham or CLP surgery. (B) Relative abundance of (B) glucose, (C) corticosterone, (D) 3-hydroxybutyrate, (E) 2-hydroxybutyrate and (F) 2-aminobutyrate measured by UPLC-MS/MS in plasma from sham and CLP
mice 30 hours post-surgery (n = 6 sham; n = 10 CLP). (G) Heatmap representation of fold change of different lipid species in plasma from sham and CLP mice 30 hours post-surgery (n = 6 sham; n = 10 CLP). * denotes p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Figure 5: PDK inhibition restores hepatic metabolism in septic mice. (A) Venn diagram of differentially expressed genes (DEGs) assessed by RNA-seq in CLP versus sham compared to CLP+DCA versus sham 30 hours post-surgery (left). Heatmap of average log2 fold change of DEGs in sham, CLP and CLP+DCA (right) (n = 4 mice per group). (B) Heatmap depiction of z-scores of top canonical pathways identified by IPA in CLP versus sham and CLP+DCA versus sham. (C) Heatmap depiction of average log2 fold change in metabolite levels involved in TCA cycle in sham, CLP and CLP+DCA 30 hours post-surgery measured by UPLC-MS/MS (n = 5 sham; n = 8 CLP and CLP+DCA). (D) Heatmap depiction of average log2 fold change in metabolite levels involved in redox balance in sham, CLP and CLP+DCA 24 hours post-surgery measured by UPLC-MS/MS (n = 5 sham; n = 8 CLP and CLP+DCA). (E) GSSG/GSH ratio in sham, CLP and CLP+DCA measured by UPLC-MS/MS. (F) Average fold change in monoacylglycerol, diacylglycerol and triacylglycerol in CLP and CLP+DCA relative to sham measured by UPLC-MS/MS. (G) Oil red O staining of frozen liver sections from sham and CLP mice 30 hours post-surgery. Scale bar is 50µm * denotes p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.
Figure 6: DCA rescues systemic energy crisis during polymicrobial infection. (A) Heatmap of average log2 fold change in plasma metabolite levels in sham, CLP and CLP+DCA (left) assessed by UPLC-MS/MS. Venn diagram of altered plasma metabolites in CLP versus sham compared to CLP+DCA versus sham 30 hours post-surgery (right) (n = 6 sham; n = 10 CLP and CLP+DCA). (C) Pathway analysis of 198 unique plasma metabolites altered in CLP mice. Left: % overlap of metabolites changed in CLP mice with metabolic pathway. Right: -log(p-value) of pathways altered. (D) Change in body weight in sham, CLP and CLP+DCA 30 hours post-surgery. Total food intake after 30 hours post-surgery in sham, CLP and CLP+DCA. (F) Respiratory exchange ratio (RER) in VO2 in sham, CLP and CLP+DCA 24 hours-post surgery. (H) Schematic representation of hepatic and systemic energy consequences of sepsis and PDK inhibition. * denotes p<0.05, ** p<0.01, *** p<0.001.
Figure 1: Sepsis Impairs Hepatic Mitochondrial Metabolism
Figure 2: Impaired Hepatic Redox Balance in Septic Mice
Figure 3: Sepsis promotes hepatic steatosis
Figure 4: Sepsis-induced anorexia increases plasma free fatty acid levels
Figure 5: PDK inhibition restores hepatic metabolism in septic mice.
Figure 6: DCA rescues systemic energy crisis during polymicrobial infection