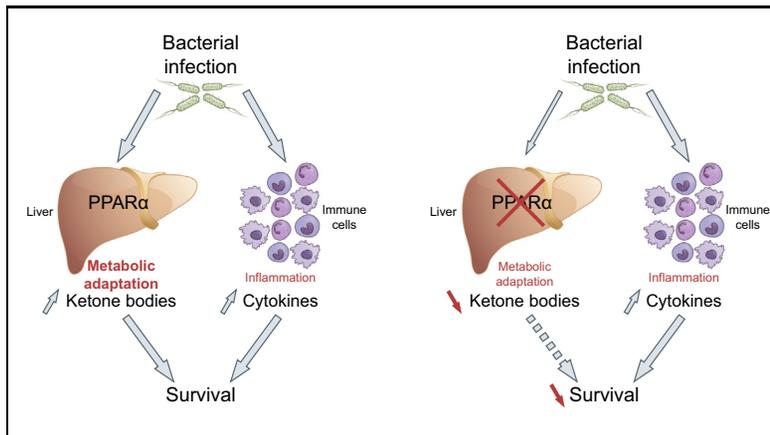


Hepatic PPAR α is critical in the metabolic adaptation to sepsis

Graphical abstract



Highlights

- Sepsis activates hepatic PPAR α .
- PPAR α plays a protective role in sepsis.
- *Ppar α* -deficiency impairs FA utilization in the liver during sepsis.
- Hepatocyte *Ppar α* -deficiency worsens the outcome of bacterial infection.
- PPAR α activity is lower in livers of non-surviving critically ill patients.

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Lay summary

As the main cause of death in critically ill patients, sepsis remains a major health issue lacking efficacious therapies. While current clinical literature suggests an important role for inflammation, metabolic aspects of sepsis have mostly been overlooked. Here, we show that mice with an impaired metabolic response, due to deficiency of the nuclear receptor PPAR α in the liver, exhibit enhanced mortality upon bacterial infection despite a similar inflammatory response, suggesting that metabolic interventions may be a viable strategy for improving sepsis outcomes.



Hepatic PPAR α is critical in the metabolic adaptation to sepsis

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Background & Aims: Although the role of inflammation to combat infection is known, the contribution of metabolic changes in response to sepsis is poorly understood. Sepsis induces the release of lipid mediators, many of which activate nuclear receptors such as the peroxisome proliferator-activated receptor (PPAR) α , which controls both lipid metabolism and inflammation. We aimed to elucidate the previously unknown role of hepatic PPAR α in the response to sepsis.

Methods: Sepsis was induced by intraperitoneal injection of *Escherichia coli* in different models of cell-specific *Ppara*-deficiency and their controls. The systemic and hepatic metabolic response was analyzed using biochemical, transcriptomic and functional assays. PPAR α expression was analyzed in livers from elective surgery and critically ill patients and correlated with hepatic gene expression and blood parameters.

Results: Both whole body and non-hematopoietic *Ppara*-deficiency in mice decreased survival upon bacterial infection. Livers of septic *Ppara*-deficient mice displayed an impaired metabolic shift from glucose to lipid utilization resulting in more severe hypoglycemia, impaired induction of hyperketonemia and increased steatosis due to lower expression of genes involved in fatty acid catabolism and ketogenesis. Hepatocyte-specific deletion of PPAR α impaired the metabolic response to sepsis and was sufficient to decrease survival upon bacterial infection. Hepatic *PPARA* expression was lower in critically ill patients and correlated positively with expression of lipid metabolism genes, but not with systemic inflammatory markers.

Conclusion: During sepsis, *Ppara*-deficiency in hepatocytes is deleterious as it impairs the adaptive metabolic shift from glucose to FA utilization. Metabolic control by PPAR α in hepatocytes plays a key role in the host defense against infection.

Lay summary: As the main cause of death in critically ill patients, sepsis remains a major health issue lacking efficacious therapies. While current clinical literature suggests an important role for inflammation, metabolic aspects of sepsis have mostly been overlooked. Here, we show that mice with an impaired metabolic response, due to deficiency of the nuclear receptor PPAR α in the liver, exhibit enhanced mortality upon bacterial infection despite a similar inflammatory response, suggesting that metabolic interventions may be a viable strategy for improving sepsis outcomes.

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Introduction

Sepsis, the systemic inflammatory response to poorly controlled infection, causes significant morbidity/mortality.¹ Sepsis is often complicated by multiple organ failure, requiring intensive care. Recently, mortality in sepsis has decreased largely due to improved supportive strategies for critically ill patients, such as mechanical ventilation, renal replacement therapy and antibiotics. While current therapeutic strategies targeting the inflammatory response have been disappointing,^{1,2} metabolic interventions, such as intensive insulin therapy³ and controlled caloric deficit through delayed administration of parenteral nutrition,⁴ have shown some promise, suggesting that appropriate adaptation of energy metabolism contributes to proper defense against pathogens.⁵

The early pro-inflammatory response to infection requires glycolysis and non-insulin-mediated glucose uptake to rapidly meet the high energy demand of innate immune cells.⁶ In this phase, hepatic gluconeogenesis increases to maintain plasma glucose concentrations.⁷ As sepsis sets in, plasma free fatty acid (FFA) and glycerol levels rise due to enhanced adipose tissue

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(AT) lipolysis.⁷ In response, organs such as the liver, muscle and heart, shift from glucose to fatty acid utilization⁸ and enhance mitochondrial activity.⁹

PPAR α is a nuclear receptor activated by fatty acids (FAs) and derivatives regulating both metabolism and inflammation.¹⁰ PPAR α is highly expressed in metabolic tissues, such as the liver, heart, kidney and muscle, the vasculature (endothelial cells, smooth muscle cells) as well as in the immune system (monocytes/macrophages, neutrophils, *etc.*).¹¹ During the fed-to-fasted transition, hepatic PPAR α expression increases¹² and is activated by the influx of AT-released FFAs, orchestrating a shift from glucose to FA utilization driving ketone body and glucose production by the liver.^{12,13}

PPAR α also exerts anti-inflammatory activities by inhibiting NF κ B and AP1 signaling.¹⁰ Consequently, *Ppara*-deficient mice display a prolonged inflammatory response upon sterile inflammation.¹⁴ Conversely, upon polymicrobial infection, *Ppara*-deficient mice display decreased survival associated with a reduced pro-inflammatory immune response,¹⁵ through mechanisms involving non-hematopoietic PPAR α .¹⁶ Although cardiac PPAR α contributes to sepsis survival by increasing cardiac performance and FA oxidation,¹⁷ the specific contribution of PPAR α in the liver has not yet been addressed.

Materials and methods

Mice

Whole body *Ppara* knockout (KO) and wild-type (WT) littermate C57BL/6J mice (gift of F. Gonzalez¹⁸) were bred at the Institut Pasteur de Lille (IPL) transgenic rodent facility (SPF status). Hepatocyte-specific *Ppara*-deficient (*Ppara fl/fl*, Albumin-Cre⁺, *Ppara*^{hepKO}) and corresponding littermate controls (*Ppara fl/fl*, Albumin-Cre⁻) mice were generated and bred at INRA's rodent facility (conventional health status, Toxalim, Toulouse, France), as described.¹⁹ In survival experiments comparing *Ppara*^{hepKO} to whole body *Ppara* KO mice, all mice were bred at the INRA transgenic rodent facility. Infection experiments were conducted on female (8–12 weeks) mice fed a standard rodent diet (Safe 04 U8220G10R) at IPL facility. All animals were housed under temperature-controlled (at 22–24 °C), 12 h light/dark cycle conditions. Experimental procedures were approved by the Nord Pas-de-Calais Ethics committee (CEEA 75, APAFIS#7738-2015121713177853 v9).

Bacterial cultures and infection

DH5 α *Escherichia coli* were grown in LB Broth at 37 °C to an OD₆₀₀ of 0.6, equivalent to 4–5 \times 10⁸ live bacteria/ml, collected by centrifugation, washed once with sterile PBS, and resuspended in cold PBS at 4–7 \times 10⁸ live bacteria/ml. Concentration and viability were confirmed by plaque assay colony counting. Mice (10–15 mice/group) were injected intraperitoneally (i.p.) with 4–7 \times 10⁸ live bacteria/mouse in 1 ml PBS and survival rates were monitored every 6 h for a week. For biochemical characterization, mice were killed by cervical dislocation 16 h after infection and serum and livers collected.

Human study

Post-mortem liver biopsies were taken from patients in an intensive care unit (n = 46), who were enrolled in a randomized controlled trial.³ All deaths occurred after multidisciplinary decision to restrict therapy when further treatment was judged to be futile. During this trial, for post-mortem tissue sampling

for academic purposes, each patient or his/her legal representative consented upon admission, via a hospital-wide information and consent procedure that required active opting-out when not consenting. Opting-out remained possible until time of death. This strategy was approved by the Institutional Ethical Review Board. Liver samples were harvested within minutes after death. Control liver biopsies from 20 demographically matched patients (written informed consent obtained prior to the procedure) undergoing an elective restorative rectal resection were obtained. All protocol and consent forms were approved by the Institutional Ethical Review Board of the KU Leuven (ML1094, ML2707). Baseline and outcome variables are indicated in Table S1. Liver biopsies were taken from liver segment IVb, snap-frozen in liquid nitrogen, and stored at –80 °C until analysis (see [supplementary information](#)).

Biochemical analysis

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Biolabo), FFA (Diasys), β -hydroxybutyrate (ketone bodies) (Thermo Fisher) were determined by colorimetric assays. Plasma TNF α , Kc/Cxcl1 and IL-6 protein levels were measured by ELISA (R&D Systems) and myeloperoxidase (MPO) activity as described.²⁰ Cytokine levels were quantified in serum of critically ill patients collected on the day of biopsy (last day alive in the intensive care unit).²¹

Liver transcriptomic analysis

RNA extraction and analysis are detailed in the [supplementary information](#). RNA microarray analysis was performed using Mouse Gene 2.0ST arrays. Array data processing was performed using Bioconductor in the R-environment (r-project.org). Gene expression was calculated after normalizing signal using robust multichip averaging in the *oligo* package.²² Differential gene expression between groups (*Ppara* WT uninfected, *Ppara* WT infected, *Ppara* KO uninfected and *Ppara* KO infected) was assessed using the *limma* package²³ with a threshold of 5% false discovery rate. Differentially expressed genes were clustered using the *hopach* package²⁴ with the cosine distance metric. Gene Ontology (GO) term enrichment of selected clusters was performed using the *clusterProfiler* package.^{25,26} For KEGG pathway analysis, data were analyzed using Partek software.

Mitochondrial respiration

Liver samples (125 mg) were minced and dounce homogenized by 8–10 strokes in ice-cold MRO5 respiratory buffer (20 mM HEPES, 10 mM KH₂PO₄, 110 mM sucrose, 20 mM taurine, 60 mM K-lactobionate, 0.5 mM EGTA, 3 mM MgCl₂·6H₂O, 1 g/L BSA (fatty acid free)). Liver homogenates (50 μ l) were introduced into O2K oxygraph chambers (Oroboros Instruments, Innsbruck, Austria) to assess oxygen consumption in presence of pyruvate (5 mM) and malate (2 mM) (state 2 respiration), followed by ADP (0.5 mM) (state 3 respiration). To measure β -oxidation, octanoylcarnitine (25 μ M) and malate (2 mM) were added, followed by ADP (0.5 mM). The respiratory control ratio was calculated as the state 3:state 2 ratio. Finally, cytochrome c (10 μ M) was added to measure mitochondrial integrity.

Histological analysis

Frozen liver samples were embedded in Frozen Section Medium (NEG-50, Richard-Allan Scientific), stained with anti-Moma2 (ab33451, abcam) or anti-Ly6G (1A8, BD Pharmingen) antibodies and counterstained with hematoxylin. Ly6G and Moma2

staining areas were determined by color detection using a Nikon Eclipse Ti microscope and a color video camera coupled to the NIS Elements software (Nikon).

Statistical analysis

Groups were compared using the Log-rank (Mantel-Cox) Test (survival test), 2-way ANOVA, 2-tailed non-paired *t* tests or non-parametric Wilcoxon tests (mouse and human studies) and expressed as means ± SEM using the GraphPad Prism software. Significance of correlations between parameters was assessed by calculation of the Pearson (*r*) correlation coefficient using GraphPad Prism software.

For further details regarding the materials and methods used, please refer to the [CTAT table](#) and [supplementary information](#).

Results

Whole body and non-hematopoietic *Ppara*-deficiency aggravate mortality upon bacterial infection

To evaluate the role of PPARα in sepsis, whole body *Ppara*-deficient (KO) and WT mice were inoculated with Gram-negative *Escherichia coli*. Three days after infection, mortality was 0% in *Ppara* WT compared to 75% in *Ppara* KO mice (Fig. 1A). As PPARα is expressed in immune cells and exerts

anti-inflammatory actions, we assessed whether restoration of PPARα in the hematopoietic compartment conferred protection to infection. Chimeric mice were generated by transplanting lethally irradiated whole body *Ppara* KO and *Ppara* WT mice with *Ppara* WT bone marrow (WTbm->KO and WTbm->WT, respectively, see [supplementary information](#)). Surprisingly, *Ppara*-deficiency in non-hematopoietic cells was still associated with increased mortality in response to infection (20% *Ppara* WTbm->WT compared to 70% *Ppara* WTbm->KO mortality 3 days after infection; Fig. S1A), revealing a protective role for non-hematopoietic PPARα in response to sepsis.

Whole body *Ppara*-deficiency results in impaired metabolic and inflammatory responses to bacterial sepsis

To understand the mechanisms of increased mortality of *Ppara* KO mice in response to sepsis, the systemic metabolic and inflammatory response to bacterial infection was characterized. Plasma FFA levels significantly increased during sepsis to a similar extent in *Ppara* WT and KO mice at 16 h after infection (Fig. 1B), when maximal changes in metabolic parameters are observed (Fig. S2). Plasma AST and ALT levels were similar in *Ppara* WT and KO mice, suggesting comparable tissue damage (Fig. 1C,D). Conversely, plasma ketone body levels increased strongly upon infection in WT, but not in *Ppara* KO mice (Fig. 1E). In addition, infection-induced hypoglycemia was more

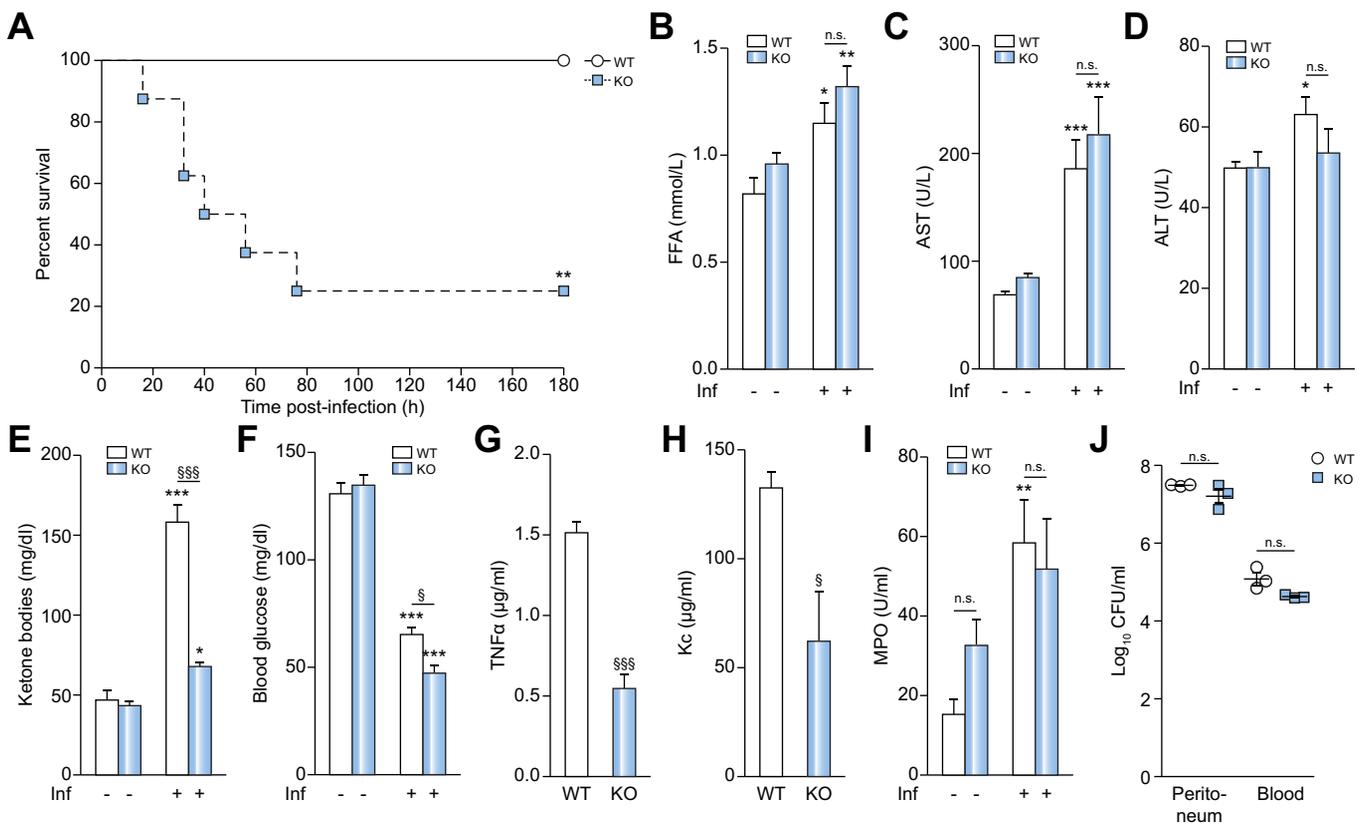


Fig. 1. Whole body *Ppara*-deficiency enhances mortality upon bacterial sepsis and impairs the metabolic and inflammatory response to bacterial infection. *Ppara* WT and KO mice were injected (ip) with vehicle (PBS) (–) or *E. coli* (4×10^8 live bacteria) (Inf) (+). (A) Survival was followed for 8 days after injection (n = 8–10 mice/group). Plasma was collected (G,H) 6 h or (B–F, I–J) 16 h after injection and (B) FFAs, (C) AST, (D) ALT, (E) ketone bodies, (F) blood glucose, (G) TNFα, (H) Kc/Cxcl1 concentrations and (I) MPO activity were measured as described in methods. (J) Bacterial levels in peritoneal fluid and blood were measured by retro-culture (n = 3 mice/group). Statistical differences are indicated (Survival test: Log-rank (Mantel-Cox) Test: ***p* < 0.01. 2-way ANOVA: ****p* < 0.001, ***p* < 0.01 and **p* < 0.05 for effect of infection; §§§*p* < 0.001; §§*p* < 0.01; §*p* < 0.05 for genotype effect; n.s.: non-significant). ALT, alanine aminotransferase; AST, aspartate aminotransferase; FFAs, free fatty acids; KO, knockout; MPO, myeloperoxidase; WT, wild-type.

pronounced in *Ppara* KO mice, suggesting defective glucose homeostasis (Fig. 1F). Interestingly, infected chimeric *Ppara* WTbm->KO mice also displayed lower plasma ketone body levels and more pronounced hypoglycemia compared to WTbm->WT controls (Fig. S1B,C). These results demonstrate that the metabolic response to sepsis depends on PPAR α expression in non-hematopoietic derived cells. In line with previous observations,¹⁵ *Ppara*-deficiency resulted in decreased, rather than increased, inflammatory responses as illustrated by lower plasma tumor necrosis factor alpha (TNF α) and chemokine Kc/Cxcl1 levels 6 h after infection (Fig. 1G,H). Since hepatic PPAR α contributes to systemic inflammation,²⁷ histological analysis of livers from infected *Ppara* WT and KO mice was performed. Surprisingly, whole body *Ppara*-deficiency did not affect infection-induced neutrophil or monocyte/macrophage recruitment in livers as assessed by Ly6G and Moma2 stainings (Fig. S3). Plasma MPO activity (Fig. 1I) and bacterial dissemination into peritoneum and blood (Fig. 1J) were also unaffected by whole body *Ppara*-deficiency.

Whole body *Ppara*-deficiency modulates the hepatic metabolic and inflammatory gene expression responses to bacterial sepsis

To identify molecular pathways regulated by PPAR α in response to sepsis, microarray analysis was performed on livers from whole body *Ppara* WT and KO mice 16 h after infection and from uninfected controls. In addition to inflammation-related pathways, the GO term "Oxidation-Reduction Process," was altered by infection in *Ppara* WT mice, suggesting altered mitochondrial function (Fig. 2A). Unbiased clustering analysis of genes affected by sepsis in either whole body *Ppara* WT or KO mice revealed 3 gene clusters (Fig. 2B,C and Fig. S4). While cluster 1 (black) and cluster 3 (purple) contained genes whose response to infection (induced or suppressed, respectively) was largely maintained in whole body *Ppara* KO mice, genes in cluster 2 (green) were either unresponsive or responded in the opposite direction in *Ppara* KO mice despite strong regulation in *Ppara* WT mice upon infection. Cluster 1 was enriched in genes associated with inflammatory response and reactive oxygen species metabolism, and cluster 3 was enriched in genes involved in cellular glucuronidation and response to xenobiotics (Fig. S4). Interestingly, Cluster 2 contained many genes related to FA metabolism and circadian rhythm, many of which are *bona fide* PPAR α targets. Indeed, carnitine palmitoyl transferase (*Cpt1a*), acyl-Coenzyme A oxidase (*Acox1*) and pyruvate dehydrogenase kinase (*Pdk4*), were induced by sepsis in *Ppara* WT mice, but either reduced or unchanged by infection in *Ppara* KO mice (Fig. 2D). Together, these results demonstrate that the most profound transcriptional differences between whole body *Ppara* WT and KO mice in response to sepsis are related to regulation of PPAR α 's metabolic targets, rather than to effects on inflammation or other pathways.

Whole body *Ppara*-deficiency impairs the hepatic metabolic shift from glucose to lipid utilization during bacterial sepsis

To further characterize the metabolic role of PPAR α during sepsis, glucose and lipid metabolism gene expression was measured in livers of infected *Ppara* WT and KO mice (Fig. S5). Sepsis increased expression of genes involved in gluconeogenesis, such as phosphoenolpyruvate carboxykinase (*Pck1*) and fructose-1,6-bisphosphatase (*Fbp1*), although the magnitude of response was lower in *Ppara* KO mice (*Ppara* WT vs. KO: *Pck1*:

3.00 vs. 1.67; *Fbp1*: 2.81 vs. 1.44-fold induction) (Fig. S5A). However, sepsis raised expression of *Pdk4*, which inhibits the final step of glycolysis, >100-fold in a PPAR α -dependent manner (Fig. S5B). Similarly, sepsis strongly increased (>12-fold) hepatic adipose triglyceride lipase (*Atgl/Pnpla2*) expression in *Ppara* WT mice only (Fig. S5C). Moreover, sepsis increased several PPAR α target genes involved in hepatic lipid metabolism such as FA uptake (e.g. *Fatp1*, *Cd36*), FA activation (e.g. *Acs11*), peroxisomal FA β -oxidation (*Acox1*) and mitochondrial FA transport and β -oxidation (e.g. *Cpt1a*, *Lcad*) (Fig. S5D). Interestingly, except for *Cpt1a*, sepsis-mediated induction of these genes was PPAR α -dependent. Moreover, expression of 3-hydroxy-3-methylglutaryl-Coenzyme A synthase (*Hmgcs2*), the rate-limiting enzyme for β -hydroxybutyrate production, was lower in *Ppara*-deficient mice (Fig. S5E). Furthermore, infected chimeric *Ppara* WTbm->KO mice, expressing *Ppara* only in hematopoietic cells, also displayed lower hepatic expression of *Pdk4*, *Cd36*, *Acox1*, *Lcad*, *Hmgcs2* and *Atgl* compared to WTbm->WT mice (Fig. S5F,G), demonstrating that the metabolic transcriptional response to sepsis in the liver depends on non-hematopoietic PPAR α .

Functional analysis of liver mitochondria also suggested impaired FA utilization in whole body *Ppara* KO mice. Unaltered citrate synthase activity indicated that *Ppara*-deficiency did not impact mitochondrial quantity (Fig. S5H). Uninfected whole body *Ppara* KO livers displayed lower respiratory control ratio values upon incubation with pyruvate and malate and, to a lesser extent, with octanoylcarnitine and malate compared to uninfected *Ppara* WT (Fig. 2E). However, bacterial infection shifted the respiration rate from pyruvate/malate to octanoylcarnitine/malate in *Ppara* WT, whereas this shift was less apparent in *Ppara* KO livers (Fig. 2E). Together, these data indicate that sepsis shifts the transcriptional and metabolic program from glucose to lipid utilization and this is impaired in whole body *Ppara* KO livers.

Hepatocyte-specific PPAR α expression is required for proper metabolic response and survival upon bacterial infection

Because the results of both transcriptomic and metabolic analysis suggested that hepatic PPAR α is activated upon sepsis, we postulated that hepatic PPAR α may be critical to the organism's response to infection. Therefore, hepatocyte-specific *Ppara*^{hepKO} were subjected to bacterial infection and their response compared to *Ppara*^{hepWT} and whole body *Ppara* KO mice. Similar to whole body *Ppara* KO mice, *Ppara*^{hepKO} showed increased mortality compared to *Ppara*^{hepWT} mice (Figs. 1A and 3A). Infected *Ppara*^{hepKO} mice also displayed lower ketone body levels compared to *Ppara*^{hepWT}, despite similar levels of plasma FFA, ALT and AST (Fig. 3B–D and Fig. S6). Liver triglyceride (TG) content increased more markedly upon bacterial infection in *Ppara*^{hepKO} vs. *Ppara*^{hepWT} mice (3.1 vs. 2.2-fold, Fig. 3F), suggesting defective hepatic lipid utilization. Surprisingly, infected *Ppara*^{hepKO} mice displayed no significant differences in glycemia (Fig. 3E), nor plasma levels of inflammatory cytokines (TNF- α , Kc and IL-6) at both 5 h and 16 h after infection (Fig. 3G,H and Fig. S7) compared to *Ppara*^{hepWT}. In addition, bacterial dissemination in peritoneum and blood did not differ between *Ppara*^{hepWT} and *Ppara*^{hepKO} mice (Fig. 3I). These data suggest that the increased mortality observed in whole body *Ppara* KO is unlikely caused by the different glycemic and systemic inflammatory responses.

Similar to whole body *Ppara* KO mice, *Ppara*^{hepKO} mice displayed major defects in sepsis-modulated regulation of several genes involved in hepatic glucose (i.e. *Pdk4*, Fig. 4A) and lipid

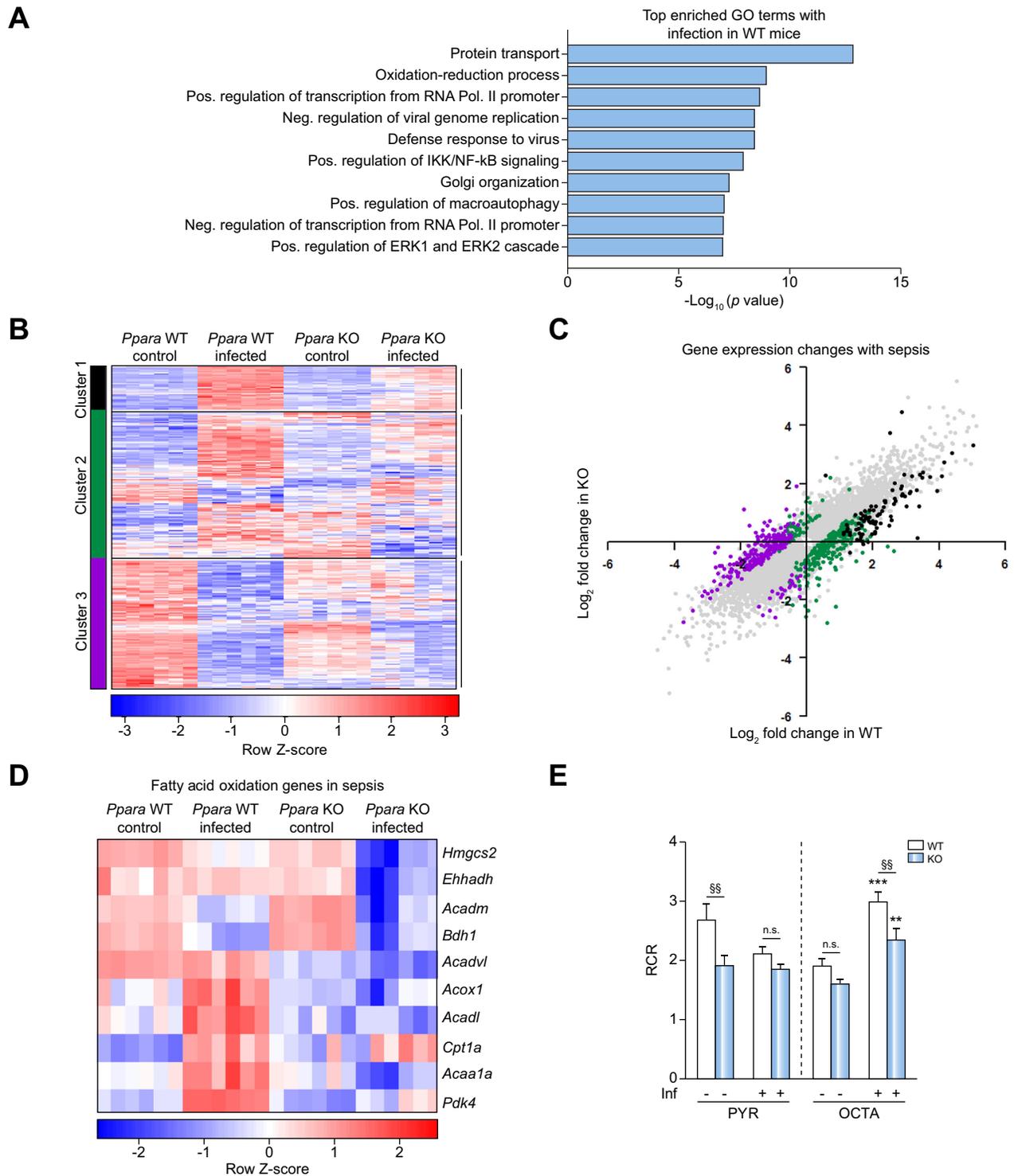


Fig. 2. Whole body *Ppara*-deficiency modulates hepatic metabolic and inflammatory transcriptional responses to infection. Livers from *Ppara* WT and KO mice injected (ip) with vehicle (PBS) (Control) or *E. coli* (4×10^8 live bacteria) (Infected) were collected after 16 h and transcriptomic analysis was performed ($n = 6$ mice/group). (A) Top enriched GO terms for genes differentially expressed comparing infection vs. control in *Ppara* WT mice. (B) Hierarchical clustering and (C) dot plot of genes affected by infection in *Ppara* WT or KO mice. (D) Selected genes from the “fatty acid oxidation pathway” GO term of Cluster 2. (E) Mitochondrial respiration measured with (PYR or OCTA) as described in methods ($n = 7-8$ mice/group). Statistical differences are indicated (2-way ANOVA: *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ for effect of infection; §§§ $p < 0.001$; §§ $p < 0.01$; § $p < 0.05$ for genotype effect; n.s.: non-significant). GO, Gene Ontology; KO, knockout; PYR, pyruvate/malate; OCTA, octanoylcarnitine/malate; WT, wild-type.

metabolism (e.g. *Cd36*, *Acox1*, *Vlca*d and *Lca*d, Fig. 4B), as well as *Ppara* itself (Fig. 4C). Interestingly, the induction of hepatic *Atgl* and *Cpt1a* expression upon sepsis was independent of hepato-

cyte PPAR α (Fig. 4B). Conversely, hepatic *Hmgcs2* expression was virtually undetectable upon sepsis in *Ppara*^{hepKO} mice (Fig. 4B). Bacterial infection increased *Atgl*, but not *Hmgcs2* or

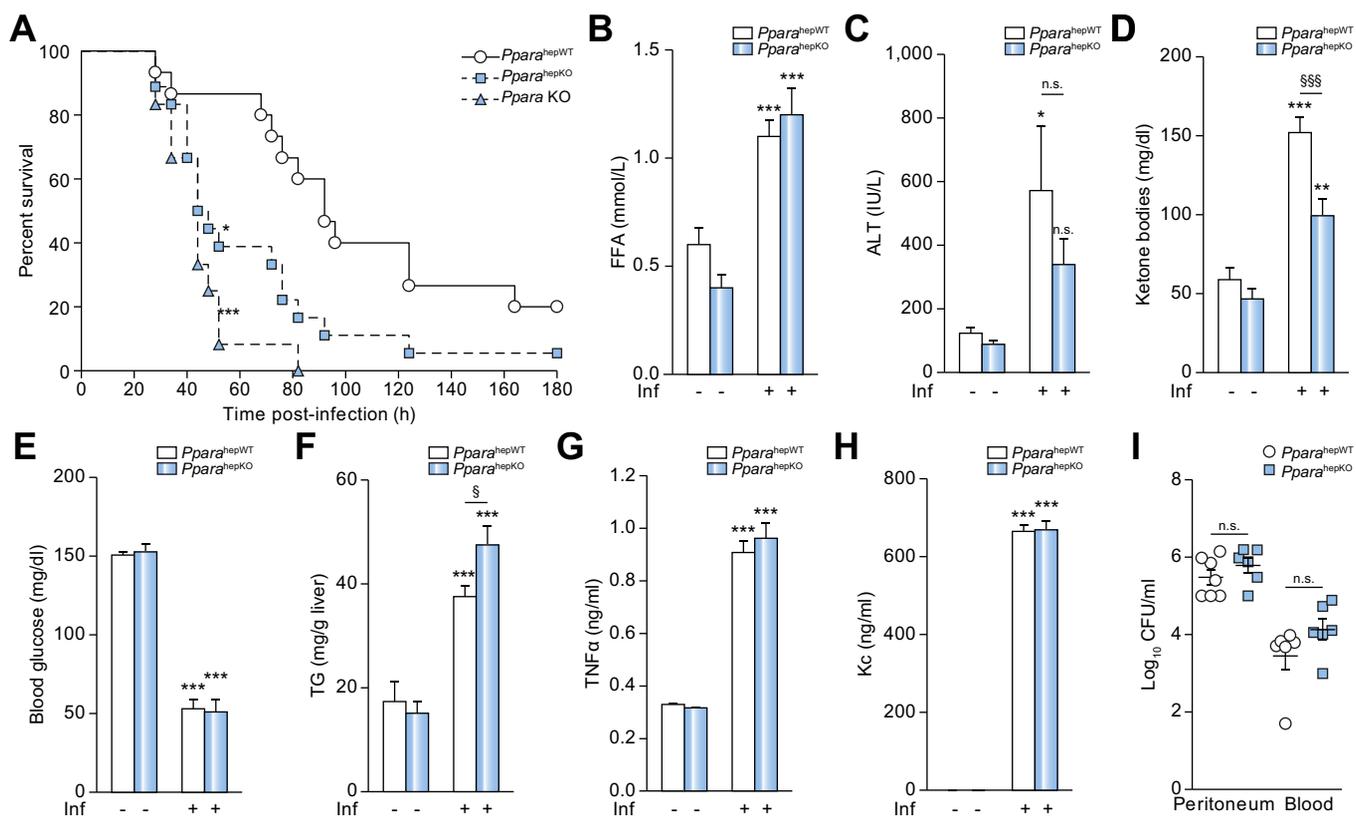


Fig. 3. Hepatocyte-specific *Ppara*-deficiency results in metabolic perturbations and aggravates mortality during bacterial infection. (A) *Ppara*^{hepWT}, *Ppara*^{hepKO}, and *Ppara* KO mice were injected (ip) with *E. coli* (7×10^8 live bacteria). Survival was followed for 8 days after bacterial infection (n = 12–18 mice/group). Plasma was collected (G, H) 5 h or (B–E) 16 h after bacterial infection and (B) FFAs, (C) ALT, (D) ketone bodies, (E) blood glucose, (G) TNF α and (H) Kc/Cxcl1 concentrations were measured as described in methods. Livers, peritoneal fluid and blood were collected 16 h after injection and (F) TG content and (I) bacterial levels were determined as described in methods (n = 7–8 mice/group). Statistical differences are indicated (Survival test: Log-rank (Mantel-Cox) Test. **p* < 0.05, ****p* < 0.001 compare to survival of *Ppara*^{hepWT}; 2-way ANOVA: §§§*p* < 0.001; §§*p* < 0.01; §*p* < 0.05 for genotype effect; n.s.: non-significant). ALT, alanine aminotransferase; FFAs, free fatty acids; KO, knockout; TG, triglycerides; WT, wild-type.

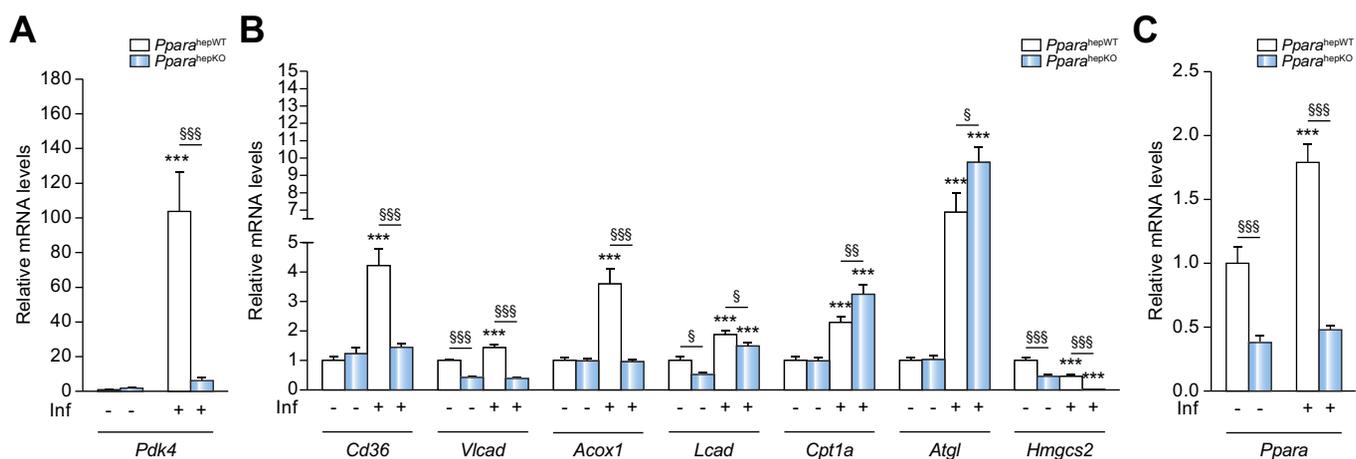


Fig. 4. Hepatocyte-specific *Ppara*-deficiency impairs the response of lipid metabolism genes to bacterial infection. *Ppara*^{hepWT}, *Ppara*^{hepKO} mice were injected (ip) with vehicle (PBS) (–) or with *E. coli* (6×10^8 live bacteria) (inf) (+). Livers were collected 16 h after infection and hepatic mRNA expression of (A) *Pdk4*, (B) genes involved in lipid metabolism and (C) *Ppara* was measured. Statistical differences are indicated (2-way ANOVA: ****p* < 0.001, ***p* < 0.01 and **p* < 0.05 for effect of infection; §§§*p* < 0.001; §§*p* < 0.01; §*p* < 0.05 for effect of genotype; n.s.: non-significant).

Dgat1 expression at the protein level (Fig. S8). Moreover, Hmgcs2 protein levels were lower in *Ppara*^{hepKO} mice both in uninfected and infected conditions. Whereas Atgl protein induction appeared less pronounced upon sepsis in *Ppara*^{hepKO} mice,

Dgat1 protein expression only increased in *Ppara*^{hepKO} mice, both consistent with increased hepatic TG content. Together, these data indicate that hepatocyte-specific PPAR α -deficiency profoundly affects the hepatic metabolic response to infection.

***Ppara*-deficiency regulates the inflammatory response, but does not promote innate immune cell recruitment or inhibit autophagy in the liver upon bacterial sepsis**

To determine the contribution of non-hematopoietic PPAR α to systemic inflammation in sepsis, expression of inflammatory genes was measured in livers and spleens from whole body *Ppara* KO, chimeric *Ppara* WTbm->KO mice and their respective controls. The induction of *Tnfa*, *Mcp1*, *Il6* and *Ifng* upon sepsis was markedly attenuated in livers (Fig. 5A), and, to a lesser extent, in spleens (Fig. S9) of whole body *Ppara* KO mice compared to controls. Likewise, induction of the vascular inflammation markers *Vcam1* and *Icam1* (Fig. 5B) and the mitochondrial anti-oxidant enzyme *Sod2* (Fig. 5C) was lower in livers of whole body *Ppara* KO mice than in their WT counterparts. By contrast, the hepatic and vascular inflammatory and anti-oxidant responses (Fig. 5D,E), as well as MPO activity (Fig. 5F) and immune cell recruitment (Fig. S10) were similar in chimeric *Ppara* WTbm->KO and WTbm->WT mice, despite differing survival outcomes (Fig. 1A and Fig. S1A). Interestingly, in *Ppara*^{hepKO} mice, the induction of inflammatory genes (Fig. 6A) upon sepsis was either higher (*Tnfa*) or unchanged (*Mcp1*, *Il6*), whereas neutrophil and monocyte/macrophage recruitment was again similar (Ly6G and Moma2 stainings, Fig. 6C-E) upon infection. Altogether, these data indicate that the attenuated inflammatory response observed in livers of whole body *Ppara*

KO mice depends on hematopoietic, but not hepatic *Ppara* expression.

Because autophagy may play a protective role during sepsis²⁸ and PPAR α mediates fasting-induced autophagy,²⁹ markers of autophagy were assessed in whole body and hepatocyte-specific *Ppara*-deficient mice. Whereas sepsis increased expression of certain autophagy genes, their regulation was not different in whole body *Ppara* KO mice (Fig. S11A). Moreover, hepatocyte-specific *Ppara*-deficiency (Fig. S11B-C) resulted in more pronounced induction of *Ulk1*, *Atg5*, *Bnip3* and *Becn1* gene expression and Lc3b-II/I protein ratio, suggestive of a compensatory induction of autophagy to combat the deleterious response to sepsis in *Ppara*^{hepKO} mice. Altogether, these data indicate that hepatocyte PPAR α expression contributes to protection against sepsis by controlling the systemic metabolic response.

PPAR α expression and activity is lower in livers of critically ill patients

To determine whether hepatic PPAR α expression is altered in critically ill humans, livers from non-surviving critically ill patients and healthy controls were analyzed for PPARA and its targets gene expression (Fig. 7A). Interestingly, PPARA expression was lower in livers of critically ill patients. Moreover, expression of genes involved in TG lipolysis (*ATGL*), glucose oxidation (*PDK4*),

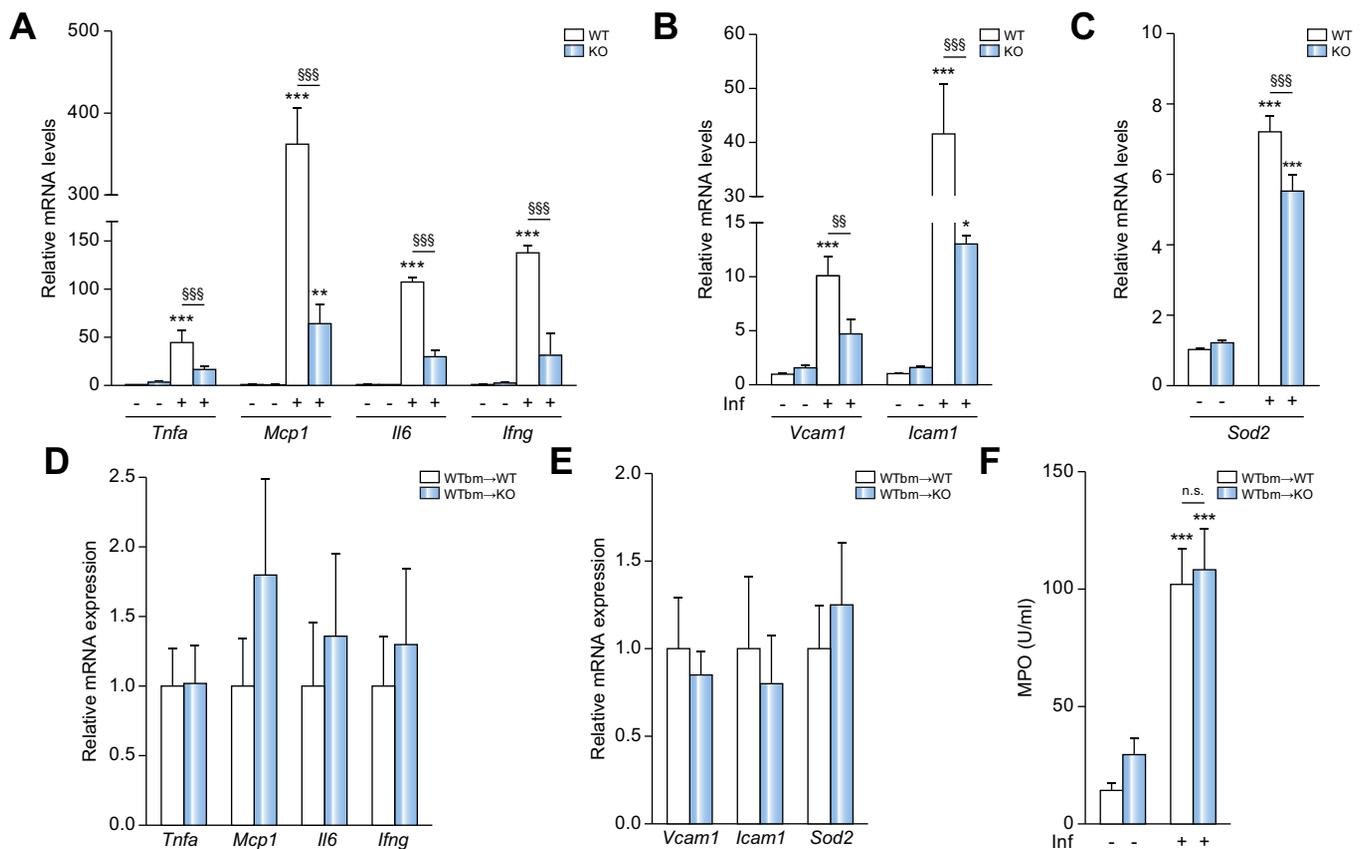


Fig. 5. The sepsis-induced inflammatory response occurs through hematopoietic PPAR α . (A-C) Whole body *Ppara* WT and KO mice or (D-F) chimeric *Ppara* WTbm->WT and WTbm->KO mice were injected (ip) with vehicle (PBS) (-) or *E. coli* (4×10^8 live bacteria) (Inf) (+). Livers were collected 16 h after infection and mRNA expression of genes involved in (A, D) inflammation, (B, E) endothelial activation, and (C, E) oxidative stress, were analyzed using RT-qPCR. (F) Plasma MPO activity was measured as described in methods (n = 8 mice/group). Statistical differences are indicated (2-way ANOVA: *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ for effect of infection; \$\$\$ $p < 0.001$; \$\$ $p < 0.01$; \$ $p < 0.05$ for genotype effect; ns: non-significant). KO, knockout; MPO, myeloperoxidase; RT-qPCR, quantitative reverse transcription PCR; WT, wild-type.

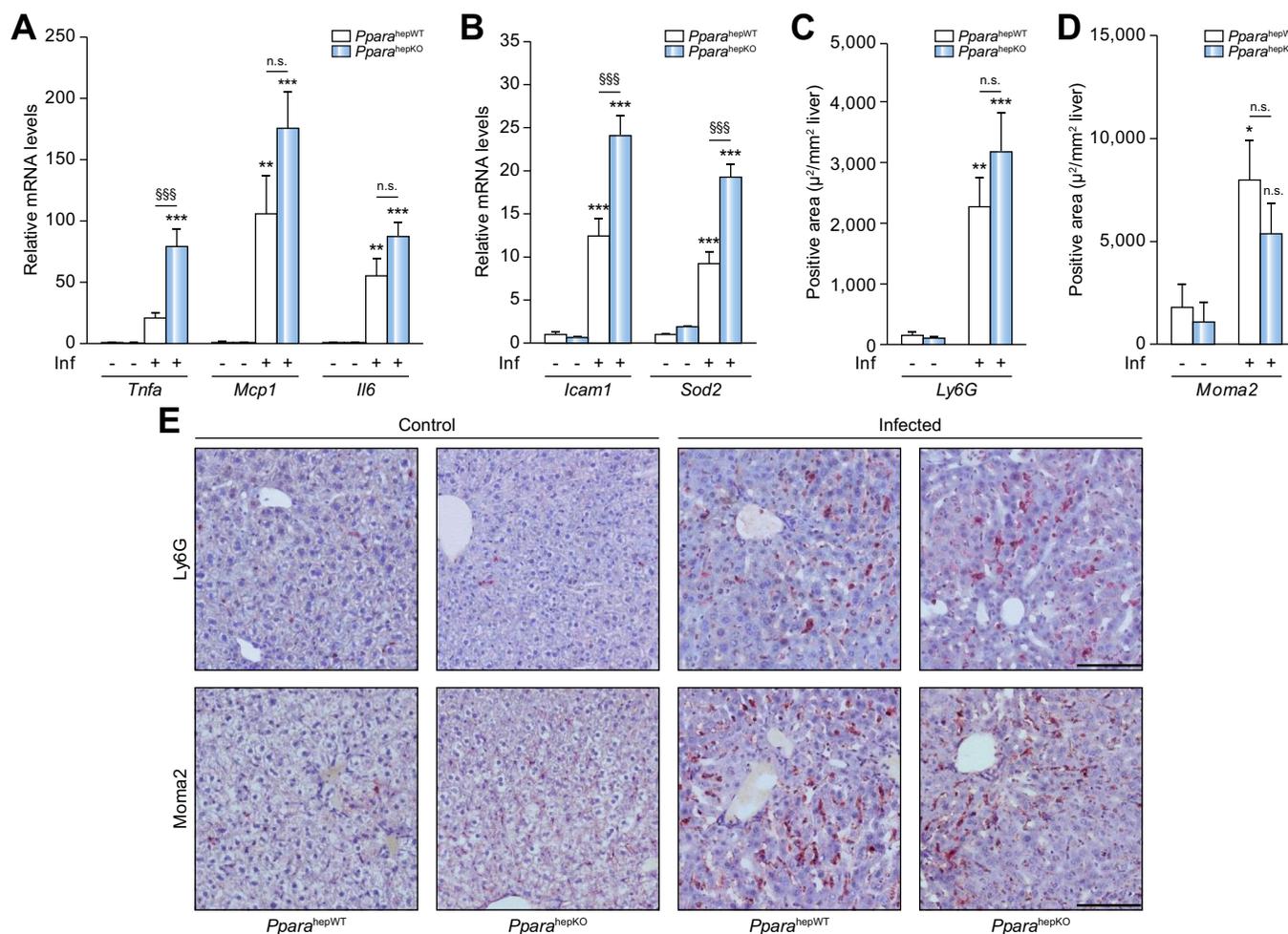


Fig. 6. Hepatocyte-specific *Ppara*-deficiency modulates the inflammatory response in the liver without affecting innate immune cell recruitment upon bacterial infection. *Ppara*^{hepWT}, *Ppara*^{hepKO} mice were injected (ip) with vehicle (PBS) (–) or *E. coli* (6x10⁸ live bacteria) (inf) (+). Livers were collected 16 h after infection and mRNA expression of genes involved in (A) inflammation, (B) endothelial activation and oxidative stress were analyzed using RT-qPCR. Liver sections stained for Ly6G (E, top panel) and Moma2 (E, bottom panel) and quantified (C, D), respectively, using NIS Element software (n = 7–8 mice/group) (Bar = 100 μm). Statistical differences are indicated (2-way ANOVA: ***p < 0.001, **p < 0.01 and *p < 0.05 for effect of infection; §§§p < 0.001; §§p < 0.01; §p < 0.05 for genotype effect; n.s.: non-significant). KO, knockout; RT-qPCR, quantitative reverse transcription PCR; WT, wild-type.

FA uptake and β-oxidation (*CD36*, *LCAD*) and ketogenesis (*HMGCS2*) were also lower and correlated with *PPARA* expression (Fig. 7A,B). Surprisingly, *PPARA* mRNA levels did not correlate with serum cytokine levels in these patients (Fig. 7B), suggesting a critical role for hepatic *PPARα* in the metabolic, but not in the inflammatory response to sepsis in critically ill human patients.

Discussion

Our results demonstrate that *PPARα* protects against sepsis primarily by controlling the metabolic response in hepatocytes, by shifting its energy utilization from glucose to FAs and increasing ketogenesis.

The host defense toward bacterial infection is a complex response involving resistance (to limit microbial burden) and tolerance (to limit tissue injury and organ dysfunction) mechanisms. These processes require metabolic reprogramming in immune and non-immune cells.³⁰ Resistance is characterized by a balance between local activation of pro-inflammatory pathways to restrain and eliminate invading pathogens and anti-inflammatory pathways required to prevent exaggerated

systemic inflammation.^{31,32} Our data show that whole body *Ppara*-deficiency attenuates organ and systemic inflammatory responses upon infection. This contrasts with previous observations in models of sterile chronic and acute inflammation in which *Ppara*-deficiency results in exacerbated inflammatory responses to endotoxemia in vascular, splenic and liver cells.³³ Accordingly, whole body *Ppara*-deficiency also resulted in a decreased pro-inflammatory response and survival in a cecal ligation sepsis model.¹⁵

Interestingly, our data and that of others indicate that non-hematopoietic *PPARα* action is an important determinant of survival. Studies by Standage *et al.* suggest that heart *PPARα* expression contributes to survival during sepsis by increasing cardiac performance and FA oxidation.^{16,17} In the present study, we demonstrate that *PPARα* expression and activation in hepatocytes, but not immune cells, contributes to protection against sepsis by promoting an appropriate metabolic response, hence improving survival. Sepsis activates hepatic *PPARα*, which results in activation of FA metabolism and ketogenesis-related target genes, elevating the level of ketone bodies in the plasma. Indeed, *Ppara*^{hepKO} mice displayed higher hepatic TG accumula-

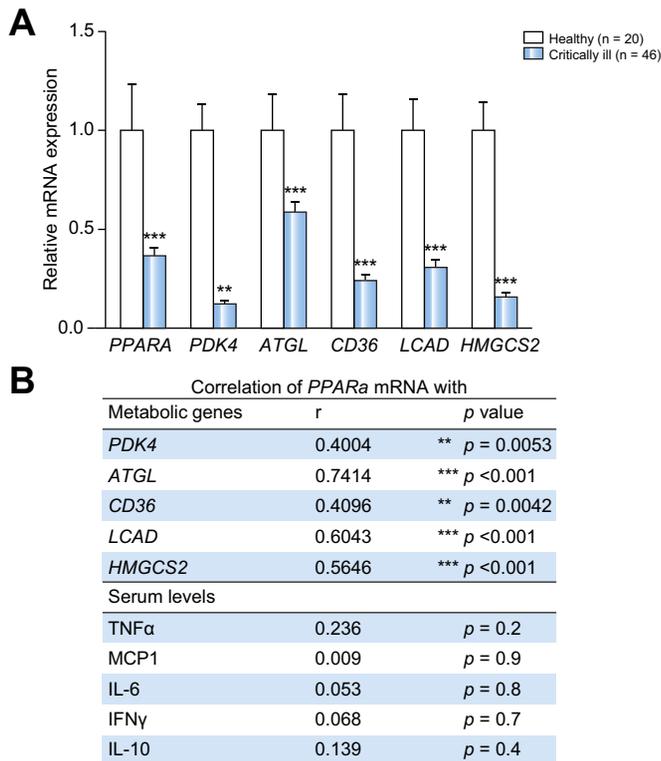


Fig. 7. PPARα gene expression in liver biopsies of critically ill patients correlates with decreased expression of fatty acid utilization genes. (A) Liver biopsies from elective surgery (n = 20) and critically ill patients (n = 46) were collected and mRNA was analyzed using RT-qPCR. Statistical differences are indicated (Wilcoxon test: ***p < 0.001; **p < 0.01; compared to healthy). Serum cytokines were quantified as described in methods. (B) Correlations of hepatic PPARα mRNA expression with metabolic gene expression or serum cytokine levels from critically ill patients were calculated. Statistical differences are indicated (Pearson (r): ***p < 0.001; **p < 0.01). RT-qPCR, quantitative reverse transcription PCR.

tion, reduced plasma ketonemia, and lower hepatic expression of FA metabolism and ketogenesis genes. Decreased ketogenesis in septic *Ppara*-deficient mice was unlikely due to defective AT lipolysis, since plasma FFA levels were unaffected by whole body and hepatocyte-specific *Ppara*-deficiency.

Defective FA oxidation and ketogenesis in septic *Ppara*-deficient mice may indirectly contribute to the aggravation of hypoglycemia and mortality. Mouse models displaying FA oxidation defects are often hypoglycemic upon lipopolysaccharide administration, e.g. *Mcad*-deficient mice^{34,35} and *Thp2*-deficient mice.³⁶ Moreover, several lines of evidence indicate that PPARα plays an important role in glucose homeostasis.³⁷ Under septic conditions, the hypoglycemia in whole body *Ppara*-deficient mice may involve increased hepatic glucose utilization. Indeed, *Ppara*-deficiency impairs the induction of *Pdk4* gene expression upon sepsis, whereas the expression of gluconeogenic genes was not affected. Still, assessment of mitochondrial respiration in *Ppara*-deficient livers suggests that infection does not increase pyruvate oxidation. Thus, the hypoglycemia might also result from increased peripheral glucose uptake by metabolic organs, such as the heart, muscle and brain, to compensate for both the inability to catabolize FA^{38,39} and reduced ketone body availability. In line, septic *Ppara*^{hepKO} mice display less pronounced hypoglycemia than whole body *Ppara* deficient mice. However, it has been shown that decreased FA oxidation and

ketogenesis as a result of *Ppara* or *Fgf21*-deficiency can lead to an inability to maintain tissue tolerance to bacterial sepsis leading to neuronal dysfunction and death.⁴⁰ Moreover, ketone body therapy protects against lipotoxicity and acute liver failure in *Ppara*-deficient mice.⁴¹ Together with our data, these data suggest that the increased mortality during sepsis may be caused by a deficiency in beneficial energetic substrates produced by FA oxidation in hepatocytes, such as ketone bodies, to maintain tissue protection.

Interestingly, in livers of non-surviving critically ill patients, PPARα mRNA levels are lower and correlate with the lower expression of genes involved in lipid and glucose metabolism, but not with plasma markers of inflammation. These data corroborate findings from a clinical metabolomic study showing that lactate, pyruvate, acetyl-carnitine and several citric acid cycle metabolites were higher in sepsis non-survivors compared to survivors, suggesting that a profound defect in FA β-oxidation, possibly as a result of mitochondrial dysfunction, is associated with the incidence of death in critical ill patients.^{42,43}

In conclusion, we have shown that during sepsis *Ppara*-deficiency in hepatocytes is deleterious, as it impairs the adaptive metabolic shift from glucose to FA utilization. While most current approaches to treat sepsis aim to harness the inflammatory response, our results might pave the way for strategies based on adaptive energy homeostasis.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Contribution to conception and design, technical support: RP, JH, NH, EB, YD, JV, CC, SAH, VL, SL, SM, PG, EV, EB, BD, HD, CG, SF, FL, AT, AM, WW, BS. Data acquisition, data analysis and interpretation: RP, JH, NH, EB, YD, DM, LL, JV, KW, SM, PG, HG, DD, BS. Drafting of the manuscript, statistical analysis: RP, JH,

NH, EB, YD, DM, LL, KW, HG, BS. Critical revision of the manuscript for intellectual content: RP, JH, DM, AT, WW, GVdB, HG, DD, BS.

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Supplementary data

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Author names in bold designate shared co-first authorship

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