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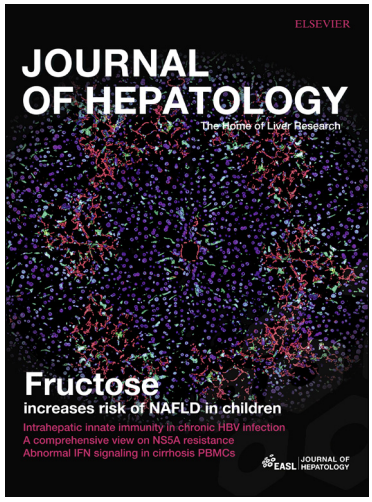

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PII: S0168-8278(19)30027-3
DOI: https://doi.org/10.1016/j.jhep.2018.12.037
Reference: JHEPAT 7238

To appear in: *Journal of Hepatology*

Received Date: 13 February 2018
Revised Date: 20 December 2018
Accepted Date: 23 December 2018

Please cite this article as: Paumelle, R., Haas, J., Hennuyer, N., Bauge, E., Deleye, Y., Mesotten, D., Langouche, L., Vanhoutte, J., Cudejko, C., Wouters, K., Hannou, S.A., Legry, V., Lancel, S., Lalloyer, F., Polizzi, A., Smati, S., Gourdy, P., Vallez, E., Bouchaert, E., Derudas, B., dehondt, H., Gheeraert, C., Fleury, S., Tailleux, A., Montagner, A., Wahli, W., Van Den Berghe, G., Guillou, H., Dombrowicz, D., Staels, B., Hepatic PPARα is critical in the metabolic adaptation to sepsis, *Journal of Hepatology* (2019), doi: https://doi.org/10.1016/j.jhep.2018.12.037

Hepatic PPARα is critical in the metabolic adaptation to sepsis

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Keywords: nuclear receptors, sepsis, metabolism, hepatocytes, inflammation.

Total word count: 5891

Number of figures and tables: 7 figures, 11 Supplementary Figures and 1 Supplementary Tables.

Conflicts of interest: none

Financial support: This work was supported by grants from European Genomic Institute for Diabetes (EGID, ANR-10-LABX-46), the Conseil régional Nord Pas-de-Calais and the Fonds européens de développement régional (FEDER). J.Haas was supported by the European Molecular Biology Organization (EMBO) Long-Term Fellowship (ALTF-277), Y.Deleye by a doctoral fellowship from the Nouvelle Société Française d'Athérosclérose, K.Wouters by European FP7 Postdoctoral fellowship (PIEF-GA-2009-235221), EFSD/GlaxoSmithKline Research and European

Atherosclerosis Society grants. D.Mesotten is a senior clinical investigator for the Research Foundation – Flanders. G.Van den Berghe receives research financing through the Methusalem program (Flemish government) and holds an “ERC Advanced Grant”. W.Wahli was supported by the Lee Kong Chian School of Medicine, Nanyang Technological University Singapore Start-Up Grant and holds a Chaire d’Excellence Pierre de Fermat (Toulouse). H.Guillou is supported by grants from Région Occitanie and ANR “Hepatokind”. B.Staels holds an “ERC advanced Grant” (694717).

Author’s 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

Running title: PPARα controls the hepatic metabolic response to sepsis

Abstract

Background and 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. However, the role of hepatic PPARα in the response to sepsis is unknown.

Methods: Sepsis was induced by intraperitoneal injection of *Escherichia coli* in different models of cell-specific *Pparα*-deficiency and their controls. The systemic and hepatic metabolic response was analysed using biochemical, transcriptomic and functional assays. PPARα expression was analysed 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 *Pparα*-deficiency in mice decreased survival upon bacterial infection. Livers of septic *Pparα*-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 PPARα 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: Metabolic control by PPARα in hepatocytes plays a key role in the host defense to infection.

Abstract word count: 234

Lay summary: As the main cause of death of 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 been mostly overlooked. Here, we show that mice with an impaired metabolic response, due to deficiency of the nuclear receptor PPARα 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.

Highlights:

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

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 therapy [3] 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 (AT) lipolysis [7]. In response, organs such as the liver, muscle and heart, shift from glucose to FA utilization [8] and enhance mitochondrial activity [9].

PPARα is a nuclear receptor activated by fatty acids and derivatives regulating both metabolism and inflammation [10]. PPARα is highly expressed in metabolic tissues, such as 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 fed-to-fasted transition, hepatic PPARα expression increases [12] and is activated by the influx of AT-released FFA, orchestrating a shift

from glucose to FA utilization driving ketone body and glucose production by the liver [12,13].

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

Materials and methods

Mice

Whole body *Pparα* knockout (KO) and wild type (WT) littermate C57BL/6J mice (gift of F.Gonzalez [18]) were bred at the Institut Pasteur de Lille (IPL) transgenic rodent facility (SPF status). Hepatocyte-specific *Pparα*-deficient (*Ppara fl/fl,* Albumin-Cre^{+}, *Pparα*^{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 *Pparα*^{hepKO} to whole body *Pparα* 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-hour 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

DH5α *E.coli* were grown in LB Broth at 37°C to an OD_{600} of 0.6, equivalent to 4-5x10^8 CFU/mL, collected by centrifugation, washed once with sterile PBS, and resuspended in cold PBS at 4-7x10^8 CFU/mL. Concentration and viability were confirmed by plaque assay colony counting. Mice (10-15 mice/group) were injected intraperitoneally (i.p.) with 4-7x10^8 CFU/mouse in 1 mL PBS and survival rates were monitored every 6hrs for a week. For biochemical characterization, mice were killed by cervical dislocation 16hrs post infection and serum and livers collected.

Human study

Post-mortem liver biopsies were taken from ICU-patients (n=46), 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 postmortem 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 Supplementary Table 1. Liver biopsies were taken from liver segment IVb, snap-frozen in liquid nitrogen, and stored at -80°C until analysis (see Supplementary Material & Methods (Suppl.M&M)).

Biochemical analysis

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Biolabo), free fatty acids (FFA) (Diasys), β-hydroxybutyrate (ketone bodies) (Thermo Fisher) were determined by colorimetic assays. Plasma Tnfa, Kc and IL-6 protein levels were measured by ELISA (R&D Systems) and 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 ICU) [21].

Liver transcriptomic analysis

RNA extraction and analysis are detailed in Suppl.M&M. 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 (RMA) in the *oligo* package [22]. Differential gene expression between groups (*Pparα* WT uninfected, *Pparα* WT infected, *Pparα* KO uninfected and *Pparα* KO infected) was assessed using *limma* package [23] with a threshold of 5% false discovery rate (FDR). Differentially expressed genes were clustered using the *hopach* package [24] with the cosine distance metric. Gene Ontology (GO) terms 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 (125mg) were minced and dounce homogenized by 8-10 strokes in ice-cold MIR05 respiratory buffer (20mM HEPES, 10mM KH_2PO_4, 110mM sucrose, 20mM taurine, 60mM K-lactobionate, 0.5mM EGTA, 3mM $MgCl_2 \cdot 6H_2O$, 1g/L BSA (fatty acid free)). Liver homogenates (50µl) were introduced into O2K oxygraph chambers (Oroboros Instruments, Innsbruck, Austria) to assess oxygen consumption in presence of pyruvate (5mM) and malate (2mM) (state 2 respiration), followed by ADP (0.5mM) (state 3 respiration). To measure β-oxidation, octanoylcarnitine (25µM) and malate (2mM) were added, followed by ADP (0.5mM). The respiratory control

ratio (RCR) was calculated as the state 3:state 2 ratio. Finally, cytochrome c (10μM) 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).

Hepatic triglyceride measurement: see Suppl.M&M.

Statistical analysis

Groups were compared using the Log-rank (Mantel-Cox) Test (survival test), 2-way ANOVA, 2-tailed non-paired t-tests or nonparametric 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.

Results

Whole body and non-hematopoietic Pparα-deficiency aggravate mortality upon bacterial infection.

To evaluate the role of *Pparα* in sepsis, whole body *Pparα*-deficient (KO) and wild type (WT) mice were inoculated with Gram-negative *Escherichia coli* (*E. coli*). Three days after infection, mortality was 0% in *Pparα* WT compared to 75% in *Pparα* 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 *Pparα* KO and *Pparα* WT mice with *Pparα* WT bone marrow (WTbm->KO and WTbm->WT, respectively, see Suppl.M&M). Surprisingly, *Pparα*-deficiency in non-hematopoietic cells was still associated with increased mortality in response to infection (20% *Pparα* WTbm->WT compared to 70% *Pparα* WTbm->KO mortality 3 days after infection; Suppl.Fig.1A), revealing a protective role for non-hematopoietic *PPARα* in response to sepsis.

Whole body Pparα-deficiency results in impaired metabolic and inflammatory responses to bacterial sepsis.

To understand the mechanisms of increased mortality of *Pparα* 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 *Pparα* WT and KO mice at 16hrs post-infection (Fig.1B), when maximal changes in metabolic parameters are observed (Suppl.Fig.2). Plasma AST

and ALT levels were similar in *Pparα* 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 *Pparα* KO mice (Fig.1E). In addition, infection-induced hypoglycemia was more pronounced in *Pparα* KO mice, suggesting defective glucose homeostasis (Fig.1F). Interestingly, infected chimeric *Pparα* WTbm->KO mice also displayed lower plasma ketone body levels and more pronounced hypoglycemia compared to WTbm->WT controls (Suppl.Fig.1B,C). These results demonstrate that the metabolic response to sepsis depends on PPARα expression in non-hematopoietic derived cells. In line with previous observations [15], *Pparα*-deficiency resulted in decreased, rather than increased, inflammatory responses as illustrated by lower plasma tumor necrosis factor alpha (Tnfa) and chemokine Kc/Cxcl1 levels 6hrs after infection (Fig.1G,H). Since hepatic PPARα contributes to systemic inflammation [27], histological analysis of livers from infected *Pparα* WT and KO mice was performed. Surprisingly, whole body *Pparα*-deficiency did not affect infection-induced neutrophil or monocyte/macrophage recruitment in livers as assessed by Ly6G and Moma2 stainings (Suppl.Fig.3). Plasma myeloperoxidase activity (Fig.1I) and bacterial dissemination into peritoneum and blood (Fig.1J) were also unaffected by whole body *Pparα*-deficiency.

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

To identify molecular pathways regulated by PPARα in response to sepsis, microarray analysis was performed on livers from whole body *Pparα* WT and KO

mice 16hrs post-infection and from uninfected controls. In addition to inflammation-related pathways, the Gene Ontology (GO) term “Oxidation-Reduction Process,” was altered by infection in *Pparα* WT mice, suggesting altered mitochondrial function (Fig.2A). Unbiased clustering analysis of genes affected by sepsis in either whole body *Pparα* WT or KO mice revealed 3 gene clusters (Fig.2B,C and Suppl.Fig.4). While cluster 1 (black) and cluster 3 (purple) contained genes whose response to infection (induced or suppressed, respectively) was largely maintained in whole body *Pparα* KO mice, genes in cluster 2 (green) were either unresponsive or responded in the opposite direction in *Pparα* KO mice despite strong regulation in *Pparα* WT mice upon infection. Cluster 1 was enriched in genes associated with inflammatory response and ROS metabolism, and cluster 3 was enriched in genes involved in cellular glucuronidation and response to xenobiotics (Suppl.Fig.4). Interestingly, Cluster 2 contained many genes related to FA metabolism and circadian rhythm, many of which are *bona fide* PPARα targets. Indeed, carnitine palmitoyl transferase (*Cpt*)*1a,* acyl-Coenzyme A oxidase (*Acox*)*1* and pyruvate dehydrogenase kinase (*Pdk*)4, were induced by sepsis in *Pparα* WT mice, but either reduced or unchanged by infection in *Pparα* KO mice (Fig.2D). Together, these results demonstrate that the most profound transcriptional differences between whole body *Pparα* WT and KO mice in response to sepsis are related to regulation of PPARα’s metabolic targets, rather than to effects on inflammation or other pathways.

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

To further characterize the metabolic contribution of PPARα upon sepsis, glucose and lipid metabolism gene expression was measured in livers of infected *Pparα* WT and KO mice (Supp.Fig.5). Sepsis increased expression of genes involved in gluconeogenesis, such as phosphoenolpyruvate carboxykinase (*Pck*)*1* and fructose-1,6-biphosphatase (*Fbp)1*, although the magnitude of response was lower in *Pparα* KO mice (*Pparα* WT vs KO: *Pck*1: 3.00vs1.67; *Fbp1*: 2.81vs1.44-fold induction) (Supp.Fig.5A). However, sepsis raised expression of *Pdk4*, which inhibits the final step of glycolysis, >100-fold in a PPARα-dependent manner (Supp.Fig.5B). Similarly, sepsis strongly increased (>12-fold) hepatic adipose triglyceride lipase (*Atgl/Pnpla2*) expression in *Pparα* WT mice only (Supp.Fig.5C). Moreover, sepsis increased several PPARα target genes involved in hepatic lipid metabolism such as FA uptake (e.g. *Fatp1, Cd36*), FA activation (e.g. *Acsl1*), peroxisomal FA β-oxidation (*Acox1*) and mitochondrial FA transport and β-oxidation (e.g. *Cpt1a*, *Lcad*) (Supp.Fig.5D). Interestingly, except for *Cpt1a*, sepsis-mediated induction of these genes was PPARα-dependent. Moreover, expression of 3-hydroxy-3-methylglutaryl-Coenzyme A synthase (*Hmgcs*)2, the rate-limiting enzyme for β-hydroxybutyrate production, was lower in *Pparα*-deficient mice (Supp.Fig.5E). Furthermore, infected chimeric *Pparα* WTbm->KO mice, expressing *Pparα* only in hematopoietic cells, also displayed lower hepatic expression of *Pdk4, Cd36, Acox1, Lcad*, *Hmgcs2* and *Atgl* compared to WTbm->WT mice (Supp.Fig.5F,G), demonstrating that the metabolic transcriptional response to sepsis in liver depends on non-hematopoietic PPARα.

Functional analysis of liver mitochondria also suggested impaired FA utilization in whole body *Pparα* KO mice. Unaltered citrate synthase activity indicated that *Pparα*-

deficiency did not impact mitochondrial quantity (Supp.Fig.5H). Uninfected whole body *Pparα* KO livers displayed lower RCR values upon incubation with pyruvate and malate (PYR) and, to a lesser extent, with octanoylcarnitine and malate (OCTA) compared to uninfected *Pparα* WT (Fig.2E). However, bacterial infection shifted the respiration rate from pyruvate/malate to octanoylcarnitine/malate in *Pparα* WT, whereas this shift was less apparent in *Pparα* 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 *Pparα* KO livers.

Hepatocyte-specific PPARα expression is required for proper metabolic response and survival upon bacterial infection.

Because the results of both transcriptomic and metabolic analysis suggested that hepatic PPARα is activated upon sepsis, we postulated that hepatic PPARα may be critical to the organism's response to infection. Therefore, hepatocyte-specific *Pparα*^{hepKO} were subjected to bacterial infection and their response compared to *Pparα*^{hepWT} and whole body *Pparα* KO mice. Similar to whole body *Pparα* KO mice, *Pparα*^{hepKO} showed increased mortality compared to *Pparα*^{hepWT} mice (Fig.1A&3A). Infected *Pparα*^{hepKO} mice also displayed lower ketone body levels compared to *Pparα*^{hepWT}, despite similar levels of plasma FFA, ALT and AST (Fig.3B-D and Supp.Fig.6). Liver triglyceride (TG) content increased more markedly upon bacterial infection in *Pparα*^{hepKO} vs *Pparα*^{hepWT} mice (3.1 vs 2.2-fold, Fig.3F), suggesting defective hepatic lipid utilization. Surprisingly, infected *Pparα*^{hepKO} mice displayed no significant differences in glycaemia (Fig.3E), nor plasma levels of inflammatory

cytokines (*Tnfa*, *Kc* and *IL-6*) at both 5hrs and 16hrs post-infection (Fig.3G,H & Supp.Fig.7) compared to *Ppar*α^{hepWT}. In addition, bacterial dissemination in peritoneum and blood did not differ between *Ppar*α^{hepWT} and *Ppar*α^{hepKO} mice (Fig.3I). These data suggest that the increased mortality observed in whole body *Ppar*α KO is unlikely caused by the different glycemic and systemic inflammatory responses.

Similar to whole body *Ppar*α KO mice, *Ppar*α^{hepKO} mice displayed major defects in sepsis-modulated regulation of several genes involved in hepatic glucose (i.e. *Pdk4,* Fig.4A) and lipid metabolism (e.g. *Cd36*, *Acox1*, *Vlcad* and *Lcad,* Fig.4B), as well as *Ppar*α itself (Fig.4C). Interestingly, the induction of hepatic *Atgl* and *Cpt1a* expression upon sepsis was independent of hepatocyte PPARα (Fig.4B). Conversely, hepatic *Hmgcs2* expression was virtually undetectable upon sepsis in *Ppar*α^{hepKO} mice (Fig.4B). Bacterial infection increased Atgl, but not Hmgcs2 or Dgat1 expression at the protein level (Supp.Fig.8). Moreover, Hmgcs2 protein levels were lower in *Ppar*α^{hepKO} mice both in uninfected and infected conditions. Whereas Atgl protein induction appeared less pronounced upon sepsis in *Ppar*α^{hepKO} mice, Dgat1 protein expression only increased in *Ppar*α^{hepKO} mice, both consistent with increased hepatic TG content. Together, these data indicate that hepatocyte-specific PPARα-deficiency profoundly affects the hepatic metabolic response to infection.

Pparα*-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 PPARα to systemic inflammation in sepsis, expression of inflammatory genes was measured in livers and spleens from whole body *Pparα* KO, chimeric *Pparα* 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 (Suppl.Fig.9) of whole body *Pparα* 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 *Pparα* 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 (Suppl.Fig.10) were similar in chimeric *Pparα* WTbm->KO and WTbm->WT mice, despite differing survival outcomes (Fig.1A, Supp.Fig.1A). Interestingly, in *Pparα*^{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 *Pparα* KO mice depends on hematopoietic, but not hepatic *Pparα* expression.

Because autophagy may play a protective role during sepsis [28] and PPARα mediates fasting-induced autophagy [29], markers of autophagy were assessed in whole body and hepatocyte-specific *Pparα*-deficient mice. Whereas sepsis increased expression of certain autophagy genes, their regulation was not different in whole body *Pparα* KO mice (Supp.Fig.11A). Moreover, hepatocyte-specific *Pparα*-deficiency (Supp.Fig.11B-C) rather 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 *Ppar*α^{hepKO} mice. Altogether, these data indicate that hepatocyte PPARα expression contributes to protection against sepsis by controlling the systemic metabolic response.

PPARα expression and activity is lower in livers of critically ill patients.

To determine whether hepatic PPARα expression is altered in critically ill human, livers from non-surviving critically ill patients and healthy controls were analysed for PPARα and target 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*), 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 the hepatocyte, by shifting its energy utilization from glucose to FA and by 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 *Pparα*-deficiency attenuates organ and systemic inflammatory responses upon infection. This contrasts with previous observations in models of sterile chronic and acute inflammation in which *Pparα*-deficiency results in exacerbated inflammatory responses to endotoxemia in vascular, splenic and liver cells [33]. Accordingly, whole body *Pparα*-deficiency also resulted in a decreased pro-inflammatory response and survival in a cecal ligation sepsis model [15].

Interestingly, our data and 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, and elevates plasma ketone body levels. Indeed, *Pparα*^{hepKO} mice displayed higher hepatic TG accumulation, reduced plasma ketonemia, and lower hepatic expression of FA metabolism and ketogenesis genes. Decreased ketogenesis in septic *Pparα*-deficient mice was unlikely due to defective AT lipolysis, since plasma FFA levels were unaffected by whole body nor hepatocyte-specific *Pparα*-deficiency.

Defective FA oxidation and ketogenesis in septic *Pparα*-deficient mice may indirectly contribute to the aggravation of hypoglycaemia and mortality. Mouse models displaying FA oxidation defects are often hypoglycemic upon LPS administration, e.g. *Mcad*-deficient mice [34,35] and *Tbp2*-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 *Pparα*-deficient mice may involve increased hepatic glucose utilization. Indeed, *Pparα*-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 *Pparα*-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 *Pparα*^{hepKO} mice display less pronounced hypoglycaemia than whole body *Pparα*–deficient mice. However, it has been shown that decreased FA oxidation and ketogenesis as a result of *Pparα* 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 *Pparα*-deficient mice [41]. Altogether, these and our 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, *PPARA* 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 *Pparα*-deficiency in hepatocytes during sepsis 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.

Acknowledgments

We thank A.Lecluse, C.Paquet, A.Lucas, C.Rommens (Inserm U1011) for technical assistance.

Bibliography

[1] Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;369:840–51. doi:10.1056/NEJMra1208623.

[2] Venkatesh B, Finfer S, Cohen J, Rajbhandari D, Arabi Y, Bellomo R, et al. Adjunctive Glucocorticoid Therapy in Patients with Septic Shock. N Engl J Med 2018;378:797–808. doi:10.1056/NEJMoa1705835.

[3] van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, et al. Intensive insulin therapy in critically ill patients. N Engl J Med 2001;345:1359–67. doi:10.1056/NEJMoa011300.

[4] Casaer MP, Mesotten D, Hermans G, Wouters PJ, Schetz M, Meyfroidt G, et al. Early versus late parenteral nutrition in critically ill adults. N Engl J Med 2011;365:506–17. doi:10.1056/NEJMoa1102662.

[5] Liu TF, Brown CM, El Gazzar M, McPhail L, Millet P, Rao A, et al. Fueling the flame: bioenergy couples metabolism and inflammation. J Leukoc Biol 2012;92:499–507. doi:10.1189/jlb.0212078.

[6] Lang CH, Dobrescu C. Gram-negative infection increases noninsulin-mediated glucose disposal. Endocrinology 1991;128:645–53. doi:10.1210/endo-128-2-645.

[7] Stoner HB. Interpretation of the metabolic effects of trauma and sepsis. J Clin Pathol 1987;40:1108–17.

[8] Cree MG, Aarsland A, Herndon DN, Wolfe RR. Role of fat metabolism in burn trauma-induced skeletal muscle insulin resistance. Crit Care Med 2007;35:S476-483. doi:10.1097/01.CCM.0000278066.05354.53.

[9] Singer M, De Santis V, Vitale D, Jeffcoate W. Multiorgan failure is an adaptive, endocrine-mediated, metabolic response to overwhelming systemic inflammation. Lancet 2004;364:545–8. doi:10.1016/S0140-6736(04)16815-3.

[10] Dubois V, Eeckhoute J, Lefebvre P, Staels B. Distinct but complementary contributions of PPAR isotypes to energy homeostasis. J Clin Invest 2017;127:1202–14. doi:10.1172/JCI88894.

[11] Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. Circulation Research 2004;94:1168–78.

[12] Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. Proceedings of the National Academy of Sciences of the United States of America 1999;96:7473–8.

[13] Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. The Journal of Clinical Investigation 1999;103:1489–98.

[14] Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPARalpha-leukotriene B4 pathway to inflammation control. Nature 1996;384:39–43.

[15] Standage SW, Caldwell CC, Zingarelli B, Wong HR. Reduced peroxisome proliferator-activated receptor α expression is associated with decreased survival and increased tissue bacterial load in sepsis. Shock 2012;37:164–9. doi:10.1097/SHK.0b013e31823f1a00.

[16] Standage SW, Waworuntu RL, Delaney MA, Maskal SM, Bennion BG, Duffield JS, et al. Nonhematopoietic Peroxisome Proliferator-Activated Receptor-α Protects Against Cardiac Injury and Enhances Survival in Experimental Polymicrobial Sepsis. Crit Care Med 2016;44:e594-603. doi:10.1097/CCM.0000000000001585.

[17] Standage SW, Bennion BG, Knowles TO, Ledee DR, Portman MA, McGuire JK, et al. PPARα augments heart function and cardiac fatty acid oxidation in early experimental polymicrobial sepsis. Am J Physiol Heart Circ Physiol 2017;312:H239–49. doi:10.1152/ajpheart.00457.2016.

[18] Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, et al. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol Cell Biol 1995;15:3012–22.

[19] Montagner A, Polizzi A, Fouché E, Ducheix S, Lippi Y, Lasserre F, et al. Liver PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD. Gut 2016;65:1202–14. doi:10.1136/gutjnl-2015-310798.

[20] Bradley PP, Christensen RD, Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood 1982;60:618–22.

[21] Langouche L, Vanhorebeek I, Vlasselaers D, Vander Perre S, Wouters PJ, Skogstrand K, et al. Intensive insulin therapy protects the endothelium of critically ill patients. J Clin Invest 2005;115:2277–86. doi:10.1172/JCI25385.

[22] Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. Bioinformatics 2010;26:2363–7. doi:10.1093/bioinformatics/btq431.

[23] Phipson B, Lee S, Majewski IJ, Alexander WS, Smyth GK. ROBUST HYPERPARAMETER ESTIMATION PROTECTS AGAINST HYPERVARIABLE GENES AND IMPROVES POWER TO DETECT DIFFERENTIAL EXPRESSION. Ann Appl Stat 2016;10:946–63. doi:10.1214/16-AOAS920.

[24] van der Laan MJ, Dudoit S, Pollard KS. Multiple testing. Part II. Step-down procedures for control of the family-wise error rate. Stat Appl Genet Mol Biol 2004;3:Article14. doi:10.2202/1544-6115.1041.

[25] Alexa A, Rahnenführer J, Lengauer T. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. Bioinformatics 2006;22:1600–7. doi:10.1093/bioinformatics/btl140.

[26] Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012;16:284–7. doi:10.1089/omi.2011.0118.

[27] Lalloyer F, Wouters K, Baron M, Caron S, Vallez E, Vanhoutte J, et al. Peroxisome proliferator-activated receptor-alpha gene level differently affects lipid metabolism and inflammation in apolipoprotein E2 knock-in mice. Arterioscler Thromb Vasc Biol 2011;31:1573–9. doi:10.1161/ATVBAHA.110.220525.

[28] Takahashi W, Watanabe E, Fujimura L, Watanabe-Takano H, Yoshidome H, Swanson PE, et al. Kinetics and protective role of autophagy in a mouse cecal ligation and puncture-induced sepsis. Crit Care 2013;17:R160. doi:10.1186/cc12839.

[29] Lee JM, Wagner M, Xiao R, Kim KH, Feng D, Lazar MA, et al. Nutrient-sensing nuclear receptors coordinate autophagy. Nature 2014;516:112–5. doi:10.1038/nature13961.

[30] Gómez H, Kellum JA, Ronco C. Metabolic reprogramming and tolerance during sepsis-induced AKI. Nat Rev Nephrol 2017;13:143–51. doi:10.1038/nrneph.2016.186.

[31] Aziz M, Jacob A, Yang W-L, Matsuda A, Wang P. Current trends in inflammatory and immunomodulatory mediators in sepsis. J Leukoc Biol 2013;93:329–42. doi:10.1189/jlb.0912437.

[32] Bosmann M, Ward PA. The inflammatory response in sepsis. Trends Immunol 2013;34:129–36. doi:10.1016/j.it.2012.09.004.

[33] Delerive P, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors in inflammation control. The Journal of Endocrinology 2001;169:453–9.

[34] Herrema H, Derks TGJ, van Dijk TH, Bloks VW, Gerding A, Havinga R, et al. Disturbed hepatic carbohydrate management during high metabolic demand in medium-chain acyl-CoA dehydrogenase (MCAD)-deficient mice. Hepatology 2008;47:1894–904. doi:10.1002/hep.22284.

[35] Spiekerkoetter U, Wood PA. Mitochondrial fatty acid oxidation disorders: pathophysiological studies in mouse models. J Inherit Metab Dis 2010;33:539–46. doi:10.1007/s10545-010-9121-7.

[36] Oka S, Liu W, Yoshihara E, Ahsan MK, Ramos DAL, Son A, et al. Thioredoxin binding protein-2 mediates metabolic adaptation in response to lipopolysaccharide in vivo. Crit Care Med 2010;38:2345–51. doi:10.1097/CCM.0b013e3181f85b2a.

[37] Peeters A, Baes M. Role of PPARalpha in Hepatic Carbohydrate Metabolism. PPAR Research n.d.;2010.

[38] Romanosky AJ, Bagby GJ, Bockman EL, Spitzer JJ. Increased muscle glucose uptake and lactate release after endotoxin administration. Am J Physiol 1980;239:E311-316. doi:10.1152/ajpendo.1980.239.5.E311.

[39] Knauf C, Rieusset J, Foretz M, Cani PD, Uldry M, Hosokawa M, et al. Peroxisome proliferator-activated receptor-alpha-null mice have increased white adipose tissue glucose utilization, GLUT4, and fat mass: Role in liver and brain. Endocrinology 2006;147:4067–78. doi:10.1210/en.2005-1536.

[40] Wang A, Huen SC, Luan HH, Yu S, Zhang C, Gallezot J-D, et al. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. Cell 2016;166:1512-1525.e12. doi:10.1016/j.cell.2016.07.026.

[41] Pawlak M, Baugé E, Lalloyer F, Lefebvre P, Staels B. Ketone Body Therapy Protects From Lipotoxicity and Acute Liver Failure Upon Pparα Deficiency. Mol Endocrinol 2015;29:1134–43. doi:10.1210/me.2014-1383.

[42] Langley RJ, Tsalik EL, van Velkinburgh JC, Glickman SW, Rice BJ, Wang C, et al. An integrated clinico-metabolomic model improves prediction of death in sepsis. Sci Transl Med 2013;5:195ra95. doi:10.1126/scitranslmed.3005893.

[43] Langley RJ, Tipper JL, Bruse S, Baron RM, Tsalik EL, Huntley J, et al. Integrative “omic” analysis of experimental bacteremia identifies a metabolic signature that distinguishes human sepsis from systemic inflammatory response syndromes. Am J Respir Crit Care Med 2014;190:445–55. doi:10.1164/rccm.201404-0624OC.

Figure Legends

Figure 1: Whole body *Pparα*-deficiency enhances mortality upon bacterial sepsis and impairs the metabolic and inflammatory response to bacterial infection. *Pparα* WT and KO mice were injected (ip) with vehicle (PBS) (-) or *E.coli* ($4x10^8$ living bacteria) (Inf) (+). (A). Survival was followed for 8 days after injection (n=8-10 mice/group). Plasma was collected 6 (G,H) or 16hrs (B-F, I-J) after injection and (B) free fatty acids (FFA), (C) aspartate aminotransferase (AST), (D) alanine aminotransferase (ALT), (E) ketone bodies, (F) blood glucose, (G) Tnfa, (H) Kc/Cxcl1 concentrations and myeloperoxidase (MPO) activity (I) 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; ns: non-significant)

Figure 2: Whole body *Pparα*-deficiency modulates hepatic metabolic and inflammatory transcriptional responses to infection. Livers from *Pparα* WT and KO mice injected (ip) with vehicle (PBS) (Control) or *E.coli* ($4x10^8$ live bacteria) (Infected) were collected after 16hrs and transcriptomic analysis was performed (n=6 mice/group). (A) Top enriched GO terms for genes differentially expressed comparing infection vs control in *Pparα* WT mice. (B) Hierarchical clustering and (C) dot plot of genes affected by infection in *Pparα* WT or KO mice. (D) Selected genes from the “fatty acid oxidation pathway" GO term of Cluster 2. (E) Mitochondrial respiration measured with (pyruvate/malate (PYR) or octanoylcarnitine/malate

(OCTA) as described in methods (n=7-8 mice/group). Statistical differences are indicated (*2way 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).

Figure 3: Hepatocyte-specific *Pparα*-deficiency results in metabolic perturbations and aggravates mortality during bacterial infection. (A) *Pparα* ^{hepWT}, *Pparα* ^{hepKO}, and *Pparα* KO mice were injected (ip) with *E.coli* ($7x10^8$ live bacteria). Survival was followed for 8 days after bacterial infection (n=12-18 mice /group). Plasma was collected 5 (G,H) or 16hrs (B-E) 16hrs after bacterial infection and (B) free fatty acids (FFA), (C) alanine amino transferase (ALT), (D) ketone bodies, (E) blood glucose, (G) Tnfa and (H) Kc/Cxcl1 concentrations were measured as described in methods. Livers, peritoneal fluid and blood were collected 16hrs 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 PPARα ^{hepWT}; *2-way ANOVA*: §§§ $p<0.001$; §§ $p<0.01$; § $p<0.05$ for genotype effect; ns: non-significant).

Figure 4: Hepatocyte-specific *Pparα*-deficiency impairs the response of lipid metabolism genes to bacterial infection. *Pparα* ^{hepWT}, *Pparα* ^{hepKO} mice were injected (ip) with vehicle (PBS) (-) or with *E.coli* ($6x10^8$ live bacteria) (inf) (+). Livers were collected 16hrs after infection and hepatic mRNA expression of (A) *Pdk4*, (B) genes involved in lipid metabolism and (C) *Ppara* was measured. Statistical differences are indicated (*2way ANOVA*: *** $p<0.001$, ** $p<0.01$ and * $p<0.05$ for

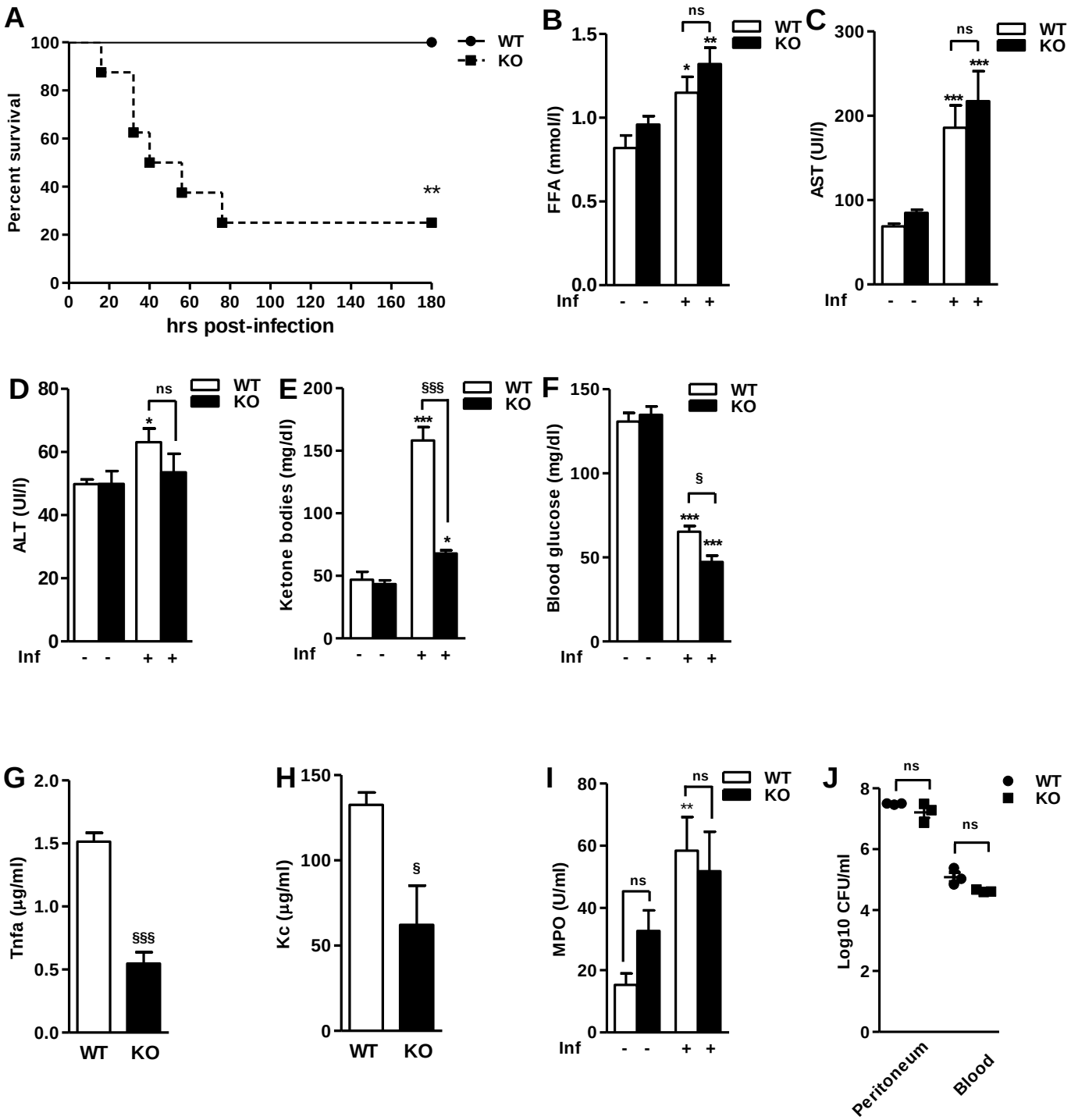
effect of infection; §§§ p<0.001; §§ p<0.01; § p<0.05 for effect of genotype; ns: non-significant).

Figure 5: The sepsis-induced inflammatory response occurs through hematopoietic PPARα. Whole body *Pparα* WT and KO mice (A-C) or chimeric *Pparα* WTbm->WT and WTbm->KO mice (D-F) were injected (ip) with vehicle (PBS) (-) or *E.coli* ($4x10^8$ live bacteria) (Inf) (+). Livers were collected 16hrs after infection and mRNA expression of genes involved in inflammation (A, D), endothelial activation (B, E), and oxidative stress (C, E), was analyzed using RT-Q-PCR. Plasma myeloperoxidase (MPO) activity (F) was measured as described in methods (n=8 mice/group). Statistical differences are indicated (*2way 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).

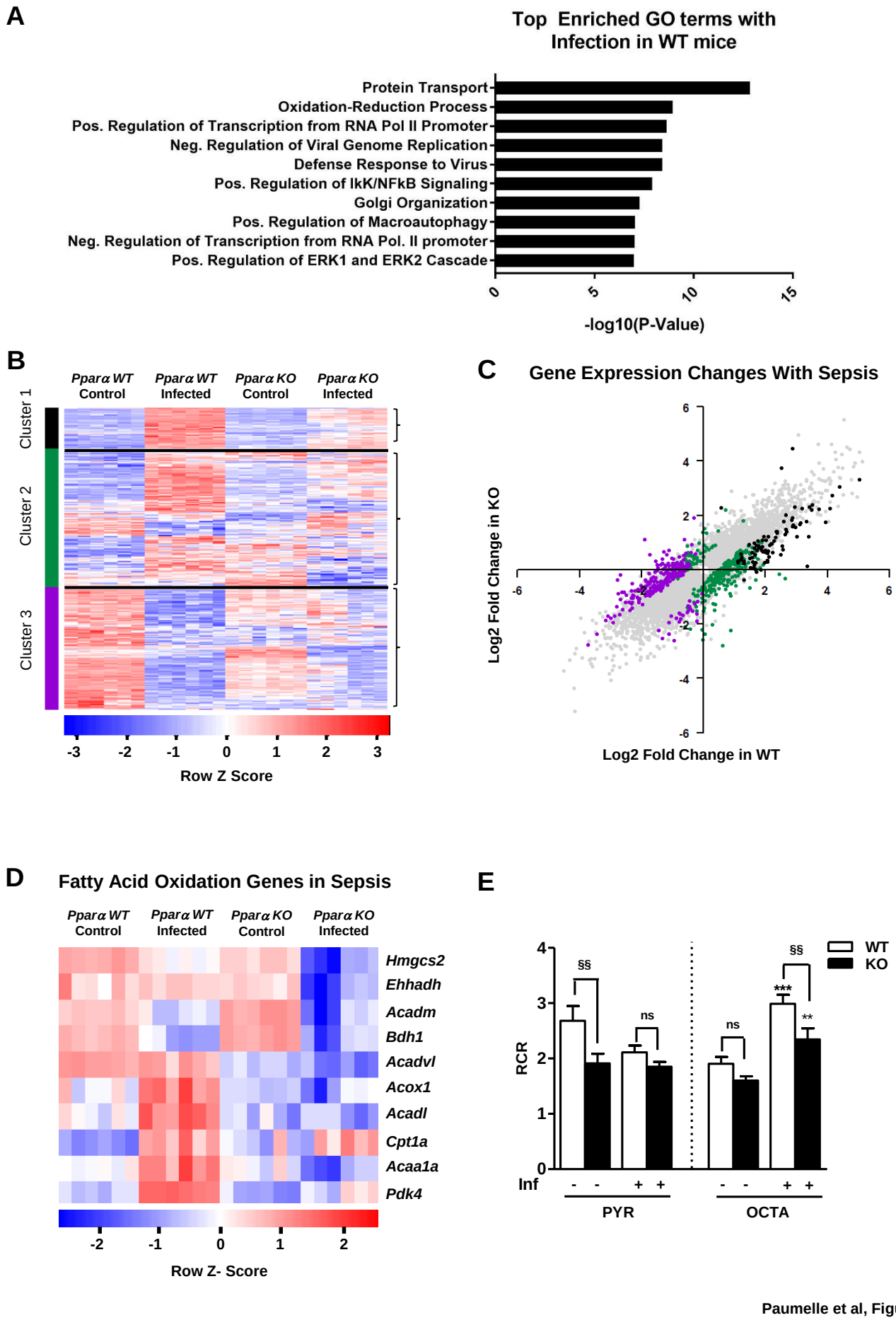
Figure 6: Hepatocyte-specific *Pparα*-deficiency modulates the inflammatory response in the liver without affecting innate immune cell recruitment upon bacterial infection. *Pparα*^{hepWT}, *Pparα*^{hepKO} mice were injected (ip) with vehicle (PBS) (-) or *E.coli* ($6x10^8$ live bacteria) (inf) (+). Livers were collected 16hrs after infection and mRNA expression of genes involved in (A) inflammation, (B) endothelial activation and oxidative stress (B) was analysed using RT-Q-PCR. 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 = 100μm). Statistical differences are indicated (*2way 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).

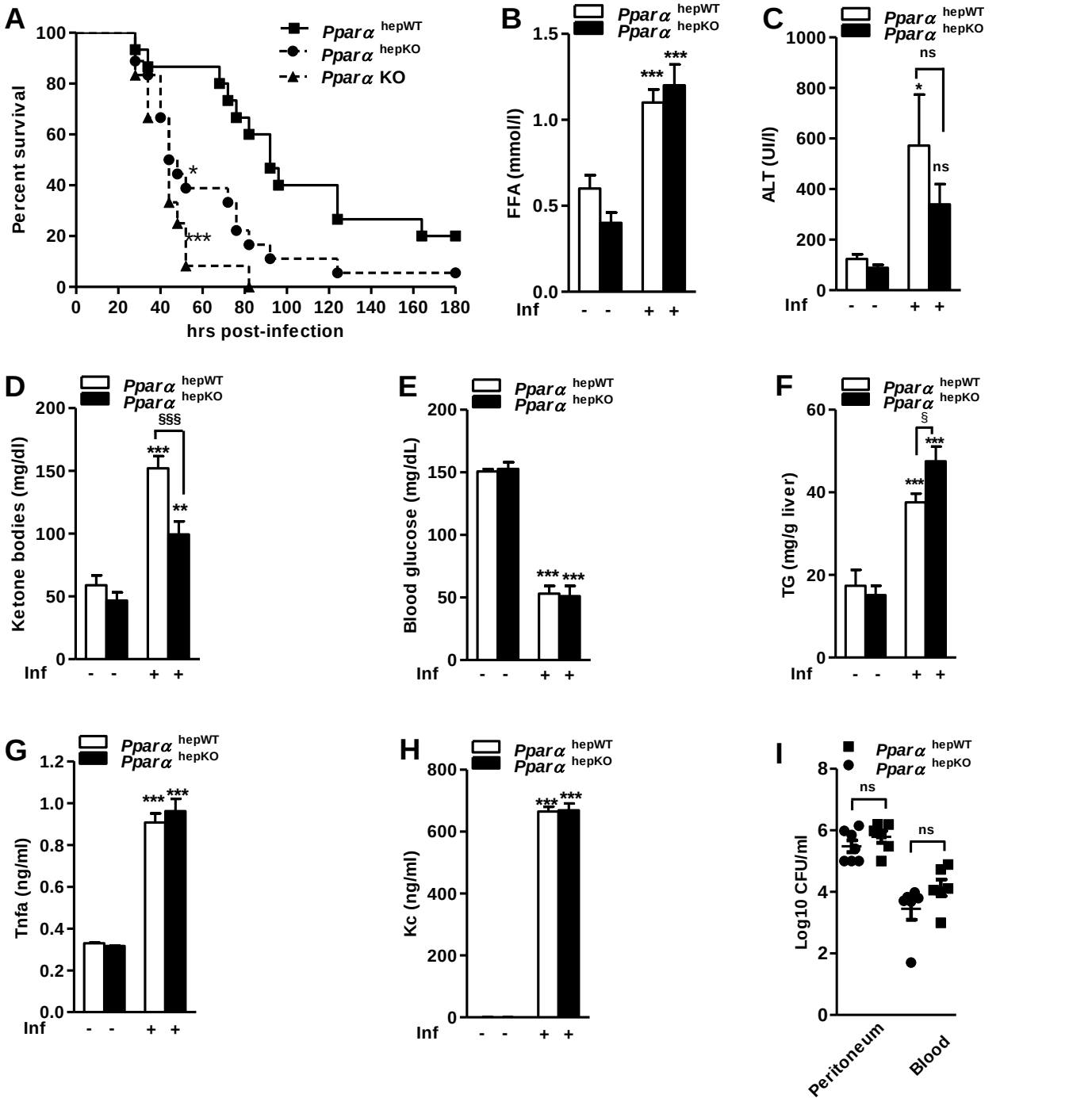
Figure 7: PPARα gene expression in liver biopsies of critically ill patients correlates with decreased expression of FA utilization genes. (A) Liver biopsies from elective surgery (n=20) and critically ill patients (n=46) were collected and mRNA was analysed using RT-Q-PCR. Statistical differences are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



Paumelle et al, Figure 1



Paumelle et al, Figure 2



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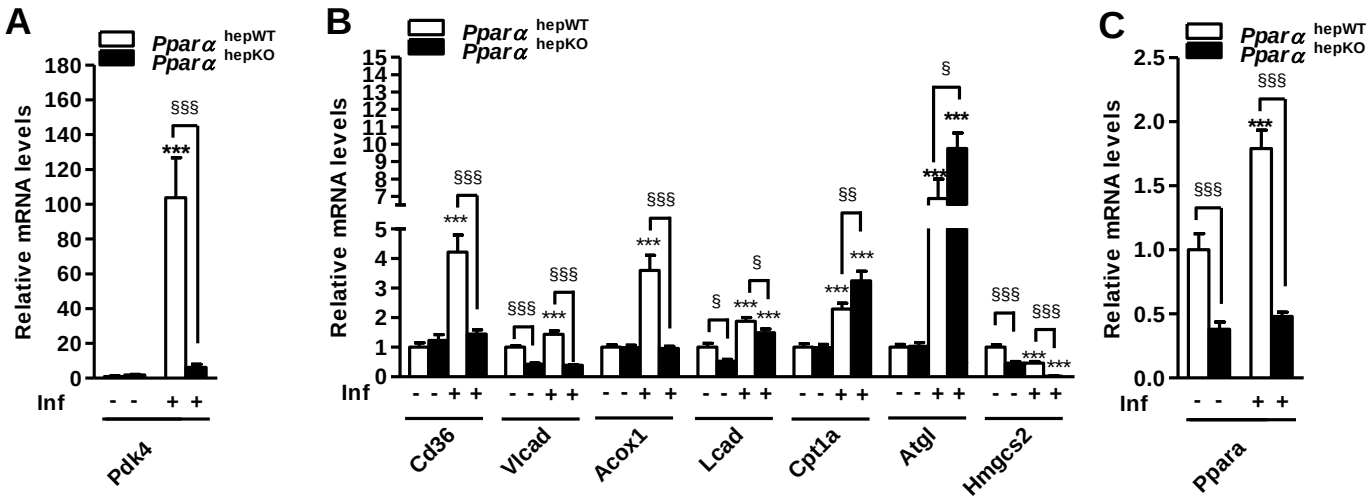
A
Pparα hepWT
Pparα hepKO
Relative mRNA levels
180
160
140
120
100
80
60
40
20
0
§§§
***
Inf
- - + +
Pdk4
B
Pparα hepWT
Pparα hepKO
Relative mRNA levels
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Inf
- - + +
§§§
***
§
§§
Cd36
Vlcad
Acox1
Lcad
Cpt1a
Atgl
Hmgcs2
C
Pparα hepWT
Pparα hepKO
Relative mRNA levels
2.5
2.0
1.5
1.0
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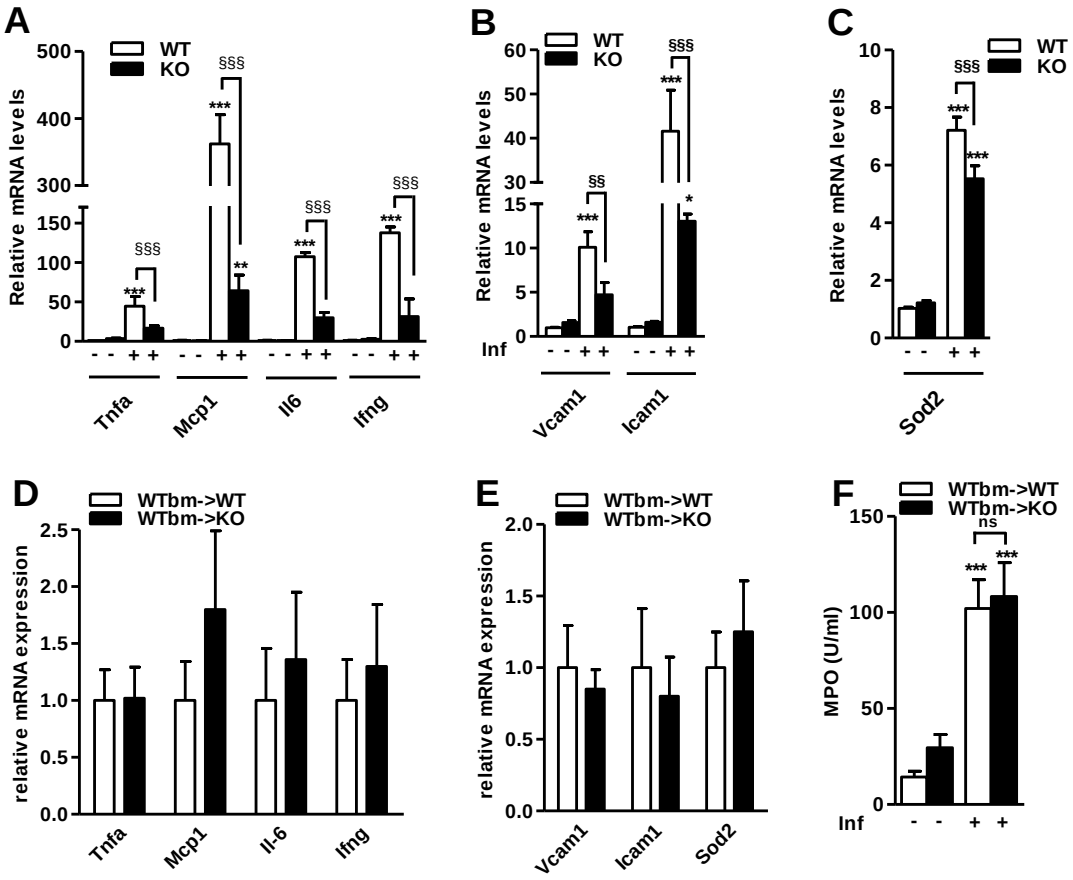
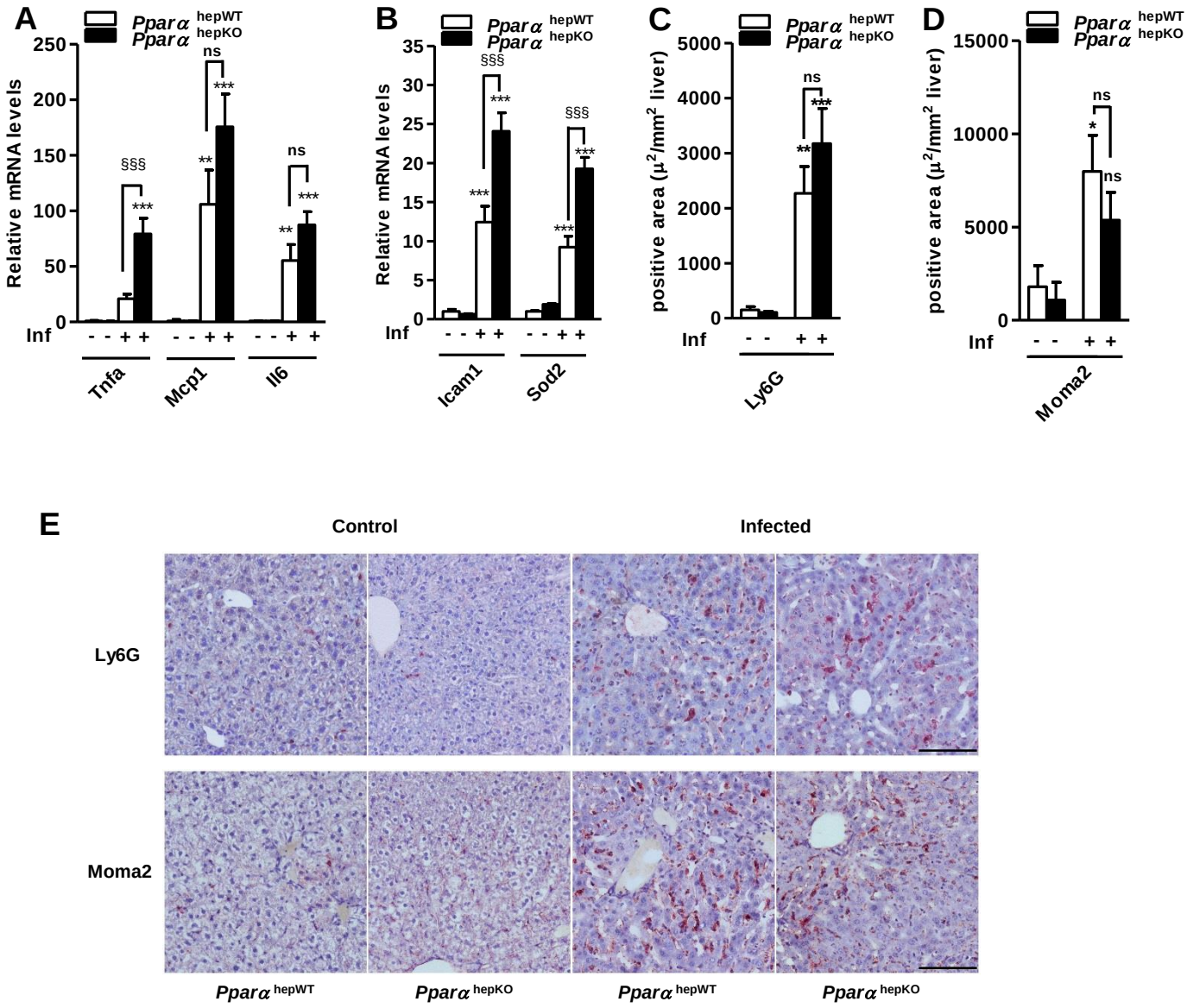
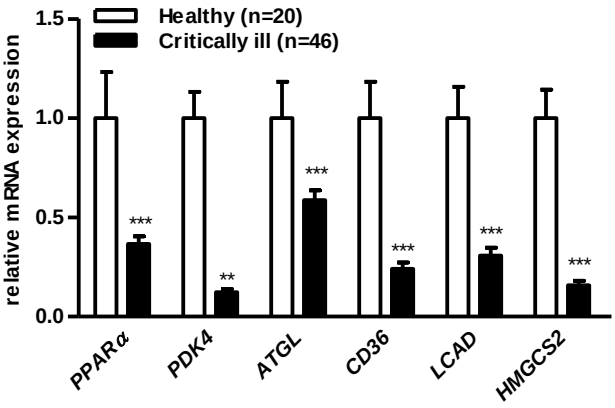


Paumelle et al, Figure 5



Paumelle et al, Figure 6

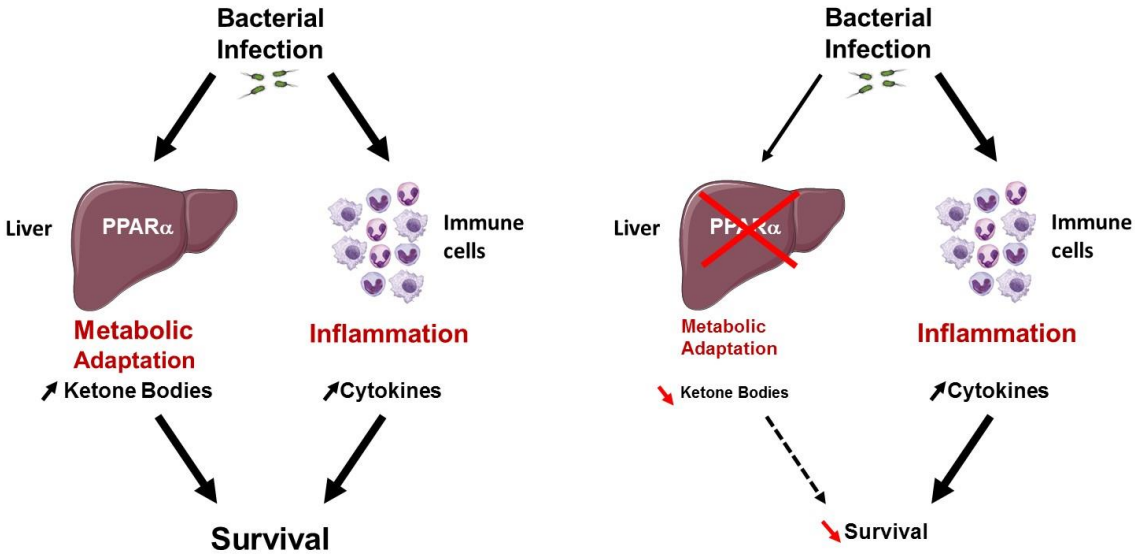
A



B

Correlation of *PPARa* mRNA with		
Metabolic genes	***r***	**P value**
PDK4	0,4004	** P=0.0053
ATGL	0,7414	*** P< 0.001
CD36	0,4096	** P=0.0042
LCAD	0,6043	*** P< 0.001
HMGCS2	0,5646	*** P< 0.001
Serum levels		
TNFa	0.236	P=0.2
MCP1	0.009	P=0.9
IL6	0.053	P=0.8
IFNg	0.068	P=0.7
IL10	0.139	P=0.4

Paumelle et al, Figure 7



Graphical abstract: PPARα expression in hepatocytes is necessary for hepatic metabolic shift from glucose to lipid utilization to protect mice against bacterial infection-induced mortality. Bacterial infection induced activation of hepatocyte-PPARα, which contributes to metabolic adaptation from glucose to lipid utilization and promotes survival. Hepatic-specific *Pparα*-deficiency decreases plasma ketone body levels without affecting systemic inflammation or immune cell activation and leads to defect in metabolic/tolerance response and increased mortality.