Published in final edited form as:

Crit Care Med. 2016 August ; 44(8): e594–e603. doi:10.1097/CCM.0000000000001585.

Non-Hematopoietic PPAR α Protects Against Cardiac Injury and Enhances Survival in Experimental Polymicrobial Sepsis

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Abstract

Objective—Peroxisome-proliferator activated receptor alpha (PPAR α) is significantly downregulated in circulating leukocytes from children with sepsis. PPAR α null (*Ppara*^{−/−}) mice have greater mortality than wild type (WT) mice when subjected to sepsis by cecal ligation and puncture (CLP). We sought to characterize the role of PPAR α in sepsis and to identify the mechanism whereby PPAR α confers a survival advantage.

Design—Prospective randomized pre-clinical study.

Setting—Laboratory investigation.

Subjects—Male C57Bl/6J and *Ppara*^{−/−} mice (B6.129S4-Ppara^{tm1Gonz/J}), aged 12–16 weeks.

Interventions—Bone marrow chimeric mice were generated and subjected to CLP. Survival was measured for seven days. Separate groups of non-transplanted mice underwent CLP and were euthanized 24 hours later for plasma and tissue analyses.

Measurements and Main Results—*Ppara*^{−/−} mice had dramatically reduced survival compared to WT mice irrespective of the PPAR α status of the bone marrow they received (3% vs. 63%, $p < 0.0001$). No difference in survival was observed between *Ppara*^{−/−} mice that received WT vs. *Ppara*^{−/−} marrow or in WT mice receiving WT vs. *Ppara*^{−/−} marrow. In septic, non-transplanted mice at 24 hours, *Ppara*^{−/−} mice had elevated cardiac troponin levels compared to WT mice. Cardiac histologic injury scores were greater in *Ppara*^{−/−} vs. WT mice. Expression of

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This study was conducted in the Center for Lung Biology, Department of Medicine, University of Washington School of Medicine, Seattle, WA.

No reprints of this manuscript will be ordered.

Conflicts of Interest: The remaining authors declared no conflicts of interest. The remaining authors have disclosed that they do not have any potential conflicts of interest.

transcription factors and enzymes related to fatty acid oxidation in the heart were profoundly downregulated in both WT and *Ppara*^{-/-} mice, but more so in the *Ppara*^{-/-} mice.

Conclusions—PPAR α expression in non-hematopoietic tissues plays a critical role in determining clinical outcome in experimental polymicrobial sepsis and is more important to survival in sepsis than hematopoietic PPAR α expression. Cardiac injury due to inadequate energy production from fatty acid substrate is a probable mechanism of decreased survival in *Ppara*^{-/-} mice. These results suggest that altered PPAR α -mediated cellular metabolism may play an important role in sepsis-related end-organ injury and dysfunction, especially in the heart.

Keywords

Sepsis; PPAR alpha; peroxisome proliferators; heart; fatty acids; lipid metabolism

Introduction

Sepsis and septic shock are significant problems in children and adults. While mortality from sepsis is decreasing, its prevalence is increasing (1–4). Despite years of investigation, no specific therapeutic agent for treating sepsis exists (1, 5).

Studying peripheral blood leukocyte genome-wide expression profiles of children with septic shock, Wong et al. identified a subset of children with higher severity of illness, greater organ dysfunction, and increased mortality (6). The genomic response of these children was characterized by profound suppression of multiple immunologic signaling pathways, including B- and T-cell receptor pathways, glucocorticoid receptor signaling, as well as natural killer cell signaling. The peroxisome proliferator activated receptor alpha (PPAR α) pathway was also one of the most significantly downregulated (6).

Peroxisome proliferator activated receptors (PPAR) PPAR α , PPAR β/δ , and PPAR γ are ligand-activated nuclear receptor transcription factors. They sit at the interface between inflammatory and metabolic pathways and regulate aspects of each (7). Endogenous PPAR ligands include specific fatty acids and their derivatives. Many exogenous PPAR agonists are used in clinical practice. The fibrate class of drugs activates PPAR α , while the glitazones function as PPAR γ agonists. PPAR α is widely expressed in the body (especially in heart, kidney, and liver). It exerts a generally anti-inflammatory influence over the immune response and also regulates fatty acid oxidation (FAO) and lipid transport (7).

In a previous study, we showed that children with sepsis and septic shock had lower levels of PPAR α expression in circulating leukocytes than children without sepsis and that PPAR α expression inversely correlated with sepsis severity (8). We also demonstrated that *Ppara*^{-/-} mice had impaired survival following cecal ligation and puncture (CLP)-induced experimental sepsis. Unexpectedly, we found that *Ppara*^{-/-} mice had lower levels of inflammatory cytokines, reduced leukocyte activation, and increased tissue bacterial load. We concluded that absence of PPAR α , presumably in leukocytes, promoted development of a functionally immunosuppressed state (8).

In the current study, we initially intended to further characterize the role of PPAR α in establishing this immunosuppression. Contrary to our initial hypothesis, we found that non-hematopoietic, rather than hematopoietic, expression of PPAR α was the critical determinant of survival in experimental murine polymicrobial sepsis. Furthermore, cardiac expression of PPAR α was necessary to protect against sepsis-induced cardiac injury.

Materials and Methods

Mouse cecal ligation and puncture

The University of Washington Office of Animal Welfare approved all animal use and procedures. Wild type (WT) C57Bl/6J mice and *Ppara*^{-/-} mice on a C57Bl/6 background (B6.129S4-*Ppara*^{tm1Gonz/J}) from Jackson Laboratory (Sacramento, California) were bred in our vivarium as homozygous congenic strains. Twelve to 16 week old, age-matched males were used for all experiments. Polymicrobial sepsis was induced via CLP as described (8, 9). Briefly, the cecum was exteriorized through a midline laparotomy and ligated with 3.0 silk suture immediately distal to the ileocecal junction. A single puncture was made with a 25-gauge needle along the anti-mesenteric aspect of the cecum and light pressure applied to express a small amount (5–10 μ L) of fecal material. The cecum was replaced in the abdomen and the abdominal wall closed. All animals were resuscitated with 20 mL/kg of normal saline solution (approximately 0.5 ml per mouse) delivered subcutaneously. Mice received subcutaneous imipenem (25 mg/kg in 10 mL/kg normal saline) 2 hours after CLP and then every 12 hours until sacrifice or until three days had elapsed. Buprenorphine (0.05 mg/kg) was also administered every 12 hours for 3 days.

Separate groups (5–10/genotype/time point) of age-matched, non-transplanted mice underwent CLP and were euthanized at time 0 (un-operated baseline), 24, 48, and 72 hours after CLP. Blood was collected via cardiac puncture and plasma separated by centrifugation. Whole tissue was collected for histologic and gene expression analyses. Plasma and tissue samples were stored at -80° C until batch analysis could be performed.

Survival studies

After CLP, mice were observed every 12 hours for the first 3 days and then every 24 hours until 7 days following CLP. A clinical scoring tool was used at each observation to monitor severity of illness. Predefined surrogate endpoints for death were established and mice were euthanized when they met those criteria (Table 1). Survivors were euthanized at 7 days.

Bone marrow chimeras

Six to 8 week old WT and *Ppara*^{-/-} mice were irradiated with 1,000 rad. The next day bone marrow was isolated from the tibias and femurs of age- and gender-matched donor mice, and 4×10^6 cells were injected into the retro-orbital venous plexus of irradiated recipient mice (Supplemental Figure 1). Eighty mice underwent bone marrow transplantation in 4 groups (Supplemental Figure 2): WT control (WT recipient transplanted with WT marrow), WT chimera (WT recipient transplanted with *Ppara*^{-/-} marrow), *Ppara*^{-/-} chimera (*Ppara*^{-/-} recipient transplanted with WT marrow), *Ppara*^{-/-} control (*Ppara*^{-/-} recipient transplanted with *Ppara*^{-/-} marrow).

Six weeks post-transplant, DNA was purified from whole blood (DNeasy Blood and Tissue Kit; Qiagen, Valencia, California) and PCR performed to confirm the genotype of the engrafted marrow (common forward primer: CACACCAAGCAGCAGACACT, PPAR α mutant reverse primer: GCTATCAGGACATAGCGTTGG, WT reverse primer: CCCATTTCGGTAGCAGGTAGTCTT). All mice demonstrated appropriate engraftment with transplanted marrow without evidence of native bone marrow (Supplemental Figure 3). Mice that were significantly underweight and appeared malnourished were excluded from the survival study. Remaining mice subsequently underwent CLP, and survival was evaluated over 7 days.

Biomarker assays

Cardiac troponin-I levels were measured by ELISA (Life Diagnostics, West Chester, Pennsylvania). Blood urea nitrogen (Pointe Scientific, Canton, Michigan), creatinine (Diazyme Laboratories, Poway, California), and alanine aminotransferase (Sigma Aldrich, St. Louis, Missouri) levels were quantified using enzyme-based colorimetric assays. Blood glucose levels were measured with the Contour Next glucometer (Bayer, Whippany, New Jersey) using 5 μ L of whole blood collected at the time of cardiac puncture.

Histological analysis

Following resection, lungs, hearts, livers, and kidneys were fixed in 10% neutral buffered formalin (Sigma Aldrich, St. Louis, Missouri). Lungs were inflated with formalin to 5 cm of pressure prior to immersion. After 48 hours, samples were transferred to 70% ethanol, routinely processed and paraffin embedded, sectioned at 5 microns, and stained with hematoxylin and eosin. Multiple tissue sections of each organ were scored by a blinded veterinary pathologist for severity of injury (Table 2).

Gene expression analysis

Total RNA was purified from blood and tissue samples (RNeasy Plus Mini Kit for blood and RNeasy Plus Fibrous tissue kit for heart; Qiagen, Valencia, California). cDNA was created using a High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, California). Gene expression analysis was performed via qRT-PCR using a Sensimix II kit (Bioline, Taunton, Massachusetts) with primer/probe sets for *Ppara*, *Ppard*, *Pparg*, medium chain acyl-CoA dehydrogenase (*Acadm*), very long chain acyl-CoA dehydrogenase (*Acadvl*), carnitine palmitoyltransferase 2 (*Cpt2*), and PPAR γ coactivator 1 alpha (*Pgc1a*) (Life Technologies). Hypoxanthine guanine phosphoribosyl transferase 1 was used as the endogenous control gene. qRT-PCR was performed on an ABI 7900HT instrument (Life Technologies, Carlsbad, California).

Protein immunoblotting

Total protein was extracted from cardiac tissue by homogenization in radioimmunoprecipitation assay buffer and then quantified using the bicinchoninic acid assay (Thermo Fisher Scientific, Waltham, Massachusetts). Proteins (20 μ g of protein per well) were resolved by gel electrophoresis on 4–12% Bis-Tris Plus Gels using the Bolt System (Life Technologies, Carlsbad, California) and transferred to PVDF membranes.

Membranes were blocked with 5% BSA in 0.1 % TBST and then incubated in primary antibody solution overnight at 4° C. Rabbit anti-ACADM and anti-Vinculin antibodies were obtained from Abcam (ab92461 and ab129002 respectively, Cambridge, Massachusetts). Rabbit anti-ACADVL was obtained from Santa Cruz Biotechnology (sc-98338, Dallas, Texas). Membranes were subsequently incubated with HRP-conjugated goat anti-rabbit antibody (Abcam, ab6721), developed using SuperSignal West Pico Chemiluminescent Substrate (Life Technologies, Carlsbad, California), and imaged on a Bio-Rad Chemidoc imager (Bio-Rad, Hercules, California).

Statistical analysis

All statistics were computed using STATA 12/IC (Stata Corp. LP, College Station, Texas). Normally distributed data were compared with the two-group mean comparison test (t-test) and one-way ANOVAs. For gene expression analysis between two genotypes over multiple time points the two-way ANOVA was used to detect interaction effects. Data not normally distributed were compared using nonparametric analyses, the two-sample Wilcoxon rank sum (Mann-Whitney) and Kruskal-Wallis tests. Survival differences were calculated using the log-rank test of equality. For all statistical analyses a p value of 0.05 was considered significant. The Bonferroni correction was used for multiple comparisons.

Results

Leukocyte PPAR α expression in experimental polymicrobial sepsis

In children with sepsis, PPAR α expression is reduced in leukocytes (8). Therefore, to determine if PPAR α expression responses in septic mice recapitulated human disease, mRNA expression of PPAR α in peripheral blood leukocytes from WT mice was evaluated by qRT-PCR. Similar to humans, PPAR α was profoundly downregulated, with decreased expression maintained 72 hours after CLP (Figure 1).

PPAR α expression in non-hematopoietic tissues is critical for survival in mice during experimental polymicrobial sepsis

Consistent with prior observations (8), sepsis-induced mortality was markedly greater in *Ppara*^{-/-} mice compared to WT mice. Most *Ppara*^{-/-} mice succumbed to sepsis within 24 to 48 hours of CLP. Thereafter, the slopes of the survival curves were similar, indicating that PPAR α dependent effects occurred early in sepsis (Figure 2A).

PPAR α is expressed broadly in both hematopoietic and non-hematopoietic tissues (10, 11). Therefore, bone marrow chimeras of WT and *Ppara*^{-/-} mice were used to evaluate if PPAR α expression in leukocytes or in non-hematopoietic tissues was critical for survival. Marrow from either genotype did not affect survival of the recipient mice (Figure 2B). For example, the survival curve of the *Ppara*^{-/-} mice transplanted with WT marrow paralleled that of the *Ppara*^{-/-} mice transplanted with *Ppara*^{-/-} bone marrow. Similarly, the WT chimera's survival curve mirrored that of the WT control. Therefore, even though previous data suggested that PPAR α influences the immune response in sepsis (7, 8), these results demonstrate that PPAR α expression in non-hematopoietic tissues is more critical in determining survival outcomes than in hematopoietic cells.

PPAR α expression protects against organ injury in experimental polymicrobial sepsis

PPAR α is highly expressed in solid organs, and organ system failure is a common feature of sepsis (1, 5, 6). Therefore, plasma biomarkers of heart, kidney, and liver injury were evaluated to assess if differences in organ injury existed between WT and *Ppara*^{-/-} mice. Cardiac troponin-I, blood urea nitrogen (BUN), creatinine, and alanine aminotransferase (ALT) activity were measured at baseline and 24 hours after sepsis initiation with CLP, when the steep decline in survival was observed in the *Ppara*^{-/-} mice (Figure 2). Plasma levels of cardiac troponin-I, BUN, and creatinine were all significantly higher in *Ppara*^{-/-} mice compared to WT mice. In contrast, plasma ALT activity was elevated to the same degree in both WT and *Ppara*^{-/-} mice (Figure 3).

As assessed by tissue histology at 24 hours after CLP, neither lung nor liver showed signs of significant tissue injury in either genotype (data not shown). However, *Ppara*^{-/-} mice had higher severity scores in heart and kidney (Figure 4). Although *Ppara*^{-/-} kidneys had zones of tubular degeneration and necrosis not seen in WT kidneys (Figure 4B), pathologic changes in *Ppara*^{-/-} cardiac muscle were most striking. We observed discrete areas of myocardial vacuolization (degeneration), hyalinization and muscle fiber loss (necrosis) in the great majority of *Ppara*^{-/-} hearts that were not evident in WT tissue. In *Ppara*^{-/-} mice, this myocardial degeneration and necrosis were consistently more severe within the left ventricular free wall and to a lesser extent the interventricular septum (IVS), resulting in a “moth-eaten” appearance (Figure 4A).

Ppara^{-/-} mice have profound downregulation of enzymes related to lipid metabolism in the heart in experimental polymicrobial sepsis

The heart depends upon FAO to provide a significant portion of its energy. Because many enzymes involved with fatty acid metabolism are regulated by PPAR α (12, 13), expression of genes related to FAO was evaluated to assess if differences in PPAR α -dependent regulation of fatty acid metabolism contributed to the injury seen in *Ppara*^{-/-} heart tissues after CLP. PPAR α expression decreased in all tissues except lungs in response to sepsis (Figure 5A). Notably, expression of PPAR α in lung was low at baseline (average lung qRT-PCR cycle threshold was 30.1 compared to 24.9 in heart, 26.4 in kidney, and 24.8 in liver). Kidney and liver PPAR α expression increased above the 24 hour time point expression nadir at 48 and 72 hours, but cardiac PPAR α expression remained low.

Because of the significant troponin elevations in *Ppara*^{-/-} mice coupled with the dramatic histologic findings in the heart and the profound and persistent downregulation of PPAR α in the heart, the relative expression of 6 genes related to PPAR α function was evaluated in the heart. Medium chain acyl-CoA dehydrogenase (*Acadm*), very long chain acyl-CoA dehydrogenase (*Acadvl*), and carnitine palmitoyltransferase 2 (*Cpt2*) are downstream transcriptional targets regulated by PPAR α and their mRNA levels indicate the degree of PPAR α activation (14). These genes encode enzymes involved in FAO and lipid transport respectively, and thus they function to provide energy to the cell. PPAR γ coactivator 1 alpha (*Pgc1a*) is a protein that interacts with transcription factors, including PPAR α , to positively regulate mitochondrial energy production and other intracellular metabolic processes (15). PPAR β/δ (*Ppard*) and PPAR γ (*Pparg*) are members of the same receptor family as PPAR α .

(*Ppara*). Their expression was measured to assess both how their levels were changed in sepsis and whether absence of PPAR α affected their expression.

In both WT and *Ppara*^{-/-} mice, cardiac mRNA expression of *Acadm* and *Acadvl* was decreased in response to sepsis. Expression of *Cpt2* did not significantly change from genotype-specific basal levels (Figure 5B). WT expression of all three genes was greater than that seen in *Ppara*^{-/-} mice at the crucial 24 and 48 hour time points after CLP. Cardiac protein expression of ACADM and ACADVL appeared to increase in sepsis, but consistent with mRNA differences between genotypes, protein levels were lower in heart tissue of *Ppara*^{-/-} mice than in WT mice at every time point evaluated (Figure 6).

In WT hearts, *Pgc1a* expression was unchanged from baseline over the first 72 hours of sepsis. In the *Ppara*^{-/-} mice, however, cardiac *Pgc1a* expression was significantly higher at 72 hours after CLP. At 24 and 48 hours after CLP, WT *Pgc1a* expression was greater than *Ppara*^{-/-} mouse expression. In both WT and *Ppara*^{-/-} mice, there was a slight increase in *Ppard* and *Pparg* expression after CLP. We did not observe any differences between the genotypes at any time tested for *Ppard*. The *Ppara*^{-/-} mice, however, had increased expression of *Pparg* at 24 hours after sepsis when compared to WT mice (Figure 5B).

Blood glucose levels are lower in *Ppara*^{-/-} mice in experimental polymicrobial sepsis

Baseline blood glucose levels were similar in WT and *Ppara*^{-/-} mice. At 24 hours after CLP both WT and *Ppara*^{-/-} mice became hypoglycemic, but the *Ppara*^{-/-} mice had significantly lower blood glucose levels (Figure 7).

Discussion

Previously, we reported that reduced gene expression of PPAR α in whole blood leukocytes in children with severe sepsis is associated with decreased survival and that *Ppara*^{-/-} mice show increased mortality in CLP-induced polymicrobial sepsis (8). However, despite our observations of a similar reduction in leukocyte PPAR α expression in septic mice the data presented in this manuscript demonstrate that PPAR α expression in non-hematopoietic tissues is most important to survival. PPAR α may modulate leukocyte function, but survival in sepsis is more dependent on the role it plays in maintaining organ function.

Plasma biomarkers of heart and kidney injury were significantly elevated in *Ppara*^{-/-} mice compared to WT mice at early time points after CLP, and tissue histology revealed worse kidney injury and dramatically more cardiac damage in the *Ppara*^{-/-} mice. Interestingly, both of these organs have high energetic demands that rely primarily on FAO for ATP production (12, 16). Neither the lung nor the liver, which are much more versatile in their utilization of metabolic substrate, showed significant injury. These findings agree with recent reports that lung and liver injury are not early features of this sepsis model (17, 18). Those investigations did not, however, specifically evaluate cardiac injury.

Cardiac dysfunction is commonly observed in patients with severe sepsis (19–25), which may be explained by inflammation-induced changes to intracellular metabolism and ATP production. Reduced contribution of fatty acid substrates to cardiac metabolism has been

reported in human subjects with septic shock (26) and many nuclear hormone receptors and their cofactors are downregulated in the heart in mice after lipopolysaccharide administration (27). Lipopolysaccharide signaling through TLR-4, JNK, and nuclear factor- κ B (NF- κ B) triggers a myocardial fuel switch by suppressing FAO (28, 29). Thus, it is evident that in sepsis, the heart shifts away from utilizing fatty acid substrates for oxidative phosphorylation towards other energy sources. In humans with heart failure and in animal heart failure models, this shift in myocardial substrate utilization has been well documented—PPAR α expression is reduced, the rate of FAO decreases, and aerobic glycolysis increases to maintain adequate ATP levels (12, 30, 31). It is unknown if this substrate shift is adaptive or maladaptive.

Whether alteration of PPAR α function contributes to cardiac dysfunction and altered energy utilization in human sepsis is unknown. However, studies in mice demonstrate the importance of PPAR α in cardiac metabolism. *Ppara*^{-/-} mice have significantly lower constitutive expression of genes related to FAO in the heart (32, 33). Gene expression changes correlated with decreased cardiac uptake of radiolabeled fatty acids and reduced fatty acid metabolism (33).

In our study, PPAR α mRNA expression in WT mice was significantly reduced in heart, kidney, and liver tissues during sepsis. Reduction of PPAR α expression was greatest and most prolonged in the heart where decreased PPAR α activity was also noted by concomitant downregulation of genes encoding enzymes involved in FAO. Similar findings have been reported previously in the endotoxemia models of sepsis (27, 29), supporting a conclusion that this observation is not a feature unique to the CLP model. Importantly, mRNA expression levels of FAO enzymes were significantly lower in *Ppara*^{-/-} mice than in WT mice.

It is interesting that protein levels of ACADM and ACADVL appear to have a discordant increase at the 24 and 48 hour time points while their mRNA levels decrease. This phenomena is more dramatic in the WT mice than in the *Ppara*^{-/-} mice. Elevated enzyme levels may be due to increased translation of mRNA already present in the cell, decreased protein degradation, or by increased transcriptional activation mediated by other transcriptional activators (34–36). The absolute quantity of FAO enzymes, however, does not determine rate of FAO, which is governed primarily by substrate availability and allosteric regulation (12, 37, 38). Myocardial substrate utilization in sepsis is an area of active, ongoing research in our laboratory.

Both mRNA and protein expression of enzymes involved with FAO pathways are lower in the *Ppara*^{-/-} mice. This likely resulted in a critical decrement of energy provision from fatty acid substrates. Using a model of heart failure, Luptak et al. demonstrated that cardiac dysfunction in *Ppara*^{-/-} mice is likely due to energy insufficiency and can be ameliorated by increasing expression of an insulin independent glucose transporter in the heart (39). In light of these findings, we propose that the increased sepsis mortality observed in *Ppara*^{-/-} mice is due to cardiovascular failure caused by insufficient energy production from fatty acid substrates. We observed more severe hypoglycemia in the *Ppara*^{-/-} mice compared to WT mice, but whether this contributed to impaired ability of *Ppara*^{-/-} myocardium to use

glucose as an alternative to FAO cannot be determined based on our current data. Evaluating this possibility is a focus of ongoing work in our laboratory.

Additionally, we evaluated cardiac expression of PPAR γ and PPAR δ to assess whether these PPAR isoforms compensated for the absence of PPAR α signaling in *Ppara*^{-/-} mice. No consistent patterns of upregulation were observed indicating that compensatory increases in expression of these genes did not occur.

PGC-1 α is a transcription factor coactivator which interacts with a host of nuclear receptors, including the PPARs, to upregulate genes involved in FAO and mitochondrial biogenesis (15). We noted that although PGC-1 α is not a transcriptional target of PPAR α , the expression of PGC-1 α is lower in the *Ppara*^{-/-} mice at 24 and 48 hours after CLP. This finding indicates that other systems of metabolic regulation are likely involved in the suppression of FAO in sepsis beyond PPAR α signaling. Additionally, lower expression levels of PGC-1 α may portend impaired mitochondrial function and altered mitochondrial numbers. Mitochondrial dysfunction has recently been discussed as a plausible, and possibly very important, mechanism of organ failure in sepsis (40, 41). The results of the current study support further investigation of the role of mitochondrial dysfunction in sepsis-related organ dysfunction, especially in the heart.

Conclusions

In summary, we have confirmed our prior observations that mortality was increased in *Ppara*^{-/-} mice in sepsis. Furthermore, that increased mortality is not dependent upon PPAR α expression in hematopoietic cells, including immune cells, but rather on PPAR α expression in non-hematopoietic tissues. Of the organs examined in our model, heart injury appears to be most prominent in the *Ppara*^{-/-} mice with sepsis. Cardiac mRNA expression of genes related to FAO is downregulated in sepsis in both WT and *Ppara*^{-/-} mice, but levels are significantly lower in *Ppara*^{-/-} mice. Protein levels of FAO enzymes are also lower in *Ppara*^{-/-} mice. We conclude that increased mortality in *Ppara*^{-/-} mice is likely due to cardiac failure resulting from inadequate energy production due to insufficient FAO. Because similar metabolic changes have been noted in both mouse models of heart failure and human heart failure, we propose that these findings could have important implications for cardiovascular function in human sepsis. Returning to our initial observation that children with severe septic shock and multisystem organ failure have decreased expression of PPAR α , we feel that a similar metabolic mechanism may contribute to their increased morbidity and mortality (6, 8). Future work will be directed at linking our observations of decreased FAO enzyme expression with impaired heart function and assessing strategies to improve cardiac performance by enhancing substrate utilization for energy production.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Alden Timme, Erika E. Thommes, and Paul D. Sampson from the University of Washington, Department of Biostatistics for consulting on the data analysis; the University of Washington Histology and Imaging Core for assistance with tissue fixation, sectioning, and staining; and the Anne Manicone laboratory for sharing reagents and equipment for protein immunoblotting.

Source of Funding: This work was supported by grants from the American Heart Association (SWS) and NIH (JKM, WCP).

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Dr. Standage's institution received grant support from the American Heart Association (Grant #: 12SDG12040342). Dr. Waworuntu is employed by Fred Hutchinson Cancer Research Center. Dr. Maskal's institution received grant support from the American Heart Association. Dr. Duffield served as board member for Regulus Therapeutics Inc and Promedior Inc, is employed by Biogen, has patents (submitted a patent for use of anti-miR-21), and has stock in Promedior Inc and Biogen. His institution received grant support from the National Institutes of Health (NIH). Dr. Parks received support for article research from the NIH. Dr. Liles' institution received grant support from the NIH (Pending NIH Grants). Dr. McGuire received support for article research from the NIH. His institution received grant support from the NIH-NHLBI.

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Figure 1.

Leukocyte PPARα expression in experimental polymicrobial sepsis. Leukocyte PPARα expression assessed by qRT-PCR is significantly depressed in septic WT mice. Expression is normalized to time 0 (overall $p = 0.035$ by Kruskal-Wallis; * $p = 0.005$ on pairwise comparison between times 0 and 72 by Wilcoxon rank-sum, medians 1.0 (IQR 0.026–9.39) and 0.013 (IQR 0.013–0.026) respectively; $n = 10$ –20/group).

Figure 2.

Sepsis survival in WT, $Ppara^{-/-}$, and chimeric mice. A) $Ppara^{-/-}$ mice have much lower survival in CLP induced sepsis than WT mice (27% vs. 72 % respectively, $p < 0.0001$). Most of the mortality difference occurs between the 24 and 48 hour time points. B) Sepsis survival is not affected by reconstitution with opposite genotype bone marrow in chimeric mice. Irrespective of the type of bone marrow received, $Ppara^{-/-}$ recipients had lower survival than WT recipients ($p < 0.0001$). $Ppara^{-/-}$ mice with WT marrow had 5% survival and $Ppara^{-/-}$

mice with *Ppara*^{-/-} marrow had 0% survival ($p = 0.27$). WT mice with WT marrow enjoyed 65% survival while WT mice with *Ppara*^{-/-} marrow had 60% survival ($p = 0.90$).

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Figure 3.

Plasma biomarkers of organ injury. Twenty four hours after CLP, median cardiac troponin-I levels were significantly higher in *Ppara*^{-/-} mice compared to WT mice (0.95 ng/mL vs. 0.39 ng/mL respectively, $p = 0.0004$). BUN was similarly elevated (103 mg/dL in *Ppara*^{-/-} mice vs. 66 mg/dL in WT mice, $p = 0.023$) and creatinine levels (16.6 mg/dL elevation in *Ppara*^{-/-} mice vs. 7.3 mg/dL in WT mice, $p = 0.012$). Plasma ALT activity, though elevated in both WT and *Ppara*^{-/-} mice (1.9 mU/mL and 2.2 mU/mL respectively, $p = 0.17$), did not differ (* $p < 0.05$, $n = 5-10$ /group).

Figure 4.

Histologic severity scoring of heart and kidney tissue 24 hours after CLP. A) Severity scores of heart tissue sections were consistently higher in *Ppara*^{-/-} mice (rank sum 120.5 vs. 15.5, $p = 0.0013$). WT cardiomyocytes have normal appearance with cross striations and viable nuclei. *Ppara*^{-/-} tissue has multifocal areas where cardiomyocytes are markedly swollen with fragmentation and loss of sarcoplasmic cross striations and karyolytic or absent nuclei, indicative of degeneration and necrosis (arrowheads). Images taken from the left ventricle at 20 $\times$, scale = 100 μ m. B) Similarly, *Ppara*^{-/-} mice have higher kidney injury scores (rank sum 88.5 vs. 31.5, $p = 0.0365$). WT renal tubules and glomeruli are within normal limits. *Ppara*^{-/-} mice demonstrate multifocal to regional areas of injury in which renal tubules have swollen, pale epithelial cells, some with pyknotic or karyorrhectic nuclei (degeneration and necrosis). Few tubules also have loss of epithelium and contain luminal sloughed cells and debris (arrows). Adjacent renal tubules appeared normal (asterisk). Images taken from the renal cortex at 20 $\times$, scale = 100 μ m. (* $p < 0.05$, $n = 5-11$ /group)

Figure 5.

Cardiac gene expression in sepsis. A) Relative PPAR α expression levels in WT tissues. PPAR α expression decreases at 24 hours in all organs but the lungs (on one-way ANOVA heart $p < 0.0001$, lung $p = 0.448$, liver $p = 0.018$, and kidney $p = 0.004$; # $p < 0.05$ for pairwise comparisons with PPAR α expression at time 0). PPAR α expression is persistently suppressed only in the heart. B) Expression of genes involved in lipid metabolism and transport decrease during sepsis. Expression of these genes is generally much lower in *Ppara*^{-/-} mice than in WT mice. *Acadm*, *Acadvl*, and *Cpt2* all demonstrated significant ($p <$

0.05) interaction effects between genotype and time point on analysis by two-way ANOVA. Expression levels of *Ppard* and *Pparg* do not change dramatically in sepsis and do not differ significantly between genotypes in most instances (# $p < 0.05$ for pairwise comparison with same genotype control at time 0, * $p < 0.05$ for pairwise comparison with WT at the same time point, $n = 5-10/\text{group}$, error bars indicate standard deviation).

Figure 6.

Protein levels of FAO enzymes. Protein expression of both ACADM and ACADVL is lower in *Ppara*^{-/-} mice than WT mice at every time point. On the ACADVL immunoblot a nonspecific band (*) appeared at a higher molecular weight than the ACADVL band of interest. Vinculin is displayed as a protein loading control. This is a representative blot of 5 separate experiments (total n = 5 – 10/group).

Figure 7.

Blood glucose levels after CLP. Glucose levels are similar between genotypes at baseline (208 vs 204 mg/dL, $p = 0.7084$). After CLP-induced sepsis, levels drop in both WT and in *Ppara*^{-/-} mice, but are lower in *Ppara*^{-/-} mice (78 vs. 53 mg/dL respectively, $p = 0.0018$) (* $p < 0.05$., $n = 9-14$ /group, error bars indicate standard deviation).

Table 1**Sepsis Monitoring Criteria**

Physical Appearance	Score
Normal	0
Lack of grooming	1
Rough coat, nasal/ocular discharge	2
Very rough coat, abnormal posture	3
Diarrhea	+1
Body weight (g)	Score
Normal	0
< 10% change	1
10–20% change	2
> 20% change	3
Temperature change	Score
No significant change	0
Small changes of minimal significance	1
Temperature of 28–34° C or 39–41° C	2
Temperature of <28° C or >41° C	3
Respiratory status	Score
Normal	0
Small changes of minimal significance	1
Respiratory rate change of < 50%	2
Estimated rate increased >50% or markedly reduced	3
Unprovoked behavior	Score
Normal	0
Small changes of minimal significance	1
Abnormal: reduced mobility, decreased alertness, inactive	2
Unsolicited vocalizations, self-mutilation, very restless, immobile, shivering, seizures *	3
Response to External Stimuli	Score
Normal	0
Minor depression/exaggeration of response	1
Moderately abnormal responses	2
Hyperexcitability, shaking, violent reactions, or comatose *	3

* indicates euthanasia criteria

Table 2**Histologic Scoring Criteria**

Heart	Score
No lesions/no changes	0
LV: rare (<10) individual vacuolated myocytes	1
LV+ RV +/- IVS, Atria: 10–25 (mild) individual +/- clusters vacuolated myocytes; rare degenerate myocytes	2
LV + RV +/- IVS, Atria: >25 vacuolated myocytes; few individual degenerate myocytes	3
LV+ RV +/- IVS, Atria: multifocal individual degenerate myocytes; few clusters degenerate myocytes	4
LV + RV +/- IVS, Atria: multifocal to coalescing regions +/- individual degenerate myocytes	5
Lung	Score
Normal lung; rare alveolar macrophages +/- congestion & scant edema	0
Mild multifocal edema fluid, multifocal (few) alveolar macrophages +/- congestion	1
Multifocal moderate edema, fibrin +/- RBC; multifocal alveolar macrophages, reactive endothelium, minimal perivascular edema	2
Multifocal to coalescing alveoli w/fibrin/fluid/RBCs; moderate (>3 per alveoli) multifocal alveolar macrophages	3
Large coalescing regions of fibrin exudation/edema & mild neutrophilic alveolitis	4
Diffuse alveolar fibrin exudation & mild to moderate neutrophilic alveolitis	5
Kidney	Score
No lesions/no changes	0
Rare (minimal) multifocal vacuolated tubular epithelial cells	1
Mild multifocal vacuolated/degenerate tubules & rare multifocal necrosis	2
Moderate multifocal vacuolated/degenerate tubules & multifocal necrosis	3
Coalescing regions of tubular degeneration & necrosis	4
Diffuse renal tubular degeneration & necrosis; acute infarcts	5
Liver	Score
No lesions/no changes	0
Rare (minimal) hepatocellular degeneration	1
Mild to moderate multifocal hepatocellular degeneration & necrosis	2
Multifocal to coalescing hepatocellular degeneration & necrosis	3

LV-left ventricle, RV-right ventricle, IVS-interventricular septum

RBC-red blood cell