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Neuropathological correlates of hyperglycemia during prolonged polymicrobial sepsis in mice

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Running head: Hyperglycemia and the brain in murine sepsis

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ABBREVIATIONS

AEC	amino-ethyl-carbazole
GFAP	glial fibrillary acidic protein
ICU	intensive care unit
ISEL	in-situ end-labelling
RCA-1	Ricinus Communis Agglutinin I
RNS	reactive nitrogen species
ROS	reactive oxygen species

ABSTRACT

Glucose toxicity may play a crucial role in evoking neurologic complications of critical illness. We studied whether the neuropathological alterations in fatal human critical illness observed under hyperglycemia are present and can be attenuated by maintaining normoglycemia in a mouse model of prolonged sepsis induced by cecal ligation and puncture. Mice were randomized to moderate hyperglycemia (>8.3 mmol/l, $n=8$) or normoglycemia (4.4 - 6.7 mmol/l, $n=8$). After 5 days, hippocampus and frontal cortex from septic mice were compared with healthy controls ($n=8$). Blood glucose was 7.8 ± 1.3 mmol/l in hyperglycemic and 6.1 ± 0.7 mmol/l in normoglycemic critically ill mice ($p=0.007$). The percentage of damaged neurons was two-fold higher in frontal cortex ($p=0.01$) and hippocampus ($p=0.06$) of hyperglycemic ill mice than that of healthy mice. In frontal cortex, neuronal damage was attenuated under normoglycemia ($p=0.04$). Critical illness reduced astrocyte density and activation status 4-fold in hippocampus ($p\leq 0.02$), but not in frontal cortex, irrespective of glycemic control. Microglia were 2-4-fold more abundant in both brain areas of hyperglycemic critically ill mice ($p\leq 0.002$), but only in frontal cortex reduced in number with normoglycemia ($p=0.0008$). The density of apoptotic cells and abundance of carbonylated proteins were significantly higher than normal in frontal cortex of hyperglycemic ill mice only ($p=0.05$). In a mouse model of prolonged polymicrobial sepsis, remarkable neuropathological changes develop with neuronal damage, impaired astrocyte activation, increased microglia, apoptosis and accumulation of carbonylated proteins. These changes were partially prevented or attenuated when hyperglycemia was prevented with insulin. Frontal cortex appeared more vulnerable to hyperglycemic insults than hippocampus.

KEY WORDS

Sepsis, hyperglycemia, insulin, neuron, astrocyte, microglia

INTRODUCTION

Acute brain dysfunction is a frequent complication of critical illness that ranges from delirium to coma and has been shown to be an independent predictor of higher 6-month mortality, longer hospital stay, and increased hospital costs (1). In survivors of critical illness, longer duration of delirium in-hospital has recently been associated with worse global cognition and executive function at 3 and 12 months after discharge (2).

Several risk factors for developing acute brain dysfunction during critical illness have been identified, but the underlying pathophysiology remains poorly understood. Human and experimental data suggested that persistent hyperglycemia likely plays a crucial role in critical illness-induced neuropathological alterations (3, 4), occurrence and persistence of delirium in the intensive care unit (ICU) (5), and long-term cognitive impairment in survivors of critical illness (6). In this regard, neuropathological studies in non-survivors of critical illness suggested that, maintaining normoglycemia, despite some episodes of hypoglycemia, reduced neuronal injury, microglia activation, and apoptosis while preserving astrocyte activation status in hippocampus and/or frontal cortex (3). In a rabbit model of hyperinflammatory critical illness, microglia activation in frontal cortex appeared the earliest event preventable by maintenance of normoglycemia versus pronounced hyperglycemia and preceded neuronal damage (3). The study also implicated reduced oxidative stress in the attenuation of neuronal injury. Neuronal damage and glial alterations were essentially driven by hyperglycemia and not by an excessive glucose load (7).

Clinical neuroprotection with maintenance of strict normoglycemia (4.4-6.1 mmol/l or 80-110 mg/dl) has been suggested by reduced intracranial pressure levels and seizures in acute brain

injury patients and a lower incidence of critical illness polyneuropathy in a general critically ill patient population, as compared with hyperglycemia tolerated to the renal threshold level (12 mmol/l or 215 mg/dl) (8-11). Although several studies failed to confirm clinical benefit, recent meta-analyses supported that maintaining normoglycemia may translate into better neurologic outcomes (12, 13). The increased risk of severe hypoglycemia as major side effect of strict glycemic control with insulin (14-17) is feared for inducing direct or indirect brain damage (18, 19) and even normal blood glucose levels have been suggested to be too low for patients with acute brain injury (20-22). However, the strict glycemic control intervention or hypoglycemic events in particular did not detectably evoke neurological damage and were not associated with worse neurocognitive outcome of critically ill children at long-term follow-up (3, 23, 24).

In the present study, we evaluated whether our recently developed mouse model of prolonged polymicrobial sepsis (25) presents with similar neuropathological alterations as critically ill patients and rabbits (3) and brain region-specific vulnerability to hyperglycemic insults. This would not only allow validation for further mechanistic studies, but also would pave the way for evaluating potential new therapeutic interventions to counteract the brain abnormalities developing in critical illness.

MATERIALS AND METHODS

Animal model

The study was performed in a mouse model of prolonged sepsis (25), after approval of the protocol (P051/2010) by the Institutional Ethical Committee for Animal Research. We anesthetized male, 24-week old, C57BL/6J mice (Janvier SAS Chassal, France) having a mean weight of 29.1 g (SD 2.0 g) with an intraperitoneal injection of ketamin (3 mg, Univet, Belgium) and xylazin (0.4 mg, VMD, Belgium) and placed them on a heating pad maintained at 25°C. Mice were breathing spontaneously and were placed under continuous inhalation of 1-2% isoflurane (Schering-Plough, UK) with oxygen (2 liter/min) through a facial mask. Surgery was performed under a microscope. A homemade sterilized microrenathane catheter (MRE025; Braintree Scientific) was inserted in the left central jugular vein, tunneled to the back and attached to a single-channel-fluid-swivel (model 375/25; Instech Laboratories), allowing continuous resuscitation and parenteral nutrition under free movement. For the induction of sepsis, a 50% ligation of the cecum was performed at half the distance between the distal pole and the base of the cecum with 3.0 silk suture (Mersilk, Ethicon, Johnson & Johnson, Belgium), followed by a single puncture through-and-through with a 20-gauge needle. The cecum was then gently squeezed to extrude feces and returned to its original position. Surgical wounds were closed with 7.0 silk sutures (Mersilk, Ethicon). After surgery, the mice received analgesia by local infiltration of the surgical wounds with ropivacaine (1%, AstraZeneca, Belgium) and subcutaneous administration of buprenorphine (9 µg, Schering-Plough, UK). Meropenem was administered subcutaneously as broad-spectrum antibiotic (0.25 mg, Meropenem, AstraZeneca, UK). Mice were assigned a randomization group (vide infra), placed in individual cages and continuous intravenous fluid-resuscitation with a 4:1

mixture of NaCl_{0.45%}/Glucose_{2.5%} and 6% hydroxyethyl starch (Voluven, Fresenius Kabi, Belgium) was started at 0.5 ml/hour and maintained for 18 hours. Thereafter, fluid resuscitation was replaced with total parenteral nutrition (Clinomel N7, Baxter, Belgium), to deliver increasing amounts of calories from 2.9 kcal/day on day 1, 5.8 kcal/day on day 2 and 8.6 kcal/day on days 3 to 5. Glucose, amino acids and lipids respectively comprised 57%, 14% and 29% of the administered calories. Every 12 hours, the mice received 0.25 mg meropenem and 9-12 µg buprenorphine subcutaneously. The cage temperature was kept at 27±1°C with heating lamps throughout the study in order to avoid hypothermia.

The mice were randomly allocated (by sealed envelopes) to receive one of two levels of blood glucose control during critical illness: hyperglycemia (>8.3 mmol/l or >150 mg/dl) or normoglycemia (4.4-6.7 mmol/l or 80-120 mg/dl). Glucose levels were measured in tail blood using an Accu-Chek glucometer (Roche, USA). In mice assigned to the normoglycemia group, continuous intravenous infusion of insulin (Actrapid Penfill, Novo Nordisk, Denmark) was started to correct the blood glucose level according to an in-house developed protocol that had been validated in a pilot experiment. More specifically, starting from the morning of day 3, when total parenteral nutrition reached the maximal infusion rate of 0.3 ml per hour, 0.1 IU insulin was added per 20 ml TPN. Blood glucose levels were measured three times per day. For glucose levels between 4.4 and 5.6 mmol/l (80-100 mg/dl) the insulin infusion dose of 0.1 IU per 20 ml TPN was maintained. The insulin dose was increased to 0.2 IU, 0.25 IU or 0.3 IU per 20 ml TPN for glucose levels of 5.6-7.8 mmol/l (100-140 mg/dl), 7.8-8.3 mmol/l (140-150 mg/dl) or above 8.3 mmol/l (>150 mg/dl), respectively. Mice allocated to the hyperglycemia group did not receive insulin. In all animals, blood glucose was quantified on day 3.

Mice that lost their catheter were excluded. The study was continued until 8 animals per group survived the preset study time of 5 days. Hence, 13 animals were needed in the hyperglycemic group (5 non-survivors) and 9 in the normoglycemic group (1 non-survivor). After 5 days, the animals were sacrificed by decapitation, the brain was removed and fixed in 4% paraformaldehyde in PBS and embedded in paraffin. No transcardial perfusion with saline was performed prior to decapitation. Critically ill mice were compared with 8 healthy control mice that received 5 g of standard chow per day (13 kcal/day).

Histology

Brains were studied at the level of hippocampus and frontal cortex. Five μm sections were stained with hematoxylin and eosin to detect damaged neurons, visible as neurons with shrunken eosinophilic cytoplasm and pyknotic nuclei. Astrocytes were immunostained for glial fibrillary acidic protein (GFAP). After blocking for endogenous peroxidases and antigen retrieval with citrate buffer, sections were blocked for 30 min in 3% normal goat serum. Sections were then incubated overnight at 4°C with a 1:5000 dilution of the primary rabbit polyclonal anti-GFAP antibody (ab7260, Abcam, Cambridge, UK). This was followed by a 1 hour incubation with a biotinylated anti-rabbit secondary antibody (1:200, Dako) and with avidin-biotin complex-peroxidase (Vector labs). Amino-ethyl-carbazole (AEC) was used as chromogen and sections were counter-stained with hematoxylin. Astrocytes were considered activated when an increase in the size of GFAP-positive cells was observed and when they presented with longer, thicker processes. Microglia were visualized with a 1:1000 dilution of biotinylated isolectin RCA-1 (B1085, Sigma, Bornem, Belgium), avidin-biotin complex-peroxidase and AEC. Apoptotic cells were determined after *in situ* end labeling (ISEL; ApopTag kit; Oncor, Gaithersburg, MD). For the staining of carbonylated proteins (26), tissue

sections were first permeabilized with 0.5% Triton X-100. Sections were then incubated with 0.01% (100 µg/ml) dinitrophenyl (DNP) in 2N HCl for 1 hr at RT in the dark to derivatize the carbonylated proteins. After an overnight blocking step with 5% BSA in PBS at 4°C, sections were subsequently incubated with a polyclonal rabbit anti-DNP antibody for 1 hr (S7150, Millipore, Billerica, MA) and with a secondary Alexa Fluor 488-conjugated anti-rabbit antibody (A21206, Life technologies, Carlsbad, CA) and with 10 µg/ml Hoechst 33342 for another hr. For the staining of nitrated proteins (26), permeabilized sections were blocked overnight at 4°C with 5% BSA in PBS, followed by 1 hr incubations with a primary anti-nitrotyrosine antibody (A21285, Life technologies), and with a secondary Alexa Fluor 488-conjugated anti-rabbit antibody (A21206, Life technologies) and 10 µg/ml Hoechst 33342.

All stainings were evaluated blinded for study groups by an operator trained in human and animal neuropathology. For the neuropathological studies, images were acquired with identical exposure on a Leica DM3000 microscope with a digital camera (Wetzlar, Germany) and analyzed with ImageJ1.36b software (Wayne Rasband, NIH, Bethesda, MD). Neuronal damage is expressed as the percentage of damaged neurons relative to the total number of neurons counted by the operator in 5 fields at 40-fold magnification per mouse. The numbers of GFAP-positive cells, cross-sectional area of GFAP staining (reflecting astrocyte activation), as well as microglia density and density of apoptotic cells were evaluated in 5 fields at 40-fold magnification. Only for cross-sectional area of GFAP staining an automated assessment was performed. The non-specific nature of RCA-1 staining, which also stains endothelial and blood cells, precluded automated analysis of microglia. For carbonylated and nitrated protein analyses, whole brain sections were scanned with a high-resolution scanner (Axio Scan Z1, Zeiss, Jena, Germany) using a Plan-Apochromat objective at 20-fold magnification. Cross-sectional areas of stained carbonylated and nitrated proteins were

quantified **by automated assessment** on a whole section of hippocampus and frontal cortex in each mouse using the Zen (Zeiss, Jena, Germany) and ImageJ1.36b softwares.

Statistical analysis

Variables are presented as mean and SD or median and interquartile range. Group differences were analyzed with Kruskal-Wallis/Mann-Whitney U test. Differences were considered statistically significant when two-tailed p-values were ≤ 0.05 . The statistical analyses were performed using Statview 5.0.1 (SAS Institute, Cary, NC). With 8 animals per group, the statistical power was higher than 80% to detect similar effects of maintaining normoglycemia on microglia in hippocampus and frontal cortex as observed in critically ill patients (3).

RESULTS

According to the protocol, blood glucose levels were significantly higher in hyperglycemic animals (7.8 ± 1.3 mmol/l or 141 ± 24 mg/dl) than in normoglycemic animals (6.1 ± 0.7 mmol/l or 111 ± 12 mg/dl, $p=0.007$).

In hippocampus, sepsis tended to increase neuronal damage above control levels (median 2.3-fold higher, $p=0.06$), irrespective of the glucose control strategy (Fig. 1). In frontal cortex, neuronal damage was significantly increased 2.5-fold in hyperglycemic animals ($p=0.01$). This increase was significantly attenuated by maintenance of normoglycemia ($p=0.04$). Astrocyte density and activation status were approximately 4-fold lower in hippocampus of critically ill animals as compared with healthy controls, irrespective of the glucose control strategy (Fig. 2). This effect was less obvious in frontal cortex. Microglia were 4.2- and 1.7-fold more abundant in hippocampus ($p=0.002$) and frontal cortex ($p=0.0008$) of hyperglycemic animals than of healthy control mice (Fig. 3). Microglia density was significantly attenuated under normoglycemia in frontal cortex only ($p=0.0008$). The density of apoptotic cells in hippocampus was comparable among healthy and critically ill mice, irrespective of glucose management (Fig. 4). In frontal cortex of hyperglycemic ($p=0.05$) but not of normoglycemic mice ($p=0.10$), the number of apoptotic cells was significantly increased above control levels.

Microglia density in hippocampus ($r^2=0.32$, $p=0.02$) and frontal cortex ($r^2=0.59$, $p=0.0008$) correlated positively with blood glucose levels of critically ill animals (Table 1). None of the other immunohistopathological markers significantly correlated with glucose levels.

Sepsis increased carbonylated proteins levels in frontal cortex of hyperglycemic animals ($p=0.01$), but not in hippocampus (Fig. 5). This increase tended to be attenuated under normoglycemic conditions ($p=0.08$), but remained significantly elevated above control levels. Nitrated protein levels did not differ significantly between septic animals and controls (Fig. 5).

DISCUSSION

The present study performed in a well-validated mouse model of prolonged polymicrobial sepsis allowed to delineate two major findings. First, in this model we confirmed the presence of similar neuropathological alterations previously observed in hyperglycemic patients who died from critical illness and in hyperglycemic critically ill rabbits, as well as the potential beneficial effects of preventing hyperglycemia with insulin on neuronal damage and microglial changes. Second, our results suggest a region-specific vulnerability for hyperglycemia-induced neuropathological lesions, as frontal cortex appeared more vulnerable to hyperglycemic insults than hippocampus.

Critical illness and sepsis are associated with important neuropathological changes. These include neuronal damage, glial changes, and increased apoptosis in various areas of the brain, as suggested by studies performed in non-survivors of critical illness (27, 28). Brain alterations in patients with sepsis have been predominantly found in areas of the brain susceptible to ischemia (including Ammon's horn, the frontal junctional cortex, the lenticular nuclei, the dentate nucleus, and the medullary olive), in the hypothalamic nuclei (supraoptic and paraventricular nuclei), the amygdala, the *locus coeruleus*, and the medullary autonomic nuclei (nucleus *tractus solitarius*, ambiguous, and parabrachial nuclei) (27, 28). These neuropathological alterations may be elicited or aggravated by various insults occurring during sepsis, including persistent inflammation, cerebral hypoperfusion, and/or hypoxia. Previous data suggested that, apart from these insults, persistent hyperglycemia is associated with increased neuronal damage and apoptosis, impaired astrocyte activation, increased microglial activation and microglial apoptosis in hippocampus and/or frontal cortex (3, 4). Indeed, such neuropathological abnormalities were most pronounced with severe,

uncontrolled hyperglycemia, less with moderate hyperglycemia and significantly attenuated or prevented under strict normoglycemia as observed in brains of patients who did not survive critical illness and in an *in vivo* rabbit model of critical illness.

In the currently presented mouse model of prolonged sepsis we observed largely similar qualitative brain pathological changes under moderate hyperglycemia. The data suggest that persistent, moderate hyperglycemia for 5 days induces significant neuronal damage and microglial changes in frontal cortex, as these alterations were prevented under normoglycemia. In contrast, neuronal damage, astrocyte loss and enhanced microglia in hippocampus of the mice were not significantly affected by glycemic control and hence appeared (largely) unrelated to hyperglycemia. Nevertheless, although hippocampus is invariably damaged during prolonged, experimentally induced severe hypoglycemia (29), the association between neuronal lesions and elevated glucose levels has been scantily described. Thus, our data also suggest that during critical illness, the hippocampus may be less vulnerable to prolonged hyperglycemic stress than the frontal cortex.

We also observed a significant accumulation of carbonylated proteins in frontal cortex of septic animals under hyperglycemia. Maintenance of normoglycemia tended to attenuate this effect, though carbonylated proteins remained significantly elevated above control levels. This suggests that other mechanisms are important to explain sepsis-induced brain damage in frontal cortex, such as hypoxia, ischemia and/or systemic inflammation. Unlike carbonylated proteins, nitrated protein levels were comparable for critically ill and healthy mice in both brain areas, confirming the presence of a strong neuro-oxidative-nitrosative stress imbalance at the level of frontal cortex only. Neuro-oxidative-nitrosative stress has been suggested as a potential molecular basis for underlying brain dysfunction in sepsis (30). Because of failure of

local antioxidant systems, sepsis-induced reactive oxygen and nitrogen species (ROS/RNS) may trigger lipid peroxidation reactions in cerebral endothelial cells and surrounding brain parenchyma. Accumulation of ROS/RNS may cause structural membrane damage, worsen brain inflammation, and scavenge nitric oxide (NO) to yield peroxynitrite (ONOO⁻). ROS/RNS can cause mitochondrial dysfunction by inhibiting the mitochondrial electron transport chain and uncoupling oxidative phosphorylation, which ultimately leads to neuronal bioenergetic failure. Free radicals may induce neuronal apoptosis in vulnerable areas of the brain, including the frontal cortex. Therefore, ROS/RNS-mediated neuronal bioenergetic failure and apoptosis may represent an important mechanism in sepsis-associated encephalopathy and cognitive decline in survivors (31).

Interestingly, the particular vulnerability of the frontal cortex to hyperglycemic insults during sepsis is in line with our previous human data and with our findings in the well-controlled setting of a rabbit model of prolonged burn injury-induced critical illness (3). Moreover, our results are in line with the neurocognitive long-term follow-up study of the critically ill children included in a large randomized controlled trial on tight glucose control to age-adjusted normoglycemia (2.8-4.4 mmol/l or 50-80 mg/dl at age <1 year and 3.9-5.6 mmol/l or 70-100 mg/dl at age 1-16 years) (24). The primary acute outcome study had shown reduced intensive care morbidity and mortality with tight glycemic control as compared with usual care, despite an increased risk of severe hypoglycemia (≤ 2.2 mmol/l or ≤ 40 mg/dl) (16). Long-term follow-up four years after inclusion in the study revealed that children who had been treated with insulin to maintain normoglycemia during ICU stay did not have a worse measure of intelligence than those who had received usual care (24). Moreover, in this study, maintaining normoglycemia improved motor coordination and cognitive flexibility (24), highlighting the potential neuroprotective effects on executive functions, in which the

prefrontal cortex has an important steering role (32-34). Finally, in this study, the risk of insulin-induced hypoglycemia could not be detected, as brief hypoglycemic episodes evoked by insulin therapy, but rapidly corrected, were not associated with worse neurocognitive outcome (24). Altogether, these data suggest that the risk of tolerating persistent hyperglycemia during prolonged critical illness overwhelms by far the risk of brief episodes of hypoglycemia associated with insulin infusion.

Microglia activation was identified as the earliest neuropathological event preventable by maintenance of normoglycemia in our rabbit model of critical illness (3). In the critically ill septic mice studied here, we also observed a positive correlation between brain inflammation, as reflected by microglial cells, and the degree of hyperglycemic stress. This confirms the relevance of this cell type in the brain alterations observed during critical illness.

The present experimental study has several limitations. First, a sham group with surgical instrumentation required for the cecal ligation and puncture model was not added. Therefore, the separate impacts of “surgical stress” and sepsis cannot be differentiated in this study. However, the key question was the additional impact of hyperglycemia during critical illness on neuropathological changes. The surgical instrumentation and the treatment aspects of the model were considered to be part of the critically ill condition and its treatment. Second, we did not perform neurological evaluation of the animals during the experiments, precluding an analysis of potential correlates between brain dysfunction and neuropathological patterns observed in sepsis. Third, we have only limited blood glucose data. These did not reveal hypoglycemia, whereas prolonged deep hypoglycemia has been observed in other CLP models (35, 36). This may relate to several differences among the models, in which the anesthesia, the more extensive surgery and the early intravenous fluid resuscitation and

feeding in our study may have been particularly important. Due to the limited availability of glucose data, the impact of any potential undetected hypoglycemic episodes and the effect of glucose variability on neuropathological changes could not be determined. However, the results of our previous studies suggested that the neuropathological impact of brief hypoglycemic episodes induced by the treatment of hyperglycemia during critical illness was not significant. Fourth, although we previously demonstrated that the benefits of strict glycemic control with intensive insulin therapy in a rabbit model of prolonged critical illness were mainly explained by the maintenance of normoglycemia (37, 38), direct neuroprotection by insulin independent of glucose control cannot be excluded in the present study. For instance, inactivation by insulin of GSK-3 β has shown to protect against brain injury and to reduce inflammation (39-41). Fifth, we used a rather aspecific staining to evaluate neuronal damage. However, all brains were sampled according to the same procedure. Hence, any difference among the groups can only be explained by the randomized intervention. Finally, as for all animal studies, caution is warranted when extrapolating to other models or to the clinical setting. However, largely similar neuropathological changes and impact of glucose control have been shown to develop in critically ill patients, rabbits and now also mice (3). Together these human data and the experimental findings in two different models may further support a neuropathological basis to explain brain atrophy (42), and long-term psychological and cognitive disorders observed in survivors of critical illness (2).

In conclusion, in a mouse model of prolonged polymicrobial sepsis, we observed neuronal damage, compromised astrocyte activation, increased number of microglia and apoptosis in hippocampus and/or frontal cortex, as previously described in brains of deceased critically ill patients and in brains of an *in vivo* rabbit model of critical illness under hyperglycemia. As in the patients and rabbits, these neuropathological changes were to a certain extent attenuated or

prevented by avoiding hyperglycemia with insulin infusion, where frontal cortex appeared more vulnerable to hyperglycemic insults than hippocampus. Hence, these data support the validity of the mouse model for further mechanistic studies on neuropathological changes in sepsis and for evaluating new therapeutic interventions to improve these alterations.

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FIGURE LEGENDS

Fig. 1. Neuronal damage in hippocampus and frontal cortex of critically ill mice five days after cecal ligation and puncture. Data are normalized to the median of the healthy mice and are presented as box plots, with the central line indicating the median, the box the interquartile range, and the whiskers the 10th and 90th percentiles. Mann-Whitney U p-values above the boxes represent the comparisons of the hyperglycemic and normoglycemic groups with healthy mice, the others the comparisons between hyperglycemic and normoglycemic critically ill mice and the comparisons between healthy and all critically ill mice. Representative pictures are shown in Supplemental Digital Content Figure 1.

Fig. 2. Astrocytes in hippocampus and frontal cortex of critically ill mice five days after cecal ligation and puncture. Data are normalized to the median of the healthy mice and are presented as box plots, with the central line indicating the median, the box the interquartile range, and the whiskers the 10th and 90th percentiles. Mann-Whitney U p-values above the boxes represent the comparisons of the hyperglycemic and normoglycemic groups with healthy mice, the others the comparisons between hyperglycemic and normoglycemic critically ill mice and the comparisons between healthy and all critically ill mice. Representative pictures are shown in Supplemental Digital Content Figure 1.

Fig. 3. Microglia in hippocampus and frontal cortex of critically ill mice five days after cecal ligation and puncture. All data are normalized to the median of the healthy mice. Data are presented as box plots, with the central line indicating the median, the box the interquartile range, and the whiskers the 10th and 90th percentiles. Mann-Whitney U p-values above the boxes represent the comparisons of the hyperglycemic and normoglycemic groups with

healthy mice, the others the comparisons between hyperglycemic and normoglycemic critically ill mice and the comparisons between healthy and all critically ill mice. Representative pictures are shown in Supplemental Digital Content Figure 1.

Fig. 4. Apoptosis in hippocampus and frontal cortex of critically ill mice five days after cecal ligation and puncture. All data are normalized to the median of the healthy mice. Data are presented as box plots, with the central line indicating the median, the box the interquartile range, and the whiskers the 10th and 90th percentiles. Mann-Whitney U p-values above the boxes represent the comparisons of the hyperglycemic and normoglycemic groups with healthy mice, the others the comparisons between hyperglycemic and normoglycemic critically ill mice and the comparisons between healthy and all critically ill mice.

Fig. 5. Protein carbonylation and nitration in hippocampus and frontal cortex of critically ill mice five days after cecal ligation and puncture. All data are normalized to the median of the healthy mice. Data are presented as box plots, with the central line indicating the median, the box the interquartile range, and the whiskers the 10th and 90th percentiles. Mann-Whitney U p-values above the boxes represent the comparisons of the hyperglycemic and normoglycemic groups with healthy mice, the others the comparisons between hyperglycemic and normoglycemic critically ill mice and the comparisons between healthy and all critically ill mice.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5