**Title:**

Acute inflammation alters energy metabolism in mice and humans: Role in sickness-induced hypoactivity, impaired cognition and delirium.

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**Abstract:**
Systemic infection can result in a spectrum of metabolic and behavioral changes, termed sickness behavior, an organismal reprioritization that suppresses activity, conserves energy, and maximizes the probability of recovery. In vulnerable individuals, acute sickness can include profound acute cognitive impairments including delirium. The molecular mechanisms driving the acute suppression of activity and the acute cognitive deficits arising remain unclear. Here, we hypothesized that disruption of energy metabolism during acute inflammation is a significant contributor to behavioral changes after bacterial endotoxin in mice, and to delirium after inflammatory trauma. LPS (250 μg/kg) and IL-1β (25 μg/kg) markedly decreased blood glucose in c57BL6J mice. LPS-induced decreases in glucose still occurred in IL-1R1$^{-/-}$ mice and in animals treated with IL-1RA (100 μg/kg). Locomotor activity correlated with blood glucose concentration and treatment with glucose (2 g/kg) prevented the suppression of spontaneous activity. Inhibition of glycolysis using 2-deoxyglucose completely suppressed locomotor activity despite preventing IL-1β synthesis. Selectively in ME7 animals with chronic hippocampal and thalamic synaptic loss, LPS (100 μg/kg) produced robust cognitive dysfunction and this could be mimicked with insulin and significantly mitigated with glucose treatment, demonstrating that reduced glucose levels are a major driver cognitive impairment in the vulnerable brain. Analysis of glycolytic metabolites in human CSF from hip fracture patients showed that there is also a significant alteration of brain energy metabolism (elevated lactate and pyruvate) during delirium. Collectively the data suggest that behavioral impacts of acute systemic inflammation are strongly influenced by disruption of energy metabolism.

**Introduction:**

Systemic infection can result in a spectrum of metabolic and behavioral changes, termed sickness behavior, which includes fever, lethargy, loss of appetite, anhedonia, and impairments in cognitive function (1). Sickness behavior is an evolutionarily conserved response to illness and is thought to represent a reprioritization by the organism to conserve energy and maximize the probability of recovery (2). Systemic administration of the bacterial endotoxin lipopolysaccharide (LPS), can induce sickness behavior in humans (3, 4) and rodents (1, 5) and although peripheral LPS does not readily cross the blood-brain barrier (6) it significantly increases systemic and central pro-inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) (1, 7, 8) and can alter resting state networks (9) and local field potential (10, 11). Therefore information about the inflammatory status of the periphery is communicated to the brain and this occurs by multiple routes: *i)* direct neural activation of the brainstem and hypothalamus via sensory afferents such as the vagus nerve; *ii)* activation of macrophages of the circumventricular organs that lack a patent BBB, leading to secretion of inflammatory mediators into the parenchyma; and *iii)* activation of brain vasculature cyclooxygenases to secrete lipophilic prostaglandins directly into the brain parenchyma (12, 13). In particular, genetic and pharmacological manipulations of prostaglandin-dependent mechanisms have revealed information on neuroanatomical pathways underpinning fever and stress responses, anorexia and sleep-wake cycle changes (14). The neuroanatomical and biochemical basis for the marked hypoactivity that occurs in the hours after LPS administration is not fully understood.

Among the features ascribed to sickness behavior is cognitive impairment. Peripheral administration of LPS or IL-1β can affect synaptic plasticity (15) and hippocampal-dependent learning and memory (15, 16), although the relative preservation of cognitive function is striking given the overt suppression of spontaneous behavior (16, 17). Clinically, peripheral bacterial infections, surgeries, or inflammatory traumas can provoke cognitive impairment but this is most problematic when inflammatory insults are either severe or when they occur on a background of advanced age or evolving dementia, to trigger delirium (18, 19), an acute onset and fluctuating syndrome characterized by inability to sustain attention, reduced awareness and perception, and profound cognitive impairment (18). Delirium affects approximately 1 in 5 hospital inpatients with rates increasing to 1 in 3 for those >80 years age (20). It is associated extended hospitalization, subsequent cognitive decline, and increased risk for dementia but the neurobiological understanding of delirium is limited. One key approach to modeling delirium is the superimposition of LPS-induced sickness behavior upon models of evolving neurodegeneration (21). In mice with chronic neurodegeneration in the ME7 strain of prion disease (22), with amyloidosis in the APP/PS1 model of Alzheimer's disease (23), or with cholinergic denervation of the hippocampus (24) we have shown acute onset and fluctuating deficits (25) in cognitive domains relevant to delirium when exposed to systemic LPS at 100 μg/kg. These LPS-associated deficits are absent in normal animals but susceptibility to LPS-induced cognitive deficits increases as a

function of the underlying degenerative state of the brain (25, 26). These LPS-induced deficits are prostaglandin-dependent (26), can be mimicked by systemic administration of either IL-1β (26) or TNF-α (27), and can be reduced by systemic administration of IL-1 receptor antagonist (IL-1RA) (16, 17). Although LPS-treated ME7 mice show higher levels of microglial IL-1β compared to LPS-treated controls, due to priming of the microglial population (28), it was systemic rather than central IL-1β that contributed to LPS-induced cognitive impairments in this model (17). This suggests that IL-1β may affect cognition via a peripheral route. One possibility is that acute sickness impinges on cerebral metabolism through systemic metabolic changes; Cerebral glucose uptake is reduced in a rat model of LPS-induced sepsis (10), carbohydrate metabolism is decreased post-LPS (29) and IL-1 has been demonstrated to reduce systemic glucose concentrations, which can be mitigated by systemic administration of IL-1RA (30). Systemic hypoglycemia impacts on central glucose levels (31, 32), which in turn can affect neuronal activity. Moreover, reduced glucose availability may be especially detrimental if the brain is already compromised during evolving neurodegenerative pathology.

Therefore, we hypothesized that LPS induced-disturbances in glucose metabolism would drive suppression of activity and cognitive impairment in mice. We assessed locomotor activity and 'escape from water' T-maze working memory to determine whether LPS-induced hypoglycemia drives sickness behavior and cognitive impairments in disease-naïve and ME7 mice. We show that changes in behavior and cognition can be achieved by lowering blood glucose using LPS or insulin, and these impairments can be attenuated with glucose and can be dissociated from changes in IL-1. Finally, we analyzed glycolytic metabolites in the cerebrospinal fluid (CSF) of inflammatory trauma patients (after hip fracture), revealing a disruption of brain carbohydrate metabolism in patients with delirium.

**Results:**

*LPS and IL-1β both robustly reduce systemic glucose concentrations*

LPS (250 μg/kg; i.p.) significantly increased plasma IL-1β levels at 2 and 6 hours post-challenge in c57BL6/J mice compared to saline-treated controls (Fig. 1B) and also reduced blood glucose levels by >50 % by 7 hours post-challenge (Fig. 1C). Basal blood glucose was slightly high but not outside the range frequently reported in the literature (30, 33-35). Blood glucose was decreased as early as 3 hours following LPS and had not fully returned to baseline levels by 24 hours. The reduction in blood glucose is not explained by suppression of feeding by LPS since blood glucose only begins to decrease after 6-12 hours of fasting in healthy c57BL6/J mice (36). As IL-1β levels peak prior to LPS's effect on blood glucose, we investigated the contribution of IL-1β signaling to LPS-induced hypoglycemia. IL-1β (25 μg/kg; i.p.) produced similarly low blood glucose as LPS but with a more rapid induction and earlier nadir (Fig. 1D). IL-1β-induced reductions in glucose were completely blocked by IL-1RA (10 mg/kg body weight; i.p.). Therefore IL-1β is sufficient to reduce systemic glucose levels. To test whether IL-1β is necessary for LPS-induced hypoglycemia, we administered LPS (250 μg/kg; i.p.) and IL-1β (25 μg/kg; i.p.) to IL-1 receptor-1 knockout (IL-1R1$^{-/-}$) mice

and c57BL6/J wild type (WT) controls. Blood glucose measurements were taken 4 hours post-challenge, a time when blood glucose was significantly decreased (Fig. 1C; 1D). LPS and IL-1β reduced blood glucose in WTs, though the IL-1β response showed greater variability. Although IL-1β treatment had no effect on blood glucose levels in IL-1R1$^{-/-}$ mice, LPS-induced reductions in glucose were statistically indistinguishable from the LPS response in WTs (Fig. 1E). Moreover, the time course of LPS-induced glucose reduction was highly overlapping in WT and IL-1RI$^{-/-}$ mice (Fig. 1F). IL-1β antagonism with IL-1RA has been reported to attenuate LPS-induced hypoglycemia (30). Here, c57BL6/J mice were challenged with LPS (250 μg/kg) with or without co-administration of IL-1RA (10 mg/kg). IL-1RA showed a very modest and temporary protective effect against LPS-induced decreases in glucose 2 hours post-challenge and no effect thereafter (Fig. 1G). Bonferroni tests, after a significant 2-way ANOVA, showed significantly lower blood glucose levels in LPS+Saline mice compared to Saline+Saline controls at 2, 4 and 6 hours whereas LPS+IL-1RA-treated mice only showed significant decreases in glucose at 4 and 6 hours post-challenge. This indicates only a modest effect of IL-1RA antagonism on glucose homeostasis post-LPS. Collectively, these data show that although systemic IL-1β is sufficient to lower blood glucose it is not essential for LPS-induced decreases in glucose, supporting the idea that other cytokines can compensate for a lack of IL-1β signaling following LPS exposure.

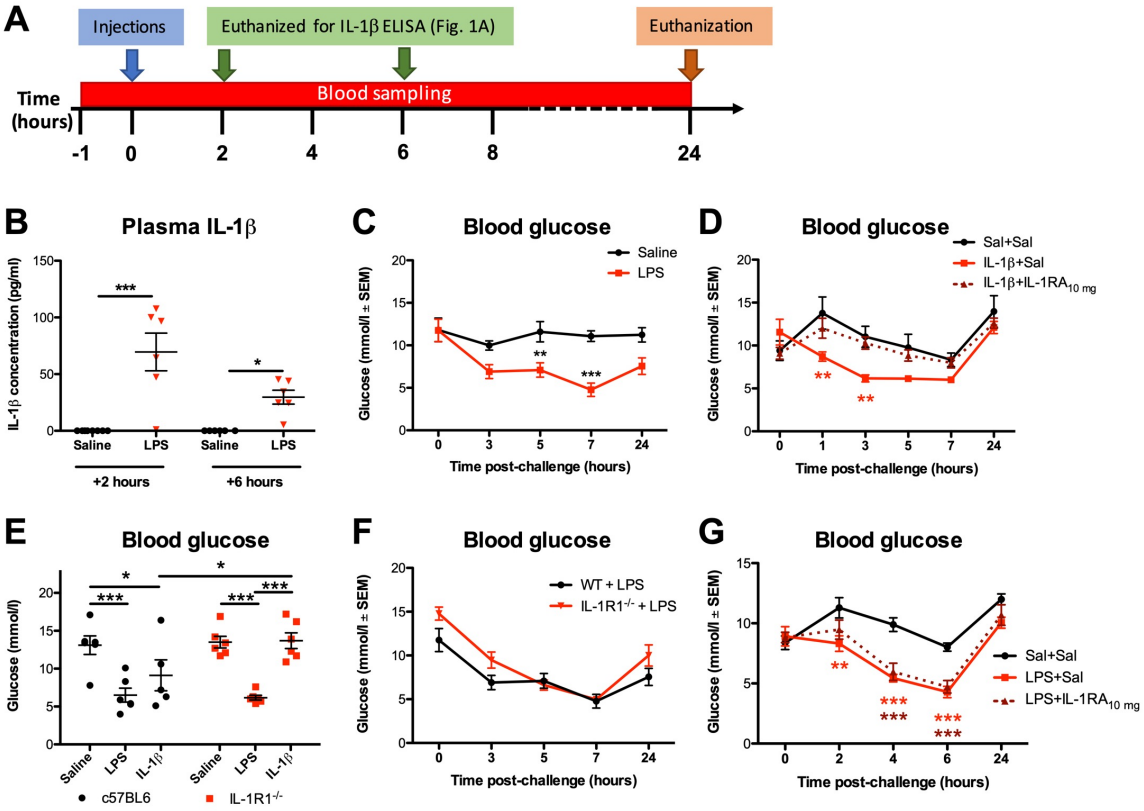


**Figure 1. LPS and IL-1β significantly lower blood glucose concentrations. A:** Timeline for treatments and sampling times. Blood sampling was from tail vein, aside from the 24 hour time point where glucose levels were measured during transcardial perfusion. In one cohort, mice were euthanized at 2 and 6 hours post-LPS challenge to collect plasma for the IL-1β ELISA. **B:** LPS treatment (250 μg/kg, i.p.) significantly increased plasma IL-1β ($F_{(1,22)}$ = 36.71; $p$ < 0.0001; n = 8 for saline/2 h group; n= 6 for other groups). **C:** LPS treatment (n = 7) significantly reduced glucose levels over 24 h compared to saline controls (0.9 %, i.p.; n = 6); main effect of treatment ($F_{(1,44)}$ = 24.10; $p$ = 0.0005). **D:** IL-1β (25 μg/kg, i.p.; n = 7) reduced systemic glucose and IL-1β's effect can be blocked using IL-1 receptor antagonist (IL-1RA; 10 mg/kg, i.p.; n = 7). Main effect of treatment ($F_{(2,85)}$ = 3.843; $p$ = 0.0420) and ** denotes significantly lower glucose levels in IL-1β+Saline-treated mice compared to controls (n = 6) at 1 and 3 h post-challenge. **E:** c57BL6/J mice, both LPS (n = 6) and IL-1β (n = 5) significantly reduced blood glucose 4 h post-challenge versus saline controls (n = 6) while in IL-1R1$^{-/-}$ mice, LPS (n = 6) but not IL-1 (n = 6) significantly reduced blood glucose versus controls (n = 6). Significant pairwise comparisons by Bonferroni *post hoc* test after a main effect of treatment ($F_{(2,29)}$ = 21.81; $p$ < 0.0001) are annotated by * (p<.05) and *** (p<0.001). **F:** Time course of changes in blood glucose in IL-1R1$^{-/-}$ (n = 5) and c57BL6J mice (n=7). There was a significant effect of genotype ($F_{(1,40)}$ = 5.673; $p$ = 0.0385) but pairwise comparisons did not reveal differences at any time point. **G:** IL-1RA (10 mg/kg, n= 12) administered immediately after LPS treatment modestly attenuated LPS-induced reductions in glucose ($F_{(2,132)}$ = 16.18; $p$ < 0.0001) but this was a transient effect ($F_{(4,132)}$ = 39.08; $p$ < 0.001). There was a significant interaction of treatment and time ($F_{(8,132)}$ = 3.502; $p$ = 0.0011) and *post hoc* tests indicated that LPS+Saline-treated mice (n = 12) had significantly lower blood glucose levels versus saline (n = 12) at 2, 4 and 6 h post-challenge while LPS+IL-1RA (n = 12) did not significantly decrease glucose levels compared to controls until 4h. All annotated Bonferroni *post hoc* tests were performed after significant main effects or interactions in ANOVA analysis: *$p$ < 0.05; **$p$ < 0.01; ***$p$ < 0.001.

*Blood glucose concentration is the major determinant of LPS-induced acute hypoactivity*

IL-1β has been reported as the major driver of LPS-induced hypoglycemia (30, 37) and of sickness behavior (38, 39). We sought to understand whether IL-1β signaling or decreases in glucose might be the proximate cause of LPS-induced hypoactivity. LPS-induced hypoactivity (squares crossed over 3 minutes) was measured in c57BL6/J mice and was significantly positively correlated with blood glucose levels (Fig. 2B): low blood glucose predicts low activity ($R^2$=0.354, p=0.0004). Thus, we hypothesized that LPS-induced hypoactivity would be attenuated by treatment with glucose (2 g/kg; i.p.) and we tested this in a 2x2 experimental design. As expected, LPS robustly produced IL-1β, reduced glucose levels, and suppression of activity. Glucose treatment had no effect on IL-1β production (Fig. 2C) but significantly improved locomotor activity (Fig. 2D) and increased circulating glucose concentration (Fig. 2E). The observed change in circulating glucose concentration was very closely mirrored in the CSF where there was significantly lowered glucose (Fig. 2F), but unaltered lactate (Fig. 2G). This CSF glucose deficit was also significantly improved by systemic glucose treatment (2 g/kg, Fig. 2F). Therefore LPS-induced reduction in systemic glucose is sufficient to produce an approximate 50 % reduction in CSF glucose concentration and this can be improved by systemic administration of glucose, with concomitant rescue of spontaneous activity. A separate group of mice was treated with LPS+2-deoxyglucose (2-DG). 2-DG has no metabolic value but inhibits glucose-6-phosphate isomerase to prevent glycolysis. In macrophages, glycolytic blockade by 2-DG impairs their ability to synthesize IL-1β (40). Here, 2-DG completely blocked IL-1β secretion and yet hypoactivity was striking. Therefore, despite LPS producing no IL-1β, animals that cannot utilize glucose show no locomotor activity and animals with high IL-1β remain spontaneously active if glucose concentration is boosted. These findings were replicated in separate cohort of mice using a separate batch of LPS (100 μg/kg; i.p.; Fig. S1). We have thus uncoupled the roles of IL-1β and reduced glucose in the generation of sickness behavior-associated hypoactivity and demonstrate that lowered blood glucose levels drive LPS-induced suppression of activity.

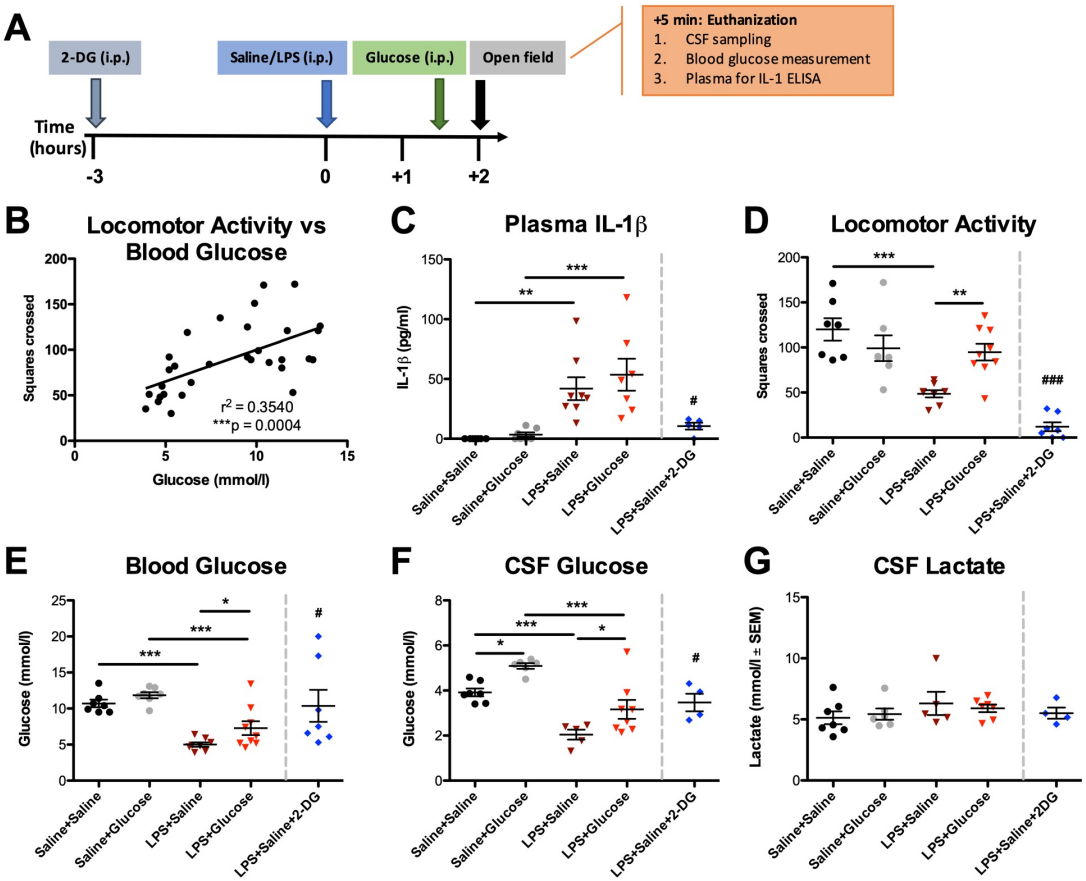


**Figure 2: Low blood glucose concentration drives LPS-induced hypoactivity. A:** Timeline for treatments and sampling times. Glucose (2 g/kg; i.p.) was administered 1.5 hours post-LPS challenge (250 µg/kg; i.p.) and open field behavior was measured 2 hours post-LPS challenge. 5 minutes following the open field test, mice underwent terminal anesthesia whereby CSF samples were taken, blood glucose levels were assessed, and plasma was collected for IL-1β ELISA. In one group of mice, 2-deoxyglucose (2-DG; 2 g/kg; i.p.) was given 3 hours prior to LPS. **B:** Linear regression analysis of locomotor activity (squares crossed/3 min) versus blood glucose concentration (mmol/l) in animals challenged with saline or LPS – n = 31; $r^2 = 0.3540$; $p = 0.0004$. **C:** LPS (250 µg/kg, i.p.; n = 8) induced IL-1β production ($F_{(1,25)} = 29.88$; $p < 0.001$), which was unaffected by glucose co-administration (n = 7; 90 minutes post-LPS) but completely suppressed by 2-DG administration (i.p., n=5, $^{\#}p = 0.0296$ versus LPS+Saline; 180 minutes pre-LPS). **D:** Locomotor activity (squares crossed/3 min) was suppressed by LPS (main effect of LPS: $F_{(1,27)} = 13.39$; $p = 0.0011$) but rescued by glucose co-administration (interaction between treatments: $F_{(1,27)} = 10.48$; $p = 0.0032$). ** denotes significant difference between LPS+Glucose (n = 9) and LPS+Saline (n = 8) and these were not significantly different to Saline+Saline (n = 7) or Saline+Glucose controls (n = 7). 2-DG completely suppressed locomotor activity ($t_{(13)} = 5.766$; $^{\#\#\#}p < 0.0001$ versus LPS+Saline). **E:** Blood glucose was suppressed by LPS (main effect: $F_{(1,27)} = 60.00$; $p < 0.0001$) and modestly increased by glucose (main effect: $F_{(1,27)} = 6.721$; $p = 0.0152$) and *post hoc* tests showed that LPS+Glucose was significantly different to LPS+Saline. **F:** CSF collected from the same animals showed a main effect of LPS ($F_{(1,22)} = 39.85$; $p < 0.0001$) and a strong main effect of glucose ($F_{(1,22)} = 14.57p = 0.0009$). LPS+Glucose was significantly different to LPS+saline in *post hoc* analysis. **G:** CSF lactate levels, in the same animals, were not altered by the treatments described. Significance levels for Bonferroni *post hoc* tests: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

*Neurodegeneration increases susceptibility to cognitive impairments due to reduced glucose availability:*

We have previously shown, using the ME7 model, that evolving neurodegeneration progressively increases susceptibility to LPS-induced transient working memory impairments on a T-maze task (Fig. 3A, (17, 22)). We hypothesized that this cognitive vulnerability in ME7 mice may be explained by a greater tendency towards metabolic insufficiency. Mice were inoculated with ME7 or NBH and, 16 weeks later, challenged with saline or LPS (100μg/kg; i.p.). LPS produced similar reductions in blood glucose in NBH and ME7 mice (Fig. 3B). There were also equivalent reductions in CSF glucose in NBH and ME7 mice following LPS (Fig. 3C), although baseline CSF glucose concentration was slightly higher in ME7 animals with respect to NBH. CSF lactate levels were similar in all 4 groups (Fig. 3D). Since ME7 mice did not show greater reduction in glucose than NBH mice, we hypothesized that cognitive function in ME7 mice might be less able to cope with limiting glucose. We tested this hypothesis by decreasing systemic glucose using insulin (11.5 IU/kg; i.p.). Insulin produced significant reductions in blood glucose in ME7 and NBH mice (Fig. 3E). Basal levels of insulin were equivalent in ME7 and NBH mice, and all mice showed similar insulin pharmacokinetics (Fig. 3F). Despite this, insulin induced significant acute working memory dysfunction in ME7 mice that was absent in NBH controls (Fig. 3G). The increased vulnerability of the ME7 mice to insulin-induced hypoglycemia was analogous to the LPS-induced cognitive deficit previously shown (Fig. 3A; (17, 22)).

Since LPS-induced sickness behavior could be reversed through the administration of glucose (Fig. 2D), we hypothesized that the LPS-induced cognitive impairment in ME7 mice might be prevented by glucose administration. ME7 mice were trained on the 'escape from water' T-maze, until criterion performance of >80 % correct was achieved. They were then treated with saline or LPS (100 μg/kg; i.p.) and, 2.5 hours after LPS, treated with saline or glucose (2 g/kg; i.p.). Neither saline- nor glucose-treated ME7 mice deviated from baseline T-maze performance in the absence of LPS, but LPS-treated ME7 mice showed robust impairment between 3-7 hours post-LPS. Those impairments in ME7+LPS+saline mice were significantly attenuated by glucose applied 2.5 hours after LPS (Fig. 3H). There was a significant interaction of LPS and glucose ($F_{(12,240)} = 3.740$; $p < 0.0001$) and *post hoc* analysis showed that ME7+LPS+glucose performed statistically significantly better than ME7+LPS+Saline mice at 5 hours post-challenge, although the protective effect of glucose in LPS-treated mice was not complete.

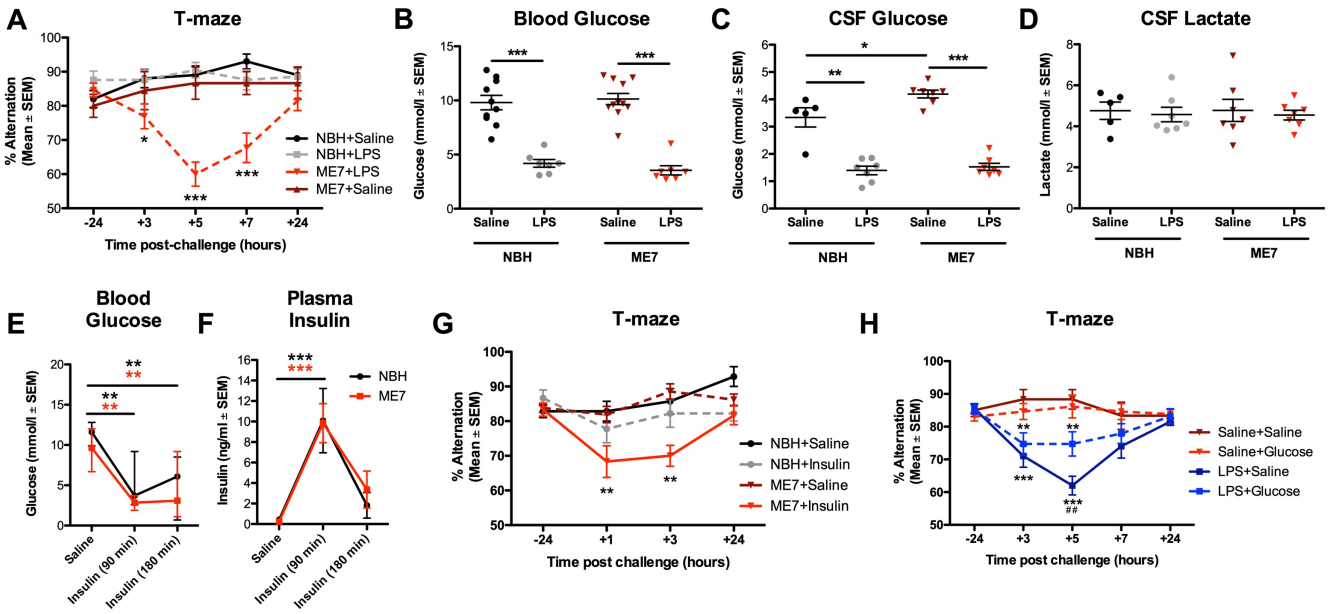


**Figure 3: Reduced blood glucose produces acute cognitive dysfunction selectively in mice with prior neurodegeneration. A:** Pooled data showing that ME7 mice have a sensitivity to LPS treatment (n = 26) that was not present in NBH mice treated with saline (n = 20) or LPS (n = 21), nor with ME7 mice treated with saline (n = 9). There was a main effect of treatment ($F_{(3,72)}$ = 19.08; $p$ < 0.0001) and an interaction between treatment and time ($F_{(12,288)}$ = 5.00; $p$ < 0.0001). **B:** After 5 hours, LPS produced equivalent decrease in glucose concentration in blood and **C:** CSF glucose in ME7 (n = 7) and NBH mice (n = 7) compared to their respective saline-treated controls (n = 11 and n = 10 respectively). There were main effects of LPS on blood glucose ($F_{(1,31)}$ = 118.3; $p$ < 0.0001) and on CSF glucose ($F_{(1,22)}$ = 146.5; $p$ < 0.0001) and also an effect of disease on CSF glucose ($F_{(1,22)}$ = 6.665; $p$ = 0.0170), with ME7+Saline > NBH+Saline by *post hoc* analysis. **D:** There were no differences in CSF lactate levels. **E:** Blood glucose (mmol/l) and **F:** Plasma insulin concentrations in saline- or Insulin-treated (11.5 IU/kg; i.p.) NBH and ME7 mice. There were similar reductions in glucose (E: main effect of insulin, $F_{(2,20)}$ = 17.11; $p$ < 0.0001) and equivalent insulin concentrations over 180 min in ME7 and NBH animals (F: main effect of insulin, $F_{(2,28)}$ = 22.86; $p$ < 0.0001). **G:** T-maze alternation in ME7 and NBH mice post-challenge with saline or insulin (+1 h = 40-160 min; and +3 h = 160-300 min post-insulin). Testing was performed earlier than in LPS-treated mice as insulin produces a more rapid decrease in blood glucose. There was a significant main effect of insulin ($F_{(3,135)}$ = 7.418; $p$ = 0.0004) and an interaction of ME7 and insulin ($F_{(9,135)}$ = 3.050; $p$ = 0.0024). ME7+insulin-treated mice (n = 12) had significantly lower alternation scores compared to NBH+saline controls (n = 7) at 1 and 3 h post-injection (NBH+insulin: n = 9; ME7+saline: n = 13). **H:** T-maze alternation in ME7 mice post-challenge with saline or LPS, co-treated with glucose (2 g/kg) or saline. LPS+Saline group (n = 20) showed robust cognitive impairment but the LPS+Glucose group (n = 19) showed significant attenuation. Two-way repeated measures ANOVA showed a main effect of LPS ($F_{(3,240)}$ = 13.75; $p$ < 0.0001) and an interaction of LPS and glucose ($F_{(12,240)}$ = 3.740; $p$ < 0.0001). LPS+Glucose mice performed significantly better than the LPS+Saline group at 5 h post-challenge ($^{\#\#}p$ < 0.01). Significance levels for Bonferroni *post hoc* tests: *$p$ < 0.05; **$p$ < 0.01; ***$p$ < 0.001.

*Human delirium triggered by acute inflammatory trauma is associated with altered carbohydrate metabolism.*

Given the demonstration that disrupting normal glucose metabolism is causal in producing acute systemic inflammation-induced acute cognitive dysfunction we sought to assess CSF concentrations of glycolytic metabolites in a cohort of acute hip-fracture patients admitted for hip fracture repair with spinal anesthesia (see Table S1 for demographic information).

Patients were assessed for delirium at the time of lumbar puncture and those with prevalent delirium were compared to hip-fracture patients without delirium on CSF glucose, lactate and pyruvate (commonly used markers of central energy metabolism disturbance in clinical populations (41, 42)). CSF glucose was not altered during delirium (Fig. 4A). Based on a previous study of all-cause delirium versus stable dementia (43), we had an *a priori* hypothesis that delirium would be associated with elevated lactate, and lactate was indeed significantly elevated during delirium (Fig. 4B; one-tailed Mann-Whitney analysis; $p$ = 0.0128). Pyruvate was not detected in all samples but it was detected significantly more often in patients with delirium (Fig. S2) and median pyruvate levels were significantly elevated in delirium (Fig. 4C). The data indicate an elevated lactate:glucose ratio (LGR) both in humans experiencing delirium after acute inflammatory trauma (Fig. 4D) and in mice cognitively impaired by acute systemic inflammation (Fig. 4E). Changes in CSF lactate and LGR levels associated with delirium were not explained by dementia status (Fig. S3). The changes in LGR observed in mice and humans differ in how they arise, the former driven by reductions in available glucose and the latter perhaps indicative of a switch from aerobic to anaerobic metabolism or a deficit in mitochondrial function. However, both mouse and human data indicate that there is a significant derangement of brain energy metabolism following these inflammatory insults and, in mice, this is clearly causal for acute cognitive dysfunction.

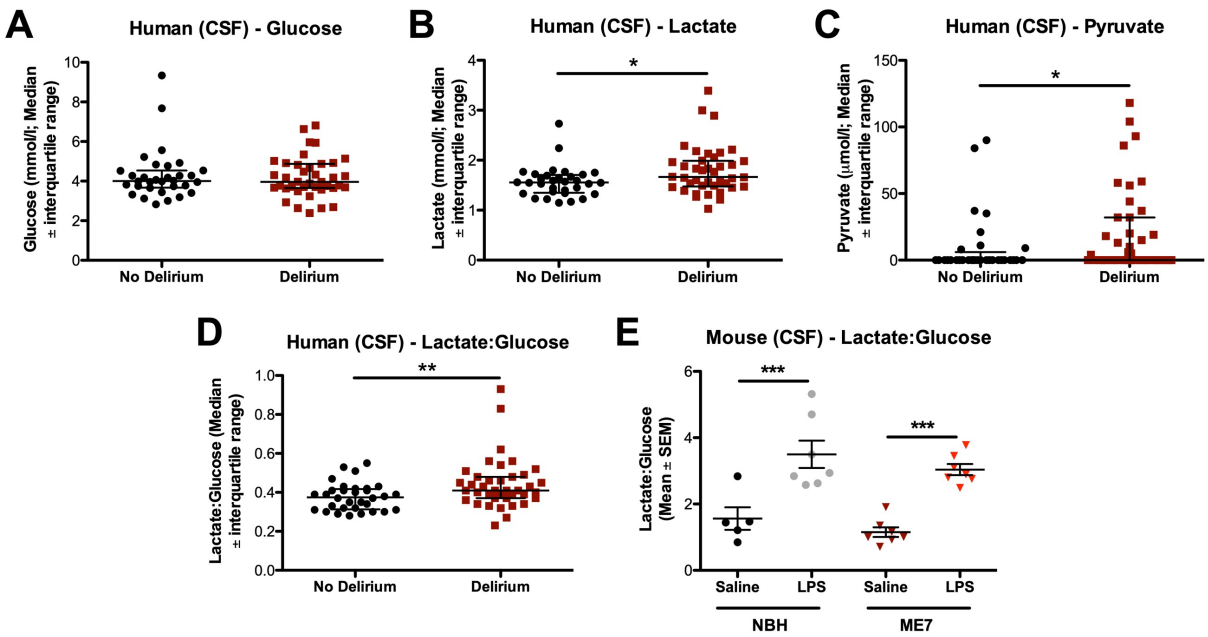


**Figure 4: Derangement of energy metabolism in human delirium.** Metabolite levels in the CSF of hip fracture patients with delirium (n = 40) at the time of CSF sampling compared to age-matched patients with no delirium at any point of their hospital stay (n = 32). **A:** Glucose levels in delirium (n = 39, 1 sample omitted due to a read error) and non-delirium cases were not significantly different (Mann-Whitney $U$ = 606.5; $p$ = 0.8442). **B:** Patients with delirium had significantly higher levels of lactate in their CSF compared to controls ($U$ = 442.5; $p$ = 0.0128). **C:** Patients with delirium showed significantly higher pyruvate levels compared to controls ($U$ = 503.5; $p$ = 0.0608). **D:** The lactate:glucose ratio (LGR) for patients with delirium (n = 39) was significantly higher compared to controls ($U$ = 399.5; $p$ = 0.0048). **E:** LPS significantly increased the CSF LGR in both ME7 (n = 7) and NBH mice (n = 7) compared to their respective saline-treated controls (n = 7 and n = 5; $F_{(1,22)}$ = 44.58; $p$ < 0.0001). Significance levels for Bonferroni *post hoc* tests: **$p$ < 0.01; ***$p$ < 0.001.

## Discussion

Here we demonstrated that LPS-induced hypoglycemia drives suppression of spontaneous activity in mice: effects of IL-1β and hypoglycemia can be uncoupled to show that glycemic status is a major determinant of spontaneous activity after LPS. Reduced glucose availability also drives cognitive impairments caused by LPS in mice with underlying neurodegeneration, and in addition the degenerating brain is more susceptible to the cognitive disrupting effects of insulin, despite equivalent reductions in blood glucose. Finally, we confirm that inflammatory trauma-induced delirium in humans is also associated with altered central energy metabolism showing elevated lactate and pyruvate.

### *Sickness Behavior*

The effects of systemic infection on spontaneous activity have largely been attributed to changes in cytokine signaling (38), with reciprocal interactions between brain and periphery occurring either via direct modulation of peripheral nerves by immune cells or via long-range action of circulating cytokines (13). IL-1 is reported to act at the endothelium or at forebrain targets to mediate LPS-induced suppression of motivated behaviors (44, 45) but

precisely how suppression of exploratory activity occurs is unclear. Sickness behavior is largely coordinated by the hypothalamus, a brain region that monitors levels of circulating IL-1β (39) and glucose (46). IL-1β is reported to shift the homeostatic set point for blood glucose in a top-down manner, with IL-1β action in the hypothalamus proposed to program the organism to operate at lower circulating glucose levels (30). There is evidence from animal models of Gram-negative bacterial infection that reducing blood glucose is adaptive for the organism in that it deprives the infectious agent of a key fuel source. Providing further glucose, in that scenario, actually increases *Listeria monocytogenes*-induced mortality (47). Nonetheless, in the acute phase, a significant decrease in blood glucose clearly reduces CSF glucose and we have shown here that this directly contributes to suppression of activity. This is consistent with prior work showing correlation between blood glucose levels and sickness behavior (5) and with studies showing that insulin-induced hypoglycemia suppresses social activity in c57BL/6 mice (48, 49). Here we show, that by directly increasing glucose availability, we prevent LPS-induced suppression of activity without reducing IL-1β (Fig. 2A-C). Moreover, 2-DG completely blocked LPS-induced secretion of IL-1β, as had been demonstrated for LPS-induced macrophage IL-1 production (40), but in preventing glucose utilization it also induced profound suppression of activity. So LPS-induced hypoactivity can be uncoupled from IL-1β to reveal the importance of the ability to take up and use glucose.

Del Rey and colleagues showed that LPS (25 μg/kg) produces increased hypothalamic activation and IL-1β produced some evidence of increased brain energy metabolism (34). Consistent with this, one study that used high dose LPS (15 mg/Kg) and microPET (FDG) supported the idea of increased hypothalamic glucose uptake to reprogram organismal glucose utilization in LPS-treated mice (47). However, another high dose LPS study revealed regional selectivity in glucose uptake: LPS (10 mg/kg i.p.) decreased glucose uptake across multiple cortical regions with less prominent decreases in subcortical areas (10). If IL-1 increases hypothalamic activity to lower the set point for glucose homeostasis, allowing animals to function efficiently at lower glucose concentrations (30, 34, 37), it is not intuitive why transiently increasing available glucose should rapidly increase spontaneous activity. Administration of glucose raises both blood and CSF glucose (Fig. 2) but this “top-up” of glucose does not override the new homeostatic set-point but instead provides a temporary and partial increase in the amount of glucose available to the mouse (Fig. S4; (30)) but this is sufficient to restore spontaneous activity and cognition. We therefore propose that while the hypothalamus may be selectively active during acute inflammation, to coordinate neuroendocrine responses to the acute threat (50), the suppression of spontaneous locomotor activity that is actually observed may be passively controlled by available glucose.

The neuroanatomical basis of LPS-induced suppression of exploratory activity is incompletely understood but correlates with suppression in cFOS in brain areas associated with positive motivation (50) and exploratory behavior (51). LPS administration results in release of norepinephrine (NE) in the hypothalamus (52) and lesioning caudal medullary NE neurons that project to the hypothalamus blocks LPS-induced suppression of activity (51).

Hypothalamic NE release can also be induced by hypoglycemia and hyperinsulinemia (53) or 2-DG treatment (54, 55) and hypoglycemia-induced social withdrawal was mediated by β-adrenoceptors (48), suggesting a potential point of convergence for how inflammation and impaired glucose metabolism may both drive changes in behavior during sickness. Whatever the neuroanatomical and neurotransmitter underpinnings, the current data strongly support the idea that available and usable glucose is a key determinant of LPS-induced suppression of activity.

This has significant implications for the very large number of studies that administer LPS systemically in order to examine the behavioral consequences of systemic infection. The levels of circulating LPS arising from bolus LPS challenges are typically much higher than anything occurring during active infection; LPS was not detectable in 57 % of patients undergoing septic shock (56). Bolus LPS treatment (2 ng/kg; i.v.) in human volunteers can produce a transient decrease in plasma glucose 90 minutes post-challenge (57) but active infection typically does not produce hypoglycemia (58). Therefore, although bolus LPS would appear to have face validity as a model of systemic infection, if key behavioral and neurophysiological changes induced by LPS in experimental subjects are underpinned by a physiological change, i.e. hypoglycemia, that rarely occurs during active infection, this necessitates a review of the generalizability of bolus LPS-induced changes to understand changes during active infection.

*Acute cognitive dysfunction and delirium*

Human data suggest that reduced glucose uptake in the medial temporal lobe associates with impaired performance in hippocampal dependent tasks (59). Remarkably, despite the robust and long lasting reductions in available glucose shown here, normal mice maintain good working memory performance (17), but the same decreases in glucose, whether caused by LPS or insulin, were sufficient to trigger dysfunction in animals with prior neurodegeneration. Speculatively, the circuitry underpinning working memory function may be operating close to thresholds for decompensation during neurodegeneration and indeed this task may recruit additional brain areas in order to maintain sufficient working memory as the brain degenerates. Although peripheral glucose administration has no effect on cognition in young, healthy rats (60), there is a cognitive enhancing effect of exogenously added glucose in aged rats (61, 62), supporting the idea that the same task may require additional metabolic support in the aged or degenerating brain. The addition of a further stressor may be sufficient to unmask age- or neurodegeneration-associated vulnerability. Humans exposed to *Salmonella Typhi*. vaccination (as a voluntary experimental inflammatory stimulus) continued to perform as well as controls on the Stroop test of executive function but appeared to recruit additional areas of the prefrontal and anterior cingulate cortex to maintain this performance during inflammation (63). If inflammation requires that increased connectivity is called upon to maintain performance, then such inflammatory insults are likely to unmask vulnerability when connectivity is impaired by evolving neurodegeneration (25). Until now, the ME7 model of delirium during dementia

has been an exemplar for an inflammatory hypersensitivity, but the current data show that the brains of these animals are also more vulnerable to bioenergetic stressors. Despite equivalent reductions in blood and CSF glucose, NBH animals are resilient to hypoglycemia-induced cognitive impairment but ME7 animals are vulnerable, whether induced by LPS or by insulin.

The brain is a metabolically demanding organ, requiring large amounts of energy to maintain consciousness (64) and in adaptive terms, it makes sense, during extreme challenges to bodily survival, to minimize energy use in the brain, perhaps constituting a 'retreat' to preserve autonomic function at the expense of higher cortical function. Engel and Romano proposed the metabolic insufficiency model of delirium in the 1950s, predicting that the electrophysiological and psychological symptoms associated with delirium are driven by a failure to meet the brain's energy requirements, regardless of the underlying cause (65). Although hypoglycemia has long been known to produce delirium in humans (66), the general applicability of the metabolic insufficiency theory has not been comprehensively tested. Slowing of the electroencephalogram (EEG) is associated with delirium (67, 68) and greater suppression correlates with delirium severity (69). Hypoglycemia is clearly not required for EEG slowing (70), but it is sufficient, alone, to produce delirium and EEG slowing (71, 72) and this can be reversed by glucose administration (73). Small CSF studies support the idea of metabolic disturbances during delirium: patients with delirium have elevated CSF lactate compared to non-delirious Alzheimer's disease controls (43) and [$^{18}$F]-fluorodeoxyglucose PET ([$^{18}$F]FDG-PET) studies reveal an overall decrease in glucose uptake, with the posterior cingulate cortex (PCC) being particularly affected (74). Given that the PCC is associated with attention and arousal, disruption of energy metabolism in this region could be important in delirium. The equivalent brain region in rodents, the retrosplenial cortex, has not been singled out in the same way but different regions of the rat cortex do show large differences in how they respond to insulin-induced hypoglycemia (75). Although hypoglycemia in mice is not precisely defined, the blood glucose concentrations in our ME7 mice remain just above the human clinical threshold for moderate hypoglycemia (3.9 mmol/l) and comfortably above the threshold for severe hypoglycemia (2.8 mmol/l) (76, 77). This is significant in the context of iatrogenic hypoglycemia, an extremely common occurrence in patients using insulin for diabetes (78). This is a major cause of emergency department admissions, especially in older patients, and 47.6% of patients admitted for adverse effects of diabetes medication show moderate to severe effects including altered mental status and seizures (79). Our ME7 data show that even when blood glucose levels do not fall into classical hypoglycemic ranges, these changes may have serious impacts on brain integrity in vulnerable patients.

It is important to stress that hypoglycemia does not occur in the hip fracture patient group studied here (Fig. 4A). Although both hypoglycemia and hyperglycemia strongly increase risk for sepsis-associated encephalopathy (80, 81), the latter appears more common but it may be significant that hyperglycemia-associated delirium typically occurs during insulin insensitivity, which impairs individuals' ability to take up and use glucose.

Insulin insensitivity occurs after LPS administration in volunteers (82), in patients with infection (83), and in post-surgical patients (84). Metabolic insufficiency could also occur due to a failure of glucose supply due to microcirculatory failure, which commonly occurs in sepsis (85) or may occur as a result of tissue hypoxia, resulting in inefficient glucose oxidation. The higher levels of lactate and pyruvate in the current study may indicate a shift from normal aerobic metabolism to anaerobic glycolysis – increases in the LGR are associated with reduced consciousness (86) and increased mortality (87). Although dementia status is a major risk factor for the development of delirium (25), the changes in lactate and the LGR observed here are not explained by the presence of dementia in these patients (Fig. S3). The data are consistent with previously reported increases in CSF lactate in patients with delirium (43) and with the observation that hypoxia is a risk factor for developing delirium (88, 89). Taken collectively, there are several reasons to now focus attention on energy metabolism in delirium.

Finally, although del Rey and colleagues (30) showed that IL-1RA protected against LPS-induced hypoglycemia, here we found only a very modest protective effect of IL-1RA. However, in the del Rey study, 300 μg/mouse IL-1RA was only partly protective and 100 μg/mouse marginally mitigated hypoglycemia, similar to what we observed here. In fact, our IL-1RA dose (200μg/mouse) effectively blocked the effect of IL-1β at 25 μg/kg (Fig 1D), which results in a blood IL-1β concentration of approximately 640 pg/ml (8). Therefore our IL-1RA dose would be sufficient to completely block LPS-induced IL-1β, but since LPS also results in the production of TNFα, which also triggers hypoglycemia (90), IL-1RA can have only a partial effect in limiting hypoglycemia. Therefore, while IL-1β might be key at 25 μg/kg LPS (30), IL-1RA barely limits hypoglycemia with LPS at 250 μg/kg (current study). We have demonstrated that i.p. IL-1RA can protect against LPS-induced T-maze impairments in ME7 mice (17) and it remains unclear how IL-1RA mediates its effect, but IL-1 has multiple potential sites of influence on brain function (44) and actions of IL-1β at the brain endothelium or blood-CSF barrier, such as induction of chemokines (8) or impacts on prostaglandin synthesis (26) may also contribute to LPS-induced cognitive deficits in ME7 mice.

In conclusion, reduced glucose availability is a major driver of LPS-induced suppression of spontaneous activity. In animals made vulnerable by evolving neurodegeneration, this decrease in glucose is now also sufficient to trigger acute cognitive dysfunction indicating that a metabolic insufficiency underlies cognitive dysfunction in this animal model resembling delirium and older patients experiencing inflammatory trauma and delirium also show a disruption of energy metabolism. Together, the findings indicate that disrupted energy metabolism is a significant contributor to both general behavioral changes associated with sickness but also to acute neuropsychiatric disorders such as delirium, which should focus attention on bioenergetic mechanisms of acute brain failure during acute illness and hospitalization in older adults.

**Materials and Methods:**

*Animals:*

Female c57BL/6 mice aged 5-8 months (Harlan, UK) were housed at 21 °C with a 12/12-hour light-dark cycle (lights on 08:00-20:00) with food and water available *ad libitum*. All animal experiments were in accordance with European Commission Directive 2010/63/EU and were performed following ethical approval by the Trinity College Dublin Animal Research Ethics Committee and licensing by the HPRA.

*ME7 prion model of neurodegeneration:*

Mice were anaesthetized using Avertin (2,2,2-tribromoethanol 50 % w/v in tertiary amyl alcohol, diluted 1:40 in $H_2O$; 20 ml/kg, i.p.; Sigma, Ireland) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The scalp of the animal was shaved, an incision was made, and holes were drilled in the skull bilaterally to infuse of 1 µl of a 10 % w/v ME7-infected c57BL/6 or a 10 % w/v normal brain homogenate in sterile PBS using a microsyringe (Hamilton, Reno, NV, USA) with a 26 G needle. Injections were made into the dorsal hippocampus at -2.0 mm (A/P); ±1.6 mm (M/L); -1.7 mm (D/V) from Bregma. The needle was left in place for 2 minutes before being slowly withdrawn to minimize reflux. Mice recovered in a heated chamber (30 °C) and then returned to their home cage where their drinking water was supplemented with sucrose (5 % w/v) and Carprofen (0.05 % v/v; Rimadyl, Pfizer, Ireland) for two days following surgery.

*Treatments:*

Mice were injected intraperitoneally (i.p.) with one or a combination of the following treatments: LPS (100µg/kg or 250 µg/kg; Sigma, Ireland), IL-1β (25 µg/kg; R&D Systems, Minneapolis, MN, USA), IL-1RA (10 mg/kg; Kineret, Biovitrum, Sweden), glucose (2g/kg; Sigma, Ireland), 2-deoxyglucose (2 g/kg; Sigma, Ireland), and insulin (11.5 IU/kg (400 µg/kg); Sigma, Ireland). All treatments were made up using sterile saline as a vehicle. LPS was administered 2 hours prior to open field behavior or 3 hours prior to the T-maze task. Glucose was administered 30 minutes before any behavioral task.

*Behavioral assessment:*

Locomotor and rearing activity was assessed in an open field. Mice were placed in a box (58 × 33 × 19 cm), and the number of rears and squares crossed were recorded over 3 minutes. Cognitive performance was assessed using an escape from water alternation task in a paddling T-maze as described previously (22). Mice were trained (2 blocks/day, 5 sessions/block, 2 trials/session) until they performed with an 80 % success rate. Mice were then challenged with saline or LPS and tested on the same T-maze task on the day of the challenge (3 blocks – corresponding to 3-5 (+3), 5-7 (+5), and 7-9 (+7) hours post-challenge) to assess the acute effects of LPS treatment. Another cohort of mice was challenged with saline or insulin and, due to the rapid action of insulin on blood glucose compared to LPS, underwent two blocks on the day of the challenge (corresponding to 40-160 min (+1 h) and

160-300 min (+3 h) post-challenge). All mice underwent recovery testing (2 blocks) on the following day.

*Blood glucose measurements:*
Mice were restrained in a plastic restrainer made from a 50 ml plastic tube, the tail vein was dilated using warm water and lanced using a 30 G needle. Glucose was measured using a veterinary glucometer (AlphaTRAK 2, Zoetis, USA). Mice were anesthetized using an overdose of Euthatal 125 minutes following LPS, blood was collected for molecular analysis and a final glucose measurement was made with the glucometer before animals were transcardially perfused with heparinized saline. Blood glucose readings were higher on the veterinary glucometer compared to a clinical glucometer (Fig. S5) but were in line with other studies (30, 33-35).

*Sampling and analysis of mouse CSF:*
CSF was collected from each mouse to determine the concentrations of glucose and lactate present centrally. Mice under terminal anesthesia were placed in a stereotaxic frame and the cisterna magna accessed by lowering the incisor bar on the animal's head to induce a downward curvature of 45° from horizontal. The position of the cisterna magna was manually determined and, using a small volume insulin syringe (BD Micro - Fine™ + 0.3ml Insulin Syringe Demi), a freehand puncture was performed slowly to avoid brain stem damage and blood contamination. On average 5 µl was collected with a success ratio of 4:5 (i.e. without any blood contamination) in 0.5 ml microcentrifuge tubes. All samples were stored at -20 °C. Samples were defrosted and centrifuged on a pulse setting before transfer to CMA Microvials (CMA Microdialysis AB, Sweden). Where sample volumes were too small (<3 µl), samples from the same treatment groups were pooled to give a workable volume for analysis. Glucose and lactate concentrations (mmol/l) were determined using a clinical CMA600 Microdialysis Analyzer (CMA Microdialysis AB, Sweden). Basal levels of CSF glucose were in the range expected from other studies (91-93), as was the case for CSF lactate (91).

*Recruitment of clinical population and analysis of human CSF:*
CSF was collected from hip fracture patients acutely admitted to Oslo University Hospital after informed consent from the patient and/or proxy (if patients were unable to consent due to cognitive impairment), as approved by the Regional Committee for Medical and Health Research Ethics (South-East Norway; REK 2009/450). CSF was collected in propylene tubes at the onset of spinal anesthesia. Samples were centrifuged, aliquoted and stored at –80° C, as previously described (94). Samples were defrosted and 15 µl of each was transferred to CMA Microvials (CMA Microdialysis AB, Sweden). Glucose, lactate, and pyruvate concentrations were then determined using a clinical CMA600 Microdialysis Analyzer (CMA Microdialysis AB, Sweden). The lower limit of detection (LOD) for each analyte was: Glucose (0.1 mmol/l); lactate (0.1 mmol/l); and pyruvate (10 µmol/l). Since the

ability to detect pyruvate is an indication of its concentration, non-detected samples were included at 0 µmol/l as a conservative measure.

*Statistical analysis:*
Preprocessing of all data was performed in Microsoft Office Excel. Statistical analysis was performed in GraphPad Prism 5 for Mac OSX and IBM SPSS Version 25 for Mac OSX. All graphs were made using GraphPad Prism 5 for Mac OSX. Pairs of groups were measured using t-tests and multiple comparisons were made using one- and two-way ANOVAs, paired and repeated measure variants were used as appropriate. Where data was found to be non-parametric, Mann-Whitney U-tests were used.

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**Supplemental Material:**

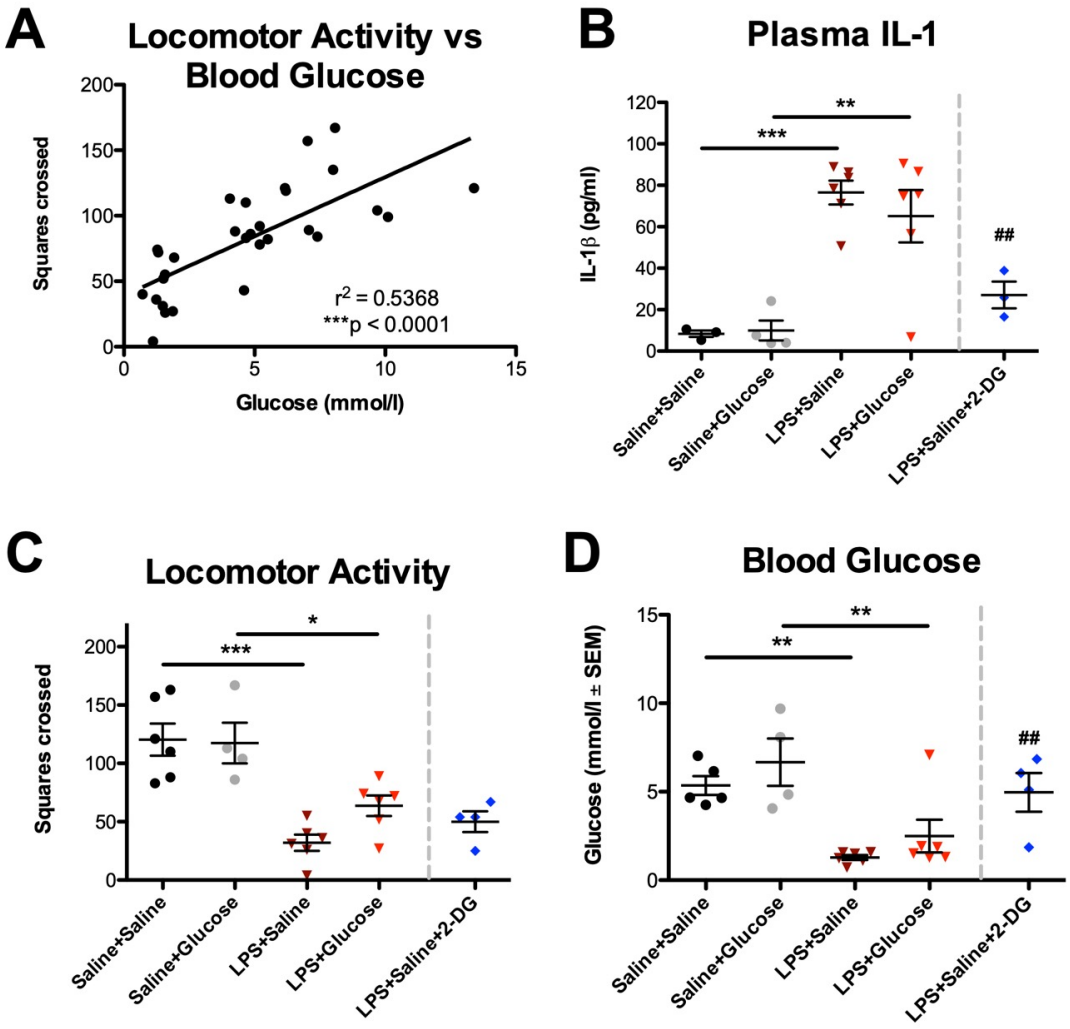


**Figure S1. Replication of findings presented in Figure 2, using different batch and different dose of LPS.** In these experiments, a more potent batch of LPS was used and animals were administered with 100 μg/kg instead of 250 μg/kg (i.p.). **A:** Linear regression analysis of locomotor activity (squares crossed in an open field) versus blood glucose concentration (mmol/l) - lower levels of glucose are correlated with reduced activity (n = 31; $r^2 = 0.5368$; $p < 0.0001$). **B:** LPS (n = 6) induced IL-1β production ($F_{(1,15)} = 40.90$; $p < 0.0001$; 125 minutes post-LPS) but IL-1β production was unaffected by co-administration of glucose (2 g/kg, i.p.; n = 6). 2-deoxyglucose (2-DG, 2 g/kg; i.p.) significantly attenuated LPS-induced IL-1β production ($t_{(7)} = 5.227$; $^{\#\#}p = 0.0012$ versus LPS+Saline). **C:** LPS significantly decreased locomotor activity ($F_{(1,18)} = 37.02$; $p < 0.0001$) but there were no significant glucose or interaction effects. Blocking glycolysis with 2-DG (n = 4) resulted in loss of locomotor activity but this was no different to LPS+Saline treatment. **D:** LPS significantly decreased blood glucose levels ($F_{(1,17)} = 27.76$; $p < 0.0001$). LPS+Saline (n = 6) and LPS+Glucose mice (n = 6) had significantly lower blood glucose levels compared to their respective Saline controls (n = 5 and n = 4 respectively). Blocking glycolysis with 2-DG (n = 4) resulted in significantly higher blood glucose levels compared to LPS+Saline treatment ($t_{(8)} = 4.175$; $^{\#\#}p = 0.0031$). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

**Table S1. Demographic information for patients recruited to the study.** [a]Based upon consensus in an expert panel. [b]Defined as 19 or 20 points on Barthel Activities of Daily Living. [c]Without information on haematocrit and arterial blood gas. [d]Mann-Whitney test and Chi-square tests depending on data distribution. APACHE II = Acute Physiology and Chronic Health Evaluation II; ASA = American Society of Anesthesiologists Physical Health Classification; CCI = Charlson Comorbidity Index score; IQR = Interquartile Range.

| | **No Delirium (n = 32)** | **Delirium (n = 40)** | **p-value[d]** |
|---|---|---|---|
| Median age, years (range) | 84.5 (60-93) | 85 (68-95) | 0.69 |
| Male, n (%) | 8 (25) | 11 (27.5) | 0.81 |
| Dementia, n (%)[a] | 2 (6.3) | 32 (80) | <0.001 |
| Independent in activities of daily living, n (%)[b] | 23 (71.9) | 8 (20) | <0.001 |
| Living in an institution, n (%) | 3 (9.4) | 20 (50) | <0.001 |
| APACHE II, median (IQR)[c] | 8 (6.3 – 9.8) | 9 (8 – 11) | 0.004 |
| CCI, median (IQR) | 1 (0 – 1.8) | 1 (0 – 2) | 0.044 |
| ASA score, median (IQR) | 2 (2-3) | 3 (3-3) | <0.001 |

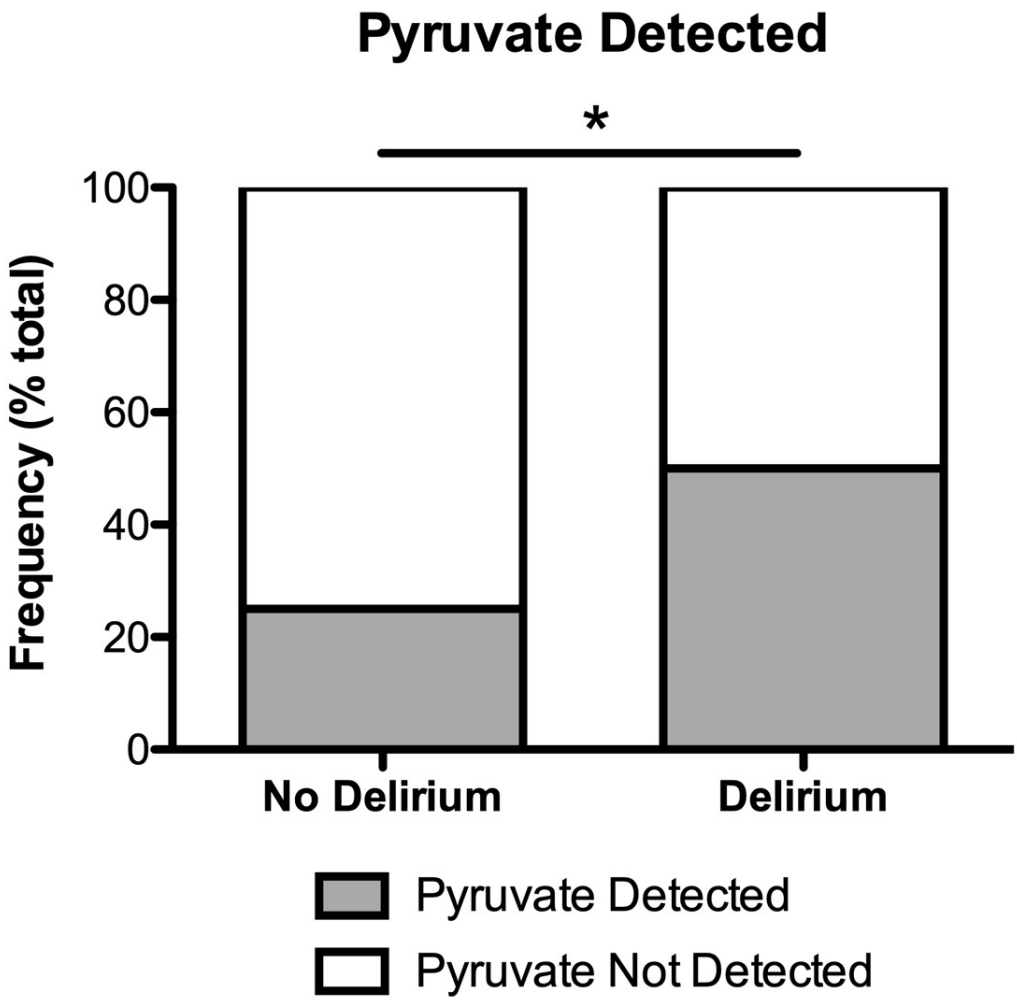


**Figure S2. Detection rates of pyruvate are higher during delirium**. Pyruvate was not detected in all CSF samples but a non-zero reading was detected in significantly more CSF samples from patients with delirium compared to controls ($U$ = 480.0; $p$ = 0.0397).

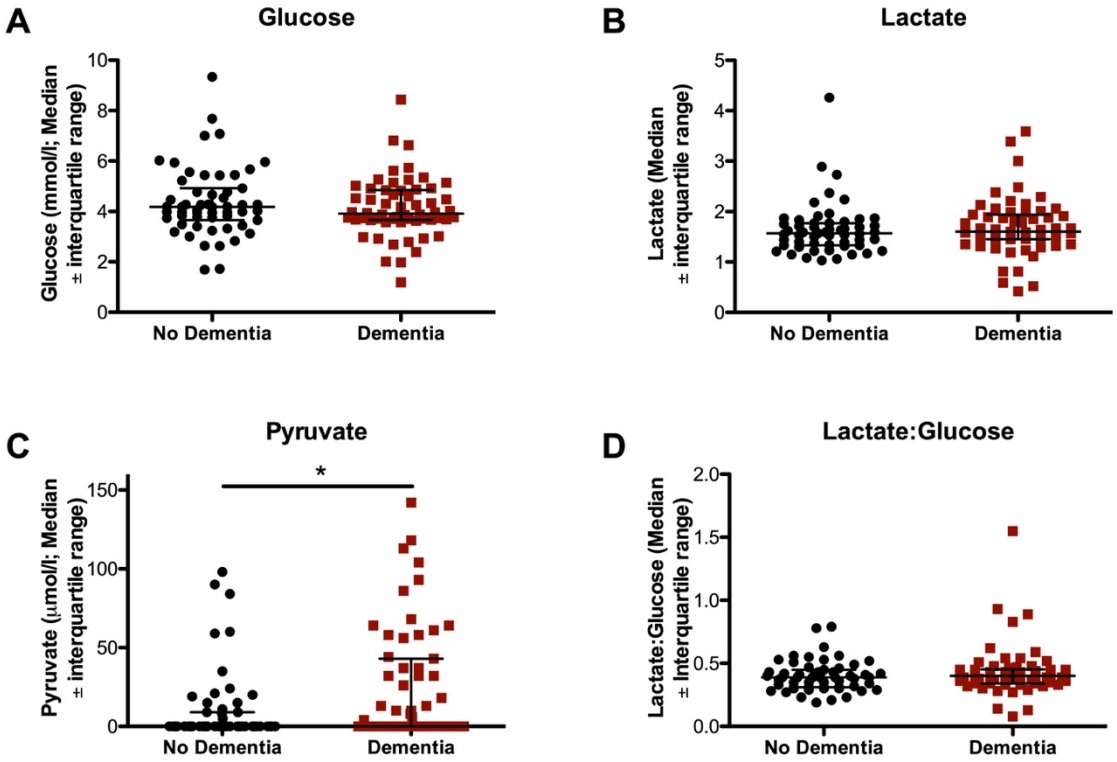


**Figure S3. Changes in CSF metabolite levels during delirium are not better explained by the presence of dementia**. Compared to No Dementia controls (n = 55), patients with dementia (n = 59) did not have significantly altered levels of CSF **A:** glucose or **B:** lactate. **C:** CSF pyruvate levels were significantly higher in patients with dementia ($U$ = 1278; $p$ = 0.0181). **D:** Dementia status had no significant effect on the CSF lactate:glucose ratio (LGR).

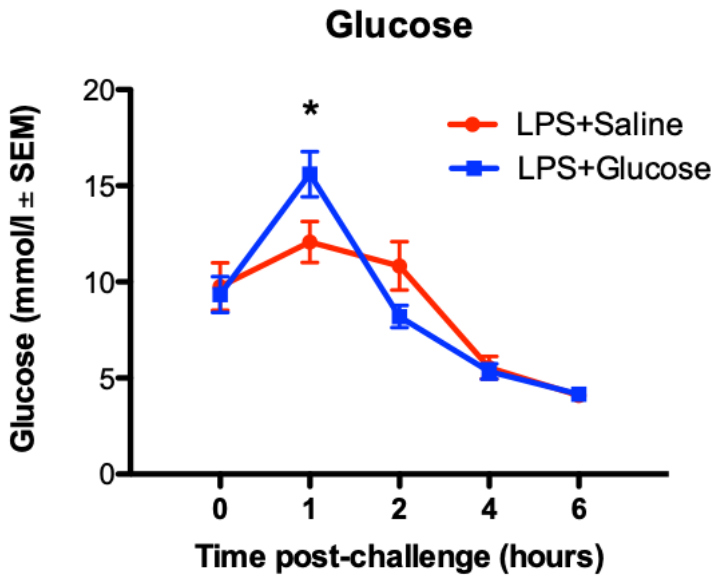


**Figure S4. Supplementing mice with glucose transiently increases blood glucose but does not override LPS-induced hypoglycemia**. Co-administration of glucose with LPS (LPS+Glucose; n = 5) causes a significant ($p < 0.05$) but transient increase in blood glucose levels but LPS-induced hypoglycemia is quickly re-established, and these animals are not different to LPS+Saline-treated controls (n = 7) from hours 2-6. When analyzing the post-challenge period (hours 1-6), there was a main effect for time ($F_{(3,30)} = 55.10$; $p < 0.0001$) and an interaction between time and treatment ($F_{(3,30)} = 4.60$; $p = 0.0092$).

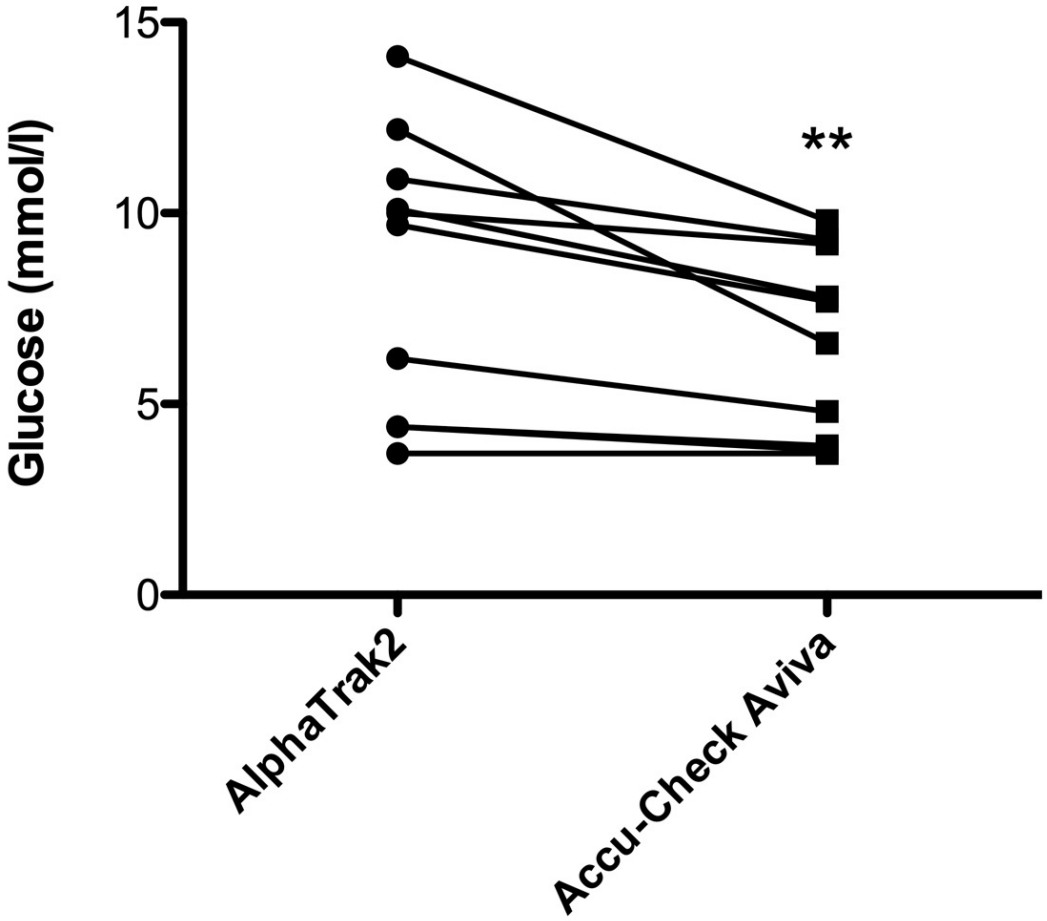


**Figure S5. Comparison of veterinary and clinical glucometers**. Upon completion of serial blood sampling studies, and observation of somewhat high glucose measures, we tested the AlphaTrak2 veterinary glucometer (Zoetis, USA) against a leading human clinical glucometer (Accu-Check Aviva, Roche, USA). These were compared, determining glucose concentrations in the same samples, at the same time. The AlphaTrak2 gave blood glucose values that were significantly higher ($t$ = 3.398; $df$ = 9; $p$ = 0.0079) than those obtained with the Accu-Check Aviva. However, all values still fell within a physiologically reasonable range (see Methods section).