Interleukin-18 Reduces Blood Glucose and Modulates Plasma Corticosterone in a Septic Mouse Model.

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博　士　論　文

Interleukin-18 Reduces Blood Glucose and Modulates Plasma Corticosterone in a Septic Mouse Model.

（Interleukin-18 はマウス敗血症モデルにおいて血糖値を下げ、

血漿中コルチコステロンを調節する）

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INTERLEUKIN-18 REDUCES BLOOD GLUCOSE AND MODULATES PLASMA CORTICOSTERONE IN A SEPTIC MOUSE MODEL

ABSTRACT

Background: Dysregulation of glucose metabolism, including hyperglycemia with insulin resistance, is commonly observed in critically ill patients. Interleukin-18 (IL-18) improves the insulin resistance associated with obesity, but the relationship between IL-18 and glucose metabolism in sepsis was unclear. The purpose of this study was to investigate the influence of IL-18 on hyperglycemia during sepsis. **Methods:** Sepsis was induced using cecal ligation and puncture (CLP) in wild-type (WT) mice, IL-18 knockout (KO) mice, and IL-18 KO mice pretreated with recombinant IL-18. Blood glucose and plasma insulin, glucagon, and corticosterone were measured. The mRNAs for gluconeogenic enzymes (*g6pc*, *pck1*) and activation of insulin signaling were also analyzed. **Results:** In both WT and IL-18 KO mice, CLP operation led to hyperglycemia that lasted longer (18 h) than after sham operation (6 h). Blood glucose levels in IL-18 KO mice were significantly higher than in WT mice, without alteration of insulin or glucagon levels. In IL-18 KO mice, insulin signaling in the liver and skeletal muscle was decreased during hyperglycemia as compared with WT mice without suppression of hepatic glucose production enzymes. Pretreatment with recombinant IL-18 reduced blood glucose levels after CLP. Additionally, corticosterone levels were higher after CLP in the presence of either endogenous or exogenous IL-18. **Conclusion:** IL-18 may reduce blood glucose by modulating insulin signaling in the liver during sepsis-induced hyperglycemia. IL-18 is an important factor associated with alterations in blood glucose during sepsis.

KEYWORDS—Glucocorticoid, hyperglycemia, IL-18, insulin resistance, sepsis

Introduction

Dysregulation of glucose metabolism is a cause of poor outcomes in critically ill patients, including patients with sepsis, independent of whether or not the patient is diabetic (1,2). Under septic conditions, adrenaline, glucagon, and glucocorticoids are increased and lead to hyperglycemia via gluconeogenesis and glycogenolysis in the liver. Pro-inflammatory cytokines, including tumor necrosis factor -α and interleukin (IL)-6, are elevated and inhibit insulin signaling pathways in the liver and peripheral tissues (3,4). Thus, septic patients exhibit poor glycemic control, hyperglycemia, and transient insulin resistance. Controlling blood glucose levels improves the prognosis of septic patients. However, because hypoglycemia is a strong risk factor for death in critically ill patients, blood glucose control should be attempted with care (5). The 2012 Surviving Sepsis Campaign guidelines state that blood glucose should be controlled to be lower than 180 mg/dL (rather than <110 mg/dL) to reduce the risk of inducing hypoglycemia (6).

IL-18 is a pro-inflammatory cytokine secreted by T-cells, macrophages, Kupffer cells, monocytes, dendritic cells, keratinocytes, intestinal epithelial cells, the pituitary gland, and the adrenal cortex (7-9). During sepsis, the production of endogenous IL-18 is increased, and increases in circulating IL-18 correlate with the severity of the disease (10-11). In fact, urinary IL-18 levels are a potentially useful biomarker for acute kidney injury during sepsis (12). Additionally, septic patients suffering acute respiratory distress syndrome (ARDS) had higher plasma IL-18 levels than septic patients without ARDS (13). In our previous report, which was the first report focusing on IL-18 and transient insulin resistance mediated by acute inflammation, we found that endogenous IL-18 attenuated transient hyperglycemia by enhancing hepatic insulin signaling during the hyperacute phase of lipopolysaccharide (LPS)-induced lethal endotoxemia (14).

Increases in circulating IL-18 predict the development of insulin resistance in patients with diabetes and obesity (15,16). Paradoxically however, experimental studies have shown that endogenous and exogenous IL-18 can mitigate insulin resistance (17,18). IL-18 enhances insulin signaling, such as Akt phosphorylation, in the liver and skeletal muscles in obese mice (18). However, there are few reports on the relationship between IL-18 and insulin signaling during sepsis. IL-18 may be a useful predictor or therapeutic target in controlling blood glucose in sepsis if the role of IL-18 in sepsis-induced transient insulin resistance or sepsis-induced hyperglycemia is better understood. We hypothesized that IL-18 suppresses sepsis-induced hyperglycemia via maintenance of insulin signaling

in the liver and skeletal muscle. To test this hypothesis, we used a mouse model of sepsis induced by cecal ligation and puncture (CLP), which is known to closely reflect the clinical situation of critically ill patients (19).

Methods

Animals

Nine to 12-week old, male C57BL/6J (wild type, WT) mice (Clea Japan, Tokyo, Japan) and IL-18 knockout (KO) mice (B6.129P2-IL18^{tm1Aki}/J; The Jackson Laboratory, Bar Harbor, ME) were used in this study. The body weights of the WT mice and IL-18 KO mice were similar at the start of the study (25.3±1.2 g and 24.8±1.0 g, respectively). All mice were kept at 22±2°C under a 12 hour light/dark cycle and allowed access to a standard diet and water ad libitum. All experiments involving mice were conducted with the approval of the Animal Care and Use Committee of Kobe University.

Sepsis model by cecal ligation and puncture

Sepsis was induced by cecal ligation and puncture (CLP) under anesthesia (0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, 5.0 mg/kg butorphanol), and the operations were performed between 8:00 am and 10:00 am in all mice. Through a 1 cm abdominal midline incision, the cecum was ligated with 3-0 silk suture and punctured with an 18-gauge needle in a through-and-through manner. The abdominal wall and skin were closed with 5-0 nylon suture in two layers. Sham-operated mice underwent laparotomy without ligation or puncture. All animals were subcutaneously given 40 mL/kg saline (37 °C) immediately after the surgery for fluid resuscitation. Prior to surgery (0 hours) and 6, 12, or 24 hours after surgery, the mice were sacrificed by cardiac puncture under general anesthesia. Samples of blood and tissue were immediately harvested, placed in the reagent necessary for the next experiment, and stored at -80°C.

When examining glucose tolerance, a “mild” CLP procedure was performed, that mimicked the standard CLP procedure for inducing sepsis except for the use of a 21-gauge needle rather than an 18-gauge needle for puncture. Glucose-tolerance tests in mice subjected to the standard CLP protocol were very difficult to perform and to interpret because the WT mice were close to death when blood glucose levels returned to the baseline after CLP. Thus, we chose to perform the glucose-tolerance tests in mice subjected to a less intense CLP protocol.

Blood analysis

Blood glucose and plasma corticosterone were measured in tail vein blood to minimize the influence of repeated stress. For the measurement of other parameters, blood samples were obtained by cardiac puncture. Blood glucose levels were measured using a portable glucose meter (Glutest NEO Sensor; Sanwa Kagaku Kenkyusho, Nagoya, Japan). Plasma insulin (Shibayagi, Gunma, Japan), glucagon (WAKO, Osaka, Japan), corticosterone (AssayPro, St. Charles, MO), IL-18 (MBL, Nagoya, Japan), and IL-6 (R&D Systems, Minneapolis, Minnesota) were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions.

Real-time reverse transcription PCR

Total RNA were extracted by TRIzol® Reagent (Invitrogen, Carlsbad, CA), and the cDNA was synthesized using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. The following cDNAs were then quantified with quantitative real-time RT-PCR analysis using SYBR Green Realtime PCR Master Mix (Toyobo, Osaka, Japan) and the primers indicated: glucose-6-phosphatase gene (*g6pc*, F: tgctgtgtctggtaggcaac, R: agaatcctgggtctccttgc), phosphoenolpyruvate carboxykinase gene (*pck1*, F: agcctgctccagctttga, R: ccctagcctgttctctgtgc), and glucokinase (*gck*, F: ggtgagctggacgagtt, R: aagattctcctctaccagctt). Relative expression of each cDNA was calculated using the ddCt method after normalization to glyceraldehyde 3-phosphate dehydrogenase (*gapdh*, F: tgtgtccgtcgtggatctga, R: ttgctgttgaagtcgcaggag) expression.

Western blotting

Anti-Akt and anti-Ser473 phosphorylated Akt were purchased from Cell Signaling Technology (Danvers, MA). Anti-β-actin antibody was obtained from Sigma-Aldrich (St Louis, MO). The liver and gastrocnemius muscle (skeletal muscle) samples were homogenized in lysis buffer (100 mM HEPES (pH 7.4), 1% Triton, 10% glycerol, 150 mM NaCl, 1 mM sodium pyrophosphate, 100 mM sodium fluoride, 2 mM EDTA, 5 mM sodium vanadate, 1 mM PMSF, 5 μg/mL aprotinin, and 5 μg/mL leupeptin). Western blotting was performed as described previously (14). Membranes were incubated overnight at 4°C with primary antibodies diluted in Can Get Signal immunoreaction enhancer solution 1 (Toyobo), then with horseradish

peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in Can Get Signal solution 2 (Toyobo) for 1 hour at room temperature. Antibody binding was detected using enhanced chemiluminescence plus reagents (GE Healthcare, Buckinghamshire, UK) and the Optima Shot CL-420a image-capturing system (Wako, Osaka, Japan). The band intensity was quantified using ImageJ ver. 1.47 (U. S. National Institutes of Health, Bethesda, Maryland).

Intraperitoneal glucose tolerance test

WT and IL-18 KO mice were intraperitoneally injected 2 g/kg D-glucose in 20 mL/kg saline at 20 hours after mild CLP. Blood glucose levels were measured using tail vein blood before (0 minutes) and 15, 30, 60, 120, and 240 minutes after mild CLP.

Recombinant IL-18 administration to IL-18 KO mice

IL-18 KO mice were injected with 1 μg recombinant mouse IL-18 (rIL-18; MBL, Nagoya, Japan) in 0.2 mL phosphate buffered saline (PBS) intraperitoneally 24 hours, 4 hours and 0.5 hours before the CLP operation. This dose of recombinant IL-18 was determined in a previous study (17). Blood glucose, plasma insulin, and plasma corticosterone were then measured using tail vein blood 0, 6, 12, 24 hours after CLP. Five mice per group were sacrificed 12 and 24 hours after CLP to analyze Akt phosphorylation in the liver and skeletal muscle.

Statistical analysis

The data were expressed as mean ± standard error of the mean. Survival was examined by Kaplan-Meier analysis and the log-rank test. T-tests were performed for examining the elevation of IL-18 after CLP. Two-factor analysis of variance (ANOVA) was performed for the time course experiments comparing WT and IL-18 KO mice. For other experiments, ANOVA with the Tukey-Kramer's post hoc test was performed. A probability level of $p<0.05$ was considered statistically significant.

Results

Mortality and plasma cytokine levels after CLP

The mortality in this septic model tended to higher in the WT mice (81.3%) as compared with IL-18 KO mice (47.1%), but this difference was not statistically

significant (p=0.069) (Fig. 1A). In WT mice, plasma IL-18 levels were elevated significantly 6 and 24 hour after CLP. As expected, IL-18 could not be detected in the IL-18 KO mice (Fig. 1B). IL-6 levels were elevated after CLP in a time-dependent manner, with no differences between the WT and IL-18 KO mice (Fig. 1C). There were no significant differences in multiple biochemical markers in the blood in response to either CLP operation or IL-18 status. (Supplemental Table 1) When lung, liver, and kidney morphology was examined after CLP, only minor differences in neutrophil accumulation were seen between the lungs of WT and IL-18 KO mice (Supplemental Figure 1). CLP-induced sepsis led to injury in the liver and kidney with no differences between the WT and IL-18 KO mice (data not shown).

Blood glucose and plasma hormones

To assess the effects of IL-18 on glucose metabolism, we measured blood glucose, insulin, glucagon, and corticosterone in WT and IL-18 KO mice after inducing sepsis using CLP or after a sham operation. Sham operation induced transient hyperglycemia in both WT and IL-18 KO mice for 6 hours, and then the blood glucose levels decreased, and the mice recovered glucose homeostasis within 15 hours after the sham operation. The hyperglycemic period was prolonged by CLP in both WT and IL-18 KO mice, and blood glucose was still high 18 hours after CLP (Fig. 2A). In addition, CLP lead to the difficulty in maintaining glucose homeostasis. During the first 24 hours after CLP, the blood glucose levels in IL-18 KO mice were significantly higher than in WT mice ($p<0.01$). By 24 hours after CLP, most of the WT mice developed hypoglycemia (<70 mg/dL glucose) (66.7%; 4/6), but the IL-18 KO mice did not (0%; 0/6). Plasma insulin levels were decreased 6 to 12 hours after CLP then sharply increased 24 hours after CLP in both WT and IL-18 KO mice (Fig. 2B). The plasma glucagon profile was also not significant altered by IL-18 deficiency (Fig. 2C). Plasma corticosterone levels were elevated after CLP, and the levels in IL-18 KO mice were significantly lower than those in WT mice 24 hours after CLP (Fig. 2D, $p<0.05$).

Gluconeogenic enzyme mRNA expression in the liver

Because gluconeogenesis and glycogenolysis in the liver increase adrenaline, glucagon, and glucocorticoid levels leading to hyperglycemia in septic patients, we examined the effects of IL-18 deletion on the glucose metabolism in the liver by

examining the expression of the mRNAs for gluconeogenic and glycolytic enzymes. *G6pc* and *pck1* (gluconeogenic enzymes) were transiently increased and then decreased after CLP, but no significant differences existed between WT and IL-18 KO mice (Fig. 3A, B). *Gck* (a glycolytic enzyme) was consistently decreased by CLP, and there were no significant differences in the WT and IL-18 KO mice (Fig. 3C). These results suggested that IL-18 modulation of blood glucose levels during sepsis was not a result of either gluconeogenesis or glycogenolysis in the liver.

Insulin signaling in the liver and skeletal muscle

To assess the effects of IL-18 deletion on insulin signaling in response to acute inflammation, we analyzed the activation of Akt. We assessed Akt phosphorylation (pAkt) 6 and 24 hours after CLP because a difference in blood glucose between IL-18 KO and WT mice was first observed 6 hours after CLP and 24 hours marked the lowest blood glucose levels after CLP. In the liver and skeletal muscle of WT mice, pAkt levels were decreased 6 hours after CLP, but recovered to baseline levels within 24 hours (Fig. 4). In IL-18 KO mice, pAkt expression was significantly lower than in WT mice before CLP and 6 hours after CLP, but was significantly higher 24 hours after CLP in both the liver and skeletal muscle.

Glucose clearance after CLP

To determine the effect of IL-18 deletion on the glucose clearance, we performed intraperitoneal glucose tolerance tests after inducing sepsis using a milder CLP procedure. Glucose clearance was significantly impaired in the IL-18 KO mice as compared with the WT mice (Fig. 5, $p<0.05$).

The effects of exogenous IL-18

To test whether the marked increase in blood glucose levels in response to CLP in IL-18 KO mice could be attenuated by exogenous IL-18, we administered rIL-18 to IL-18 KO mice before the CLP operation. Administration of rIL-18 before CLP remarkably reduced blood glucose levels 12 hours after CLP in IL-18 KO mice (Fig. 6A) as compared with control mice given PBS. Plasma insulin was increased 12 and 24 hours after CLP regardless of rIL-18 pretreatment (Fig. 6B). Plasma corticosterone levels increased 12 and 24 hours after CLP regardless of rIL-18 pretreatment, but the increase in

rIL-18-pretreated IL-18 KO mice was significantly higher than in PBS-treated IL-18 KO mice (Fig. 6C, $p<0.05$). Akt phosphorylation in the liver was increased by rIL-18-treatment (Fig. 6D); Akt phosphorylation was not increased in the skeletal muscle.

Discussion

This study showed that IL-18 influences blood glucose levels during sepsis-induced hyperglycemia. The mechanisms underlying IL-18 modulation of blood glucose levels was independent of insulin- and glucagon-dependent regulatory systems. Corticosterone, which is a main glucocorticoid in rodents, might be modulated by IL-18 during sepsis because corticosterone levels were decreased in the absence of IL-18 and increased when rIL-18 was administered. In addition, deletion of IL-18 prolonged insulin signaling in the liver and skeletal muscle, but did not influence gluconeogenesis or glycogenolysis during hyperglycemia. Thus, IL-18 may improve the uptake of glucose by the tissues and help maintain plasma glucocorticoid levels during sepsis-induced hyperglycemia.

Our finding here of higher blood glucose levels in IL-18 KO mice after CLP-induced sepsis was similar to the findings of our previous study using LPS-induced endotoxemia in mice (14). The result was also supported by Akt activation in the liver and skeletal muscle and the slower glucose clearance in IL-18 KO mice compared with WT mice shown in this study. IL-18 may decrease blood glucose by modulating insulin signaling in multiple tissues. The finding that blood glucose levels after CLP are reduced when IL-18 KO mice were treated with recombinant IL-18 strengthened this notion.

The influence of IL-18 on liver function may be small. In this study, gluconeogenic enzymes were not altered by IL-18 status. In studies of obesity and diabetes, IL-18 was found to be involved in energy homeostasis, including food intake, glucose tolerance disorder, and fatty acid oxidation (17,18). The current study suggests that IL-18 may regulate glucose homeostasis during critical illness without suppression of hepatic glucose synthesis. The plasma insulin levels in this study were not high enough to be classified as hyperinsulinemia, whereas in our previous study, hyperinsulinemia accompanied hyperglycemia in IL-18 KO mice with LPS-induced endotoxemia. The fact that sepsis was induced by an abdominal surgery procedure in the CLP model may be the cause of this difference. Insulin secretion from rat pancreatic islet can be impaired by

surgical stress (20).

To our knowledge, this is a first report that plasma corticosterone levels may be modulated by IL-18 during septic conditions. This result does not conflict with a previous report that elevation of serum corticosterone by stress (water immersion and restraint) was suppressed in the absence of IL-18 in mice (21). During sepsis, the hypothalamic-pituitary-adrenal (HPA) axis and glucocorticoid production are initially activated as an inflammatory response, then become repressed later (22). Glucocorticoids play an anti-inflammatory role as inducers of apoptosis and inhibitors of the cellular proliferation of immune cells, and glucocorticoids are often administrated to septic patients as a supplemental therapy for adrenal insufficiency (23). Lower corticosterone levels in the IL-18 KO mice may have contributed to neutrophil activation and accumulation in the lungs of the IL-18 KO mice after CLP. This is agreement with a previous study that demonstrated increased lung injury in a CLP-induced rat model of sepsis when plasma corticosterone was reduced using metyrapone (24). On the other hand, rodents subjected to CLP showed high plasma corticosterone levels, indicating adrenal insufficiency, and some interventions that suppress the elevation of corticosterone improve morbidity (25,26). Because corticosterone is metabolized in the liver, liver damage may impair corticosterone clearance. Boonen and colleagues showed that critically ill patients had decreased expression of cortisol-metabolizing enzymes in the liver and impaired cortisol clearance (27). However, our results showed similar liver function in WT and IL-18 KO mice after CLP. Thus, in this model, the elevation of corticosterone by IL-18 may reflect suppression of the inflammatory response during sepsis and not adrenal insufficiency.

We saw a trend toward improved survival in the absence of IL-18 in this CLP model, but could not correlate this with changes in a systemic inflammatory marker (IL-6), blood biochemistry, or tissue morphology. Hypoglycemia is commonly observed in mice with sepsis induced by CLP and may have been the cause of death in the WT mice (28). Hypoglycemic episodes in patients with critical illnesses have deleterious effects, such as systemic inflammation, neuroglycopenia, and cerebral vasodilatation, which contribute to mortality (29). It is likely that blood glucose in the IL-18 KO mice was not metabolized due to insulin resistance that developed 6 hours after CLP, which may have prevented hypoglycemia. Further study is needed to elucidate the contribution of IL-18 dependent pathways to mortality.

It is currently debated whether IL-18 is beneficial or harmful in sepsis. We believe that an appropriate level of IL-18 is important to resolve sepsis. Our results showed that IL-18 was important for the attenuation of insulin resistance. Although survival tended to low in the presence of IL-18, hypoglycemia (suspected as a cause of death) could be avoided by monitoring blood glucose carefully in the clinical setting. Kinoshita and colleagues reported that IL-18 therapy was beneficial for preventing bacterial infection after severe critical illness (30). In addition, we demonstrated that endogenous IL-18 suppressed testicular germ cell apoptosis during the recovery phase of endotoxemia (31). In contrast, we also reported IL-18 KO mice have improved survival and less lung inflammation using a mouse model of lethal endotoxemia (32), and Van den Berghe and colleagues reported that double inhibition of IL-1 and IL-18 improved survival and body temperatures in several mouse models of sepsis (33). Excessive IL-18 could lead to negative outcomes. We believe that 500-1,000 pg/mL is likely the upper limit of IL-18 to avoid adverse effects in mice. We are currently researching the relationship between IL-18 and hyperglycemia in the clinical setting using patient samples and have obtained encouraging results.

Our study has some limitations. First, because we focused on glucose homeostasis in this study, the effects of IL-18 on the HPA axis remain unclear. IL-18 acts on the pituitary gland and adrenal cortex and inhibits corticotropin-releasing hormone release from hypothalamus (8,9,34). The mechanisms of IL-18-induced glucocorticoid production are still unclear and may involve the HPA axis. We are continuing to investigate the effects of IL-18 on the HPA axis. Second, our septic mice did not receive nutritional therapy or insulin therapy. Exogenous glucose infusion stimulates pancreatic insulin secretion from β cells, and exogenous insulin treatment is common in patients with sepsis-induced hyperglycemia. We cannot make clear conclusions regarding the influence of IL-18 on clinical outcomes based on this study.

Conclusions

IL-18 has an important role in suppressing insulin resistance and does not aggravate organ damage in mice with CLP-induced sepsis. Therefore, IL-18 is an important factor associated with alterations in blood glucose levels during sepsis. In addition, our findings provide new insight that IL-18 elevates glucocorticoid production during sepsis.

List of Abbreviations

ANOVA	analysis of variance
ARDS	acute respiratory distress syndrome
CLP	cecal ligation and puncture
ELISA	enzyme-linked immunosorbent assay
IL	Interleukin
KO	knockout
LPS	lipopolysaccharide
pAkt	phosphorylated Akt
PBS	phosphate buffered saline
WT	wild type

Competing Interests: None

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Authors' contributions

HY and MI drafted this manuscript. HY, MI, and TI carried out this study and analyzed samples. YU advised about histophathological analysis. HY, MI, MU and JK involved in the design of this study.

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References

1. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R: Intensive insulin therapy in critically ill patients. *N Engl J Med* 345(19): 1359–1367, 2001.
2. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R: Intensive insulin therapy in the

medical ICU. *N Engl J Med* 354(5): 449–461, 2006.

3. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271(5249): 665–668, 1996.
4. Senn JJ, Klover PJ, Nowak IA, Mooney RA: Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 51(12): 3391–3399, 2002.
5. Finfer S, Liu B, Chittock DR, Norton R, Myburgh JA, McArthur C, Mitchell I, Foster D, Dhingra V, Henderson WR, *et al.*: Hypoglycemia and risk of death in critically ill patients. *N Engl J Med* 367(12): 1108–1118, 2012.
6. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, *et al.*: Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 39(2): 165–228, 2013.
7. Sedimbi SK, Hägglöf T, Karlsson MCI: IL-18 in inflammatory and autoimmune disease. *Cell Mol Life Sci* 70(24): 4795–4808, 2013.
8. Wang N, Sugama S, Conti B, Teramoto A, Shibasaki T: Interleukin-18 mRNA expression in the rat pituitary gland. *J Neuroimmunol* 173(1-2): 117–125, 2006.
9. Conti B, Sugama S, Kim Y, Tinti C, Kim H, Baker H, Volpe B, Attardi B, Joh T: Modulation of IL-18 production in the adrenal cortex following acute ACTH or chronic corticosterone treatment. *Neuroimmunomodulation* 8(1): 1–7, 2000.
10. Endo S, Inada K, Yamada Y, Wakabayashi G, Ishikura H, Tanaka T, Sato S: Interleukin 18 (IL-18) levels in patients with sepsis. *J Med* 31(1-2): 15–20, 2000.
11. Zaki Mel-S, Elgendy MY, El-Mashad NB, Farahat ME: IL-18 level correlates with development of sepsis in surgical patients. *Immunol Invest* 36(4): 403–411, 2007.
12. Bagshaw SM, Langenberg C, Haase M, Wan L, May CN, Bellomo R.: Urinary biomarkers in septic acute kidney injury. *Intensive Care Med* 33(7): 1285–1296, 2007.
13. Dolinay T, Kim YS, Howrylak J, Hunninghake GM, An CH, Fredenburgh L, Massaro AF, Rogers A, Gazourian L, Nakahira K, *et al.*: Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am. J. Respir. Crit Care Med* 185(11): 1225–1234, 2012.
14. Yamashita H, Aoyama-Ishikawa M, Takahara M, Yamauchi C, Inoue T, Miyoshi M, Maeshige N, Usami M, Nakao A, Kotani J: Endogenous interleukin 18 suppresses

hyperglycemia and hyperinsulinemia during the acute phase of endotoxemia in mice. *Surg Infect (Larchmt)* 16(1): 90–96, 2015.

15. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, Illig T, Martin S, Herder C: Elevated levels of interleukin-18 predict the development of type 2 diabetes: Results from the MONICA/KORA Augsburg study, 1984-2002. *Diabetes* 54(10): 2932–2938, 2005.
16. Hung J, McQuillan BM, Chapman CM, Thompson PL, Beilby JP: Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. *Arterioscler Thromb Vasc Biol* 25(6): 1268–1273, 2005.
17. Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, Tack CJ, van Krieken H, Kim SH, Stalenhoef AF, *et al.*: Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nat Med* 12(6): 650–656, 2006.
18. Lindegaard B, Matthews VB, Brandt C, Hojman P, Allen TL, Estevez E, Watt MJ, Bruce CR, Mortensen OH, Syberg S, *et al.*: Interleukin-18 activates skeletal muscle AMPK and reduces weight gain and insulin resistance in mice. *Diabetes* 62(9): 3064–3074, 2013.
19. Rittirsch D, Hoesel LM, Ward PA: The disconnect between animal models of sepsis and human sepsis. *J Leukoc Biol* 81(1): 137–143, 2007.
20. Hirano T, Manabe T, Ando K, Yamaki K, Yoshimura T, Tobe T: Effect of surgical stress on glucose-stimulated insulin release from isolated perfused rat pancreas. *Int Surg* 76(4): 250–252, 1991.
21. Seino H, Ueda H, Kokai M, Tsuji NM, Kashiwamura S, Morita Y, Okamura H: IL-18 mediates the formation of stress-induced, histamine-dependent gastric lesions. *Am J Physiol Gastrointest Liver Physiol* 292(1): G262–G267, 2007.
22. Kanczkowski W, Sue M, Zacharowski K, Reincke M, Bornstein SR: The role of adrenal gland microenvironment in the HPA axis function and dysfunction during sepsis. *Mol Cell Endocrinol* 408: 241–248, 2014.
23. Schwartzman RA, Cidlowski JA: Glucocorticoid-induced apoptosis of lymphoid cells. *Int Arch Allergy Immunol* 105(4): 347–354, 1994.
24. Incerpi EK, Oliveira LM, Pereira EM, Soncini R: Inhibition of endogenous glucocorticoid synthesis aggravates lung injury triggered by septic shock in rats. *Int J Exp Pathol* 96(3): 133–139, 2015.

25. Zhao T, Li Y, Bronson RT, Liu B, Velmahos GC, Alam HB: Selective histone deacetylase-6 inhibition attenuates stress responses and prevents immune organ atrophy in a lethal septic model. *Surgery* 156(2): 235–242, 2014.
26. Lee JK, Jung JS, Park SH, Sim,YB, Suh HW: Deficiency of alpha-calcitonin gene-related peptide induces inflammatory responses and lethality in sepsis. *Cytokine* 64(2): 548–554, 2013.
27. Boonen E, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, Vanwijngaerden YM, Spriet I, Wouters PJ, Vander Perre S, *et al.*: Reduced cortisol metabolism during critical illness. *N Engl J Med* 368(16): 1477–1488, 2013.
28. Maitra SR, Wang S, Brathwaite CE, El-Maghrabi MR: Alterations in glucose-6-phosphatase gene expression in sepsis. *J Trauma* 49(1): 38–42, 2000.
29. Egi M, Finfer S, Bellomo R: Glycemic control in the ICU. *Chest* 140(1): 212–220, 2011.
30. Kinoshita M, Miyazaki H, Ono S, Inatsu A, Nakashima H, Tsujimoto H, Shinomiya N, Saitoh D, Seki S: Enhancement of neutrophil function by interleukin-18 therapy protects burn-injured mice from methicillin-resistant Staphylococcus aureus. *Infect Immun* 79(7): 2670–2680, 2011.
31. Inoue T, Aoyama-Ishikawa M, Kamoshida S, Nishino S, Sasano M, Oka N, Yamashita H, Kai M, Nakao A, Kotani J, *et al.*: Endogenous interleukin 18 regulates testicular germ cell apoptosis during endotoxemia. *Reproduction* 150(2): 105–114, 2015.
32. Takahara M, Aoyama-Ishikawa M, Shuno K, Yamauhi C, Miyoshi M, Maeshige N, Usami M, Yamada T, Osako T, Nakao A, *et al.*: Role of endogenous IL-18 in the lung during endotoxin-induced systemic inflammation. *Acute Med Surg* 1(1): 23–30. 2014.
33. Vanden Berghe T, Demon D, Bogaert P, Vandendriessche B, Goethals A, Depuydt B, Vuylsteke M, Roelandt R, Van Wonterghem E, Vandenbroecke J, *et al.*: Simultaneous targeting of IL-1 and IL-18 is required for protection against inflammatory and septic shock. *Am J Respir Crit Care Med* 189(3): 282–291, 2014.
34. Tringali G, Pozzoli G, Vairano M, Mores N, Preziosi P, Navarra P: Interleukin-18 displays effects opposite to those of interleukin-1 in the regulation of neuroendocrine stress axis. *J Neuroimmunol* 160(1-2): 61–67, 2005.

Figure Legends

Fig. 1. Survival and plasma cytokine levels after CLP. A. Survival of mice after CLP in WT mice (n=16) and IL-18 KO mice (n=17). B. Alteration of plasma IL-18 after CLP. (n=3 at 0 hour; n=6 for other time points). C. Alteration of plasma IL-6 after CLP. (n=3 at 0 hour; n=6 for other time points). WT CLP, filled square and solid line; KO CLP, open square and dashed line. *, $p<0.05$ vs. 0 hour by t-test.

Fig. 2. Blood glucose and plasma hormones. A. Blood glucose levels. (n=6) B. Plasma insulin levels. (n=3 at 0 hour; n=6 for other time points) C. Plasma glucagon levels. (n=3 at 0 hour; n=6 for other time points) D. Plasma corticosterone levels. (n=6) *, $p<0.05$; **, $p<0.01$ vs. WT CLP during 24 hours. ##, $p<0.01$ vs. sham group at each time point. WT sham, filled triangle and solid line; KO sham, open triangle and dashed line; WT CLP, filled square and solid line; KO CLP, opened square and dashed line.

Fig. 3. Expression of hepatic gluconeogenic and glycolytic enzymes. A. *g6pc* mRNA expression. B. *pck1* mRNA expression. C. *gck* mRNA expression. (n=3 at 0 hour; n=6 for other time points) WT CLP, filled square and solid line; KO CLP, open square and dashed line.

Fig. 4. Akt activation. A. Akt activation in the liver. B. Quantitation of Akt phosphorylation in the liver. C. Akt activation in the skeletal muscle. D. Quantitation of Akt phosphorylation in the skeletal muscle. n=4; *, $p<0.05$; **, $p<0.01$.

Fig. 5. Glucose clearance during an intraperitoneal glucose tolerance test after mild CLP. n=3; *, $p<0.05$ between WT CLP and KO CLP by two-factor ANOVA. WT CLP, filled square and solid line; KO CLP, open square and dashed line.

Fig. 6. The effects of administering recombinant IL-18 to IL-18 KO mice. A. Blood glucose levels. B. Plasma insulin levels. C. Plasma corticosterone levels. (n=5) *, $p<0.05$ between PBS-treated KO and rIL-18-treated KO mice by two-factor ANOVA. Recombinant IL-18-treated KO CLP, filled diamond and solid line; PBS-treated KO CLP, open diamond and dashed line. D. Akt phosphorylation in rIL-18 and PBS mice in the liver and skeletal muscle. n=4.

Figure 1

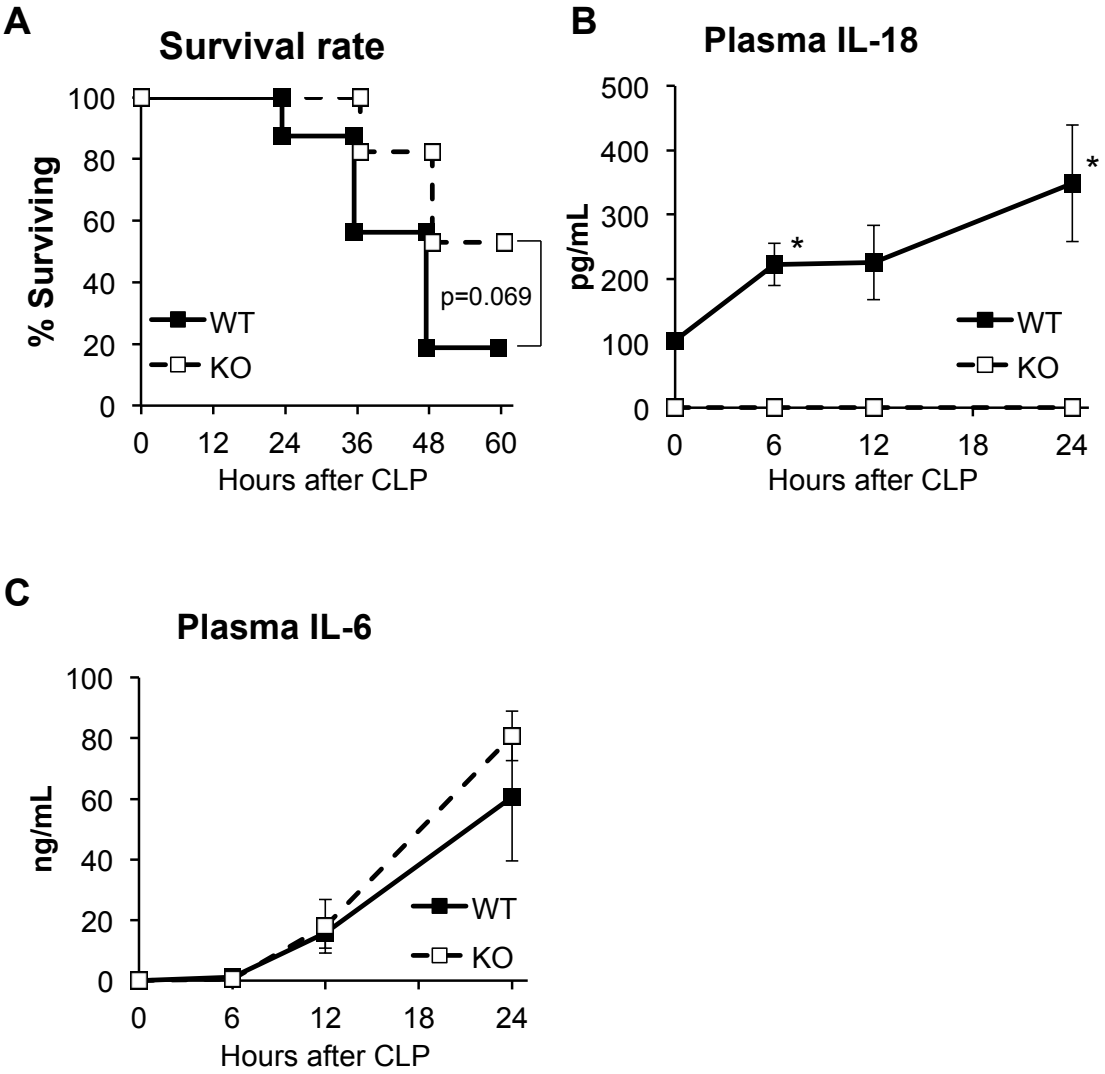

A
Survival rate
% Surviving
100
80
60
40
20
0
0
12
24
36
48
60
Hours after CLP
WT
KO
p=0.069
B
Plasma IL-18
pg/mL
500
400
300
200
100
0
0
6
12
18
24
Hours after CLP
WT
KO
*
*
C
Plasma IL-6
ng/mL
100
80
60
40
20
0
0
6
12
18
24
Hours after CLP
WT
KO

Figure 2

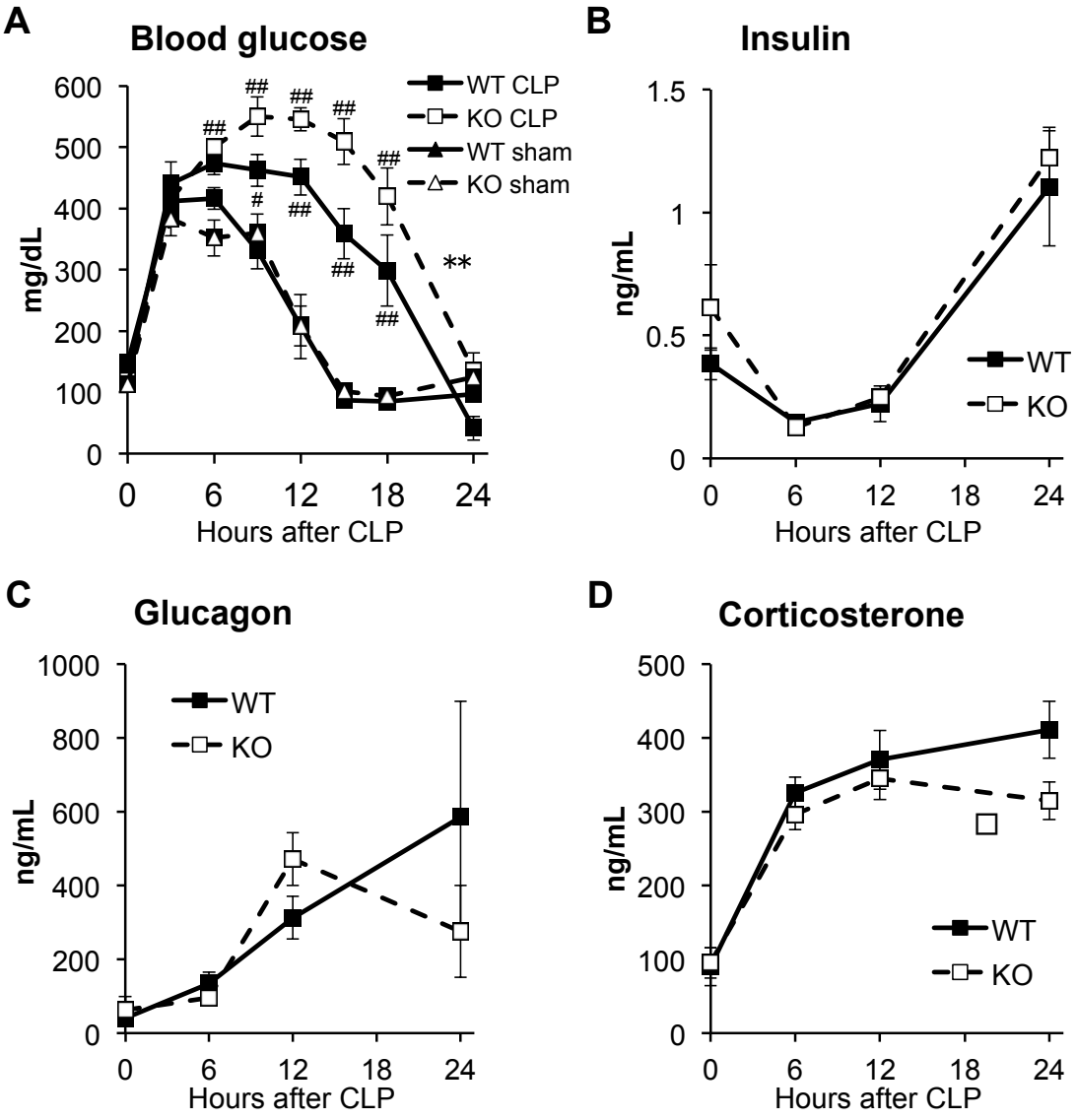

A
Blood glucose
mg/dL
600
500
400
300
200
100
0
0
6
12
18
24
Hours after CLP
WT CLP
KO CLP
WT sham
KO sham
##
#
**
B
Insulin
ng/mL
1.5
1
0.5
0
WT
KO
C
Glucagon
ng/mL
1000
800
600
400
200
0
D
Corticosterone
500
400
300
200
100

Figure 3

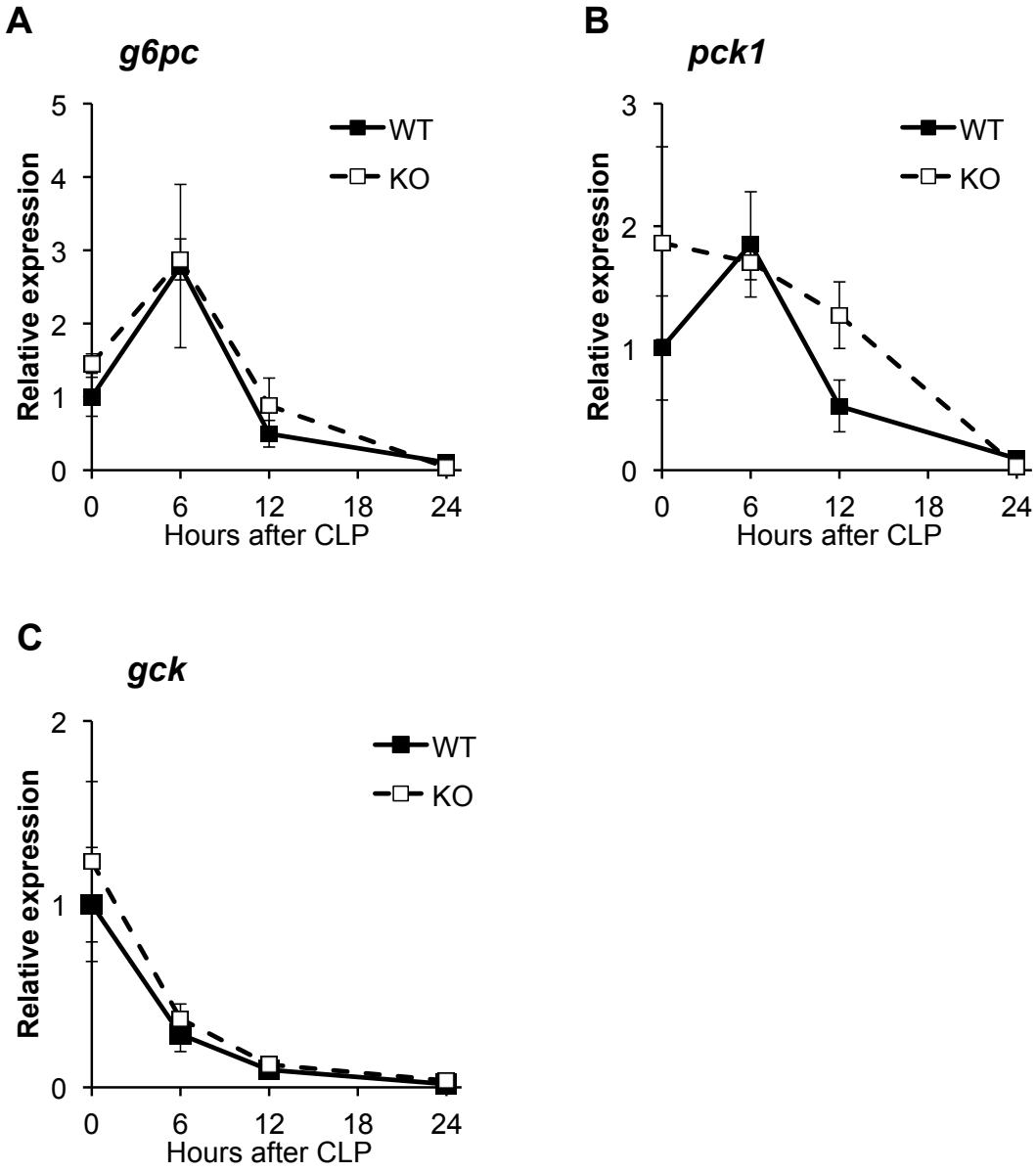

A
g6pc
Relative expression
WT
KO
Hours after CLP
B
pck1
Relative expression
WT
KO
Hours after CLP
C
gck
Relative expression
WT
KO
Hours after CLP

Figure 4

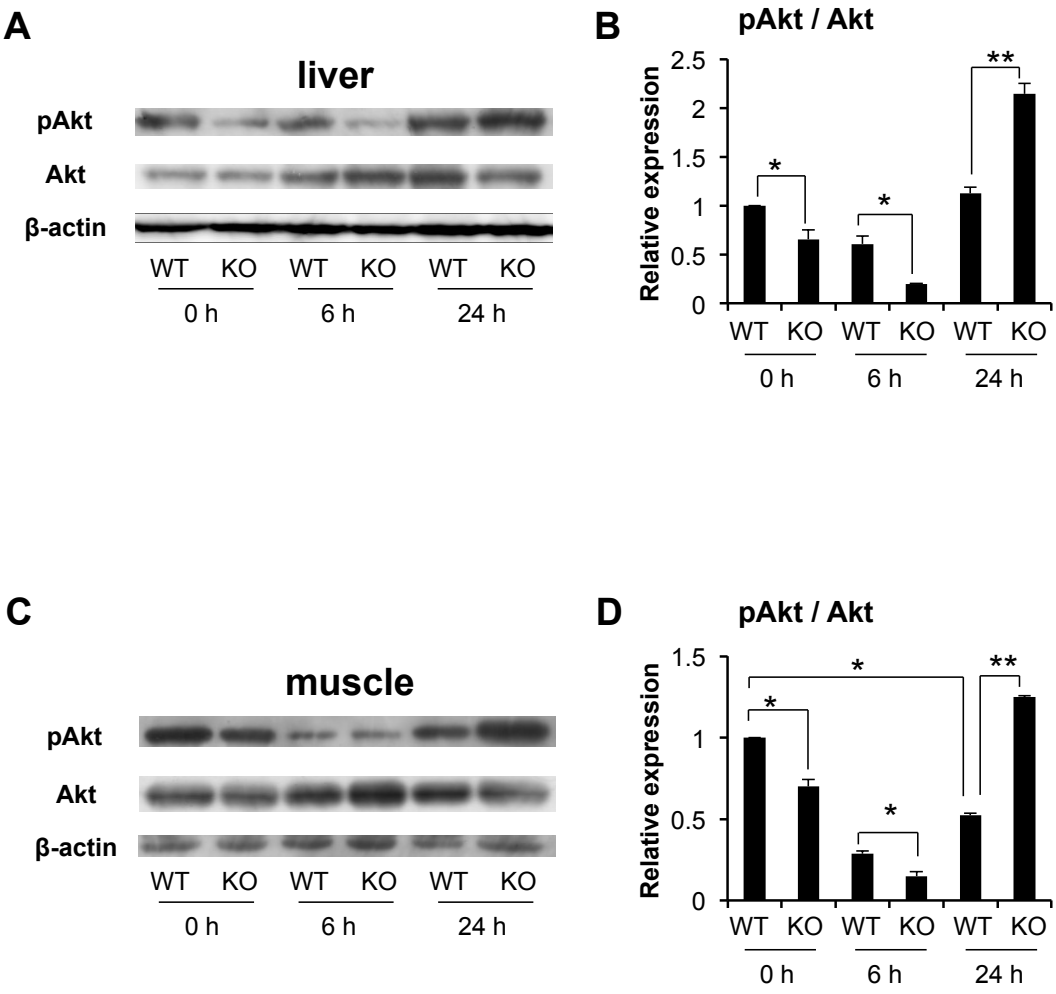

A
liver
pAkt
Akt
β-actin
WT KO WT KO WT KO
0 h 6 h 24 h
B
pAkt / Akt
Relative expression
2.5
2
1.5
1
0.5
0
*
*
**
WT KO WT KO WT KO
0 h 6 h 24 h
C
muscle
pAkt
Akt
β-actin
WT KO WT KO WT KO
0 h 6 h 24 h
D
pAkt / Akt
Relative expression
1.5
1
0.5
0
*
*
*
**
WT KO WT KO WT KO
0 h 6 h 24 h

Figure 5

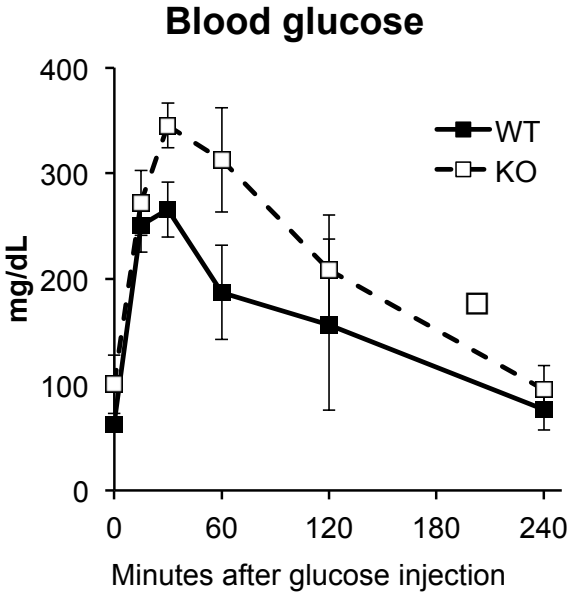
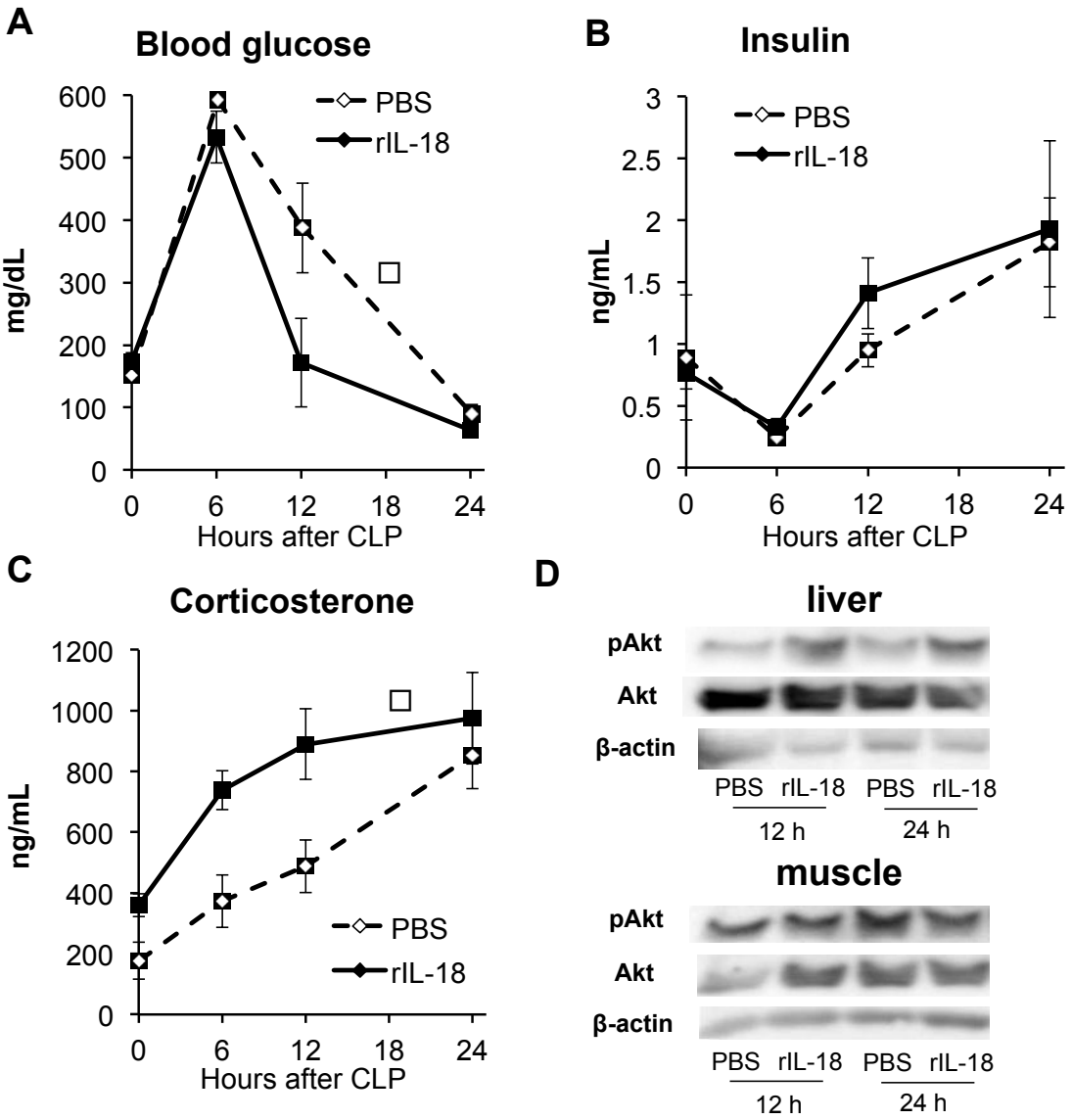

Blood glucose
400
300
200
100
0
mg/dL
WT
KO
0
60
120
180
240
Minutes after glucose injection

Figure 6


A
Blood glucose
mg/dL
600
500
400
300
200
100
0
PBS
rIL-18
0
6
12
18
24
Hours after CLP
B
Insulin
ng/mL
3
2.5
2
1.5
1
0.5
0
PBS
rIL-18
0
6
12
18
24
Hours after CLP
C
Corticosterone
ng/mL
1200
1000
800
600
400
200
0
PBS
rIL-18
0
6
12
18
24
Hours after CLP
D
liver
pAkt
Akt
β-actin
PBS rIL-18 PBS rIL-18
12 h
24 h
muscle
pAkt
Akt
β-actin
PBS rIL-18 PBS rIL-18
12 h
24 h

Supplemental Methods

Blood analysis

Blood samples were obtained by cardiac puncture. Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cre), urea nitrogen (UN), lactate dehydrogenase (LD), and creatine kinase (CK) levels were measured by an outside laboratory (LSI Medience Corporation; Tokyo, Japan).

Histological pathology

Lung samples taken before cecal ligation and puncture (CLP) or 24 hours after CLP were immersed in fixative solution (4% paraformaldehyde in PBS) for 24 hours, dehydrated through graded alcohol dilutions, and embedded in paraffin. Tissue was sectioned and stained with hematoxylin and eosin (H&E) using standard protocols. The average alveolar septal thickness in the lungs was quantified by measuring 50 septa at 400X magnification. Polymorphonuclear neutrophils were stained using a naphthol AS-D chloroacetate esterase staining kit (Sigma Diagnostics) and identified by nuclear morphology stained in bright red. All histological analyses were performed in 10 high power fields (HPFs) per sample with the samples' identities masked.

Supplemental Results

Tissue histopathology

To assess the effect of interleukin (IL)-18 deletion on organ damage in multiple organs, biochemical tests (AST, ALT, LD, CK, Cre, UN) and morphological assessments were performed 24 hours after CLP operation. There were no significant differences in the AST, ALT, LD, CK, and Cre levels in the blood in response to either CLP operation or IL-18 status (Supplemental Table 1). UN levels were significantly increased by CLP both in the wild type (WT) and IL-18 knockout (KO) mice (Supplemental Table 1).

Next, we examined lung morphology after CLP in the presence and absence of IL-18 (Supplemental Figure 1). Prior to CLP, there was no difference in lung morphology between WT and IL-18 KO mice. After CLP, there was neutrophil accumulation and septal thickening in both the WT and IL-18 KO mice, with more neutrophil accumulation in the lungs of the IL-18 KO mice than in the WT mice ($p<0.01$).

List of Abbreviations

ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
CK	creatine kinase
CLP	cecal ligation and puncture
Cre	creatinine
H&E	hematoxylin and eosin
HPFs	high power fields
IL	Interleukin
KO	knockout
LD	lactate dehydrogenase
UN	urea nitrogen
WT	wild type

Supplemental Figure Legends

Supplemental Figure. 1. Lung morphology after CLP. A. Naphtol AS-D chloroacetate esterase staining. Black arrowheads showed alveolar septal thickening, and black arrows showed a polymorphonuclear neutrophils. B. Alveolar septa thickness. C. The number of neutrophil per high power field. **, $p<0.01$ by ANOVA with the Tukey-Kramer post hoc test.

Supplemental Table 1: Blood biochemistry after induction of sepsis by CLP

	CK (IU/L)	LD (IU/L)	AST (IU/L)	ALT (IU/L)	Cre (mg/dL)	UN (mg/dL)
WT 0 hours	1295±572	463±171	257±91	20±2	0.23±0.03	28±3
KO 0 hours	1303±108	483±23	307±24	38±15	0.30±0.06	26±3
WT 24 hours	1502±608	634±184	503±125	86±15	0.52±0.10	85±14*
KO 24 hours	3912±1009	1838±490	538±110	157±45	0.40±0.14	101±10**

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CLP, cecal ligation and puncture; Cre, creatinine; KO, knockout; LD, lactate dehydrogenase; UN, urea nitrogen; WT, wild type. *, $p<0.05$; **, $p<0.01$ vs. 0 hour by ANOVA with the Tukey-Kramer *post hoc* test.

Supplement figure 1